19.0429.04000

FISCAL NOTE

Requested by Legislative Council
03/20/2019

Amendment to: HB 1417

1 A. **State fiscal effect:** *Identify the state fiscal effect and the fiscal effect on agency appropriations compared to funding levels and appropriations anticipated under current law.*

	2017-2019 Biennium		2019-2021 Biennium		2021-2023 Biennium	
	General Fund	Other Funds	General Fund	Other Funds	General Fund	Other Funds
Revenues						
Expenditures		$30,000				
Appropriations						

1 B. **County, city, school district and township fiscal effect:** *Identify the fiscal effect on the appropriate political subdivision.*

	2017-2019 Biennium	2019-2021 Biennium	2021-2023 Biennium
Counties			
Cities			
School Districts			
Townships			

2 A. **Bill and fiscal impact summary:** *Provide a brief summary of the measure, including description of the provisions having fiscal impact (limited to 300 characters).*

The bill authorizes an enhanced amount for dried leaves or flowers to a patient with a debilitating medical condition of cancer. Removes the additional authorization for dried leaves or flowers, changes the definition of a written certification, and increases the purchase amount to 4,000 milligrams.

B. **Fiscal impact sections:** *Identify and provide a brief description of the sections of the measure which have fiscal impact. Include any assumptions and comments relevant to the analysis.*

All sections of the bill have a fiscal impact. The changes to the written certification form, the addition of an enhanced amount of dried leaves or flowers, and the removal of the additional authorization for dried leaves or flowers, and increasing the limit all require programming changes to the information technology system.

3. **State fiscal effect detail:** *For information shown under state fiscal effect in 1A, please:*

A. **Revenues:** *Explain the revenue amounts. Provide detail, when appropriate, for each revenue type and fund affected and any amounts included in the executive budget.*

B. **Expenditures:** *Explain the expenditure amounts. Provide detail, when appropriate, for each agency, line item, and fund affected and the number of FTE positions affected.*

Due to the emergency clause in this bill an estimated $30,000 is required in the 2017-19 biennium to complete necessary program and coding changes to the information technology system. This would be a payment made to the information technology vendor. The Department of Health would use special funds derived from fees to pay for the costs associated with the changes.

C. **Appropriations:** *Explain the appropriation amounts. Provide detail, when appropriate, for each agency and fund affected. Explain the relationship between the amounts shown for expenditures and appropriations. Indicate whether the appropriation or a part of the appropriation is included in the executive budget or relates to a continuing appropriation.*

N/A

Name: Brenda M Weisz

Agency: ND Department of Health

Telephone: 701-328-4542

Date Prepared: 03/21/2019

19.0429.03000

FISCAL NOTE

Requested by Legislative Council
02/13/2019

Amendment to: HB 1417

1 A. **State fiscal effect:** *Identify the state fiscal effect and the fiscal effect on agency appropriations compared to funding levels and appropriations anticipated under current law.*

	2017-2019 Biennium		2019-2021 Biennium		2021-2023 Biennium	
	General Fund	Other Funds	General Fund	Other Funds	General Fund	Other Funds
Revenues						
Expenditures				$30,000		
Appropriations						

1 B. **County, city, school district and township fiscal effect:** *Identify the fiscal effect on the appropriate political subdivision.*

	2017-2019 Biennium	2019-2021 Biennium	2021-2023 Biennium
Counties			
Cities			
School Districts			
Townships			

2 A. **Bill and fiscal impact summary:** *Provide a brief summary of the measure, including description of the provisions having fiscal impact (limited to 300 characters).*

The bill authorizes an enhanced allowable amount for dried leaves or flowers for a qualifying patient with a debilitating medical condition of cancer. The bill also removes the additional authorization for dried leaves or flowers and changes the definition of a written certification.

B. **Fiscal impact sections:** *Identify and provide a brief description of the sections of the measure which have fiscal impact. Include any assumptions and comments relevant to the analysis.*

All sections of the bill have a fiscal impact. The changes to the written certification form, the addition of an enhanced amount of dried leaves or flowers, and the removal of the additional authorization for dried leaves or flowers all require programming changes to the information technology system.

3. **State fiscal effect detail:** *For information shown under state fiscal effect in 1A, please:*

A. **Revenues:** *Explain the revenue amounts. Provide detail, when appropriate, for each revenue type and fund affected and any amounts included in the executive budget.*

B. **Expenditures:** *Explain the expenditure amounts. Provide detail, when appropriate, for each agency, line item, and fund affected and the number of FTE positions affected.*

An estimated $30,000 is required to complete necessary program and coding changes to the information technology system. This would be a payment made to the information technology vendor. The Department of Health would use special funds derived from fees to pay for the costs associated with the changes.

C. **Appropriations:** *Explain the appropriation amounts. Provide detail, when appropriate, for each agency and fund affected. Explain the relationship between the amounts shown for expenditures and appropriations. Indicate whether the appropriation or a part of the appropriation is included in the executive budget or relates to a continuing appropriation.*

All fees received under the Medical Marijuana Program are deposited into a special fund. The program operates under a continuing appropriation as established in NDCC. No appropriation is required.

Name: Brenda M Weisz

Agency: ND Department of Health

Telephone: 701-328-4542

Date Prepared: 02/13/2019

19.0429.02000

FISCAL NOTE

Requested by Legislative Council
01/18/2019

Bill/Resolution No.: HB 1417

1 A. **State fiscal effect:** *Identify the state fiscal effect and the fiscal effect on agency appropriations compared to funding levels and appropriations anticipated under current law.*

	2017-2019 Biennium		2019-2021 Biennium		2021-2023 Biennium	
	General Fund	Other Funds	General Fund	Other Funds	General Fund	Other Funds
Revenues						
Expenditures				$30,000		
Appropriations						

1 B. **County, city, school district and township fiscal effect:** *Identify the fiscal effect on the appropriate political subdivision.*

	2017-2019 Biennium	2019-2021 Biennium	2021-2023 Biennium
Counties			
Cities			
School Districts			
Townships			

2 A. **Bill and fiscal impact summary:** *Provide a brief summary of the measure, including description of the provisions having fiscal impact (limited to 300 characters).*

The bill authorizes an enhanced allowable amount for dried leaves or flowers, changes the definition of bona fide provider-patient relationship and written certification, adds to the list of debilitating medical conditions, and removes the additional authorization for dried leaves or flowers.

B. **Fiscal impact sections:** *Identify and provide a brief description of the sections of the measure which have fiscal impact. Include any assumptions and comments relevant to the analysis.*

All sections of the bill have a fiscal impact. The changes to the written certification form, the addition of an enhanced amount of dried leaves or flowers, and the removal of the additional authorization for the use of dried leaves or flowers all require programming changes to the information technology system.

3. **State fiscal effect detail:** *For information shown under state fiscal effect in 1A, please:*

A. **Revenues:** *Explain the revenue amounts. Provide detail, when appropriate, for each revenue type and fund affected and any amounts included in the executive budget.*

B. **Expenditures:** *Explain the expenditure amounts. Provide detail, when appropriate, for each agency, line item, and fund affected and the number of FTE positions affected.*

An estimated $30,000 is required to complete necessary program and coding changes to the information technology system. This would be a payment made to the information technology vendor. The Department of Health would use special funds derived from fees to pay for the costs associated with the changes.

C. **Appropriations:** *Explain the appropriation amounts. Provide detail, when appropriate, for each agency and fund affected. Explain the relationship between the amounts shown for expenditures and appropriations. Indicate whether the appropriation or a part of the appropriation is included in the executive budget or relates to a continuing appropriation.*

All fees received under the Medical Marijuana Program are deposited into a special fund. The program operates under a continuing appropriation as established in NDCC. No appropriation is required.

Name: Brenda M Weisz

Agency: ND Department of Health

Telephone: 701-328-4542

Date Prepared: 01/21/2019

2019 HOUSE HUMAN SERVICES

HB 1417

2019 HOUSE STANDING COMMITTEE MINUTES

Human Services Committee
Fort Union Room, State Capitol

1/23/2019
HB 1417
31290
☐ Subcommittee
☐ Conference Committee

Committee Clerk: Elaine Stromme

Explanation or reason for introduction of bill/resolution: Relating to medical marijuana written certifications; and to amend and reenact subsections relating to access to medical marijuana.

Testimony: 1 - 8

Chairman Weisz: Opened Hearing on HB 1417: then **Vice Chairman Rohr** took over;

Representative Vetter: 3:51 –14:58 Introduced the HB1417. (Testimony #1)
I would like an amendment (Testimony #2) Based on the testimony from many different people.

Chairman Weisz: Thank You.

Representative Porter: There seems to be a conflict in your testimony.

Representative Vetter: I am scaling it back slightly so it is more accessible.

Representative Porter: In lines 27 – 29 that one of the requirements on the Health Care Provider is that they have a reasonable expectation that the individual seeking the treatment will come back for follow-up care**.** They are not there just to get a Medical Marijuana Card.

Representative Vetter: On page 6, I did add in that if you see a doctor once or twice that you have a patient doctor relationship or is there a certain amount of time that I have to see them? Or see them afterwards?

Representative Porter: On the original ballot language we seem to go back and forth.
It says that a physician is to manage the well-being of a qualified patient. You are now taking the language out that we came up with in the last session to fit that ballot language, was that relationship and that ongoing relationship. It is really making it less of a patient provider relationship.

Representative Vetter: I am just taking out parts of it, I am not taking out the full patient provider relationship. I am just taking out these added parts, that the provider maintains records, the provider maintains care, I don't see an issue.

Representative Westlind: They would only have to go to a Doctor once to get a medical marijuana card. Most Medical providers say they cannot have a good relationship with a patient unless they have multiple visits. I think this would be a problem that we are taking out too many regulations here.

Representative Vetter: I don't see how this would be a problem to allow more people to have access. If you see him one or two times, I don't see a problem at all.

Representative Westlind: Also the patient needs to come back for follow-up care and there is no provision for that in here.

Representative Vetter: ok.

Representative Westlind: You are allowing for an enhanced allowable amount and of course that is authorized by the Health Care Provider, if they aren't treating the condition which was taken out how does that Health Care Provider know how much the patient should have.

Representative Vetter: The enhanced amount that you see on there, is kind of confusing, Actually this section has to do with the condition of cancer.

Chairman Weisz: They don't have to treat the condition.

Representative Vetter: This just has to do with the medical condition of cancer.

Representative Skroch: Does the person measuring out the dosage have any pharmaceutical training?

Representative Vetter: Talking about the doctor patient relationship the care provider probably knows more about the cannabis than the doctor does, that is kind of one of the issues too having to protect doctors.

28:00 – 35:00 **David Owen:** I am the chairman of legalized ND**;** Page 2 line 19 – 21. I would like to answer some of the questions, you brought up, like the doctor patient relationship. The healthcare provider has concluded a medical assessment. It doesn't take many meetings to determine this. Also the Federal Law states that doctors cannot write a prescription for medical marijuana for a patient. You have to move it to a conditional certification system. What this does is stops the fear that doctors have that they are breaking federal law as a Result they cannot give their patient the go ahead to move forward. This bill fixes that problem.

Chairman Weisz : Any further support?

Steven James Peterson: of Compassionate Care of ND (Testimony #3)
Christopher Howell: Representing the Veterans for safe access to cannabis.(Testimony# 4

Chairman Weisz: Under the current law how would you go about getting the doctors recommendation?

Christopher Howell: I can't, it is impossible for someone at the VA to get it. Because they are not allowed to issue recommendation, or speak about it. We need an alternative.

Representative Tveit: If you would see a civilian doctor would that jeopardize your relationship with the VA?

Chris Howell: It could the way the federal law is written, but currently the Justice Department and the VA is not going after anyone for using medical cannabis outside of the VA system. That is inclusive in 30 different states.

Chairman Weisz: Further testimony in support?

41:26 **Jodi Vetter**: I am in support of this bill (Testimony #5)

Halely Wiley: I have 3 children, I get migraines the only help I can get is from Medical Marijuana.

Chairman Weisz: Any other support?

Waylen Pretends Eagle: I am representing the Committee for Compassionate Care, I just have one comment to add. This is only a plant, and we should be helping people.
Doctors would like protection from the federal law. I support this bill.

Robert Efrmenko: I am a user; I am a Vietnam vet. I would like to see a law that would take care of all of us.

Chairman Weisz: Support?

Steven Schalger: I am from Elgin, ND, I have been diagnosed with a form of leukemia**.** My doctor is in favor of Medical Marijuana but I would have to be in severe pain, this is coming. So anything you can do to help would be appreciated.

Chairman Weisz: Support?

Chris Nolden: I am not a doctor, but I would like to see this bill pass.

Chairman Weisz: None, thank you. Any further support? Opposed?

60:00 – 1:01 **Jason Wahl:** Director of the division of Medical Marijuana with the Department of Health – Testimony # 6

Testimonies 7 & 8 were handed in for lack of time, no oral testimony.

Chairman Weisz: Hearing on HB 1417 Closed.

2019 HOUSE STANDING COMMITTEE MINUTES

Human Services Committee
Fort Union Room, State Capitol

HB 1417
2/6/2019
32328

☐ Subcommittee
☐ Conference Committee

Committee Clerk Signature Nicole Klaman

Explanation or reason for introduction of bill/resolution:

Relating to medical marijuana written certifications and relating to access to medical marijuana

Minutes:

Chairman Weisz: 1417 is allowing enhanced condition for cancer.

Rep. Todd Porter: Move to amend removing sections 2,3,4,7 and 8.

Chairman Weisz: Strictly will deal with enhanced amount for cancer.

Rep. Gretchen Dobervich: If we leave out section 5, it would allow for enhancing for anything not just cancer.

Chairman Weisz: The bill will become section 1, 5 and 6
1 sets up enhanced means and can possess. 5 says what qualifies which is cancer. 6 to treat or alleviate medical condition for cancer. Everything else will go away.

Chairman Weisz: Amended bill will be 1,5 and 6 and removing the overstrike Section 5 line 25 and 26.

Rep. Westlind: Section 5 line 22 and 23, 24, Line 18 states can use an enhanced amount. Do we need section 6?

Rep. Schneider: Per department, it's on the card. This is why we need section 5

Voice Vote: Adopt Amendment remove section 2,3,4,7 and 8.
Motion to amend carries.

Rep. M Ruby: Did you not want to bring pediatric?

Chairman Weisz: That is in 1519. Is that what we want to do?

Rep. Westlind: we spoke about this, and felt that because a minor can be 10-200lbs. They added 12% THC would benefit up to the provider.

Rep. Dobervich: Would that be considered dosing?

Rep. Porter: when down to the specifics of milligram it is dosing.

Rep. Porter: Move to amend Section 5 line 25 and 26, remove overstrikes

Rep. Rohr: Second

Voice Vote: Motion Carries to further Amend

Derrick Wilke, Chief Operating Officer Health Dept: Trying to provide information on that but not a stance.

Chairman Weisz: What about dosing?

Derrick Wilke : Not discussed

Chairman Weisz: add 6-12%

Rep. Skroch: These extra provisions can be explained on the floor as to why. To have the option for a child dying of a brain tumor or late stages of autism. I feel it's important to include it.

Rep. Porter: Last session, pediatric component. All testifying experts defined the percent, going over that you are getting into a different part of the drug. I'm not comfortable going to the doubling of the percentage at this point.

Rep. Schneider: Recalling testimony, the level was too low for the autistic boy. Why go through the trouble if it's not going to work Can we find flexibility to allow for the law?

Chairman Weisz: The language is trying to allow for the flexibility.

Rep. Westlind: Should we drop it to 8%

Rep. Porter: I was going back to what experts said last session. They stated 6% was a safe and effective dose.

Rep. Skroch: If we do the enhanced dose, it's still left in the hands of the physician. Aren't they going to have the ability to discern?

Rep. Westlind: It's in the law right now, it would move it up.

Rep. Skroch: It's giving them an opportunity, if needed.

Rep. Schneider: Page 2 line 25 and 26; this allows the doctor discretion or expanded discretion?

Chairman Weisz: We are really divided on this. Either we want to make the allowance for enhanced.

Rep. Tveit: Somewhere in all this we have to trust the physician to make these decisions.

Rep. Dobervich: Do we have to put an amount in? There's no actual dosing guidelines

Rep. Rohr: There's no research.

Rep. Dobervich: The individual is already in the hands of a provider. Can we leave the parameters out to get around the dosing?

Chairman Weisz: I think to get the support it's best not to leave it blank.

Rep. Rohr: I think in the terminally ill child, medical marijuana isn't going to be the treatment being used.

Tara Brandner, Office of the Attorney General: Nothing in the written certification involves an amount.

Chairman Weisz: If the provider would give us an amount would that be helpful?

Tara Brandner: Hard question to answer for a number of reasons. First, completely open there are no parameters. Compromising 6%-12% may receive pushback due to doctor responsibility.

Tara Brandner: I think once you say above a range or below one, you get into dosing.

Rep. Schneider: Specificity came from where? Why do we have these? Who suggested we have them?

Chairman Weisz: Currently it's at 6%, which is safe to protect brain development.

Rep. Schneider: There's just so much specificity, and this is why were are stumbling.

Rep. M Ruby In 1417, that is representative Vetter's language and we were going to move to 1519, but that didn't happen.

Rep. Dobervich: If we are going to have a specific amount, 12% or under, does this change it?

Chairman Weisz: Let's not discuss the enhanced amount. Let's determine if we should have an enhanced amount for pediatric?
We aren't making forward movement.

Rep. Westlind: I wonder if we put this in 1417, will the enhanced amount get the 66% we need. Maybe just leave the enhanced amount for cancer patients.

Rep. M. Ruby: I think we should leave 1417 as amended. We are saying 6 is enough and we haven't tried it yet. **I move a Do pass as amended on HB 1417.**

Rep. Porter: Seconded.

Roll Call Vote: Yes: 11 No 2 Absent 1.

Do Pass As Amended Carries.

Rep. Westlind: Carrier

Chairman Weisz: Closes meeting.

Adopted by the Human Services Committee

February 6, 2019

PROPOSED AMENDMENTS TO HOUSE BILL NO. 1417

Page 1, line 1, remove "create and enact a new section to chapter 19-24.1 of the North Dakota"

Page 1, line 2, remove "Century Code, relating to medical marijuana written certifications; and to"

Page 1, line 3, remove ", 3, 15, 38,"

Page 1, line 3, after the fifth comma insert "and"

Page 1, line 3, remove the second ", and"

Page 1, line 4, remove "subsection 3 of section 19-24.1-11"

Page 2, remove lines 15 through 31

Page 3, remove lines 1 through 29

Page 4, remove lines 1 through 15

Page 4, line 25, remove the overstrike over "~~A written certification may not be~~"

Page 4, remove the overstrike over line 26

Page 6, remove lines 10 through 20

Renumber accordingly

Date: 2-6-19
Roll Call Vote #: 1

2019 HOUSE STANDING COMMITTEE
ROLL CALL VOTES
BILL/RESOLUTION NO. 1417

House Human Services Committee

☐ Subcommittee

Amendment LC# or Description:

Recommendation: ☒ Adopt Amendment
☐ Do Pass ☐ Do Not Pass ☐ Without Committee Recommendation
☐ As Amended ☐ Rerefer to Appropriations
☐ Place on Consent Calendar

Other Actions: ☐ Reconsider ☐

Motion Made By Rep. Porter Seconded By Rep. Westlind

Representatives	Yes	No	Representatives	Yes	No
Robin Weisz - Chairman			Gretchen Dobervich		
Karen M. Rohr – Vice Chairman			Mary Schneider		
Dick Anderson					
Chuck Damschen					
Bill Devlin					
Clayton Fegley					
Dwight Kiefert					
Todd Porter					
Matthew Ruby					
Bill Tveit					
Greg Westlind					
Kathy Skroch					

Total (Yes) No

Absent

Floor Assignment

If the vote is on an amendment, briefly indicate intent:

Motion to amend passes
Remove Section 2, 3, 4, 7 + 8

Date: 2-6-19
Roll Call Vote #: 2

2019 HOUSE STANDING COMMITTEE
ROLL CALL VOTES
BILL/RESOLUTION NO. 1417

House Human Services Committee

☐ Subcommittee

Amendment LC# or Description: _____

Recommendation: ☒ Adopt Amendment
☐ Do Pass ☐ Do Not Pass ☐ Without Committee Recommendation
☐ As Amended ☐ Rerefer to Appropriations
☐ Place on Consent Calendar

Other Actions: ☐ Reconsider ☐ _____

Motion Made By Rep. Porter Seconded By Rep. Rohr

Representatives	Yes	No	Representatives	Yes	No
Robin Weisz - Chairman			Gretchen Dobervich		
Karen M. Rohr – Vice Chairman			Mary Schneider		
Dick Anderson					
Chuck Damschen					
Bill Devlin					
Clayton Fegley					
Dwight Kiefert		✓	✓		
Todd Porter					
Matthew Ruby	✓				
Bill Tveit					
Greg Westlind					
Kathy Skroch					

Total (Yes) _____ No _____

Absent _____

Floor Assignment _____

If the vote is on an amendment, briefly indicate intent:

Section 5 Line 25 : 26
Remove Overstrikes

Motion Carries to Further Amend.

Date: 2-6-19
Roll Call Vote #: 3

2019 HOUSE STANDING COMMITTEE
ROLL CALL VOTES
BILL/RESOLUTION NO. 1417

House Human Services Committee

☐ Subcommittee

Amendment LC# or Description: _____

Recommendation: ☐ Adopt Amendment
☒ Do Pass ☐ Do Not Pass ☐ Without Committee Recommendation
☒ As Amended ☐ Rerefer to Appropriations
☐ Place on Consent Calendar

Other Actions: ☐ Reconsider ☐ _____

Motion Made By Rep. Ruby Seconded By Rep. Porter

Representatives	Yes	No	Representatives	Yes	No
Robin Weisz - Chairman	X		Gretchen Dobervich	X	
Karen M. Rohr – Vice Chairman	X		Mary Schneider		X
Dick Anderson	X				
Chuck Damschen		X			
Bill Devlin	X				
Clayton Fegley	X				
Dwight Kiefert	—	—			
Todd Porter	X				
Matthew Ruby	X				
Bill Tveit	X				
Greg Westlind	X				
Kathy Skroch	X				

Total (Yes) 11 No 2

Absent 1

Floor Assignment Rep. Westlind

If the vote is on an amendment, briefly indicate intent:

REPORT OF STANDING COMMITTEE

HB 1417: Human Services Committee (Rep. Weisz, Chairman) recommends **AMENDMENTS AS FOLLOWS** and when so amended, recommends **DO PASS** (11 YEAS, 2 NAYS, 1 ABSENT AND NOT VOTING). HB 1417 was placed on the Sixth order on the calendar.

Page 1, line 1, remove "create and enact a new section to chapter 19-24.1 of the North Dakota"

Page 1, line 2, remove "Century Code, relating to medical marijuana written certifications; and to"

Page 1, line 3, remove ", 3, 15, 38,"

Page 1, line 3, after the fifth comma insert "and"

Page 1, line 3, remove the second ", and"

Page 1, line 4, remove "subsection 3 of section 19-24.1-11"

Page 2, remove lines 15 through 31

Page 3, remove lines 1 through 29

Page 4, remove lines 1 through 15

Page 4, line 25, remove the overstrike over "~~A written certification may not be~~"

Page 4, remove the overstrike over line 26

Page 6, remove lines 10 through 20

Renumber accordingly

2019 SENATE HUMAN SERVICES

HB 1417

2019 SENATE STANDING COMMITTEE MINUTES

Human Services Committee
Red River Room, State Capitol

HB 1417
3/5/2019
Job # 33194

☐ Subcommittee
☐ Conference Committee

Committee Clerk: Justin Velez

Explanation or reason for introduction of bill/resolution:

Relating to medical marijuana written certifications and relating to access to medical marijuana.

Minutes: Attachment #1-6

Madam Chair Lee opens the hearing on HB 1417.

(00:20-09:25) Representative Steve Vetter, District 18. Introduces HB 1417 and provides testimony. Please see **Attachment #1** for testimony. Representative also supplied written testimony from **Aaron Basting Citizen of North Dakota included in Attachment #1**

Senator Hogan: Your condition was originally on HB 1519. Why would you like to add it to this bill versus that bill?

Representative Vetter: The reason is, is because the minor changes that are in mind with this bill is increasing the amounts for adults with cancer. HB 1519 has become the bill where all it does is list conditions, I wanted to keep everything on topic.

Senator Hogan: So this is the bill that includes enhanced amounts?

Representative Vetter: Correct.

Senator O. Larsen: You had listed the three trials that are taking place across the country, are they covered by insurance and how in depth are we into those trials?

Representative Vetter: I don't know if I have the best answer for you there is someone coming up after me that would be able to answer that question for you. A lot of these studies are about 2-3 years old because it hasn't really been studied until after it was legalized in a couple of states.

Senator Anderson: This study is based on growing these self-cultures in a petri dish and putting THC or CBD in with those self-cultures. It has nothing to do with taking oral THC and

pretending that they are going to cause cell death. I guess I'm a little confused about this bill here allowing you to eat cookies and this one which says we are going to put these cells in a petri dish and see what kills them. You just can't make that connection across.

Representative Vetter: I don't have a good answer for you. When it comes to some of the medical stuff it is somewhat foreign to me, I apologize.

Senator K. Roers: My background is oncology nursing; I keep seeing reference to an enhanced amount but I don't see the definition of what that means. Also one of the challenges which was one of the favorite parts of the original bill was removing the provider from having to certify that the patient would benefit from this because as Senator Anderson is pointing out in that study, there is very little robust research because it is still a Schedule 1. How would a provider even know how much a patient needs for efficient dosing?

Representative Vetter: I have heard from doctors at the University of Minnesota where they do a lot of these experiments that you were asking about. There is some research done but at the same time, I think the way to law currently is it just has the arbitrary number where you can only have 6% of THC and I don't know where that came from, with that low bar of only having 6% THC a lot of these different treatments, especially the treatments that would be used for, with minors you are going to have your more severe cases like the one I kind of showed you so it doesn't work the way it currently is because of the low bar. The doctors from the University of Minnesota claim that this arbitrary number doesn't work because we think for certain treatments we should use more and less comparison.

Senator K. Roers: I'm seeing that the current standard is 2.5 ounces purchased per month and a total of 3 ounces for the possession limit and then the enhanced amount increases that from 2.5oz. to 6oz. and from 3oz. to 7.5oz. Am I reading that correctly?

Representative Vetter: I believe so. Basically, the bill before you it just deals with enhanced for adults with cancer.

Senator K. Roers: The amendment doesn't necessarily change the amount it just allows the pediatric patient to be a part of that enhanced group?

Representative Vetter: What the amendment does is it allows the doctor, it doesn't set a certain standard of you can have this much or that much, it lets the doctor determine whether or not they need an enhanced amount.

Senator K. Roers: I think that is a noble goal but I think that you are going to find that, that will be a trip up point in the fact that we are struggling to get providers to even certify that people can have a card. I think execution may be more difficult than that.

Senator Anderson: I think here we are trying to bridge between a clinical trial where a physician is deciding how much the patient should have and really over the counter marijuana stuff that you can go in the store and buy. There is a big gap between the clinical trial being reproducible and a doctor saying that we should use this in a clinical trial when we can't even get a physician to say this might help you. That is why they don't want to say go get a card because the physician without the clinical trial and the proven track record is not willing to

say that this is going to work for this patient because he doesn't have the research to back it up. Increasing the amount in the cookies, the physician is still not going to prescribe the cookies for this person because it's not a part of a clinical trial. There are clinical trials but like Senator K. Roers says we are getting way ahead of ourselves when we are trying to go home and treat ourselves because some clinical trial is working on this project someplace else.

Representative Vetter: I see your point there. On one sides of things we don't want you to name the condition then here comes Representative Vetter with an amendment saying that well maybe for these minor we should raise the amount, if we could set an amount I would be ok with that it's just that nobody really knows that max amount should be. I know that most doctors aren't going to want to even make that recommendation but there are a few that will from I have researched. The doctors that I talked about earlier from the University of Minnesota they say that they want that ability to do that and I guess they have that ability in Minnesota, that is why I'm trying to add this amendment that is the purpose of it.

Senator Clemens: This is very close to what the previous two committee people have asked you about. The medical marijuana program has just been introduced within the last month and it is just getting started. What is really the reasoning behind a program that has just started and going to enhanced doses?

Representative Vetter: I would see your concern. The purpose of some of those things is that they were finding that a lot of the doctors didn't want to give the recommendations, so that was the purpose of the discussion of the access. I know that certain hospitals have said that they weren't going to allow the people and some of them said yes they will be getting that access was one of the driving forces.

Madam Chair Lee: That is separate from what we are talking about.

Representative Vetter: From what I have read with cancer patients, the amount that they use is considerably more than some of these other diseases and mainly because the biggest treatment for cancer is with chemo-therapy. I have personally seen it with people that have went through chemo-therapy and telling me personally that it is night and day from being sick to having the drive to eat, more energy, and not nauseous. That was one of the real driving forces behind cancer and if you notice that is why it is just cancer, we aren't increasing the amounts for everybody, we just want to make sure that the cancer patients have enough.

Madam Chair Lee: There are also legal drugs that would deal with the side effects of chemo-therapy. Two things I wanted to mention. FDA trials of drugs those that we can't count on to be pure because there have trials by the FDA start out with experimental processes in the lab then will use the drugs on healthy volunteers then use it on individuals for the certain diseases and we don't have any of that here. The Right to Try federal legislation that passed enables somebody at the end of life to have the option to negotiate with the manufacturer of that drug that has not yet reached the third step to be able to try it because if they are going to die anyways why wouldn't they try something that would have this potential. There were also articles written when the federal law was passed about the fact that there were children who were given some of these drugs under right to try and the terrible side effects that they had from them was an unfortunate problem that no one had anticipated. I don't know that we

can recognize any drug as not having some kind of side effects to it whether it is for children or adults. We have a particular responsibility to kids to make sure that they are not going to be harmed in all of this. I understand the system and the disease, if we open it up to cancer then somebody else with a debilitating disease is going to come and say why not me.

Representative Vetter: I would say to that yes, maybe all the Right to Try trials and FDA stuff hasn't been done but I look at it as when you have two sides of the equation where you have all these people who want these medical drugs and then I think what are the medications they are currently taking, they are getting opioids and other drugs that can essentially harm and kill them so what choice do we have?

Madam Chair Lee: There are meds that aren't opioids that will interfere, reduce, and eliminate the risk of nausea after chemo-therapy and some of those other conditions, there are medications out there that can currently be used to deal with those issues. The idea that medical marijuana is going to be all end all but it is just one more option and that is what your bringing to us and that is what we are going to be discussing.

Representative Vetter: On the medical marijuana side versus some of these other drugs you don't have the people dying from it and you don't have the addiction problems from it.

(28:53-29:55) Steven James Peterson lobbyist for The Committee for Compassionate Care of North Dakota. Testifying in support of HB 1417. Please see **Attachment #2** for testimony.

Senator Anderson: You are exactly making our point, that the patients who want this should enroll in a clinical trial and in this country the FDA does accept work done in Australia for drug approvals so you are making our point that we should stick with our clinical trials until we get resolution of it and then the practitioner can prescribe these things for the patient.

(31:18-37:00) Chris Noblen, Bismarck Cititzen. Testifying in support of HB 1417. Testimony is as follows: I do stand for the ability for cancer patients to have an enhanced amount. I don't have that exact number for you but in the amount of reading research I think that there is research across the world maybe not as much as we would like to see but, at this point these programs have been legal since 1996 in America. I do have some serious concerns about how this bill is written, I know what the intention is but I don't know if I see that in the writing. I know that the intention of the bill is to basically leave the rest of it alone and then give an enhanced amount to cancer patients. I am kind of concerned because right now how it sits, when a person applies for their card a doctor has to check a box and that box then allows the patient to have access to the natural flower form and if you look in the bill you can see where there are a couple of place where that seems to get crossed out. A person could construe this a couple of ways. How it is written now every adult patient would now have access to 2.5oz. of flower then you go down further and it says a health care provider may authorize, it crosses out "the use" so now we are crossing that out and replacing it with just an "enhanced amount of leaves" so what happened to the whole using flower part is that getting clarified anywhere in here that all adult patients qualify for the 2.5oz.? Personally, in all of the discussions that I have had, I have been trying to clarify that the whole way along. I personally believe that all adult medical program patients should have access

to the natural flower. I don't think that is a check mark that the doctor wants to deal with checking.

Senator K. Roers: As I read it I think in the process in trying to add this cancer part, you have actually made it so that the only way you can use the dried leave and flowers is for cancer.
Chris Noblen: There is that wording and Senator K. Roers I thank you for clarifying exactly what I am trying to say. I would also like to quick answer what I know about the pediatric amendment. I can tell you right now, that 6% number is not a mystery number. There is a study to show that for children of a certain height and weight that 6% is probably the max you want to go without possibly causing mental damage. There needs to be a determination between pediatric and adult because someone who is less than 19 years old isn't necessarily a pediatric patient. There are patients that are going to need this that may be 15,16, or 17 years old and they are like full grown adults. My son he is 220lbs and 3-4 inches taller me so if he were to need access to any of this medicine for any of the reasons on the list, chances are pediatric medicine wouldn't work so good for him. I don't want to give a 10lb baby 100% pure cannabis medicine, I think that is foolish. I do not want this pass if this is going to affect adversely adult patients access to the most natural form of the medicine which is the flower.

(43:05-48:26) Alexa Johnson, West Fargo Citizen. Testifying in support of HB 1417. Testimony is a follows: Two of my boys have autism and my nine year olds behavior are getting quite sever with violence. The second portion of HB 1519 which is now this amendment which is what I want to directly address with my idea. Our concern for the possibility of autistic children not being able to access all of the THC that they might need. I just want to clarify that this amendment addresses minors and not just cancer patients. Throughout my research to obtain better research for my children, I came across an international organization called Whole Plant Access for Autism. I became associated with two women Jenni Mai (**Attachment #3** for testimony) and her son is Nate, he has very severe autism and in the state of Missouri they were at the point where they could do nothing but institutionalize him, he was at the point that he was biting through his own lip. When the family moved to California and got access to medical cannabis within a span of 7 months Nate went from having to take 18 pills a day to zero. I want you to understand that this enhanced amount, I do have some specific numbers for you. My concern for autistic children is in milligrams for example, Nate started using cannabis at 18 which would be considered a minor in the state of North Dakota, sometimes on bad days he need up to 500 milligrams of THC a day and a lot of that was due to the fact that his mother was weening him off of very strong pharmaceuticals so that high THC amount not only helped him get off the pharmaceuticals it also helped him treat the withdrawal symptoms.

(46:26) Madam Chair Lee: Was she weening him off pharmaceuticals with the collaboration of a physician or was she just doing on her own.

Alexa Johnson: Yes. All parents that are using medical cannabis are very cautious about how hard it could be on our kid's systems.

(46:54) Alexa Johnson continues her testimony: If Nate Mai was 18 years old in the state of North Dakota he would have blown through his allowed amount of THC within two weeks. Another woman from California Sandy Christianson, (**Attachment #4** for testimony) who has

a young son. He uses THC for effective seizure control and also has autism, he absolutely needs to have 250 milligrams a day of THC to control his seizure so it is a very black and white figure, I want you to be aware of that too. His mother told me that we may have even given up on cannabis altogether and would have locked my son up in an institution or even be dead because of the amount of seizures he was having, that would have been if they did not have adequate access to enough THC. The reality of it is that they need the cannabis the most and I really feel like we need to be open minded about that and recognize that you have two checkpoints here before these kids get a card. The physician is going to have to feel good enough about checking that box saying that they can have more THC and the Division of Medical Marijuana has the final say over that card. (Please see **Attachment #5** for additional provided testimony from Tanya Stueve, Williston Citizen)

Senator O. Larsen: The individual seeking treatment in California with the increased amount of THC, what is the yearly costs of that therapy?

Alexa Johnson: It would range from child to child, I don't have numbers for you regarding that.

Madam Chair Lee: From your standpoint, the amendments provided by Representative Vetter expands that beyond cancer to conditions like autism and you support that?

Alexa Johnson: Absolutely.

(49:46-55:31) Jason Wahl, Director of the Division of Medical Marijuana for the Department of Health. Testifying in support of HB 1417. Please see **Attachment #6** for testimony.

(52:51) Madam Chair Lee: I visited with the folks at the Attorney General's office and they have some things that they are looking at as well so if you would in the end funnel that all through Jenifer Clark (Legislative Council) that would be great.

Jason Wahl: We can absolutely do that.

(53:10) Jason Wahl continues his testimony.

Senator K. Roers: If all of these bills passed and we got rid of the requirement for the provider to say that they would benefit from this would the dried leaves and flowers now become the enhanced amount?

Jason Wahl: That is the most simplistic way I would envision that.

Senator K. Roers: Would you have two different cards?

Jason Wahl: Right now the registry identification card has on there if they are authorized for dries leaves and flowers. One of the amendments that you will see is we need to change the section of the law about what is on a registry identification card because it says right now it needs the state whether to authorize for dried leaves and flowers, we would need to get language in there in regards to whether or not they are authorized for the enhanced amount

of dried leave and flowers. What that would look like we are going to have to work on but the card would be the identifier but the dispensary is required to validate the card every time that they come through the door.

Senator K. Roers: Do they have an online resource that they match?

Jason Wahl: They take the number that is on the card and enter it in the system.
Senator K. Roers: I just want to make sure I heard correctly so what you are saying is, based on other parts of code by changing that one section to add of cancer, you don't believe that negates others from being allowed because of other changes?

Jason Wahl: That enhanced amount for cancer patients would be specifically for cancer patients, all adult qualifying patients would now have access to dried leaves and flowers without any additional authorization.

Senator Clemens: Why weren't the amounts that we are talking about here not included in the original medical marijuana bill two years ago?

Jason Wahl: I wasn't associated with the program at that time but every time a new program is introduced to the state the Department of Health attempts to identify information from other states, have discussions in other states and attempt to setup amounts as best they can give the information at that time. Obviously the knowledge base about medical marijuana programs with the Department of Health given the short time period of when the initiated measure passed in November and coming to session in January, it was a limited time. We now have had two years to sit down and go through a lot more information, now that we are dispensing, seeing the product types, having manufacturers, and lean on the industry side more in relation to getting more information and ourselves becoming better educated about these programs. I think we are bringing forward certain things to legislator's attention that what we know now, we believe this is better information then what we had at the time. I think the state could in relation to the number of changes that were needed to that Compassionate Care Act in a very short period of time to get that done. If you recall, the senate did vote on for example, the maximum number of plants a manufacturing facility could have and changed that to be able to meet patient demand. We supported that change and it is a good change, back then 1,000 plants I think was maybe a reasonable number, knowing what we know now it's not the best number for the program. There will probably be in 2021 you will be hearing me answer something again in relation to how come we are changing this now and why are we looking at those things, just because things evolve and become more knowledgeable and we see the program being implemented we identify where changes are needed.

Senator K. Roers: Have you seen an increase in demand since the dispensary opened?

Jason Wahl: We certainly have seen an increase in demand on us returning phone calls and talking to qualifying patients. We receive several calls daily in regards to the program with the dispensary opening these past few days it has been a little more difficult to get back, we always attempt to get back to individuals the day that they call, that has been nearly impossible for us given our staffing level and the number of calls received. We have seen that since the dispensary opened Friday, we have not seen a big spike in applications at this point yet.

Madam Chair Lee closes the hearing on HB 1417

19.0429.03003
Title.04000

Adopted by the Senate Human Services Committee

March 13, 2019

3/13/19
1 of 3

PROPOSED AMENDMENTS TO ENGROSSED HOUSE BILL NO. 1417

Page 1, line 1, after "2" insert ", 38,"

Page 1, line 1, remove the third "and"

Page 1, line 2, after "19-24.1-03" insert ", subdivision a of subsection 5 of section 19-24.1-05, subsection 7 of section 19-24.1-10, section 19-24.1-11, subsection 4 of section 19-24.1-21, and subsection 10 of section 19-24.1-32"

Page 1, line 3, after "marijuana" insert "; and to declare an emergency"

Page 2, line 12, overstrike "two" and insert immediately thereafter "four"

Page 2, after line 12, insert:

"**SECTION 2. AMENDMENT.** Subsection 38 of section 19-24.1-01 of the North Dakota Century Code is amended and reenacted as follows:

38. "Usable marijuana" means a medical marijuana product or the dried leaves or flowers of the plant of the genus cannabis in a combustible delivery form. However, the term does not include ~~the dried leaves or flowers unless authorized through a written certification and does not include~~ a cannabinoid edible product. In the case of a registered qualifying patient who is a minor, "usable marijuana" is limited to pediatric medical marijuana."

Page 4, after line 4, insert:

"**SECTION 5. AMENDMENT.** Subdivision a of subsection 5 of section 19-24.1-05 of the North Dakota Century Code is amended and reenacted as follows:

a. The department receives documentation the minor's health care provider has explained to the parent or legal guardian with responsibility for health care decisions for the minor the potential risks ~~and benefits~~ of the use of pediatric medical marijuana ~~to treat or alleviate the debilitating medical condition~~; and

SECTION 6. AMENDMENT. Subsection 7 of section 19-24.1-10 of the North Dakota Century Code is amended and reenacted as follows:

7. A registered qualifying patient's certifying health care provider ~~shall~~may notify the department in writing if the health care provider's registered qualifying patient no longer has a debilitating medical condition ~~or if the~~. The health care provider ~~no longer believes the patient will receive therapeutic or palliative benefit from the medical use of marijuana~~may notify the department if a bona fide provider-patient relationship ceases to exist. The qualifying patient's registry identification card becomes void immediately upon the health care provider's notification of the department and the registered qualifying patient shall dispose of any usable marijuana in the cardholder's possession within fifteen calendar days, in accordance with rules adopted under this chapter.

SECTION 7. AMENDMENT. Section 19-24.1-11 of the North Dakota Century Code is amended and reenacted as follows:

19-24.1-11. Registry identification cards.

1. The contents of a registry identification card must include:

   a. The name of the cardholder;

   b. A designation as to whether the cardholder is a qualifying patient, designated caregiver, or compassion center agent;

   c. A designation as to whether a qualifying patient is a minor;

   d. A designation as to whether a qualifying patient or a designated caregiver's qualifying patient is authorized to use ~~the~~an enhanced amount of dried leaves or flowers of the plant of the genus cannabis to treat or alleviate the patient's debilitating medical condition of cancer;

   e. The date of issuance and expiration date;

   f. A random ten-digit alphanumeric identification number containing at least four numbers and at least four letters which is unique to the cardholder;

   g. If the cardholder is a designated caregiver, the random identification number of the qualifying patient the designated caregiver is authorized to assist;

   h. A photograph of the cardholder; and

   i. The phone number or website address at which the card can be verified.

2. Except as otherwise provided in this section or rule adopted under this chapter, a registry identification card expiration date must be one year after the date of issuance.

3. If a health care provider ~~states in the written~~limits certification ~~that the qualifying patient would benefit from the medical use of marijuana~~ until a specified date, less than one year, the registry identification card expires on that date.

SECTION 8. AMENDMENT. Subsection 4 of section 19-24.1-21 of the North Dakota Century Code is amended and reenacted as follows:

4. A dispensary or agent of the dispensary may not dispense usable marijuana unless the dispensary first uses the verification system to confirm the registered qualifying patient or registered designated caregiver identification card is valid. A dispensary or agent of the dispensary:

   a. May not dispense usable marijuana to a person other than a registered qualifying patient or a registered qualifying patient's registered designated caregiver. If a registered qualifying patient is a minor:

(1) The dispensary or agent of the dispensary may not dispense usable marijuana to a minor; and

(2) The usable marijuana dispensed to the minor's designated caregiver must be in the form of pediatric medical marijuana.

b. May not dispense to a registered qualifying patient or registered designated caregiver more than the allowable amount of usable marijuana and may not dispense an amount if it is known that amount would cause the recipient to purchase or possess more usable marijuana than is permitted under this chapter.

~~c. May not dispense to a registered qualifying patient or registered designated caregiver the dried leaves or flowers of the plant of the genus cannabis in a combustible delivery form unless the registry identification card and verification system authorize this form of usable marijuana.~~

SECTION 9. AMENDMENT. Subsection 10 of section 19-24.1-32 of the North Dakota Century Code is amended and reenacted as follows:

10. A health care provider is not subject to arrest or prosecution or the denial of any right or privilege, including a civil penalty or disciplinary action by a court or occupational or professional regulating entity, solely for providing a written certification or for ~~otherwise~~ stating in the health care provider's professional opinion a patient is likely to receive therapeutic or palliative benefit from the medical use of usable marijuana to treat or alleviate the patient's debilitating medical condition or for refusing to provide written certification or a statement. This chapter does not release a health care provider from the duty to exercise a professional standard of care for evaluating or treating a patient's medical condition.

SECTION 10. EMERGENCY. This Act is declared to be an emergency measure."

Renumber accordingly

Date: 3/18/19
Roll Call Vote #: 1

2019 SENATE STANDING COMMITTEE
ROLL CALL VOTES
BILL/RESOLUTION NO. 1417

Senate Human Services Committee

☐ Subcommittee

Amendment LC# or Description: .03002, two to four, shall to may

Recommendation: ☒ Adopt Amendment
☐ Do Pass ☐ Do Not Pass ☐ Without Committee Recommendation
☐ As Amended ☐ Rerefer to Appropriations
☐ Place on Consent Calendar

Other Actions: ☐ Reconsider ☐ _____

Motion Made By Sen. Hogan Seconded By Sen. K. Roers

Senators	Yes	No	Senators	Yes	No
Sen. Judy Lee	X		Sen. Kathy Hogan	X	
Sen. Oley Larsen	X				
Sen. Howard C. Anderson		X			
Sen. David Clemens		X			
Sen. Kristin Roers	X				

Total (Yes) 4 No 2

Absent 0

Floor Assignment _____

If the vote is on an amendment, briefly indicate intent:

Date: 3/13/19
Roll Call Vote #: 2

2019 SENATE STANDING COMMITTEE
ROLL CALL VOTES
BILL/RESOLUTION NO. 1417

Senate Human Services Committee

☐ Subcommittee

Amendment LC# or Description: _____

Recommendation: ☐ Adopt Amendment
☒ Do Pass ☐ Do Not Pass ☐ Without Committee Recommendation
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☐ Place on Consent Calendar

Other Actions: ☐ Reconsider ☐ _____

Motion Made By Sen. K. Roers Seconded By Sen. Hogan

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Sen. Oley Larsen	X				
Sen. Howard C. Anderson		X			
Sen. David Clemens		X			
Sen. Kristin Roers	X				

Total (Yes) 4 No 2

Absent 0

Floor Assignment Sen. K. Roers

If the vote is on an amendment, briefly indicate intent:

REPORT OF STANDING COMMITTEE

HB 1417, as engrossed: Human Services Committee (Sen. J. Lee, Chairman) recommends **AMENDMENTS AS FOLLOWS** and when so amended, recommends **DO PASS** and **BE REREFERRED** to the **Appropriations Committee** (4 YEAS, 2 NAYS, 0 ABSENT AND NOT VOTING). Engrossed HB 1417 was placed on the Sixth order on the calendar.

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   e. The date of issuance and expiration date;

   f. A random ten-digit alphanumeric identification number containing at least four numbers and at least four letters which is unique to the cardholder;

   g. If the cardholder is a designated caregiver, the random identification number of the qualifying patient the designated caregiver is authorized to assist;

   h. A photograph of the cardholder; and

   i. The phone number or website address at which the card can be verified.

2. Except as otherwise provided in this section or rule adopted under this chapter, a registry identification card expiration date must be one year after the date of issuance.

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Renumber accordingly

2019 CONFERENCE COMMITTEE

HB 1417

2019 HOUSE STANDING COMMITTEE MINUTES

Human Service Committee
Fort Union Room, State Capitol

HB 1417
4/8/2019
34606

☐ Subcommittee
☒ Conference Committee

Committee Clerk Signature Nicole Klaman

Explanation or reason for introduction of bill/resolution:

Relating to medical marijuana written certifications; and to amend and reenact subsections relating to access to medical marijuana.

Minutes:

Chairman Greg Westlind: Opened meeting

Chairman Westlind: Senators, please present explanation of your amendments.

Senator Judy Lee: I will start, but ask my colleagues to chime in on anything that I miss.

On page 2 we changed the cumulative total of both to 4000 mg, as we had been advised this was appropriate.

We eliminated the provider having to notified the cessation of a bona fide provider patient relationship and said "may inform". The reason for this was because there maybe situations when the patient moves out of state without the provider's knowledge. Other kinds of reasons for that, but at the time of the card renewal there would have to be continuation of care whether with the establishing provider or through a new provider.

Section 7 a designation on "D" that "the patient may use an enhanced amount of dry leaves or flower to treat or alleviate the patients debilitating medical condition of cancer", so that was added in.

Section 7 sub 3, if the healthcare provider limits the certification until a specific date less than 1 year, then the card expires on that date. This is a little different because the providers no longer have to say that there is a benefit recognized.
We added the emergency measure, to take effect as soon as possible.

Chairman Westlind: Page 6, subsection C; struck out "May not dispense dry leaves or flowers unless the registry has authorized. In other words, everyone who has a medical marijuana card has the option of smoking it or not smoking it they have the option of smoking it or not smoking it.

Senator Roers: Correct, that was a change that makes dry leaves and flowers available to all who are registered. The enhanced amount would replace the check box that is currently for dry leaves and flowers would be changed too.

Rep. Westlind: What was the reason for removing that?

Senator Roers: I believe it came from Rep. Vetter. He had brought us a large amount of amendments and that was the one we opted for, I think.

Senator Anderson: I think this kind of goes along with taking the doctor out and saying he doesn't have to tell you that this might help you. All the providers want to say is that you have a qualifying condition.

Rep. Westlind: My concern is whether this is going to bring it closer to recreational marijuana or is it a form of enhancement?

Senator Anderson: The intention of the committee was that it was an expansion of the medical marijuana. They still have to have the medical marijuana card if they are smoking it. Most of the people who initiated the measure were thinking about smoking it. It does bring it closer to recreational, but remember the medical marijuana card is still required.

Rep. Westlind: The Medical Marijuana Division is for this change. They said 75% of the people with cards have the distinction of being able to smoke it.

(0:07:07)
Senator Roers: I did write a note to myself stating that Jason Wall was in favor of eliminating that box to certify the leaves and flowers. I think it was a combination of everyone is getting it checked anyway and removing the physician to decide what format when they don't know the differences between each.

Rep. Fegley: Section 6, line 7; The health care provider "may" notify the department in writing if it's no longer needed. If it's may how does the department disqualify the card if they don't know that happened?

Senator Lee: I briefly mentioned that when we first went through it. It's not always possible to do that. My example of a patient moving out of state without the providers knowledge. Replacing "shall" with "may" leaves it optional and not required.

Senator Roers: Each card has a 1 year expiration, it's not an open ended date. We also looked at what is the penalty if you don't do it on a "shall" vs a "may", on the provider if they didn't know the patient moved out of state. How do you enforce that?

Rep. Fegley: The health care provider has to come up with an idea that the person no longer qualifies, so they must have seen that patient to make that determination, which would allow for him to notify the department.

Senator Roers: I do not disagree, I'm stuck on what the enforcement is. What's the teeth if they don't do it?

Senator Lee: I separated it a bit. First Section in 6 and second section in 6. I agree, it's one thing to say they no longer have the debilitating condition and I don't think any of us would disagree that we would like to see it severed at that point.
The second sentence, "they may notify the department if the relationship ceases to exist". This is the part we saw really challenging. The patient could have moved to a different provider or maybe from Fargo to Washburn. It just seemed as something that was going to be tough to follow up on and since part of the challenge has been the reluctance of providers due to us putting a lot of responsibility on them by to say "this will benefit" and we are trying to make them think about whether or not there is some potential use a patient will have for the medical marijuana and get providers, in appropriate circumstances, to write that recommendation in order for the individual to get the card. Some of these things get a little tougher but it can't cause any of us to lose a lot of sleep at night. We will find something that works for all of us.

Senator Anderson: Full disclosure here, I'm here because I voted against this. I wasn't particularly in favor of letting the providers off the hook. My contention is that the measure would not have passed if they wouldn't have the doctors giving people advice about it. Now there are others that would disagree.

Chairman Westlind: Rep. Schneider, would you like to add anything?

Rep. Schneider: Those were good explanations.

Rep. Westlind: Any further comments? Seeing none, we will discuss this information and meet again.

Closes the meeting.

2019 HOUSE STANDING COMMITTEE MINUTES

Human Services Committee
Fort Union Room, State Capitol

HB 1417
#34653
4/10/19
☐ Subcommittee
☒ Conference Committee

Committee Clerk: Nicole Klaman *~typed by* Jeanette Cook

Explanation or reason for introduction of bill/resolution:

Relating to medical marijuana written certifications; and to amend and reenact subsections relating to access to medical marijuana.

Minutes:

Chairman Westlind: Opened the conference committee on HB 1417.

Senator Lee: It would be good to know what the House's concerns were again.

Chairman Westlind: One concern was changing the word "Shall" to "May". We had lengthy discussions with Jason Wahl on that. Most of our committee does agree that the language is fine. There were concerns about practitioners making a business out of issuing medical marijuana cards. We are fine with the division of marijuana that they are very much aware of that. If they see a provider that comes up with six different recommendations for people to get a card in a week, it will be investigated to make sure it is legitimate.

Senator Lee: Are there any other changes that your members think are important?

Representative Westlind: That was most of our discussion. It was mentioned in committee that you were recommended raise the amount from 2000 mg to 4000 mg. What was the reasoning behind that?

Senator Roers: It had to do with the packaging that the manufacturer made. It made it easier for them be able to sell the product being manufactured. It wasn't that we wanted everyone to have larger amounts.

Senator Anderson: We also heard from practitioners, and they advised higher amounts to treat some chronic diseases to keep people from having to go back so often to get prescriptions.

Representative Westlind: I know from researching this that in certain varieties the THC can vary considerably. One cultivar can have way more than other.

Rep. Schneider: I think those were the concerns that were raised and addressed. I'm good with the changes that the Senate made.

Rep. Fegley: I concur with that.

Representative Westlind moved an amendment that the House accede to the Senate Amendments on HB 1417.
Representative Fegley seconded the motion.

A roll call vote was taken: Yes 6 No 0 Absent 0
The motion carried.

The committee was adjourned.

Date: 4/8/2019
Roll Call Vote #: 1

2019 HOUSE CONFERENCE COMMITTEE
ROLL CALL VOTES

HB 1417 as (re) engrossed

House Human Services Committee

Action Taken
- ☒ **HOUSE accede to Senate Amendments**
- ☐ **HOUSE accede to Senate Amendments and further amend**
- ☐ **SENATE recede from Senate amendments**
- ☐ **SENATE recede from Senate amendments and amend as follows**

- ☐ **Unable to agree**, recommends that the committee be discharged and a new committee be appointed

Motion Made by: Rep. Westlind Seconded by: Rep. Fegley

Representatives	4/8	4/10		Yes	No	Senators	4/8	4/10		Yes	No
Rep. Westlind, Chairman	x	x		x		Senator Judy Lee, Chairman.	x	x		x	
Rep. Fegley	x	x		x		Senator Anderson	x	x		x	
Rep. Schneider	x	x		x		Senator Roers	x	x		x	
Total Rep. Vote						Total Senate Vote					

Vote Count Yes: 6 No: 0 Absent: 0

House Carrier Rep. Westlind Senate Carrier Sen. K Roers

LC Number _____ . _____ of amendment

LC Number _____ . _____ of engrossment

Emergency clause added or deleted

Statement of purpose of amendment

REPORT OF CONFERENCE COMMITTEE

HB 1417, as engrossed: Your conference committee (Sens. J. Lee, Anderson, K. Roers and Reps. Westlind, Fegley, Schneider) recommends that the **HOUSE ACCEDE** to the Senate amendments as printed on HJ pages 1309-1312 and place HB 1417 on the Seventh order.

Engrossed HB 1417 was placed on the Seventh order of business on the calendar.

2019 TESTIMONY

HB 1417

HB-1417 #1 1-23-19 P1

Fwd: Medical Marijuana Question from Pediatric Oncology Patient

Vetter, Steve M.

Fri 1/18/2019 10:27 PM

To:Steve Vetter <steve@eappraisaloffice.com>;

Sent from my iPhone

Begin forwarded message:

From: Aaron Basting <aaron.basting@gmail.com>
Date: January 18, 2019 at 11:49:30 AM CST
To: smvetter@nd.gov
Subject: Medical Marijuana Question from Pediatric Oncology Patient

CAUTION: This email originated from an outside source. Do not click links or open attachments unless you know they are safe.

Steve,

My name is Aaron Basting and me and my family are residents of rural Thompson, ND. I know I'm not in your district but have seen that you have proposed a change in the medical marijuana law and feel that you may have a good understanding of some of the changes needed.

In December of 2017 my two year old daughter Emma was diagnosed with high risk Neuroblastoma, a rare form of a solid tumor pediatric cancer. Emma's tumor wrapped her spine and within two weeks of diagnosis it compressed her spine paralyzing her from the chest down.

Since then Emma has been through 6 rounds of chemo, three major surgeries, a stem cell transplant, 20 rounds of radiation, and is about to start her 5th of 6 total rounds of immunotherapy, almost wrapping up the Childrens Oncology Group's frontline protocol for high risk Neuroblastoma. Emma's case is unique as during her surgery they weren't able to remove all of her tumor so residual disease remains in the crevices of her spine, which would potentially cause further nerve damage if they tried removing it. With that said once her frontline protocol is finished we will have scans every three months in hopes that the tumor remaining will not grow and will stay in a dead or matured state. the Children's Oncology Group has no further protocol set to attack this disease.

All of Emma's treatments have taken place in MN and on top of the toxic cancer treatments she has had to take a number of dangerous medications for nausea and pain. Unfortunately because Emma is a ND resident she has not had access to medical marijuana like other kids being treated for similar and same cancers.

We've been frustrated with the time and complications that have been involved to get medical marijuana available in ND. I understand there is not adequate data that dates back years with information as to how much the drug can help with specific cancers but there is information out there that both THC and CBD can stop and slow cancer growth....specifically with Emma's cancer there is an in vitro in vivo study that has been done on mice to show that both THC and CBD can kill cancerous neuroblastoma cells. See links below to information on studies....

https://www.ncbi.nlm.nih.gov/pubmed/27022310
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4791143/
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5473390/

After going through the century code I'm extremely concerned that the state has plans to limit the THC in pediatric medical marijuana to 6%. In Emma's case most parents and oncologists nationwide have felt that a 50-50 ratio of CBD to THC is appropriate for Neuroblastoma. We want to remain ND residents but at the same time we want our daughter to have access to the best medications for her condition. With the program not currently actively working and in place it she does not have this access. And when it is place if she is limited by ND law to 6% THC, she still won't have access to the best options that are available in most other states.

Thank you for your consideration on this and I look forward to speaking with you further on this in hopes of changing the pediatric medical marijuana rule.

Aaron Basting
2074 6th Ave NE
Thompson, ND 58278

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page 3

JPPT The Journal of Pediatric Pharmacology and Therapeutics
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Cannabinoids in Pediatrics

Christopher T. Campbell, PharmD,[✉] Marjorie Shaw Phillips, MS, and Kalen Manasco, PharmD

✉Corresponding author.

Department of Pharmacotherapy and Translational Research (CTC, KM), University of Florida College of Pharmacy, Gainesville, Florida; Department of Pharmacy (CTC, KM), University of Florida Health Shands Children's Hospital, Gainesville, Florida; Department of Pharmacy (MSP), Augusta University Medical Center, Augusta, Georgia; Department of Clinical and Administrative Pharmacy (MSP), University of Georgia College of Pharmacy, Augusta, Georgia

Correspondence Christopher T. Campbell, PharmD; campbellc@cop.ufl.edu

Abstract

Despite its controversial nature, the use of medical marijuana and cannabis-derived medicinal products grows more popular with each passing year. As of November 2016, over 40 states have passed legislation regarding the use of either medical marijuana or cannabidiol products. Many providers have started encountering patients experimenting with cannabis products for a wide range of conditions. While the debate continues regarding these agents for both medicinal and recreational use in the general population, special consideration needs to be made for pediatric use. This review will deliver the history of marijuana use and legislation in the United States in addition to the currently available medical literature to equip pediatric health care providers with resources to provide patients and their parents the best recommendation for safe and appropriate use of cannabis-containing compounds.

Keywords: CBD, cannabidiol; cannabis; epilepsy; pediatrics; pharmacy

Introduction

Over the past several years, medical marijuana use has become a controversial topic not only within the medical community but also at state and national legislative levels. Although marijuana and its derivatives are currently Schedule 1 substances per the federal Controlled Substances Act (CSA), many states have relaxed their legislation to allow use. More recently, the use of cannabidiol (CBD) products in pediatrics has sparked additional debate, and pediatric providers have started encountering patients experimenting with these products in their daily practice, necessitating an understanding of the history and available medical literature on this topic.

Many of the misconceptions regarding medical marijuana in the pediatric population stem from negative connotations associated with the term *marijuana* owing to its psychoactive effects. Therefore, it is important to define the various terms associated with products that are currently being used by the public as well as by pediatric researchers. *Cannabis* is a general term that refers to the 3 species of hemp plants (*Cannabis sativa*, *Cannabis indica*, *Cannabis ruderalis*).[1] *Marijuana* is a term that describes the dried leaves, flowers, stems, and seeds from the hemp plant that are often smoked for recreational and medicinal use. Marijuana contains various different chemicals called *cannabinoids*.

Cannabinoids are the chemicals found within cannabis that interact with specific receptors, namely, cannabinoid (CB) receptors, within the body. The over 60 types of cannabinoids currently identified differ by the degree to which they are psychoactive.[2] While delta-9-tetrahydrocannabinol (THC), the cannabinoid most commonly associated with marijuana as a drug of abuse, is psychoactive, other cannabinoids including *CBD* are not. THC has been linked to the development of schizophrenia, and a contributor to neurodevelopment deficits in adolescents.[3,4] Different marijuana strains will have varying amounts of both THC and CBD, and thus the concentrations and ratios of these different cannabinoids within a product, especially for pediatric use, has been a subject of interest not only for medical professionals but also for state legislators as well.

History and Regulation

Dating back as far as 2000 BC, hemp plants had been used for various medicinal and industrial purposes. In 1851, the United States Pharmacopeia (USP) classified marijuana as a legitimate medical compound and many physicians supported its use for conditions such as epilepsy, chronic migraines, and pain.[5] Reports of Victorian-era neurologists using Indian hemp to treat epilepsy were also promising.[6] However, when phenobarbital and phenytoin came to the market in the early 1900s, the use of marijuana-based products declined.

In the 1930s, political propaganda sought to associate marijuana use, specifically by minority and low-income populations, with psychosis, addiction, and violent crime. Many believe this was influenced by several prominent businessmen in competing synthetic fiber industries in attempts to reduce the size of the growing hemp industry.[5] Marijuana soon became labeled as a drug of abuse and to discourage its use, Congress passed the Marijuana Tax Act of 1937 placing a heavy tax on cannabis and hemp use for both medicinal and industrial purposes. Despite opposition from the American Medical Association (AMA) and physicians who believed in the medical efficacy of marijuana, by 1941, all cannabis preparations were removed from the USP and National Formulary.

In the 1960s and early 1970s, marijuana soon became associated with recreational use by anti-establishment groups further adding to the stigma associated with its usage. By 1970, the CSA labeled cannabis as a Schedule 1 substance. This relatively short era of recreational marijuana use has influenced how the public perceives the drug. Since that time, there have been repeated unsuccessful attempts to reconsider its Schedule 1 status to allow for easier investigation.[5] The AMA and the American Academy of Pediatrics (AAP) have reaffirmed their opposition to the legalization of medical and recreational marijuana use outside of any US Food and Drug Administration (FDA) regulatory process.[7]

The AAP also supports further research into the indications and correct dosage for cannabinoids in addition to developing policy around how to verify purity and formulations.[8] In the meantime, the AAP has suggested good practices to follow when considering the use of marijuana, recreationally or medically (Table 1).

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Table 1.

Recommendations from the American Academy of Pediatrics[8]

Research and development should be conducted of pharmaceutical cannabinoids. The AAP recommends changing marijuana from a DEA Schedule 1 to a DEA Schedule 2 to facilitate this research.
The federal and state governments should establish robust health surveillance regarding the impact of marijuana, particularly on children and adolescents.
In states that have legalized marijuana for recreational use, the AAP strongly recommends strict enforcement of rules and regulations that limit access, marketing, and advertising to youth.
Where marijuana is sold legally, either for medicinal or recreational purposes, it should be contained in child-proof packaging to prevent accidental ingestion.
The AAP discourages adults from using marijuana in the presence of children because of the influence of role modeling by adults on child and adolescent behavior.

AAP, American Academy of Pediatrics; DEA, Drug Enforcement Administration

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To date, however, 8 states and the District of Columbia have passed legislation to legalize recreational marijuana use, with an additional 20 states allowing for some form of medical cannabis. Fourteen nonmedical marijuana states have specific legislation regarding CBD (Figure).[9–11] The changing legislative and regulatory landscape has significantly impacted the use of cannabinoid products in this country. Discussion about the safe and efficacious use of these products in a responsible way that protects vulnerable populations, including pediatrics, is necessary.

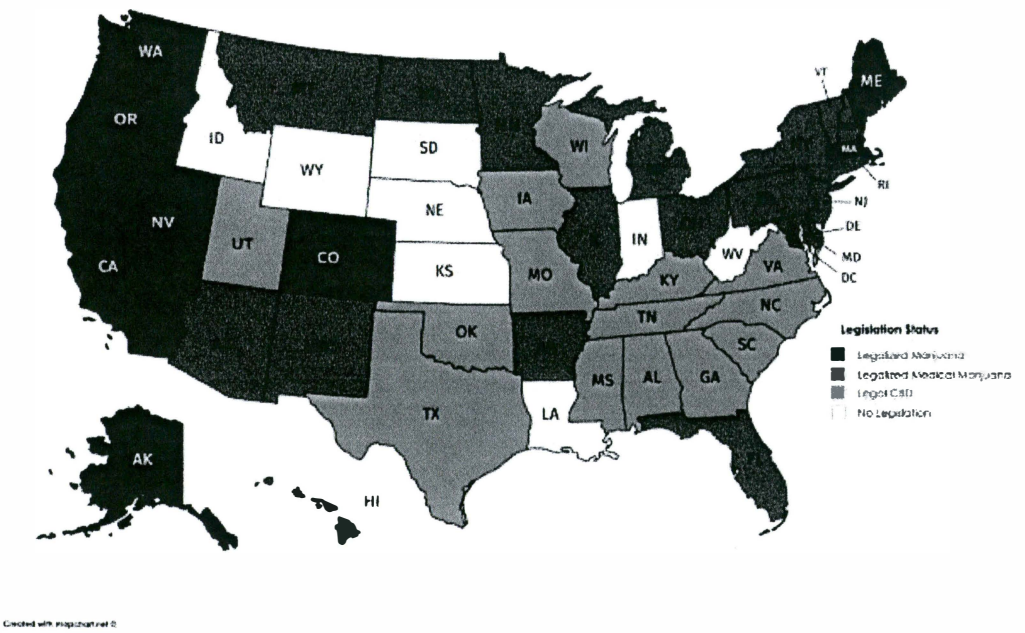

Figure.

2017 Legislative status of marijuana in the United States

Pharmacology

Similar to endogenous opioids, a human's central nervous system is impregnated with cannabinoid receptors and endocannabinoids. In the early 1990s, 2 receptors were discovered, cannabinoid type 1 (CB1) and cannabinoid type 2 (CB2). Both CB1 and CB2 are G-coupled protein receptors located presynaptically and control the release of neurotransmitters at both inhibitory and excitatory synapses. CB1 is mostly expressed on presynaptic peripheral and central nerve terminals and is believed to be responsible for psychologic effects on pleasure, memory, thought, concentration, sensory and time perceptions, and coordinated movement. CB2 receptors, concentrated in peripheral tissues and immune cells, may play an anti-inflammatory and immunosuppressive role. In addition to directing the release of various neurotransmitters, this receptor regulates the release of certain cytokines. Innervation of both these receptors results in both physiological (tachycardia, hypertension, dry mouth and throat) as well as psychological (elation, euphoria, heightened perception, irritability, poor coordination and balance) effects.[3,5]

Additionally, endocannabinoids N-arachidonoylethanolamine (anandamide) and 2-arachidonoylglycerol, both arachidonic acid derivatives, bind with CB1 and CB2. While the function of these endogenous ligands is not fully understood, their action may be attributed as antiemetic, antianalgesic, and anti-inflammatory. Endocannabinoids can also play a role in excitation of the neuronal networks, thus having effect on the quality of a seizure. Previous studies have documented deficiencies in endocannabinoids in temporal lobe epilepsy patients as well as a rise in anandamide concentrations post seizures in mice, suggesting an antiseizure activity profile.[6]

The 2 most studied exogenous cannabinoids include THC and CBD. THC is a partial agonist at both CB1 and CB2 receptors and achieves its psychoactive properties likely through modulation of gamma-aminobutyric acid (GABA) and glutamine. THC seems to possess antiseizure activity but may be a proconvulsant in certain species.[12] CBD, however, does not appear to bind to either CB1 or CB2 but does possess neuroprotective and anti-inflammatory effects.[5] Several possible mechanisms of CBD have been proposed: inhibition of cyclooxygenase and lipoxygenase, inverse agonism at CB1/CB2 receptors, and enhancement of anandamide.[3] It is proposed that CBD may be effective in epilepsy through modulation of the endocannabinoid system. CBD halts the degradation of the endocannabinoid anandamide, which may have a role in inhibiting seizures. Additionally, research demonstrates that CBD may play a role with the regulation of T-type calcium channels and nuclear peroxisome proliferator-activated receptor-γ, both of which have been implicated in seizure activity.[12] Because CBD is one of the most abundant cannabinoids within cannabis resin and its mechanism is still unclear, there is peaked interest in the possible clinical indications that it could treat including epilepsy, pain, and inflammatory disorders.

Several other synthetic forms of cannabinoids have been available for use in some countries, including dronabinol, nabilone, and nabiximols (Table 2). These products are being used to treat nausea and vomiting associated with chemotherapy, anorexia and weight loss in patients with acquired immune deficiency syndrome (AIDS), and relief of spasticity and neuropathic pain associated with multiple sclerosis (MS).[13–16] Epidiolex (GW Pharmaceuticals, Cambridge, United Kingdom) is a CBD product currently in clinical trials.[17]

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Table 2.

Synthetic Cannabinoid Products

Drug	Component(s)	FDA Approval Status	Indication
Dronabinol[13,15]	Synthetic THC	FDA approved – 1985	Treatment of nausea and vomiting associated with cancer chemotherapy in patients who have failed to respond adequately to conventional antiemetic therapy.
			Treatment of anorexia associated with weight loss in patients with acquired immune deficiency syndrome.
Nabilone[14,15]	Synthetic dimethylheptyl analogue of THC	FDA approved – 1985	Treatment of nausea and vomiting associated with cancer chemotherapy in patients who have failed to respond adequately to conventional antiemetic therapy.
Nabiximols[15,16]	Tetranabinex (plant-derived THC) and Nabidiolex (plant-derived CBD)	Not FDA approved Available in 16 countries outside of the United States	Symptomatic relief of spasticity in adults with multiple sclerosis who have not responded adequately to other therapy and who demonstrate meaningful improvement during an initial trial of therapy.
			Symptomatic relief of neuropathic pain in patients with multiple sclerosis. Intractable cancer pain.
Epidiolex[17]	Plant-derived CBD	Undergoing clinical trials	Clinical trials ongoing for epilepsy conditions.

CBD, cannabidiol; FDA, US Food and Drug Administration; THC, delta-9-tetrahydrocannabinol

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Pharmacokinetics

Historically, patients and recreational users have inhaled or vaporized marijuana, resulting in a quick onset and higher peak concentrations. Owing to first-pass metabolism, the enteral route decreases the bioavailability of THC to from 5% to 20% and CBD to from 6% to 19% and increases the time to onset.[3,18,19] Differences in absorption between various age groups, populations, and individual people make it difficult to recommend a one-size-fits-all dosage strategy. Interpatient variability may affect which blood concentrations will be effective, and tolerance is known to occur owing to downregulation of CB1 receptors.[3,18]

Both THC and CBD are highly lipophilic with long half-lives, 30 hours versus 9 to 32 hours, respectively.[3,18,20] CBD is also highly protein bound and is both metabolized by and a potent inhibitor of the CYP450 enzymes (2C19, 3A4), potentially causing significant medication interactions.[3,18,20,21] While CYP inducers such as phenytoin and carbamezapine may decrease CBD concentrations, CBD is known to increase concentrations of clobazam, an antiepileptic drug approved by the FDA in 2011 for the treatment of Lennox-Gastaut syndrome (LGS). CBD inhibits CYP3A4 and CYP2C19, preventing the degradation of clobazam and its active metabolite, N-desmethylclobazam. In an expanded access trial, patients with concomitant clobazam and CBD use had increases in clobazam concentrations of > 60% and N-desmethylclobazam, of 500%.[22] At this time it is not clear what other drug interactions may exist and what dosage manipulations may be necessary.

Clinical Data

The debate about the use of cannabinoid products in pediatric patients has persisted owing to the lack of well-developed and published randomized controlled trials. There has been a wide variety of mostly case series and international studies for adult indications, such as chronic pain, MS, headache, and various neuropsychiatric disorders, which are beyond the scope of this review but have been reviewed elsewhere.[20] The pediatric literature lacks the same breadth owing to public stigma and restrictions on investigational use. This has resulted in retrospective and parentally reported data in epilepsy and behavioral conditions. Despite the overall lack of published data on CBD in pediatric patients, most of the literature is devoted to its use in epilepsy. Current large prospective trials are underway for different epilepsy indications, and recent animal studies researching use in perinatal brain injury and neuroblastoma may open new avenues to consider cannabinoids for pediatrics.

Epilepsy. The data in pediatric epilepsy have been surrounding the use of CBD products as well as unregulated THC/CBD products from private dispensaries. A Cochrane review[23] was conducted in 2012 to assess the safety and efficacy of cannabinoid use in patients with epilepsy. The authors included blinded and unblinded randomized controlled trials. Only 4 studies met their criteria, including 1 abstract and 1 letter to the editor (Table 3). All 4 trials were of low quality with small sample sizes and variations in product, dose, frequency, and duration.[24–27] The authors summarized the finding that a CBD dose of 200 mg to 300 mg daily was safely administered over a short period. The only reasonable conclusion made was that the efficacy of CBD use could not be confirmed, but the rate of adverse reactions in each of the studies was low over a short period.

Table 3.

Included Studies in Cochrane Review[23]

Study	Patient Demographics	Intervention	Results
Mechoulam et al[24]	Nine patients with uncontrolled temporal lobe epilepsy who had failed multiple medications	Group 1: CBD 200 mg daily (n = 4) Group 2: Placebo daily (n = 5) Duration: 3 mo	Group 1: 2 patients seizure free entire 3 mo; 1 patient with partial improvement (not defined) Group 2: no patient improvement
Cunha et al[25]	Fifteen patients (11 female) with temporal lobe irritative activity; ages: 14–49 yr	Group 1: CBD 200–300 mg daily (n = 8) Group 2: Placebo (n = 7) Duration: 18 wk	Group 1: 50% (n = 4) of patients had complete improvement Group 2: 14% (n = 1) of patients with complete improvement
Ames[26]*	Twelve institutionalized, mentally retarded patients with frequent seizures	Group 1: CBD 300 mg daily for 1 wk, then CBD 200 mg daily (n = ?) Group 2: Placebo (n = ?)	No statistically significant difference in seizure frequency between the 2 groups
Trembly[27]†	Twelve patients with incompletely controlled epilepsy	Each patient served as his or her own control on the following schedule: • 3 mo of normal outpatient epileptics • 6 mo of placebo • 6 mo of CBD 100 mg 3 times a day	"No discernable effect" on MMPI, Beck depression inventory, trail-making test, and finger-tapping test

CBD, cannabidiol; MMPI, Minnesota Multiphasic Personality Inventory

* Letter to the Editor only available, resulting in incomplete data

† Abstract only available, resulting in incomplete data and lack of results

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The American Academy of Neurology conducted a systematic review in 2014 which included 34 studies that used medical marijuana to treat MS, epilepsy, and movement disorders.[28] The authors included 2 studies to assess the role of cannabinoids in decreasing seizure frequency.[25,26] Of note, these

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studies were also evaluated in the Cochrane review. The authors[28] concluded that "data are insufficient to support or refute the efficacy of cannabinoids for reducing seizure frequency" and thus, there is not sufficient evidence to advise patients to use cannabinoid products in epilepsy.

Despite this, parents and patients are making the decision to use these products for 3 reasons according to Cilio et al:[12] 1) prominent Internet and nation media attention; 2) reports of cases of children successfully treated with CBD products; and 3) the belief that treatments derived from natural products are safer or more effective.[12] National attention has been on those patients who have moved to states where CBD use is legal and researchers have sought to gather data from parental observations. It is important to note that the following studies are based on parental perceptions and thus we cannot draw definitive conclusions.

The most famous case was presented on a CNN special, "Weed."[29] Charlotte is a little girl from Colorado who was diagnosed with Dravet syndrome at the age of 3 months. She suffered from frequent status epilepticus. Charlotte failed multiple medications, and at 5 years of age, she had significant cognitive delay and required help with all of her activities of daily living.[30] Her parents sought out a group in Colorado that created an oral, liquid, high-concentration CBD-to-THC strain of cannabis. Once her parents started giving her this strain, dubbed "Charlotte's Web", within 3 months Charlotte had a > 90% reduction in her seizure frequency and by month 20, Charlotte was able to perform most of her daily activities independently with only 2 to 3 nocturnal tonic-clonic seizures per month. Stories like Charlotte's have prompted parents across the country in similar situations to move their families across the country to gain access to these products.

In a retrospective chart review of 75 children and adolescents younger than 18 years who were given oral cannabis extracts for treatment of their epilepsy, 57% of parents reported some improvement in seizure frequency with 33% reporting a >50% reduction in seizures.[31] Dosage information was not reported and parents used various formulations and concentrations of CBD and THC. Parents also described improvements in behavior and alertness (33%), language (11%), and motor skills (11%). Major adverse effects noted were somnolence (12%) and gastrointestinal symptoms (11%).

Investigators at Stanford University administered a survey to 150 parents on Facebook to identify parentally reported effects of CBD on their child's seizures.[32] Of 19 respondents aged 2 to 16 years, 18 had treatment-resistant epilepsy for more than 3 years before CBD use. Based on parental response, 84% reported a reduction in child's seizure frequency with 50% having a greater than 80% reduction in seizure frequency. Twelve of these 19 patients were also able to be weaned from another antiepileptic drug. In addition, parents reported overall better mood, increased alertness, and better sleep. Parents reported oral CBD dosages of 0.5 mg/kg/day to 28.6 mg/kg/day and THC of 0 to 0.8 mg/kg/day.

In a similar Facebook survey administered by researchers at the University of California, Los Angeles, the authors[33] similarly reported an 85% reduction in seizure frequency among 117 respondents, with an average age of 6 years. Most patients (86%) conveyed that changes in frequency occurred within 14 days. As with previous surveys, dosage and formulations were varied but based on parental report of formulation used. Overall, most parents (83.5%) reported using an oral CBD product with at least a 15:1 ratio of CBD to THC. Of the 40% of respondents who provided dosages, the median weight-based dose of CBD was 4.3 mg/kg/day given in 2 to 3 oral doses. As mentioned above, these surveys should be evaluated carefully given the inability to verify dose, formulation, and response. The conclusion that can be made is that there is a rather strong positive parental perception regarding the efficacy of cannabinoids, specifically CBD.

Most orphan drug designations for CBD are for pediatric seizure disorders (Table 4).[34] A search of ClinicalTrials.gov in November 2016 identified 4 completed Phase 2 and Phase 3 protocols for pediatric seizure disorders, as well as 14 ongoing treatment trials, including intermediate-size expanded

access protocols (up to 50 patients each). Published findings from open-label use of CBD for treatment-resistant epilepsy under an expanded-access program at 11 epilepsy centers in the United States suggest that CBD might reduce seizure frequency and might have an adequate safety profile in children and young adults with this condition.[35] Congressional testimony in June 2015 indicated that 20 intermediate-size expanded access Investigational New Drug Applications had been authorized to treat approximately 420 children with 1 CBD product; most of these are not listed on ClinicalTrials.gov.[36]

Table 4.

Orphan Drug Designations for Cannabidiol in the Treatment of Pediatric Conditions[34] *

Orphan Designation	Designation Date	Sponsor
Dravet syndrome'	November 14, 2013	GW Pharma Ltd
Lennox-Gastaut syndrome	February 24, 2014	GW Pharma Ltd
Lennox-Gastaut syndrome	June 23, 2014	Insys Development Company Inc
Dravet syndrome'	July 1, 2014	Insys Development Company Inc
Pediatric schizophrenia (pediatrics is defined as 0 through 16 years of age)	November 17, 2014	Insys Development Company Inc
Neonatal hypoxic ischemic encephalopathy'	April 22, 2015	GW Pharma Ltd
Infantile spasms	July 23, 2015	Insys Development Company Inc
Fragile X syndrome	February 23, 2016	Zynerba Pharmaceuticals Inc
Tuberous sclerosis complex	April 19, 2016	GW Pharma Ltd
Infantile spasms	June 13, 2016	GW Research Ltd

FDA, US Food and Drug Administration

* None of these products are FDA approved for orphan or any other indication

' Granted FDA Fast Track designation for this indication. A drug development program with Fast Track designation is afforded greater access to the FDA for the purpose of expediting the drug's development, review, and potential approval to get important new drugs to the patients earlier

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After announcing positive results from 2 pivotal randomized, double-blind, Phase 3 trials for the treatment of seizures related to LGS, and a third for seizures associated with Dravet syndrome in 2016, GW Pharmaceuticals expects to submit a single New Drug Application for both indications to the FDA in the first half of 2017 for its proprietary pharmaceutical-grade CBD product Epidiolex.[37] In the second LGS study, patients randomized to the investigational product 20 mg/kg/day (n = 76) or 10 mg/kg/day (n = 73) added to their current antiepileptic treatment, experienced a median reduction in monthly drop seizures of 42% and 37%, compared to 17% in the placebo group (n = 73); a difference that was statistically and clinically significant ($p = 0.0047$ and $p = 0.0016$, respectively).[37] These data confirmed the results of the first LGS trial in which 86 patients receiving Epidiolex 20 mg/kg/day achieved a 44% mean reduction in monthly drop seizures as compared to 22% for 85 patients in the placebo group ($p = 0.0135$).[38] Patients with Dravet syndrome receiving the GW Pharmaceuticals' CBD in addition to their baseline antiepileptic regimen (n = 61) achieved the primary endpoint of a significant reduction in convulsive seizures ($p = 0.01$, median reduction of 39%) assessed over the 14-week treatment period as compared with the addition of placebo (n = 59).[39] Insys Therapeutics (Phoenix, AZ) has reported that their synthetic pharmaceutical CBD in a nonalcoholic, medium-chain triglyceride-based formulation was generally well tolerated in a completed Phase 1/Phase 2 safety and pharmacokinetic study in 61 pediatric patients with treatment-resistant epilepsy at total daily doses up to 40 mg/kg.[40]

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Behavioral Conditions. Cannabinoids and CBD use in this patient population is a growing interest on social media sites. While the data for these indications are limited to case reports using dronabinol, some of the benefits of CBD on behavior and motor skills reported in the aforementioned retrospective studies in epilepsy may be transferable to this population as well. A 6-year-old patient with early infant autism received enteral dronabinol drops titrated up to 3.62 mg/day. He had improvements in hyperactivity, irritability, lethargy, stereotype, and speech.[41] In a published abstract, Kruger et al[42] report on the effect of dronabinol use in treating self-injurious behavior in 10 mentally retarded adolescents. The dronabinol dose ranged from 2.5 mg twice daily to 5 mg 4 times a day. Seven of the 10 patients had significant improvement in their self-injurious behavior that lasted through the follow-up at 6 months. Two of the 10 patients experienced agitation and the drug was discontinued. An Israeli single-center, double-blind, placebo-controlled cross-over trial of CBD and THC in a 20:1 mixture for behavioral problems in children with autistic spectrum disorder is scheduled to start in January 2017.[43]

Perinatal Brain Injury. Perinatal brain injury can be induced by neonatal asphyxia, stroke-induced focal ischemia, and neonatal hypoxia-ischemic encephalopathy, among other things. These conditions lead to long-lasting functional impairment due to neuroinflammation, apoptotic-necrotic cell death, and brain lesions.[44] Several adjunctive medication therapies in addition to hypothermia, include magnesium sulfate and minocycline which may play a role in modulating neuroinflammation and apoptosis. The endocannabinoid system responds early to neuronal damage, working to prevent glutamate excitotoxicity and regulate the inflammatory response. While there are no current human studies, results from mice and pig models demonstrate that CBD can reduce the density of necrotic neurons and modulate cytokine release.[45,46]

Neuroblastoma. Most recently, researchers have reported on the use of CBD in both *in vitro* and *in vivo* animal studies of neuroblastoma (NBL), a common childhood cancer.[47] Investigators are proposing that antitumor activity is achieved by action at vanilloid and peroxisome proliferator-activated receptors. *In vitro*, they found that both CBD and THC reduced the viability of NBL cells in a dose- and time-dependent manner. When comparing the two, CBD had a significantly better response in reducing viability of NBL cells than THC. They next treated mice with daily intraperitoneal injections of THC, CBD, or ethanol, or gave no treatment. Tumor growth in both the THC and CBD groups was significantly reduced.

What's The Harm?

Worldwide, marijuana is the most commonly abused illegal substance and adolescent daily use is on the rise.[18] Adolescents perceive that marijuana use is not as much of a risk owing to legalization and decriminalization, leading to its use both recreationally and to self-treat anxiety and other psychiatric conditions. Unfortunately, the neurocognitive and behavioral effects of marijuana use in pediatric patients, including its effects on psychological dysfunction, amotivation syndrome, and carcinogenic risk, have been widely reported.[4,21]

Evolving legislation and the increased use of cannabinoid products outside of investigational studies have also impacted our health care delivery and emergency resources. The state of Colorado has been on the forefront of the medicinal and recreational use of cannabis debate. Wang et al[48] reported the occurrences of pediatric emergency department visits associated with marijuana exposure before and after changes in drug enforcement in 2009. A total of 1378 patients younger than 12 years were evaluated for unintentional ingestions from January 1, 2005, to December 31, 2011. Before 2009, no patients (0/790; 0%) sought care at this emergency department for accidental marijuana ingestions as compared with 14 patients (14/588; 2.4%) after 2009 ($p < 0.001$). Patients ranged in age from 8 months to 12 years and presented with symptoms of lethargy, ataxia, and respiratory insufficiency. While the dosages were not reported, 7 patients ingested a marijuana edible. Eight of the 14 patients were

admitted to the hospital with 2 admissions to the pediatric intensive care unit. Prior to diagnosis, these 14 patients received routine testing such as urinalyses, complete blood counts, and complete metabolic panels. Some of these patients also received more invasive testing including computed tomography, activated charcoal, lumbar punctures, and intravenous antibiotics. All of these contribute to higher hospital and emergency room costs, increased lengths of stay, and potential harm to the patients.

In addition to increased emergency room visits, from 2005 to 2011, the call volume at Poison Control Centers for pediatric marijuana exposures had increased by 30.3% in states where marijuana has been decriminalized as compared to a steady rate in states that have not adopted marijuana decriminalization legislation.[19] While marijuana and CBD products are becoming more available, these products remain in DEA (Drug Enforcement Administration) Schedule 1 status and are therefore not regulated in manufacturing, packaging, and labeling outside of clinical trials. As seen in the Colorado case study, 50% of the unintentional ingestions were secondary to an edible, which children can easily mistake for food if not supervised by parents. None of these products are required to have safety packaging to prevent accidental ingestion by children. In addition, no warning labels or verification of product ingredients is required, leaving the medical community caught between providing safe medical care and allowing patient autonomy. As mentioned previously, the AAP has published recommendations to limit the access of marijuana to children.

Pharmacist's Role

In 2007, amidst medical marijuana legalization in several states, Seamon et al[21] identified that pharmacists needed to be attentive to the legislative changes going on at the state and federal levels. Pharmacists are uniquely poised to understand the medicinal chemistry as well as the practical implications associated with decriminalization and legalization. Pharmacists can continue to educate both medical professionals and lay people about the differences among cannabinoids, and help to remove the stigma around appropriate and legal use of CBD products. At the same time, medical professionals need to remember the documented deleterious effects of acute marijuana intoxication on neurocognitive development and psychiatric issues.

Many health care facilities are working through processes that address patient use of these medications. Because use of cannabis products outside of approved clinical trials is not legal under federal law, thus not permitted under Centers for Medicare & Medicaid Services (CMS) Conditions of Participation, there are significant challenges in managing hospitalized patients. Whatever the state and situation, pharmacists need to be aware of the external factors associated with allowing a patient to use CBD in an inpatient setting.

Pharmacists are also poised to participate in the design and evaluation of current and future research in this area. The importance of drug interactions between CBD and other antiepileptics remains uncertain both for the efficacy and safety of CBD products. The difference in concentrations, dosages, and formulations of various products sold at private dispensaries is not standardized or regulated. Differences in state legislation on allowable concentrations and amounts can be confusing for patients and their families, and pharmacists can help to provide that information. Various organizations have been helpful in updating and summarizing this information.[9]

Conclusions

Cannabis and its ingredients have had a fascinating history over the past 4000 years, but lack of published data precludes fully recommending its use for medicinal purposes in pediatrics. Further study is underway and will add to our knowledge of the efficacy and safety of CBD in pediatrics. Long-term

studies to assess neurocognitive development with CBD will need to be assessed as well. As pharmacists, it is our duty to provide our patients and their parents with the most accurate, safe, and legally appropriate advice.

Abbreviations

AAP	American Academy of Pediatrics
AIDS	acquired immune deficiency syndrome
AMA	American Medical Association
CB	cannabinoid
CB1	cannabinoid type 1 receptor
CB2	cannabinoid type 2 receptor
CBD	cannabidiol
CMS	Centers for Medicare & Medicaid Services
CNN	Cable News Network
CSA	Controlled Substances Act
DEA	Drug Enforcement Administration
FDA	US Food and Drug Administration
GABA	gamma-aminobutyric acid
LGS	Lennox-Gastaut syndrome
MS	multiple sclerosis
NBL	neuroblastoma
THC	delta-9-tetrahydrocannabinol
USP	United States Pharmacopeia

Footnotes

Disclosures The authors declare no conflicts or financial interest in any product or service mentioned in the manuscript, including grants, equipment, medications, employment, gifts, and honoraria. Of note, both Augusta University (ClinicalTrials.gov Identifier: NCT02397863) and the University of Florida (ClinicalTrials.gov Identifier: NCT02461706) are sponsors of expanded access clinical trials of cannabidiol and drug-resistant epilepsy in children.

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In vitro and in vivo efficacy of non-psychoactive cannabidiol in neuroblastoma.

Fisher T[1], Golan H[2], Schiby G[3], PriChen S[4], Smoum R[5], Moshe I[1], Peshes-Yaloz N[6], Castiel A[6], Waldman D[2], Gallily R[7], Mechoulam R[5], Toren A[8].

Author information

Abstract

BACKGROUND: Neuroblastoma (nbl) is one of the most common solid cancers in children. Prognosis in advanced nbl is still poor despite aggressive multimodality therapy. Furthermore, survivors experience severe long-term multi-organ sequelae. Hence, the identification of new therapeutic strategies is of utmost importance. Cannabinoids and their derivatives have been used for years in folk medicine and later in the field of palliative care. Recently, they were found to show pharmacologic activity in cancer, including cytostatic, apoptotic, and antiangiogenic effects.

METHODS: We investigated, in vitro and in vivo, the anti-nbl effect of the most active compounds in Cannabis, Δ(9)-tetrahydrocannabinol (thc) and cannabidiol (cbd). We set out to experimentally determine the effects of those compounds on viability, invasiveness, cell cycle distribution, and programmed cell death in human nbl SK-N-SH cells.

RESULTS: Both compounds have antitumourigenic activity in vitro and impeded the growth of tumour xenografts in vivo. Of the two cannabinoids tested, cbd was the more active. Treatment with cbd reduced the viability and invasiveness of treated tumour cells in vitro and induced apoptosis (as demonstrated by morphology changes, sub-G1 cell accumulation, and annexin V assay). Moreover, cbd elicited an increase in activated caspase 3 in treated cells and tumour xenografts.

CONCLUSIONS: Our results demonstrate the antitumourigenic action of cbd on nbl cells. Because cbd is a nonpsychoactive cannabinoid that appears to be devoid of side effects, our results support its exploitation as an effective anticancer drug in the management of nbl.

KEYWORDS: Neuroblastoma; apoptosis; cannabidiol; non-psychoactive cannabinoids; tumour xenograft models; Δ9-tetrahydrocannabinol

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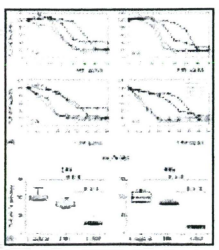
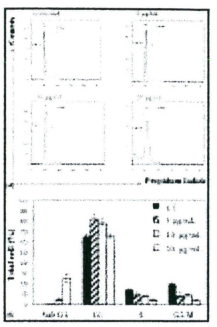
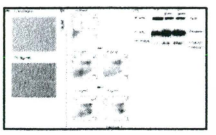
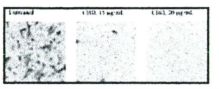
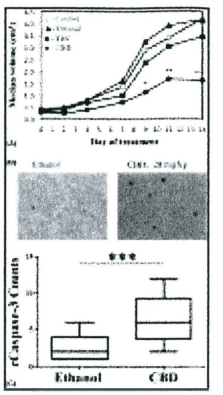

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In vitro and *in vivo* efficacy of non-psychoactive cannabidiol in neuroblastoma

T. Fisher, PhD,[*,a] H. Golan, MD,[*†,a] G. Schiby, MD,[‡] S. PriChen, PhD,[§] R. Smoum, PhD,[‖] I. Moshe, MSc,[*] N. Peshes-Yaloz, PhD,[#] A. Castiel, PhD,[#] D. Waldman, MD,[*†] R. Gallily, PhD,[**] R. Mechoulam, PhD,[‖] and A. Toren, MD PhD[†††]

[*]Pediatric Hemato-Oncology Research Laboratory, Sheba Cancer Research Center
[†]Department of Pediatric Hemato-Oncology, The Edmond and Lily Safra Children's Hospital
[‡]Department of Pathology, The Chaim Sheba Medical Center, Tel-Hashomer, Israel;
[§]Pediatric Stem Cell Research Institute, The Chaim Sheba Medical Center, Tel-Hashomer, Israel;
[‖]Institute for Drug Research, Hebrew University of Jerusalem, Jerusalem, Israel;
[#]Cancer Research Center, The Chaim Sheba Medical Center, Tel-Hashomer, Israel;
[**]The Lautenberg Center for General and Tumour Immunology, Hebrew University of Jerusalem, Jerusalem, Israel;
[††]Sackler School of Medicine, Tel-Aviv University, Tel-Aviv, Israel.
[a]These authors contributed equally to the preparation of this manuscript.
Correspondence to: Amos Toren, Department of Pediatric Hemato-Oncology, The Edmond and Lily Safra Children's Hospital, The Chaim Sheba Medical Center, Tel-Hashomer 52621 Israel. E-mail: amost@post.tau.ac.il

Abstract

Background

Neuroblastoma (NBL) is one of the most common solid cancers in children. Prognosis in advanced NBL is still poor despite aggressive multimodality therapy. Furthermore, survivors experience severe long-term multi-organ sequelae. Hence, the identification of new therapeutic strategies is of utmost importance. Cannabinoids and their derivatives have been used for years in folk medicine and later in the field of palliative care. Recently, they were found to show pharmacologic activity in cancer, including cytostatic, apoptotic, and antiangiogenic effects.

Methods

We investigated, *in vitro* and *in vivo,* the anti-NBL effect of the most active compounds in *Cannabis*, Δ^9-tetrahydrocannabinol (THC) and cannabidiol (CBD). We set out to experimentally determine the effects of those compounds on viability, invasiveness, cell cycle distribution, and programmed cell death in human NBL SK-N-SH cells.

Results

Both compounds have antitumourigenic activity *in vitro* and impeded the growth of tumour xenografts *in vivo.* Of the two cannabinoids tested, CBD was the more active. Treatment with CBD reduced the viability and invasiveness of treated tumour cells *in vitro* and induced apoptosis (as demonstrated by morphology changes, sub-G1 cell accumulation, and annexin V assay). Moreover, CBD elicited an increase in activated caspase 3 in treated cells and tumour xenografts.

Conclusions

Our results demonstrate the antitumourigenic action of CBD on NBL cells. Because CBD is a nonpsychoactive cannabinoid that appears to be devoid of side effects, our results support its exploitation as an effective anticancer drug in the management of NBL.

Keywords: Neuroblastoma, cannabidiol, Δ^9-tetrahydrocannabinol, apoptosis, tumour xenograft models, non-psychoactive cannabinoids

INTRODUCTION

Neuroblastoma (NBL) is the most frequent extracranial solid tumour in childhood. It accounts for approximately 8% of childhood cancers and is characterized by variable clinical behaviour, reflecting molecular differences in the tumour[1]. Using current risk stratification criteria, approximately 40% of NBL tumours are classified as high-risk. Treatment for children with high-risk NBL involves multimodality therapy, including chemotherapy, autologous stem-cell transplantation, surgery, radiation therapy, and immunotherapy using differentiation therapy. Despite that aggressive approach, children with NBL have very poor outcomes, and the survivors experience serious side effects related to treatment toxicity[2]. Hence, the need for new and less-toxic therapeutic strategies to treat the disease is urgent.

For millennia, *Cannabis sativa* has been used in folk medicine to alleviate pain, depression, amenorrhea, inflammation, epilepsy, and numerous other medical conditions[3]. In cancer patients specifically, cannabinoids are well known to exert palliative effects; their best-established use is the inhibition of chemotherapy-induced nausea and vomiting, but they are also applied for pain alleviation, appetite stimulation, and attenuation of wasting[4].

Recently, increasing evidence suggests that Δ^9-tetrahydrocannabinol (THC) and cannabidiol (CBD), major components of *Cannabis sativa,* and synthetic cannabinoids and the endocannabinoid anandamide have antitumour activity[5,6]. Many adult cancer types (lung cancer, glioma, thyroid cancer, lymphoma, skin cancer, pancreatic cancer, uterine cancer, and breast and prostate carcinoma) have been reported to be sensitive to the antiproliferative action of cannabinoids in a wide variety of experimental models, including cancer cell lines in culture, xenograft mouse models, and genetically engineered mice[6].

Cannabinoids act chiefly by activating the specific cannabinoid receptors CB1 and CB2[6]. However, it is now well-established that these molecules also have effects that are CB receptor–independent; other receptors, such as vanilloid receptor 1[7] and the peroxisome proliferator–activated receptors[8], could be responsible for their action.

The mechanisms involved in the antitumour effects of cannabinoids include proliferation inhibition and growth arrest[9], induction of apoptosis[10,11], stimulation of autophagy[12,13], angiogenesis inhibition[14], and anti-metastatic effects[15–17]. However, the antitumourigenic mechanism of action of CBD is as yet unknown[18].

At the molecular level, cannabinoids have been shown to trigger changes in various signalling pathways, including Akt/mammalian target of rapamycin complex 1[19], ERK, upregulation of stress-associated transcription factor p8[19,20], downregulation of matrix metalloproteinase 2[21], and vascular endothelial growth factor signalling[22]. Nevertheless, studies exploring the putative antitumourigenic properties of cannabinoids in pediatric tumours are still limited, and the molecular mechanisms underlying the antitumourigenic effect are poorly understood. Recently published data demonstrated the antitumourigenic activity of cannabinoids—mainly THC and synthetic cannabinoids—on alveolar rhabdomyosarcoma and osteosarcoma by inducing apoptosis[23] and triggering the endoplasmic reticular stress and autophagy process[24].

Our study aimed to characterize both the *in vitro* and *in vivo* effects of cannabinoids on another pediatric tumour, NBL, and to unravel the mechanism responsible for those effects. Given our positive results, we suggest that non-THC cannabinoids such as CBD might provide a basis for the development of novel therapeutic strategies in high-risk NBL, without the typical psychotropic effects of THC and without the strong side effects associated with chemotherapeutic agents.

METHODS

Cannabinoids

Δ^9-Tetrahydrocannabinol was supplied by Prof. Raphael Mechoulam, Institute for Drug Research, Medical Faculty, The Hebrew University, Ein Kerem Campus, Jerusalem, Israel. Cannabidiol was supplied by THC Pharm GmbH, Frankfurt, Germany.

Cell Cultures

The human NBL cell lines SK-N-SH[25] and IMR-32[26] were purchased from ATCC (Manassas, VA, U.S.A.) and the European Collection of Authenticated Cell Cultures (Salisbury, U.K.) respectively. The NUB-6[27] and LAN-1 cell lines were kindly provided by Dr. Shifra Ash, Schneider Children's Medical Center of Israel[28].

SK-N-SH cells were cultured in Eagle minimum essential medium (ATCC), supplemented with 10% fetal bovine serum (FBS) and 100 U/mL penicillin–streptomycin (Gibco, Paisley, U.K.). IMR-32 cells were cultured in Eagle basal medium (Sigma–Aldrich, St. Louis, MO, U.S.A.) supplemented with 2 mmol/L glutamine, 1% non-essential amino acids, 10% FBS, and 100 U/mL penicillin–streptomycin. LAN-1 and NUB-6 cells were cultured in RPMI-1640 (Gibco) supplemented with 10% FBS and 100 U/mL penicillin– streptomycin. All the cell lines were cultured at 37°C in a humidified atmosphere containing 5% CO_2.

MTT Test

An MTT assay (Biological Industries, Kibbutz Beit-Haemek, Israel) was used to evaluate the effect of CBD and THC on NBL cell viability. SK-N-SH, LAN-1, IMR-32, and NUB-6 cells (5×10^3 cells/mL) were plated (200 µL) in triplicate in flat-bottom 96-well plates in the appropriate medium. The cells were allowed to adhere to the plate surface overnight and were then cultured with increasing doses of THC or CBD (0–50 µg/mL) for 24, 48, and 72 hours. Cell viability was then determined by MTT assay, which measures the reduction of MTT to formazan by the mitochondria of viable cells[29]. Formazan was measured spectrophotometrically by absorption at 560 nm in a PowerWaveX plate reader (BioTek, Winooski, VT, U.S.A.). All experiments were repeated at least 3 times. Cell morphologies were assessed daily by light microscopy.

Microscopy Analysis

One day before treatment, SK-N-SH cells were plated (1×10^6 cells per 9-cm plate). After 48 hours of incubation with CBD (10 μg/mL), cell morphology changes were assessed by light microscopy (Olympus CKX41: Olympus, Tokyo, Japan).

Cell-Cycle Analysis

One day before THC or CBD treatment, SK-N-SH cells were plated (1×10^6 cells per 9-cm plate). After 24, 48, and 72 hours of treatment, the cells were washed in phosphate-buffered saline (PBS: Biological Industries), detached using a solution of 0.1% trypsin (Biological Industries), and spun at 1100 rpm. The resulting pellet was resuspended in 250 μL cold PBS, and the cells were fixed overnight with 5 mL cold 75% ethanol (Sigma–Aldrich) and PBS at −20°C. The pellet was then washed twice with cold PBS (followed by centrifugation at 1100 rpm for 7 minutes). Distribution of the cells in G1, S, and G2/M phases of the cell cycle were monitored after nuclei had been stained with 50 μg/mL propidium iodide (Sigma–Aldrich) containing 125 U/mL protease-free RNase (Sigma–Aldrich) in 0.5% Triton (Bio-Lab, Jerusalem, Israel) and had been PBS-buffered for 30 minutes in the dark. The cells were analyzed using an Epics XL-MCL flow cytometer and the FlowJo software application (Beckman Coulter, Brea, CA, U.S.A.).

Apoptotic Cell Death

Annexin V Assay One day before CBD treatment (7.5 μg/mL and 10 μg/mL for 24 hours and 48 hours), cells were plated (1×10^6 cells per 9-cm plate). Cells treated with 1–10 μmol/L staurosporine (Sigma –Aldrich) for 24 hours served as a positive control; untreated cells served as a negative control. Treated cells, untreated cells, and positive control cells were harvested, and after the annexin V assay [human recombinant annexin V (APC conjugate, catalogue no. ALX-209–252: Enzo Life Sciences, Ann Arbor, MI, U.S.A.); annexin V binding buffer, no. 556454 (BD Pharmingen, San Diego, CA, U.S.A.); and 7-aminoactinomycin D, no. 559925 (BD Pharmingen)] were analyzed using an Epics XL-MCL flow cytometer and the FlowJo software application.

Caspase Assay One day before CBD treatment (7.5 μg/mL and 10 μg/mL for 24 hours), cells were plated (1×10^6 cells per 9-cm plate). Cells were harvested, and proteins were extracted with radioimmunoprecipitation assay buffer (Sigma–Aldrich). Protein concentrations were calibrated using the BCA Protein Assay Reagent Kit (Pierce, Rockford, IL, U.S.A.).

Samples were separated on 12% SDS-PAGE (Bio-Rad, Rishon LeZion, Israel) and transferred onto nitro filters (Schleicher and Schuell Bioscience, Dassel, Germany). The blots were reacted using caspase 3 (8G10) rabbit monoclonal antibody (Cell Signalling Technology, Danvers, MA, U.S.A.) as the primary antibody. The secondary antibody, horseradish peroxidase conjugated goat anti-rabbit antibody (Jackson ImmunoResearch Laboratories, Farmington, CT, U.S.A.), was detected by chemiluminescence. Signals were detected using an ECL Kit (Amersham Pharmacia, Little Chalfont, U.K.) and visualized by exposure to radiography film.

Invasion Assay

Tumour cell invasion was assayed in Transwell chambers (Transwell 3422: Corning, Corning, NY, U.S.A.) pre-coated with Cultrex Basement Membrane Extract (Trevigen, Gaithersburg, MD, U.S.A.). Membrane filters were placed in 24-well tissue-culture plates according to manufacturer guidelines. After 24 hours of treatment with CBD (15 μg/mL and 20 μg/mL), cells were harvested, and 2×10^5 cells suspended in 200 μL serum-free medium were added to the upper surface of each chamber. The bottom of the chamber was filled with 750 μL medium with 10% FBS. After 24 hours in which cells were

allowed to migrate to the underside of the membrane, the invaded cells were fixed with paraformaldehyde (Electron Microscopy Sciences, Hatfield, PA, U.S.A.) and stained with crystal violet (Sigma–Aldrich).

In Vivo Studies

All experiments involving mice were approved and performed according to the guidelines issued by the Sheba Medical Center Research Committee for the Care and Use of Laboratory Animals (permit no. 803/12).

To study the *in vivo* antitumour activity of cannabinoids, NBL tumours were induced in nonobese diabetic immunodeficient (NOD/SCID) mice by subcutaneous injection. Briefly, 1×10^7 SK-N-SH cells suspended in 100 μL serum-free medium and Cultrex (1:1) were injected subcutaneously into the rear flank of 5- to 8-week-old NOD/ SCID mice. Mice were maintained in a pathogen-free environment and monitored weekly for tumour growth. Secondary tumours were detected by palpation and were measured with external callipers. Volume was calculated as $(\text{width})^2 \times (\text{length}) \times 0.52$. When tumours had reached an average size of 400 mm^3, the mice were randomly assigned to treatment and control groups (each $n = 12$). They were then injected intraperitoneally for 14 days with THC (20 mg/kg daily), CBD (20 mg/kg daily), or vehicle (ethanol) or were left untreated. At the end of the treatment period, the mice were euthanized, and the tumours were excised and processed for further analyses.

Histology Formalin-fixed tissues were dehydrated, embedded in paraffin, and sectioned at 4 μm. The slides were warmed to 60°C for 1 hour and then processed by a fully automated protocol. Immunostainings were calibrated on a Benchmark XT staining module (Ventana Medical Systems, Tucson, AZ, U.S.A.). Briefly, after sections had been dewaxed and rehydrated, a CC1 Standard Benchmark XT pre-treatment for antigen retrieval (Ventana Medical Systems) was selected for active caspase 3. Active caspase 3 antibody (Epitomics, Burlingame, CA, U.S.A.) was diluted 1:10 with Antibody Diluent (Ventana Medical Systems) and incubated for 1 hour at 37°C. Detection was performed using an ultraView detection kit (Ventana Medical Systems) and counterstained with hematoxylin (Ventana Medical Systems). After the run on the automated stainer was completed, the slides were dehydrated in 70% ethanol, 95% ethanol, and 100% ethanol (10 s each). Before cover-slipping, the sections were cleared in xylene (10 s) and mounted with Entellan (EMD Millipore, Billerica, MA, U.S.A.). The stained sections were reviewed under light microscopy and analyzed by a pathologist.

Statistical Analysis

Unless otherwise specified, results are shown as means or medians ± standard deviation. A Kruskal–Wallis test, followed by a post hoc Mann–Whitney test, was used to evaluate significant differences in the viability of cell lines, the growth rate of xenografts, and the counts of positive cleaved caspase 3 cells for the various treatment groups. A p value less than 0.05 was considered statistically significant. All analyses were performed using the IBM SPSS Statistics software application (version 21: IBM, Armonk, NY, U.S.A.).

RESULTS

Viability of NBL Cell Lines *In Vitro*

We used an MTT assay to assess the effect of THC and CBD on the viability of the SK-N-SH, NUB-6, IMR-32, and LAN-1 NBL cell lines [Figure 1(A)]. *In vitro,* after 24 hours of treatment, CBD and THC had already effectively reduced the viability of NBL cell lines in a dose- (0–50 μg/mL) and time-dependent manner, with CBD having the better effect. Better response to treatment was observed in the

SK-N-SH and NUB-6 cell lines, as demonstrated by a 50% reduction in cell viability at lower CBD or THC concentrations (5 μg/mL and 15 μg/mL for SK-N-SH and NUB-6 respectively, compared with >20 μg/mL for IMR-32 and LAN-1). The same trend was found, and even enhanced, after treatment with THC and CBD for 48 hours [Figure 1(A)]. More importantly, the response after treatment of SK-N-SH cells with CBD (10 μg/mL) was better than the response after treatment with the same concentration of THC [Figure 1(B), $p = 0.0004$ for 24 and 48 hours of treatment].

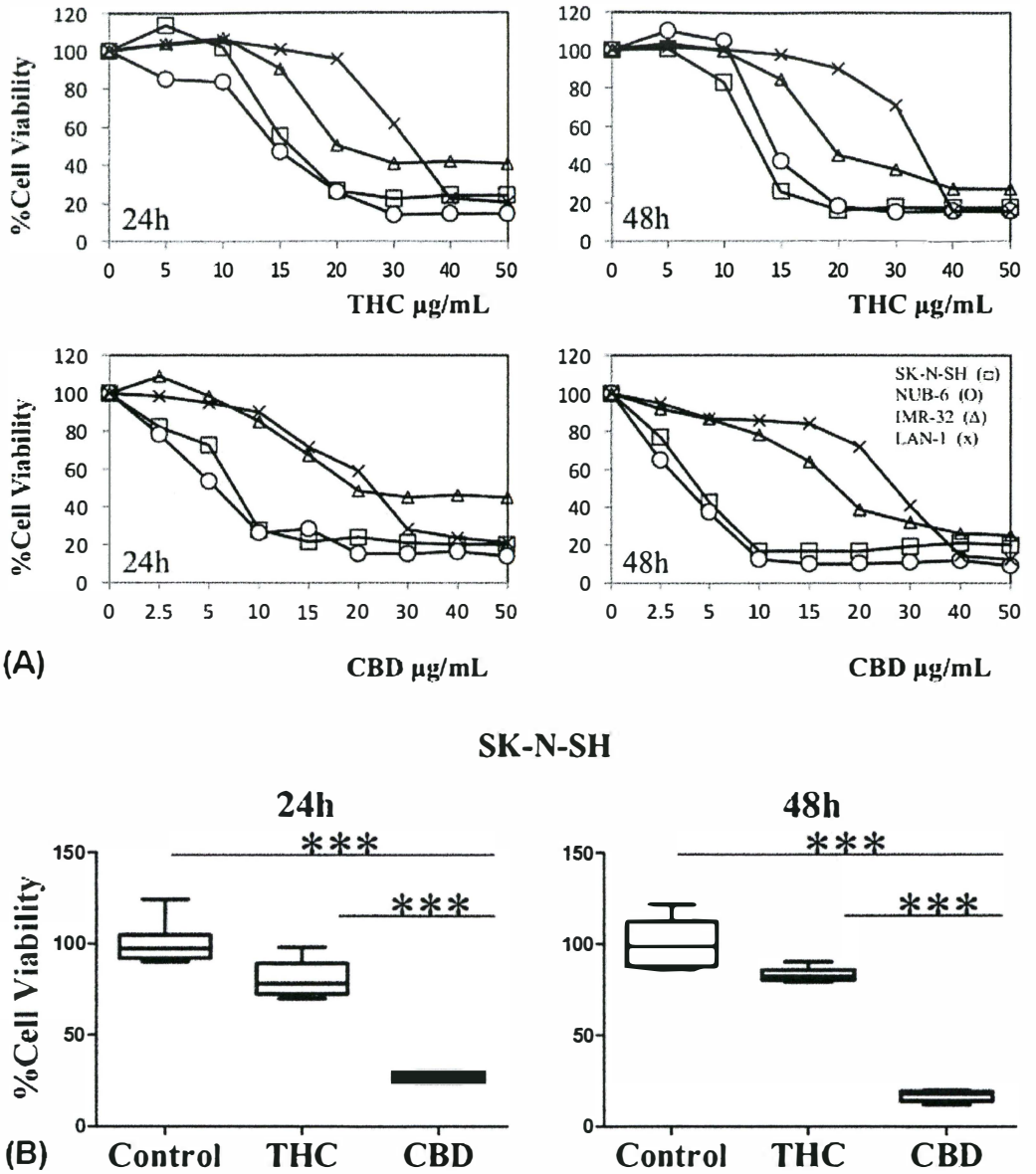

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FIGURE 1

Δ^9-Tetrahydrocannabinol (THC) and cannabidiol (CBD) reduce viability of neuroblastoma (NBL) cell lines *in vitro,* with CBD having a better effect. (A) Cell lines SK-N-SH (open squares), NUB-6 (open circles), IMR-32 (open triangles), and LAN-1 (crosses) were incubated with increasing concentrations (0–50 µg/mL) of THC or CBD for 24 hours and 48 hours. Viability was measured by MTT assay. (B) Mean ± standard deviation of SK-N-SH cell viability after incubation with 10 µg/mL THC or CBD for 24 and 48 hours. *** Denotes a significant change relative to control ($p = 0.0004$). Data are expressed as a percentage of the vehicle control and are the mean of pooled results from experiments performed in triplicate.

The foregoing data indicate that anti-NBL activity is better with CBD than with THC in all NBL cell lines tested. Because cell lines showed varying sensitivity to CBD, we chose the most sensitive SK-N-SH cell line to confirm the antiproliferative effect of CBD in further *in vitro* and *in vivo* experiments.

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Cell-Cycle Analysis

We next studied the effect of 48 hours of treatment with increasing doses of CBD (5–20 μg/mL) on cell-cycle progression [Figure 2(A)]. During treatment with CBD (5 μg/mL), the percentage of SK-N-SH cells sequestered in the G1 compartment rose to 82.4% from 65.8% in untreated control cells [Figure 2(B)]. Accordingly, the percentages of cells in G2 and S phase were found to be decreased, indicating that those cell populations had undergone G1 phase arrest (similar results were obtained when NUB-6 cells were treated with 10 μg/mL CBD; data not shown). Furthermore, an accumulation of SK-N-SH cells in sub-G1 phase [Figure 2(B)] was detected when that line was incubated with 10 μg/mL CBD (4.27%) and 20 μg/mL CBD (25.3%), indicating the possibility that treatment with CBD induced apoptosis in a dose-dependent manner.

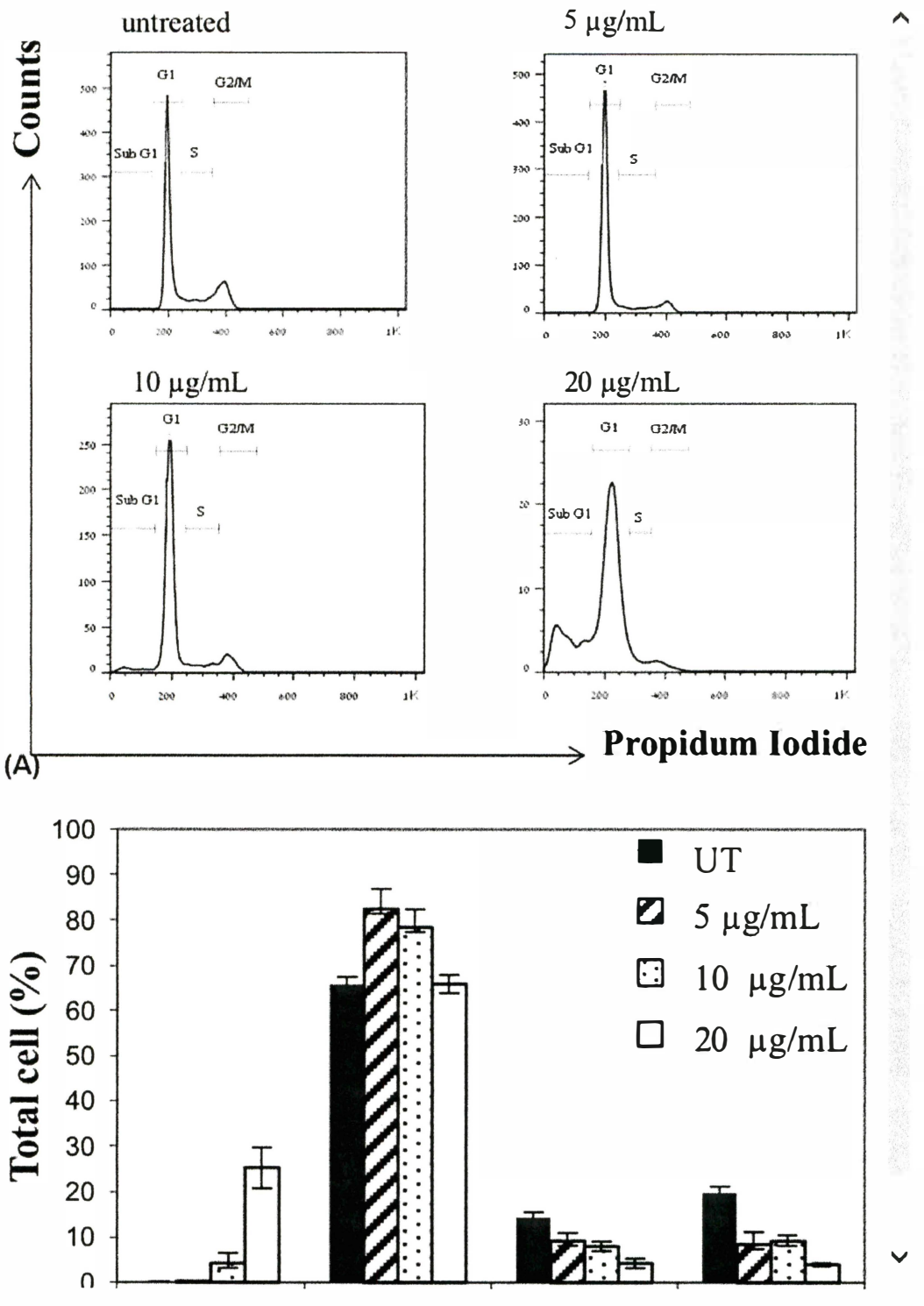

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FIGURE 2

Alteration of SK-N-SH cell cycle progression induced by cannabidiol (CBD). (A) Cell cycle analysis in untreated SK-N-SH cells and in cells treated with increasing concentrations of CBD for 48 hours. (B) Change in cell accumulation percentages during cell cycle progression after incubation with CBD for 48 hours. UT = untreated.

Apoptotic Cell Death

To verify our hypothesis that the reduction in NBL cell viability associated with CBD treatment was indeed attributable to apoptotic cell death, we first examined morphology changes after CBD treatment. Microscopic analysis showed that treatment with 10 μg/mL CBD affected cell morphology; the number of cells that had lost their normal shape, becoming rounded and swollen, and that floated in the medium increased [Figure 3(A)]. Those results confirmed that CBD treatment might induce the appearance of typical features of apoptosis.

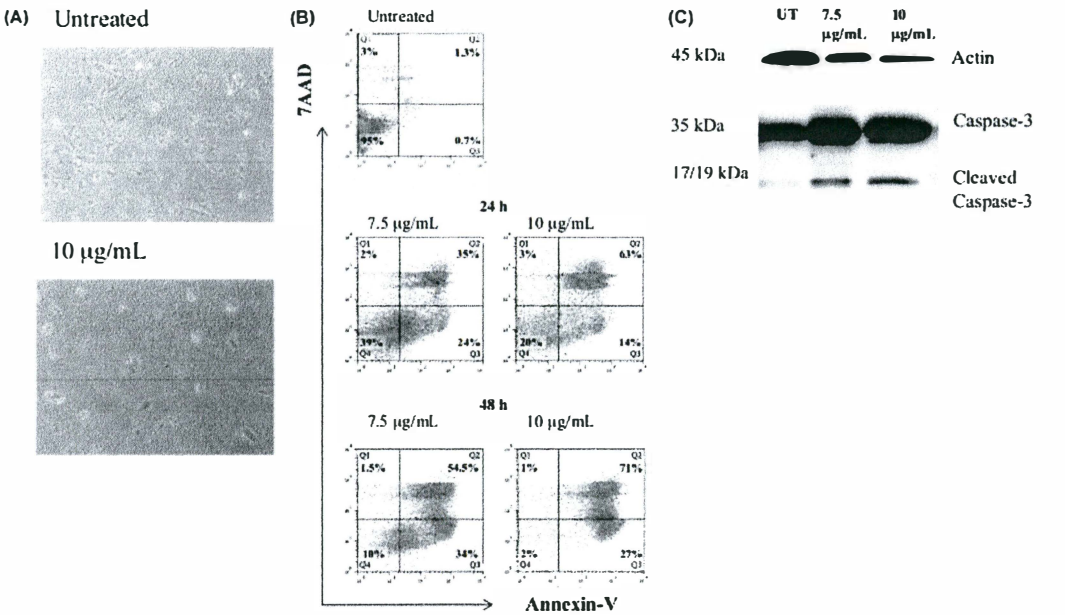

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FIGURE 3

Apoptotic effects of cannabidiol (CBD) on SK-N-SH cells. (A) Changes in SK-N-SH cell morphology: untreated cells compared with cells treated with 10 μg/mL CBD for 48 hours. (B) Apoptotic effects of CBD on SK-N-SH cells analyzed by annexin-V assay. Cells were treated with CBD in a dose- and time-dependent manner (7.5 μg/mL, 10 μg/mL; 24 hours, 48 hours) and were stained with annexin-V and 7-amino actinomycin D (7AAD). Q1 = percentage of dead cells; Q2 = percentage of cells in late apoptosis; Q3 = percentage of cells in early apoptosis; Q4 = percentage of live cells. (C) Apoptotic effects of CBD on SK-N-SH cells analyzed by caspase-3 assay. Cells were treated with increasing doses of CBD (7.5 μg/mL, 10 μg/mL) for 24 hours.

Next, we used an annexin V assay to measure the percentage of cells undergoing apoptosis after CBD treatment [Figure 3(B)]. Staurosporine-treated cells were used as a positive control. Treatment of SK-N-SH cells with CBD (7.5 μg/mL and 10 μg/mL) for 24 hours and for 48 hours resulted in an increase in the early apoptotic cell population (annexin V–positive and 7-aminoactinomycin D–negative) in a time-dependent manner. A dose- and time-dependent increase in the late apoptotic cell population was also demonstrated (annexin V–positive and 7-aminoactinomycin D–positive). Early apoptosis was demonstrated in 24% of cells incubated for 24 hours with CBD (7.5 μg/mL), but in only 0.7% of

untreated cells. As Figure 3 shows, the proportion of late apoptotic cells increased to 63% from 35% after 24 hours of treatment with increasing concentrations of CBD (10 μg/mL and 7.5 μg/mL respectively)

Finally, to further confirm the apoptotic effects of CBD on SK-N-SH cells, we measured apoptosis by caspase 3 assay [Figure 3(C)]. After 24 hours of treatment with increasing doses of CBD (7.5 μg/mL and 10 μg/mL), a dose-dependent cleavage of caspase 3 was found as evaluated by the appearance of activated p17 and p19 fragments on Western blot analysis.

Altogether, the foregoing results confirm that treatment with CBD induces apoptosis in the SK-N-SH NBL cell line.

Cell Invasiveness

As shown in Figure 4, cell invasion in Transwell chambers was dramatically decreased for SK-N-SH NBL cells treated for 24 hours with CBD (15 μg/mL and 20 μg/mL) than for untreated cells.

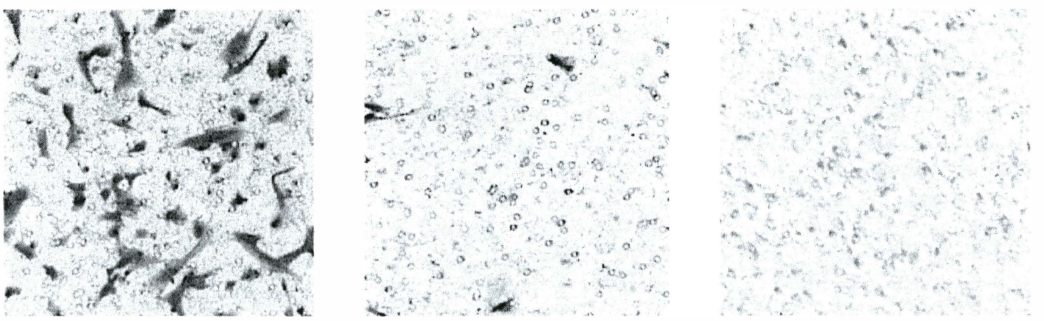

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FIGURE 4

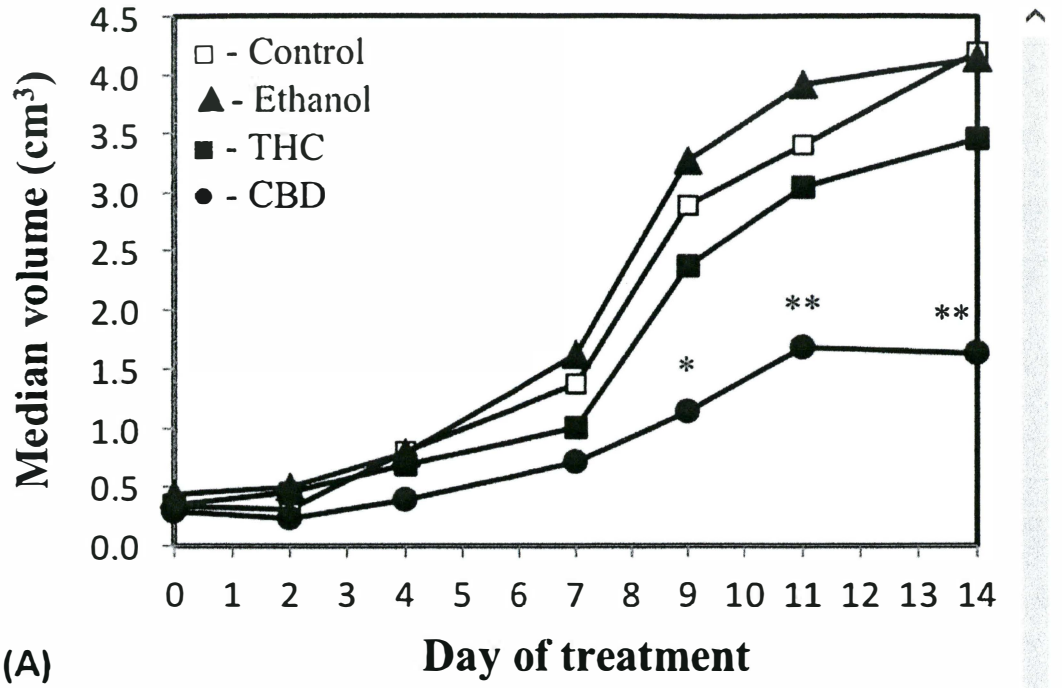
Anti-invasiveness effect of cannabidiol (CBD) on SK-N-SH cells. The invasion assays were performed using cell cultures (2×10^5 cells/well) treated with CBD (15 μg/mL, 20 μg/mL) for 24 hours; results were compared with those for untreated cells (2×10^5 cells/well). For each well (treated or untreated cells), 10 fields were examined by light microscopy.

Tumour Growth Rate in Mouse Xenograft Model

Because tumour regression in an animal xenograft model represents an important endpoint of clinical relevance, we evaluated the ability of cannabinoids to reduce NBL tumour growth *in vivo*. Tumour xenografts were first generated by subcutaneous injection of SK-N-SH cells into NOD/SCID mice. The mice were then treated with daily intraperitoneal injections of 20 mg/kg THC, 20 mg/kg CBD, or ethanol vehicle (control), or were left untreated for 14 days.

Tumour growth was significantly reduced in THC- and CBD-treated mice than in the vehicle-treated or untreated mice [Figure 5(A)]. Interestingly, response to treatment was observed to be better in the group treated with CBD than in the group treated with THC: Median xenograft volume at the end of treatment

was 2.31 cm^3 in the CBD-treated group compared with 4.28 cm^3 in the untreated group ($p = 0.029$) and 4.31 cm^3 in the vehicle-treated group ($p = 0.036$). In the THC-treated group, median volume was 3.46 cm^3, which was significant only compared with the untreated group ($p = 0.039$).



(A)

(B)

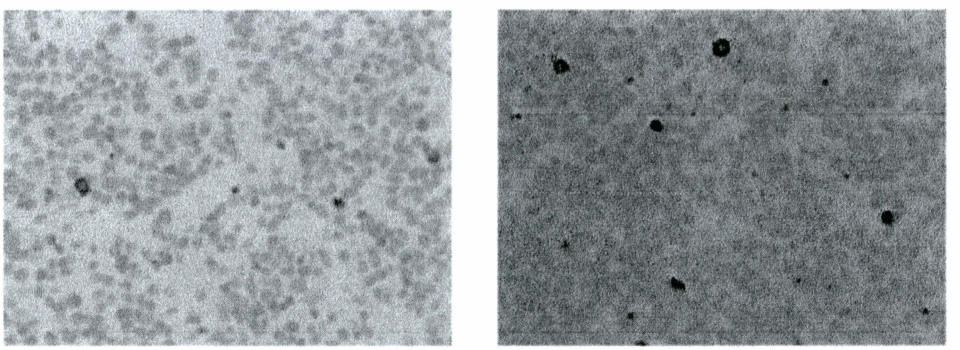


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FIGURE 5

Cannabidiol (CBD) suppresses tumour growth in a mouse xenograft model and increases cleaved caspase-3 staining in treated xenografts. (A) Growth rate of SK-N-SH cell–derived tumour xenografts treated for 14 days with intraperitoneal injections of ethanol-vehicle (n = 12, closed triangles), 20 mg/kg Δ^9-tetrahydrocannabinol (n = 12, closed squares), 20 mg/kg CBD (n = 12, closed circles) and untreated

controls ($n = 12$, open squares). Data represents tumour volume during 14 days of treatment. [a] $p < 0.05$ and [b] $p < 0.01$ for CBD compared with ethanol treatment (Mann–Whitney U-test). (B) Activated caspase-3 immunostaining in SK-NS-H cell–derived tumour xenografts treated with CBD 20 mg/kg or ethanol vehicle for 14 days. (C) Counts of cleaved caspase-3 immunoreactive cells in 18×10 lens fields from xenografts of CBD- and ethanol-treated mice. [a] $p < 0.0001$ compared with ethanol.

To further define the *in vivo* effect of CBD treatment with respect to apoptosis induction, we analyzed tissue obtained from tumour xenografts. Tumours were excised after the last day of treatment, and paraffin-embedded sections were analyzed immunohistochemically with the apoptosis indicator cleaved caspase 3. Cells positive for cleaved caspase 3 were detected with significantly greater frequency in sections of xenografts from CBD-treated mice [Figure 5(B)] than in sections from ethanol-treated mice [$p < 0.001$, Figure 5(C)].

To summarize, THC and CBD both suppressed the SKN-SH tumour xenograft growth rate, with CBD treatment demonstrating a better effect. Moreover, the better efficacy of CBD and its effect on the induction of activated caspase 3 are consistent with the results obtained *in vitro*.

DISCUSSION

In recent years, interest in the role of cannabinoids, mainly THC, in cancer therapy has been renewed because of the ability of these molecules to limit tumour cell proliferation and to induce selective cell death[5,6,30]. The response to treatment with cannabinoids has been investigated and demonstrated in a wide variety of adult tumours[30]; however, the effect has been studied in only a few pediatric tumours[23,24]. We therefore investigated the role of cannabinoids in a pediatric tumour, NBL, which is the most frequent extracranial solid tumour of childhood and which still carries a very poor prognosis[1].

We focused only on the major compounds in cannabis, THC and CBD. The results obtained in the *in vitro* studies can be summarized as follows:

- Both molecules—and CBD in particular—reduced the viability of NBL cells.
- The effect of CBD seemed to be mediated by apoptotic cell death, as demonstrated by morphology changes, accumulation of sub-G1 cells, annexin V assay, and increased expression of cleaved caspase 3.
- The invasiveness of NBL cells was also reduced with CBD treatment.

Based on that first set of results, we studied the effect of CBD and THC on xenograft tumours generated in NOD/SCID mice from SK-N-SH cells that had already demonstrated the greatest sensitivity to the effect of those molecules. In accord with the findings from the *in vitro* experiments, THC and CBD both reduced the xenograft growth rate, with CBD showing a superior effect.

Our *in vitro* data suggesting that CBD inhibits the proliferation of, and induces apoptosis in, NBL cells—together with its remarkable effect on NBL xenografts—are, to the best of our knowledge, the first to show an antitumour effect of CBD on NBL cells. Moreover, the results obtained in our study indicate that, of the two cannabinoids tested, CBD was more effective on the SK-N-SH cell line and on xenografts than was the more-studied THC. Those results accord with recently emerging data showing an effect of CBD on other tumours such as glioblastoma and breast, lung, prostate, and colon cancer[18,31–34].

Δ^9-Tetrahydrocannabinol, the second most abundant cannabinoid in *Cannabis sativa,* has been shown to induce apoptosis and to inhibit tumour cell viability and invasiveness in various tumours[34–38], as our study also demonstrated. Recently, CBD was also reported to enhance the production of reactive oxygen species in cancer cells[39], to downregulate the metastatic factor Id1, and to upregulate the pro-differentiation factor Id2[16,40].

The mechanism by which CBD produces the observed effects has not yet been completely clarified, but seems to be independent of the CB1 and CB2 receptors. Various studies have demonstrated that CBD acts as an agonist for vanilloid receptor 1 and for the TRPV2, TRPA1, PPARG, and 5-HT1A receptors, and as an antagonist for the TRPM8 and GPR55 receptors[41]. However, CBD's antitumourigenic molecular mechanisms of action have not been studied in NBL. A hint can be found in several reports showing that various NBL cell lines express the foregoing receptors shown to be involved in the action of CBD[42–44]. Several studies have demonstrated that activation of those receptors with agonists other than CBD mediates cell death in a variety of NBL cell lines, including SK-N-SH[45,46], the CBD-responsive cell line in our study.

As a potential therapeutic agent, CBD could have many advantages, especially compared with psychoactive THC. Because most—if not all—of the psychoactive effects of cannabinoids are produced by activation of the central CB1 receptors[47], CBD, which has been shown to act independently of CB1, is devoid of psychoactive effects[48] and can serve as a more suitable treatment, especially in children. Additionally, it shares the palliative properties and low toxicity profile described for other cannabinoids, has none of the strong side effects associated with chemotherapeutic agents[10,18,49], and might have synergistic activity with well-established antineoplastic substances[10,50].

The most widely used route of cannabinoid administration is smoking—an unattractive clinical option, particularly in children. Our work indicates that systemic (intraperitoneal) administration of CBD effectively reduces tumour growth, and use in a clinical setting can therefore be based on other routes of administration, such as in oral or oromucosal treatments.

CONCLUSIONS

Our findings about the activity of CBD in NBL support and extend previous findings about the antitumour activities of CBD in other tumours and suggest that cannabis extracts enriched in CBD and not in THC could be suitable for the development of novel non-psychotropic therapeutic strategies in NBL. Use of CBD either as single agent or in combination with existing compounds and chemotherapy agents is a possibility. Combination therapy might improve the antitumourigenic effects of other treatments and allow for a reduction in the chemotherapy dose, minimizing toxicity and long-term sequelae. Future studies are needed to highlight the pathways involved in the antitumourigenic effects of CBD in NBL as demonstrated in the present work.

CONFLICT OF INTEREST DISCLOSURES

We have read and understood *Current Oncology*'s policy on disclosing conflicts of interest, and we declare that we have none.

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Endocannabinoid involvement in endometriosis

Natalia Dmitrieva,[a] Hiroshi Nagabukuro,[a,1] David Resuehr,[a,2] Guohua Zhang,[a,3] Stacy L. McAllister,[a] Kristina A. McGinty,[a] Ken Mackie,[b] and Karen J. Berkley[a]

[a]Program in Neuroscience, Florida State University, Tallahassee, FL 32306
[b]Department of Psychological and Brain Sciences and the Gill Center, Indiana University, Bloomington IN 47405
Corresponding author: Karen J. Berkley, Ph.D, Program in Neuroscience/Psychology, Florida State University, 1107 W. Call St., Tallahassee, FL 32306-4301, Tel; 850.644.5741. Fax: 850.644.9874, kberkley@psy.fsu.edu
[1]Current address: Merck Research Laboratories, Boston MA 02115
[2]Current address: Vanderbilt University Medical Center, Nashville TN 37232
[3]Current address: Shanghai Jiaotong University School of Medicine, Shanghai, China 200025

Abstract

Endometriosis is a disease common in women that is defined by abnormal extrauteral growths of uterine endometrial tissue and associated with severe pain. Partly because how the abnormal growths become associated with pain is poorly understood, the pain is difficult to alleviate without resorting to hormones or surgery, which often produce intolerable side effects or fail to help. Recent studies in a rat model and women showed that sensory and sympathetic nerve fibers sprout branches to innervate the abnormal growths. This situation, together with knowledge that the endocannabinoid system is involved in uterine function and dysfunction and that exogenous cannabinoids were once used to alleviate endometriosis-associated pain, suggests that the endocannabinoid system is involved in both endometriosis and its associated pain. Here, using a rat model, we found that CB1 cannabinoid receptors are expressed on both the somata and fibers of both the sensory and sympathetic neurons that innervate endometriosis's abnormal growths. We further found that CB1 receptor agonists decrease, whereas CB1 receptor antagonists increase, endometriosis-associated hyperalgesia. Together these findings suggest that the endocannabinoid system contributes to mechanisms underlying both the peripheral innervation of the abnormal growths and the pain associated with endometriosis, thereby providing a novel approach for the development of badly-needed new treatments.

Keywords: visceral pain, neural sprouting, neuroplasticity, neurovascular, transplant, sensory-sympathetic coupling

1. Introduction

Endometriosis, a disease common in women of childbearing age, is defined by abnormal growths of uterine endometrial tissue outside the uterus. Symptoms include dysmenorrhea, dyspareunia (vaginal hyperalgesia), chronic pelvic/abdominal musculoskeletal and other types of severe pain that are often difficult to alleviate without resorting either to hormonal treatments that produce often-intolerable side effects of hypoestrogenicity or to surgery that frequently fails to help [16,21,27,16,48]. New treatment strategies are badly needed. Although it is recognized that both inflammatory and neuropathic mechanisms contribute to endometriosis-associated pains, progress in developing new treatment strategies is impeded by a poor understanding of how the growths, which do not correlate with the pains, contribute to them [16,21,27,48].

A rat model of surgically-induced endometriosis (ENDO), in which ectopic uterine cysts form in the abdomen [51], develops pain symptoms similar to those of women, including vaginal hyperalgesia (10) and vaginally-referred abdominal muscle hyperalgesia [38]. The ENDO model's surgical control (shamENDO) develops neither cysts nor pain symptoms [10,38]. The ectopic growths in both the rat model and in women recruit their own sensory and sympathetic nerve supply [4,5]. This innervation in the rat model, diagrammed in Fig. 1b, consists of branches of axons that sprout from pre-existing sensory neurons whose cell bodies are located in thoracic dorsal root ganglia (DRG) and from sympathetic neurons whose cell bodies are located in the coeliac ganglion (CG). Findings from the rat model and clinical studies strongly support the conclusion that this innervation contributes to endometriosis-associated pain [21,45,48].

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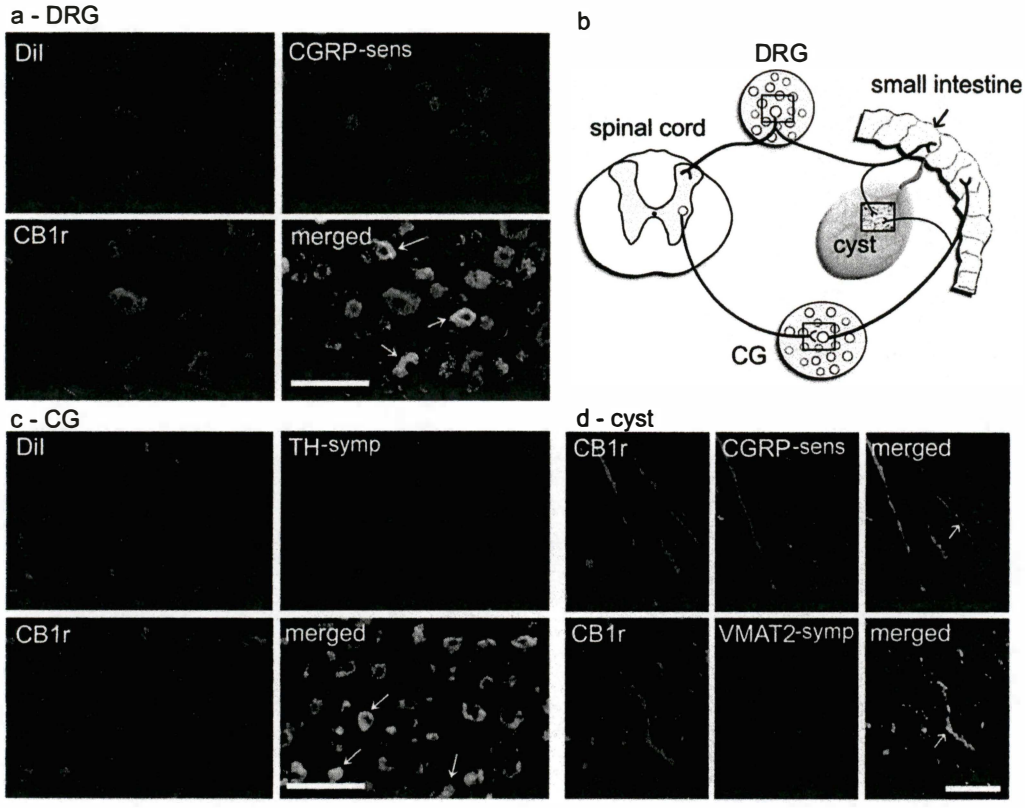

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Figure 1

CB1 co-localization on sensory and sympathetic axons in ectopic endometrial cysts, and on cyst-innervating sensory and post-ganglionic sympathetic ganglion cells in thoracic dorsal root ganglion (DRG) and coeliac ganglion (CG), respectively. Boxed areas in **b** show approximate locations of photomicrographs in the other panels. **Panel a** shows cyst-innervating neurons in a T10 DRG retrogradely-labeled with Dil dye (blue), some of which co-label with both CB1 (red) and sensory-calcitonin gene related peptide (CGRP-sens; green) antibodies. Tripe-labeled neurons appear white. **Panel c** shows cyst-innervating neurons in a CG, some of which co-label with both CB1 (red) and sympathetic-tyrosine hydroxylase (TH-symp; green) antibodies. Tripe-labeled neurons appear white. **Panel d** shows CB1-labeled (red) either CGRP-sens or vesicular monoamine transporter 2-sympathetic (VMAT2-symp)-labeled fibers (both green) in the cyst. Double-labeled fibers appear yellow. Arrows in all merged images point to examples of either tripe-labeled ganglion cells (**panels a** and **c**) or double-labeled axons (**panel d**). See Methods for details of image acquisition and preparation used to generate this figure. Calibration bars, 100 μm.

The CB1 cannabinoid receptor protein (CB1) is found in the rodent uterus and blastocyst where the receptor contributes to normal fetal implantation and implantation failure [41]. These findings implicate involvement of the endocannabinoid system in reproductive function and dysfunction. The endocannabinoid system plays a key role in pain mechanisms [42], and, previously, cannabinoids were long used by women to alleviate dysmenorrhea [45].

Together the findings suggest that the endocannabinoid system is involved in endometriosis and its associated pain via CB1 receptors and innervation of the ectopic growths. Using the rat model, we performed a combination of immunohistochemical and pharmacological studies to test this hypothesis and assess the endocannabinoid system's potential as a target for new therapies. Studies were done when the rat was in proestrus, the estrous stage when hyperalgesia in the ENDO model is most severe [10,45,38].

2. Materials and methods

2.1. Animals and vaginal cytology

Adult female Sprague-Dawley rats (Charles River) were used. They weighed 175–200g at study start and 300–350g when euthanized. They were maintained on a 12-h light/dark cycle (lights on 07:00), housed individually in a temperature-controlled room (22.2°C) in plastic cages lined with wood chip bedding, with *ad libitum* access to Purina rat chow and water. Reproductive status was determined by histological examination of cells extracted by daily vaginal lavage done ~2h after lights on [3]. All rats cycled regularly (4-day cycle) during the study. Assessments were done when the rat was in proestrus (traditional nomenclature; [3]) 3 – 8 h after lights on. The studies were approved by Florida State University's Animal Care and Use Committee and adhered to the guidelines of the Committee for Research and Ethical Issues of IASP [53].

2.2. Endometriosis (ENDO) and control (shamENDO) surgeries

Surgery was done under aseptic precautions using methods developed by Vernon and Wilson [51]. Rats in diestrus were anesthetized with a ketamine hydrochloride and xylazine mixture (K/X; 73 mg/kg and 8.8 mg/kg, respectively, i.p.) and put on a heating pad to maintain body temperature ~37°C. A midline incision was made to expose the uterus. A ~l-cm segment of left uterine horn and associated fat was removed and put in warm sterile saline. Four pieces of uterine horn (~2 mm × 2 mm) or, for shamENDO, four similarly-sized pieces of fat were cut from this segment. Using 4.0 nylon sutures, the pieces were sewn around alternate cascade mesenteric arteries starting from the caecum, and the wound closed in layers. Postoperative recovery was uneventful; regular estrous cyclicity resumed within a few days. All assessments were done > 6 wks after the surgery; i.e., when ENDO-induced hyperalgesia is fully-developed and stable [10,45,38].

2.3. Sample harvesting and tissue preparation for immunohistochemical studies

On the day of euthanization, each rat (in proestrus, 6 – 10 wks after ENDO surgery) was anesthetized with urethane (1.2 g/kg, i.p.) and perfused transcardially with 4% paraformaldehyde in 0.1M phosphate buffer. The cysts, a 1-cm section of the mid-right (healthy eutopic) uterine horn, dorsal root ganglia (DRG) from T3-S1, and the coeliac ganglion (CG) were harvested and post-fixed in the same fixative for 4h. Samples were cryoprotected in 30% sucrose and stored at −80°C until sectioned. Cysts were embedded in Histo Prep medium (Fisher Scientific), cut serially in 20μm-thick sections on a cryostat, and thaw mounted on subbed slides.

2.4. Single CB1 immunostaining of cysts

The CB1 receptor antibody was raised in rabbits using a glutathione-S-transferase fusion protein containing the last fifteen amino acids of rat CB1. This same fusion protein was used for affinity purification (details in [6]). The specificity of this antibody has been demonstrated by a lack of immunoreactivity in CB1 receptor knockout mice [6]. Antigen was retrieved in 10 mM sodium citrate (pH 9.0), 80°C for 5 min. Sections were quenched (l% NaHB4, 10 min; 0.3% H_2O_2, 15 min), then blocked in 0.3% Triton X-100 in 0.05 M Tris-NaCl with 10% normal goat serum (NGS) and avidin (2

drops/ml), 1 h. Sections were incubated with rabbit anti-CB1 primary antibody (1:3000) in 0.3% Triton X-100 in 0.05 M Tris-NaCl, including 2% NGS and biotin (2 drops/ml) at 4°C for 48 h. Sections were incubated in biotinylated goat anti-rabbit IgG (1:300; Vector, at room temperature (RT) for 2 h, then incubated in avidin-biotin-peroxidase complex (Elite kit, Vector) for 1.5 h. Staining was visualized with 3,3'-diaminobenzidine (DAB kit, Vector). Controls were omission of primary or secondary antibody and absorption of primary antiserum with its respective antigen prior to use. There was no labeling in control sections.

2.5. Quantification of CB1-positive fibers in cysts and uterus

Sections of cysts and uterus (n = 8) harvested from the same rats were analyzed. An image of an entire DAB-stained section through the middle of each cyst or mid-uterine horn was captured with an Optronics Microfire camera and Neurolucida software (MBF Bioscience). Using a protocol described previously [52], a region in each section around the hilus of the cyst or an equivalent region of the uterus was demarcated (Fig. 2a, b), and its area calculated with the Stereo Investigator program. Two observers, blinded to group and consistent ($r > 85\%$) independently counted nerve fibers in this area using criteria described elsewhere [52]. After decoding, data were analyzed with unpaired Student's *t*-tests.

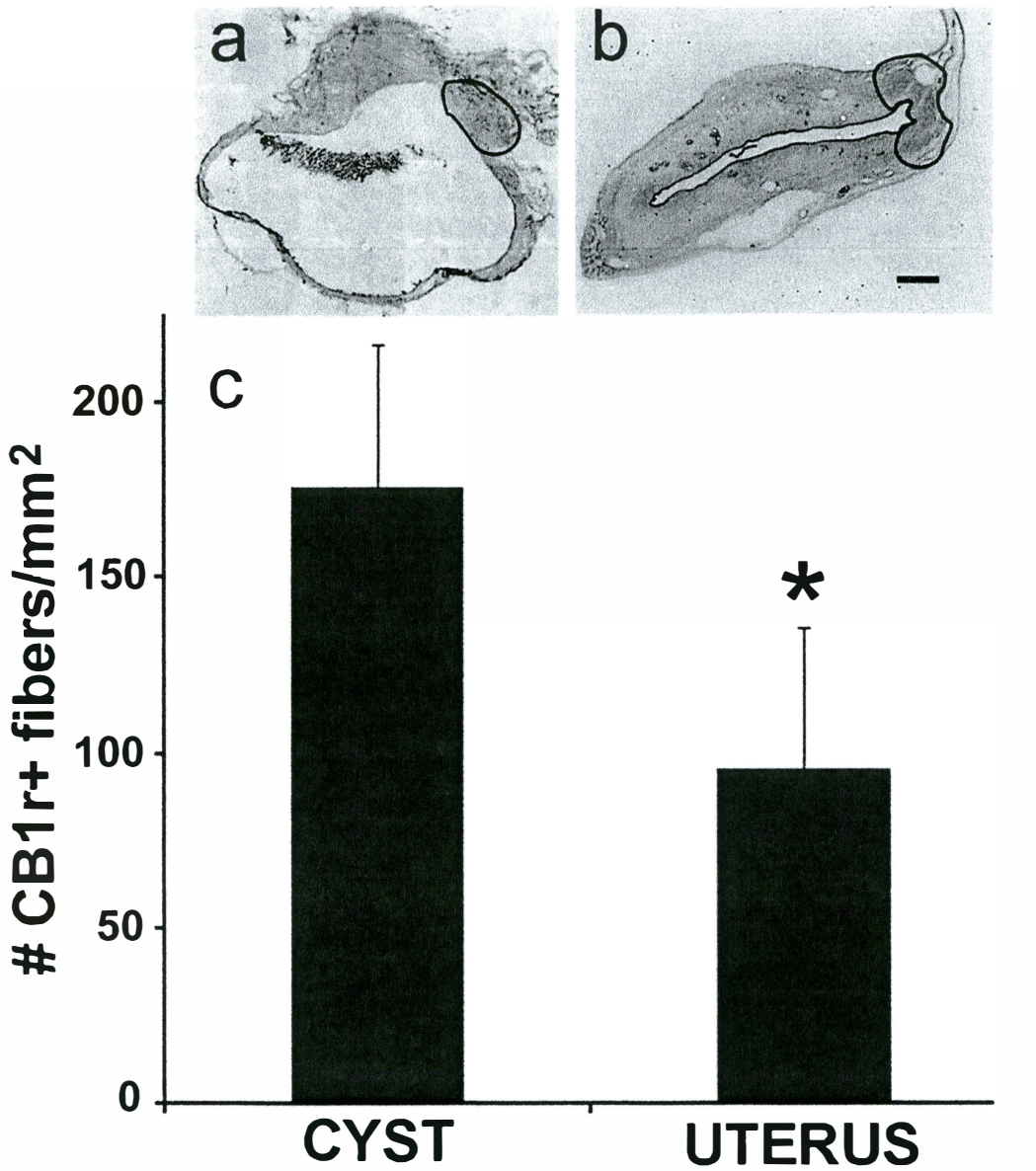

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Figure 2

Density of CB1-positive fibers is greater in the cysts and uterine horn. The regions outlined in the photomicrographs (**a, b**) show the approximate area in which counts were made in each tissue (i.e., the hilus of the cyst; the region in the uterine horn at the entrance of the uterine artery). Note that the size of this area did not differ between the two tissues ($P = 0.89$, unpaired Student's t-test). For the graph in **c**, *, $P = 0.024$; unpaired Student's t-test. Data shown as means and s.e.m. Calibration bar = 0.5 mm for both photomicrographs.

2.6. Double-immunostaining of cysts for CB1 and either calcitonin gene related peptide (CGRP) or vesicular monoamine transporter 2 (VMAT2)

Sections were immunostained with primary antibodies for CB1 (rabbit CB1-L15; from [6]) and either rabbit CGRP or rabbit VMAT2 (Chemicon: CGRP, AB5920, polyclonal; VMAT2, AB1598, raised against 13 amino acid C-terminal domain). Sections were washed in phosphate buffered saline (PBS), treated with Histo-VT-one antigen retrieval reagent (Nacalai) for 20min at 70°C, then washed in PBS and quenched in 0.3% H_2O_2 in PBS for 1h. First blocking was in 5% horse serum (HS) in 0.3% PBS-Tween-20 (PBST) for 1h at RT. Incubation in CB1 primary antibody (1:10,000) was done in 2% HS for 2h at RT, then overnight at 4°C. Sections were washed in 0.1% PBST and incubated in ImmPress (Vector) secondary antibody for 30 min at RT. After washing in 0.1%PBST then PBS, amplified signals were generated with Perkin Elmer Cy3 Tyramide Signal Amplification substrate (Perkin Elmer) at 1:50 in manufacturer's buffer for 10min. Sections were washed, blocked in normal goat serum (NGS) and incubated with either CGRP or VMAT rabbit primary antibody (1:4,000 dilution each) overnight in 0.3% PBST containing 2% NGS. After washing in PBST, sections were incubated in Cy2 Goat anti-rabbit secondary antibody (1:400, Jackson Immuno) for 2h, washed and mounted (ProLong, Invitrogen) for fluorescent image acquisition. Controls were omission of either primary antibody (no labeling observed), and assurance that CB1 fluorescence was visible only after amplification. A semi-quantitative assessment of CB1 co-labeling with either CGRP (sensory fibers) or VMAT2 (sympathetic fibers) was done on 6 sections from 6 rats. Double-labeling was characterized as "all (> ~75%)," "many (~75-50 %)," "few (~50-25 %)," or "none."

2.7. Retrograde labeling of ganglion cells in dorsal root ganglia (DRG) and coeliac ganglia (CG)

Six to 10 wks after ENDO surgery, 5 rats were anesthetized with K/X. Under aseptic conditions, the cysts were localized and isolated from surrounding tissue. Crystals of the neurotracer DiI (1.2 – 2 mg) were put inside each cyst through a small incision in its wall. The incision was closed with a suture, and rats recovered for ~2 wk before euthanization (in proestrus) and tissue harvesting. Cryostat-cut sections were first analyzed by epi-fluorescent microscopy to select sections containing retrogradely-identified ganglion cells labeled adequately for later confocal image acquisition to examine triple labeling with DiI and antibodies specific for sensory and sympathetic fibers, described next.

2.8. Immunohistochemical processing of DRGs and CGs

DRGs containing ganglion cells retrogradely-labeled with DiI were double-immunostained using primary antibodies for CB1 and CGRP (specified in Sections 2.4 and 2.6). CGs containing ganglion cells retrogradely-labeled with DiI were double-immunostained using primary antibodies for CB1 and for sympathetic neurons (rabbit a-tyrosine hydroxylase; TH; Chemicon, AB152, polyclonal, purified, SDS-denatured TH was used as the immunogen). Immunohistochemistry was done as described above with the following modifications: amplified signals for CB1 were generated with Perkin Elmer Cy5 Tyramide Signal Amplification substrate at 1:500 in amplification buffer for 15min. After washing and subsequent blocking in 5% NGS, sections were incubated with either CGRP (for DRGs) or TH (for CGs) rabbit primary antibody (1:4,000 dilution each) overnight in 0.3% PBST/2% NGS. After washing in PBST, sections were incubated in Cy2 Goat anti-rabbit secondary antibody (1:400, Jackson Immuno) for 2h, washed and mounted (ProLong) for confocal image acquisition. Controls were: (a) omission of CB1 antibody followed by TSA amplification and subsequent verification that the CB1 antibody signal could only be detected when amplified, (b) omission of either second primary antibody (CGRP/TH), and (c) omission of Cy2 secondary antibody.

Confocal images were captured using a Leica TCS SP2 AOBS laser confocal microscope. Monochromatic lasers at minimal excitement (Cy2 = 488 nm; DiI = 543 nm, Cy5 = 647 nm) and optimal photomultiplier settings for each of the three fluorescent dyes were used. Image stacks of 10 images at 2-μm intervals were acquired for each dye. Averaged overlays of the three separate captures

were generated with Leica LCS suite software and exported for contrast and brightness correction in Adobe Photoshop, v. 6 (Adobe Systems). For clarity in Fig. 1, the original dye colors were changed using Adobe Photoshop CS4 Extended (http://www.microscopyu.com/articles/confocal/threecolorconfocal.html). Grey scale image data from each dye capture was placed into a single channel of a Red-Green-Blue (RGB) image file. Image capture from DiI (originally red) was placed into the Blue channel, showing only blue. Image capture from CB1 (originally magenta) was placed into the Red channel, showing only red. Image captures from CGRP and TH (originally green) were placed into the Green channel; showing only green. The three differently-colored images were then aligned and merged, and the contrast and brightness of the merged images globally adjusted.

2.9. Assessment of triple-labeled ganglion cells in DRG and CG

T9 or T10 DRGs and CGs from four rats were chosen based on image quality of their retrograde labeling and immunohistochemical labeling with two antibodies. Singe sections were randomly selected from the middle of each DRG or CG. For each section, the original separate confocal images of fluorescent labeling for each of the three markers in (DiI, CB1 and either CGRP or TH) were coded and quantified using the Stereo Investigator program from MBF Bioscience. The three images from each section were overlaid and counted individually, allowing the experimenter to count neurons labeled with CB1, DiI, and either TH (in CGs) or CGRP (in DRGs), and then the double- and triple-labeled cells. The area and diameter of each counted cell in the DRGs was measured using the same program. Numbers of cells labeled by CB1, DiI, and either TH or CGRP within each ganglion, and the cell sizes were exported to spreadsheets to calculate the number of single-, double-, and triple-labeled DRG and CG cells, and the average sizes of the differently-labeled DRG cells.

2.10. Studies of visceromotor reflex (VMR) to vaginal distention, general

Before initiating VMR measures, and > 10 wks after ENDO surgery, each rat was acclimatized to the testing box (a clear acrylic chamber 65 mm wide × 75 mm high × 220 mm long) and to receiving vaginal distention in the box. Rats were put in the box for 10 min daily for 4–5 days. During the last 2 –3 days of this period, a distendable latex balloon (8 mm long by 1.5 mm wide uninflated) was inserted in the mid-vaginal canal and distended in 0.05 ml increments over ~1-min period via a hand-held 1.0-ml syringe filed with water to a 1.0 ml maximum unless the rat struggled. This distention stimulus was identical to that used in previous studies of vaginal nociception in awake rats [10,45]. Note that in these earlier behavioral studies, we found that the probability of the rat's making escape responses increases as the distention volume increases. Most naive rats begin making responses when distention volume reaches 0.35 ml; the probability of escape increases to 100% by 0.85–1.0 ml of distention [10,45].

2.11. Electrode implantation; recording electromyographic (EMG) activity during vaginal distention

Each fully-acclimated rat was anesthetized with K/X. Under aseptic surgical conditions, sterilized Teflon-coated stainless steel wires (AS633, Cooner Wire) were threaded into the left external abdominal oblique muscle and exteriorized at the back of the neck. After 7 to 10-days recovery, the rat was put in the testing box and its electrodes connected to measure EMG activity. EMG signals were amplified, filtered (low, 3 kHz; high, 10 Hz), converted to digital data using a Micro 1401 processor and sampled at 1 kHz using Spike2 software (Cambridge Electronics Design). The balloon was inserted in the vaginal canal, and connected via a catheter to a pressure transducer (COBE Cardiovascular) and to a water-filled syringe connected to a syringe pump. After a stable baseline EMG activity was recorded, the balloon was inflated at a steady rate (0.3 ml/min). The maximum distention was either the

volume that evoked enough EMG activity so that a threshold could later be clearly defined offline or a top volume of 0.825 ml. (The threshold volume; i.e., the VMRth, is described below in the data analysis section.) Note that if the rat struggled or tried to remove the balloon, the balloon was immediately deflated.

2.12. Drug treatment

Before drugs were delivered, the VMR was measured twice (10–15 min interval). The drug solution was then injected i.p. Thirty minutes later, the VMR was recorded again. In the WIN2, dose-response study (Fig. 3b), rats were treated with different doses (1 mg/kg, 3 mg/kg) of (R)-(+)-WIN 55,212-2 mesylate salt (WIN2) using a within-rat, cross-over design. The interval between doses was 4 days. To determine if this dosing scheme produced tolerance, the effects of single and multiple doses were compared. As seen in Fig. 4, this dosing scheme did not produce tolerance. In the antagonist study (Fig. 3c), rats were treated with: (a) WIN2 (3mg/kg), (b) WIN2 (3mg/kg) combined with the CB1 antagonist (AM251, 3 mg/kg) or with the CB2 antagonist (AM630, 1 mg/kg), (c) the antagonists alone (same doses), or (d) vehicle alone. Each rat was tested 2 to 6 times with a different treatment each time, at intervals greater than 4 days.

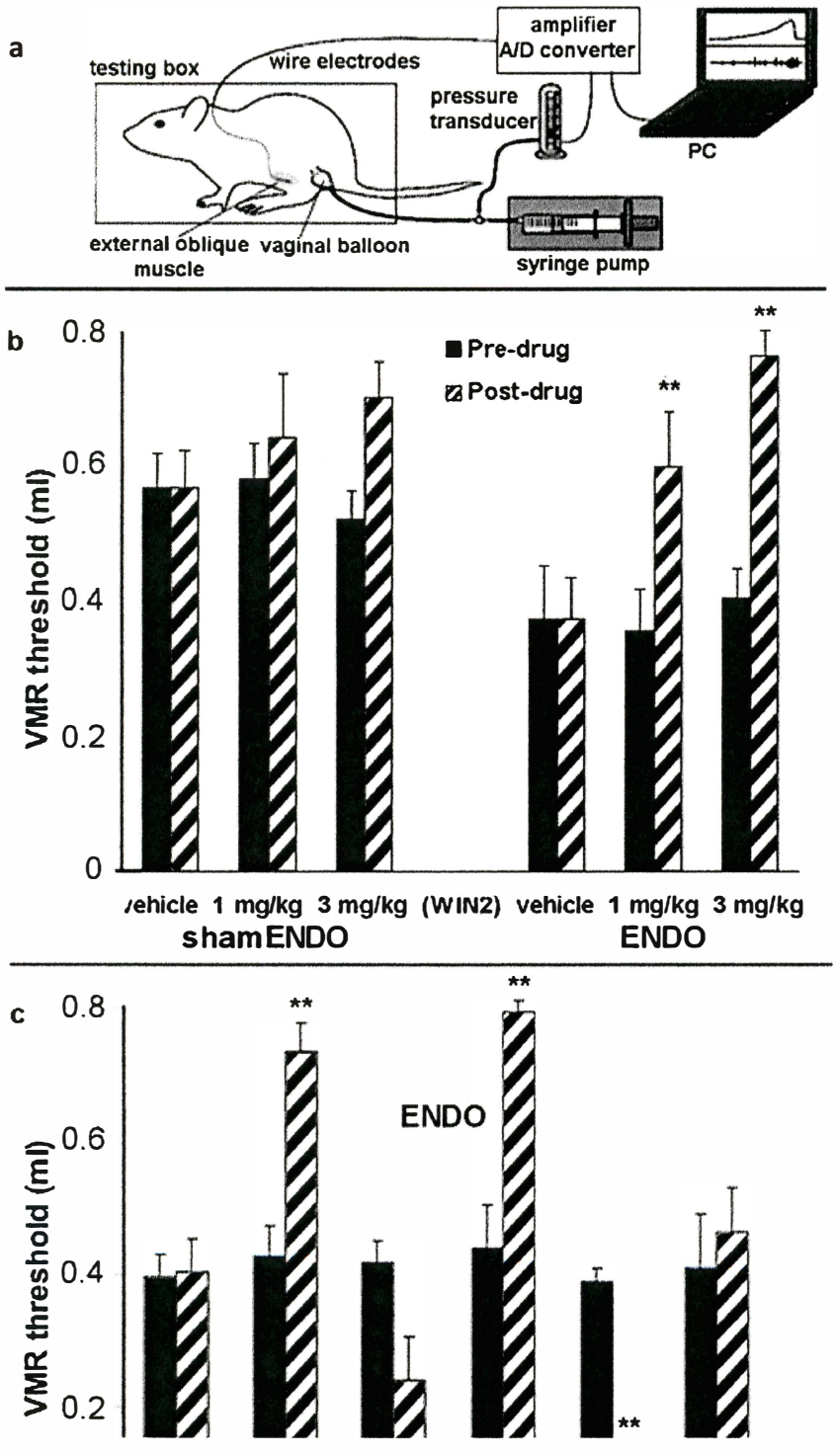

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Figure 3

Effects of WIN2 (CB1/CB2 agonist), AM 251 (CB1 antagonist), AM 630 (CB2 antagonist) on the visceromotor reflex threshold (VMRth) to vaginal distention. The diagram in **a** shows the testing set-up. The graph in **b** compares the effect of 2 doses of WIN2 on the VMRth in shamENDO and ENDO rats. Comparing only the back bars shows that the pre-drug VMRth was significantly greater in shamENDO

than in ENDO rats: $P = 0.00025$, unpaired Student's *t*-test, which confirms earlier studies that ENDO produces referred muscle hyperalgesia [38]. In the shamENDO group, WIN2 had no significant affects: ANOVA ($P = 0.353$). In contrast, in the ENDO group, both doses of WIN2 increased the VMRth significantly. $^{**}P < 0.01$ compared with pre-drug; ANOVA followed by post-hoc paired Student's t-tests. Thus, WIN2 alleviates referred muscle hyperalgesia in the ENDO group. The graph in **c** illustrates, in ENDO rats, the effects of WIN2 when combined with antagonists AM 251 (CB1-antagonist; CB1 ant) and AM630 (CB2 antagonist; CB2-ant) and with the antagonists delivered alone. $^{**}P < 0.008$ compared with pre-drug; ANOVA followed by post-hoc Student's *t*-tests, with alpha set at 0.008 (Bonferroni correction). The CB1, but not the CB2 antagonist prevented the effect of WIN2, which indicates that WIN2 acts via the CB1 receptor. The CB1, not the CB2, antagonist alone significantly reduced the VMRth (increases nociception), indicating that endocannabinoids normally suppress endometriosis-induced hyperalgesia. Data shown as means and ± s.e.m.

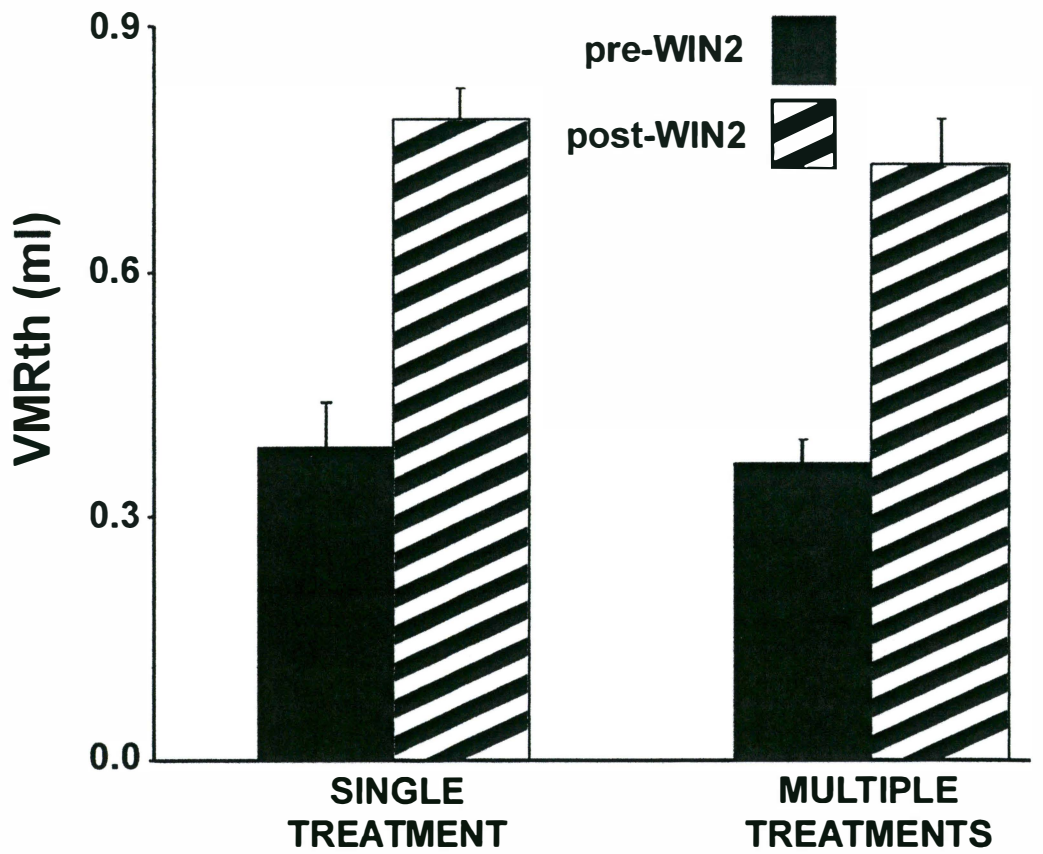

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Figure 4

Lack of tolerance to repeated administration of WIN2 over 8 days. The graph compares the effect on the VMRth of a single treatment of WIN2 (3 mg/kg) with multiple treatments in ENDO rats. In the multiple group, WIN2 was given three times at four-day intervals. No values between single-and multiply-dosed groups differed significantly; i.e., pre drug (P = 0.775), post-drug (P = 0.437), or the change in VMRth from pre to post drug (P = 0.711, not shown on graph); Students t-tests. Data are shown as means ± s.e.m.

2.13. Drugs and doses

WIN2 and AM 251 trifluoroacetate salt were purchased from Sigma. AM 630 was purchased from Tocris. Drugs were dissolved with 5% dimethyl sulfoxide-distilled water and administered at 2 ml/kg. The doses for WIN2, AM251 and AM630 were chosen based on effective doses in similar studies by others [19,32]. Note that the lower dose of the CB2 antagonist is unlikely to account for the lack of effectiveness of this antagonist in the present studies (Fig. 3c) because others have shown in rats that a pharmacological effect on a visceral organ that had been induced by a selective CB2 agonist was abolished by the same dose of AM 630 used here (1 mg/kg) [33]; i.e., this dose is sufficient to produce a selective CB2 antagonism.

2.14. Data analysis for VMR studies

As previously [38], area-under-the-curve (AUC) values of the VMR were used for statistical analyses. AUCs of the EMG were calculated for every 15-sec period beginning with a baseline period 15 sec before inflation of the balloon. The VMR threshold value (VMRth) was defined as the distention volume that induced twice (200%) the baseline AUC. Changes in VMRth values from before to after drug administration were then calculated.

SPSS/PASW version 17 software (IBM) was used for statistical analyses. For the two WIN2-dosing studies (ENDO group, shamENDO group, Fig. 3b), the data were analyzed using two-way ANOVAs. The ENDO group's ANOVA was significant [$F(5,24) = 6.928$, $P = 0.000$], with significant effects of dose ($P = 0.010$), pre-post ($P = 0.001$), and dose/pre-post interactions ($P = 0.027$). The ANOVA was therefore followed by post-hoc Student's *t*-tests for pre-drug versus post-drug effects, significance set at 0.05. The shamENDO group's ANOVA was not significant [$F(5,24) = 1.168$, $P = 0.353$], and therefore not followed by post-hoc tests. For the antagonist study (Fig. 3c), the two-way ANOVA was significant [$F(11,48) = 13.010$, $P = 0.000$], with significant effects of treatment ($P = 0.000$) and treatment/pre-post interaction ($P = 0.000$). This ANOVA was therefore followed by Student's *t*-tests, with significance set at 0.008 (Bonferroni correction).

3. Results

3.1. CB1 receptors are located on sympathetic and sensory fibers that innervate the ectopic growths

If the endocannabinoid system is involved in endometriosis and its associated pain, then CB1 receptors should be located on the axonal fibers innervating the cysts. With double-labeling fluorescence immunohistochemistry, most (> 75%) sympathetic fibers (vesicular monoamine transporter 2, VMAT2-positive) and many (50 – 75%) sensory fibers (calcitonin gene related peptide, CGRP-positive) in the cysts co-labeled with an antibody for CB1 receptors (CB1; [6]) (Fig. 1d). The fibers were associated mainly with blood vessels and muscle fibers in the walls of the cysts, and were seen less often in the endometrial layer. Although some CB1-labeled fibers were observed in the eutopic uterus of the same rats, notably, the density of the fibers was significantly higher in the cysts than uterus (Fig. 2).

3.2. CB1 receptors are located on neuronal somata from which axons sprout to innervate the ectopic growths

Support for CB1 involvement in endometriosis-associated pain would be strengthened by evidence that CB1 receptors are located on the somata of sensory and sympathetic neurons that innervate the cysts. Results of tripe-labeling fluorescence immunohistochemical experiments examining neuronal somata were consistent with the fiber labeling. For cyst-projecting CG neurons (identified by the retrogradely-labeled fluorescent marker DiI), 52% co-labeled with CB1, many of which (68%) were additionally

labeled with an antibody identifying them as sympathetic neurons (tyrosine hydroxylase, TH). For DiI-identified cyst-projecting DRG neurons, 47% were labeled with the CB1, some of which (24%) were also CGRP-labeled. Overall, cyst-projecting CB1-positive DRG neurons were small to medium-sized (diameter range and median:, 22.9 – 41.5 μm; 33.6 μm). Those DRG neurons that were additionally-labeled by CGRP were mainly small (diameter range and median: 22.9 – 30.4 μm; 27.7 μm). These sizes are within ranges reported by others for DRG neurons containing either CB1 [20,46] or CGRP [31].

3.3. Pharmacological studies

Support for the involvement of CB1 receptors in endometriosis-associated pain and for the endocannabinoid system as being a potential treatment target would be strengthened by evidence that exogenous cannabinoid agents reduce ENDO-induced hyperalgesia. Three studies were done to address this issue by testing the effects of cannabinoid agents on referred nociception in ENDO or shamENDO rats. Referred nociception was measured by distending the vaginal canal and recording the volume threshold at which electrical activity occurred in the external abdominal oblique musculature (Fig. 3a) [38]. This muscle activity is called the visceromotor reflex (VMR; [39]) and its threshold, the VMRth.

3.4. WIN 55212-2 (WIN2), a CB1/CB2 agonist, alleviates referred muscle hyperalgesia in ENDO rats

The effects of different doses of the CB1/CB2 agonist, WIN 55212-2 (WIN2) [22], on vaginal distention-induced referred abdominal muscle nociception in conscious ENDO and shamENDO rats were tested using a within-rat cross-over design. The VMRth was significantly lower in ENDO than shamENDO rats, confirming the existence of vaginally-referred muscle hyperalgesia in unanesthetized ENDO rats [38]. WIN2 treatment had no significant effect on shamENDO rats, but significantly increased the VMRth in ENDO rats (Fig. 3b). No tolerance was apparent after multiple treatments (Fig. 4).

3.5. WIN2 alleviates referred muscle hyperalgesia in ENDO rats in a CB1 receptor-dependent manner

To determine which receptor was involved in the alleviation of hyperalgesia, we tested the effects on the VMRth of combining WIN2 with a CB1 or CB2 receptor antagonist (AM 251 or AM 630, respectively; [22]). Co-administration of AM 251, but not of AM 630, with WIN2 prevented WIN2's reduction of ENDO-induced hyperalgesia (Fig. 3c).

3.6. The *endocannabinoid system* is normally engaged in ENDO's induction of vaginal hyperalgesia

To examine the potential role of endogenous cannabinoids in ENDO-induced vaginal hyperalgesia, the CB1 or CB2 receptor antagonists were given alone to ENDO rats. The CB1 (AM 251), but not the CB2 (AM 630) antagonist, increased the referred hyperalgesia (i.e., decreased the VMRth; Fig. 3c). Because AM251 competitively inhibits endocannabinoid activation of CB1 receptors [18], this finding, together with the immunohistochemical results above, suggests that endocannabinoids tonically suppress ENDO-induced hyperalgesia.

4. Discussion

4.1. Ectopic growths, their innervation, and CB1 receptors

We used a rat model to test the hypothesis that the endocannabinoid system contributes via the nervous system to both the ectopic growths that define endometriosis and the hyperalgesia associated with it. Two findings support the first part of this hypothesis, endocannabinoid involvement in innervation of the growths. First, CB1 receptors were abundantly found not only on sensory and sympathetic fibers that had sprouted to innervate the ectopic growths (cysts), but also on retrogradely-identified somata of DRG and CG neurons from which the sprouted fibers originate. This finding indicates that CB1 receptors are strategically located for endogenous cannabinoids to influence functioning of cyst-innervating neurons. Second, CB1 labeling was significantly denser in the cysts than in the eutopic uterus. This finding indicates an upregulation of CB1 receptors in the ectopic uterine tissue relative to the healthy uterine tissue, which suggests that the endocannabinoid system is involved in the development and functioning of the abnormal sprouted innervation.

Another relevant aspect of our findings is that both sensory and sympathetic fibers invade the ectopic growths [4], creating direct two-way communication between the growths and the central nervous system. The fact that both types of fibers and their cell bodies are invested with CB1 receptors suggests that the endocannabinoid system contributes to coordinating and maintaining this communication. Furthermore, the physical proximity between sensory and sympathetic fibers within the cysts suggests a functional coupling between them [28,36]. The investment of these peripheral axons with CB1 receptors further suggests that the endocannabinoid system can regulate this coupling.

4.2. CB1 involvement in ENDO-associated vaginal hyperalgesia

Two findings support the second part of our hypothesis, endocannabinoid system involvement in ENDO-associated vaginal hyperalgesia via CB1, which in turn suggests a new approach to alleviate endometriosis-associated pain. First, treating ENDO rats with a CB1 (AM251) but not a CB2 (AM630) receptor antagonist increased the rats' ENDO-induced referred hyperalgesia, suggesting that CB1 but not CB2 receptors normally act to reduce the hyperalgesia. Because AM251 can act as an inverse agonist, however, further support for endocannabinoid involvement would come from testing drugs that increase endocannabinoid levels such as fatty acid amide hydrolase or monoacylglycerol lipase inhibitors [22]. Second, treatment of ENDO rats with a CB1/CB2 receptor agonist alleviated the ENDO-induced hyperalgesia in a CB1 receptor-dependent manner. Importantly, rats that were multiply-dosed with the CB1/CB2 agonist did not show tolerance, suggesting that repeated treatment with cannabinoids would continue to alleviate ENDO-induced hyperalgesia without losing efficacy.

4.3. Mechanisms underlying treatment efficacy in ENDO-associated pains and their potential association with the endocannabinoid system

The finding here that a cannabinoid agent alleviates hyperalgesia in a rat model of endometriosis is consistent with the known efficacy of cannabinoid agents in women with endometriosis [45] and supports a growing body of previous work in numerous contexts demonstrating the considerable potential of the endocannabinoid system as a target for the development of new therapies to alleviate pain [14,17].

Although mechanisms by which the endocannabinoid system and exogenous cannabinoid agents act to influence pain are under intense study [14,17], little is known in the specific context of endometriosis-associated pain. One obvious possibility in the rat model, and perhaps women, is that exogenous cannabinoid agents act directly on the sensitized peripheral nociceptive afferents, as demonstrated in other inflammatory and neuropathic models using CB1 knockout mice [1].

Other possibilities relate to mechanisms underlying the efficacy of clinical treatments currently used to alleviate endometriosis-associated pain. As reviewed by Giudice [16], in addition to surgical removal of the ectopic growths, medical treatments effective for some women include a variety of agents targeted

on "minimizing inflammation" (e.g., non-steroidal anti-inflammatory drugs, NSAIDs). Other more reliable medical treatments are targeted on "interrupting or suppressing cyclic ovarian hormone production, inhibiting the action and synthesis of estradiol, and reducing or eliminating menses" (e.g., progestins, GnRH agonists, aromatase inhibitors, Danazol, and combined oral contraceptives).

Regarding NSAIDs, some, like naproxen, are of debatable efficacy in endometriosis-associated pain (e.g., 2,26). It is generally concluded, however, that the efficacy of NSAIDs likely depends on their influence on the inflammatory environment of the ectopic growths or peritoneal fluid [30,21,48]. For example, one purported mechanism of endometriosis-associated dysmenorrhea involves increased peritoneal fluid levels of prostaglandins and many other pro-inflammatory molecules [21,29]; effective NSAIDs would reduce these molecules and thus be acting peripherally.

On the other hand, there is a well-recognized extensive and complex association between the endocannabinoid and inflammatory systems. It is therefore possible that some NSAIDs might act to reduce endometriosis-associated pain via the endocannabinoid system. For example, COX-2 converts the endocannabinoid 2-arachidonoylglycerol (2-AG) into prostaglandin E2 glycerol ester (PGE2-G), which is pro-nociceptive because, when administered into the footpad, it induces mechanical allodynia and thermal hyperalgesia [23]. Thus, specific COX-2 inhibitors, which alleviate endometriosis-associated pain [12], could, as has recently been shown, act centrally to reduce hyperalgesia by preventing 2-AG breakdown into pro-nociceptive molecules [7,47,50].

Regarding hormonal therapies, mechanisms by which they alleviate endometriosis-associated pain are poorly understood [16,48]. One mechanism could involve opioid receptors. Matsuzaki and colleagues [34] found that muopioid receptors exist in sampels of deep-infiltrating endometriosis (associated with severe pain). However, this group also found that while treatment with a GnRH agonist or oral progestin eliminated the receptors, their function is unclear. Furthermore, there is a paucity of clinical data regarding the efficacy of opioids in treating endometriosis-associated pain. [16,27,48]. A second mechanism could involve the influence of hormonal therapies on the complex association between prostaglandins and estradiol. For example, PGE_2 increases estradiol concentrations in ectopic growths [9], which could increase sprouting of nociceptors into them [11], but this possibility has not yet been studied directly in the context of pain.

A third mechanism could involve a direct influence of hormonal therapy on the ectopic growths' innervation. In the rat model, the proestrous-to-estrous stage reduction in both plasma estradiol and ENDO-associated hyperalgesia is in turn associated with a reduction in both sympathetic fiber density and growth factors within the ectopic growths [52]. This finding suggests that hormonal therapy-induced hypoestrogenicity could act directly on the sympathetic-sensory coupling mechanism discussed in section 4.1. Consistent with this possibility are recent findings from a comprehensive clinical review that hormonal therapy is related to a reduction in nerve fiber density in ectopic endometrial growths [37].

Finally, studies regarding the association between hormonal or reproductive status and the endocannabinoid system are in their infancy; most focus, not on pain, but on healthy reproductive function [e.g., 49]. Even less is known regarding interactions between reproductive/hormonal status and the potential efficacy of cannabinoid agents for pain. Results so far are inconclusive. Kalbasi and colleagues [25] found that WIN2's efficacy on the thermal tail flick test in mice is *reduced* by estradiol, whereas Craft and Leitl [13] found that Δ^9-tetrahydrocannabinol's efficacy on tail withdrawal and paw pressure tests in rats is *enhanced* by estradiol. In contrast, two other rodent studies suggest that hormonal therapies in endometriosis might affect how cannabinoids influence central pain mechanisms; both studies observed ovarian cyclical changes in cannabinoid receptor density or endocannabinoid content in brain areas potentially associated with nociception, [8,44].

4.4. Endocannabinoid involvement in neurovascular coordination

Previous findings from our laboratory suggest that the innervation of ectopic uterine growths by sensory and sympathetic fibers in the rat model likely derives from axonal branches that sprout from innervated blood vessels that are themselves simultaneously branching to vascularize the abnormal tissue as it grows [4]. Investment of the sprouted fibers with CB1 receptors supports the suggestion that the endocannabinoid system is involved in this neurovascular coordination [43], which has implications for conditions other than endometriosis. Thus, such a process could be common to other conditions that similarly involve growth of pathological tissue (e.g., benign or malignant tumors) or in which tissue is transplanted. Indeed, evidence is accumulating for endocannabinoid involvement in some of these conditions [15,24,40], but association of this involvement with neurovascular coordination has not yet been considered or studied.

4.5. Summary and conclusions

These studies in a rat model of endometriosis provide evidence that endocannabinoids might regulate the innervation of the disease's abnormal growths and that exogenous cannabinoid agents can be effective in reducing endometriosis symptoms. The fact that CB1 receptor expression is greater in the cysts than healthy uterus from the same rats suggests that treatments to activate CB1 receptors (either directly by CB1 agonists or indirectly by increasing relevant endocannabinoid levels) could be developed with minimal effects on uterine function. Although the rat model parallels many aspects of endometriosis in women, there are of course significant differences [48]. However, when considered together with the past history of successful use of cannabinoids for alleviation of gynecological pains [45], and insofar as findings in rats can model mechanisms of endometriosis-related signs and symptoms, the present results suggest that approaches targeted at the endocannbinoid system represent a promising new direction for developing badly-needed new treatments for pain suffered by women with endometriosis.

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Footnotes

The authors declare no conflicts of interest.

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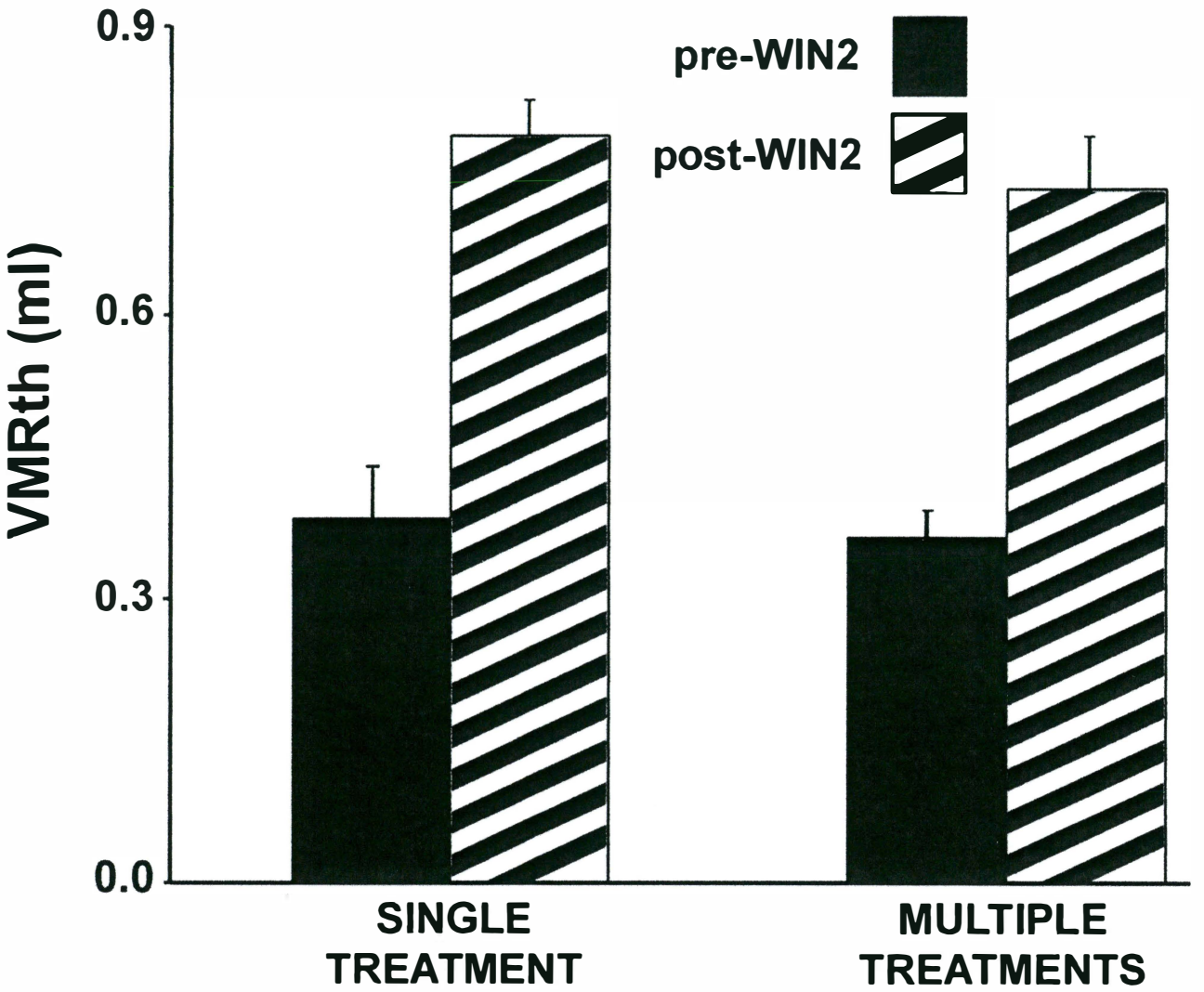
Figure 4



Lack of tolerance to repeated administration of WIN2 over 8 days. The graph compares the effect on the VMRth of a single treatment of WIN2 (3 mg/kg) with multiple treatments in ENDO rats. In the multiple group, WIN2 was given three times at four-day intervals. No values between single-and multiply-dosed groups differed significantly; i.e., pre drug ($P = 0.775$), post-drug ($P = 0.437$), or the change in VMRth from pre to post drug ($P = 0.711$, not shown on graph); Students t-tests. Data are shown as means ± s.e.m.

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Neuropathy #1 1-23-19

Br J Pharmacol. 2017 Sep;174(17):2832-2841. doi: 10.1111/bph.13887. Epub 2017 Jul 27.

Single and combined effects of Δ^9 -tetrahydrocannabinol and cannabidiol in a mouse model of chemotherapy-induced neuropathic pain.

King KM[1], Myers AM[1], Soroka-Monzo AJ[1], Tuma RF[1], Tallarida RJ[1], Walker EA[2], Ward SJ[1].

Author information

Abstract

BACKGROUND AND PURPOSE: The non-psychoactive phytocannabinoid cannabidiol (CBD) can affect the pharmacological effects of Δ^9 -tetrahydrocannabinol (THC). We tested the possible synergy between CBD and THC in decreasing mechanical sensitivity in a mouse model of paclitaxel-induced neuropathic pain. We also tested the effects of CBD on oxaliplatin- and vincristine-induced mechanical sensitivity.

EXPERIMENTAL APPROACH: Paclitaxel-treated mice (8.0 mg·kg^{-1} i.p., days 1, 3, 5 and 7) were pretreated with CBD (0.625-20.0 mg·kg^{-1} i.p.), THC (0.625-20.0 mg·kg^{-1} i.p.) or CBD + THC (0.04 + 0.04-20.0 + 20.0 mg·kg^{-1} i.p.), and mechanical sensitivity was assessed on days 9, 14 and 21. Oxaliplatin-treated (6.0 mg·kg^{-1} i.p., day 1) or vincristine-treated mice (0.1 mg·kg^{-1} i.p. days 1-7) were pretreated with CBD (1.25-10.0 mg·kg^{-1} i.p.), THC (10.0 mg·kg^{-1} i.p.) or THC + CBD (0.16 mg·kg^{-1} THC + 0.16 mg·kg^{-1} CBD i.p.).

KEY RESULTS: Both CBD and THC alone attenuated mechanical allodynia in mice treated with paclitaxel. Very low ineffective doses of CBD and THC were synergistic when given in combination. CBD also attenuated oxaliplatin- but not vincristine-induced mechanical sensitivity, while THC significantly attenuated vincristine- but not oxaliplatin-induced mechanical sensitivity. The low dose combination significantly attenuated oxaliplatin- but not vincristine-induced mechanical sensitivity.

CONCLUSIONS AND IMPLICATIONS: CBD may be potent and effective at preventing the development of chemotherapy-induced peripheral neuropathy, and its clinical use may be enhanced by co-administration of low doses of THC. These treatment strategies would increase the therapeutic window of cannabis-based pharmacotherapies.

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1-23-19
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Neuropathy

PubMed

Format: Abstract

Full text links

LinkOut to related resource

Cleve Clin J Med. 2018 Dec;85(12):943-949. doi: 10.3949/ccjm.85a.17115.

Cannabis for peripheral neuropathy: The good, the bad, and the unknown.

Modesto-Lowe V[1,2,3], Bojka R[2], Alvarado C[3].

Author information

Abstract

Cannabis may be an effective alternative or adjunctive treatment for peripheral neuropathy, an often debilitating condition for which standard treatments often provide little relief. Most studies show moderately improved pain from inhaled cannabis use, but adverse effects such as impaired cognition and respiratory problems are common, especially at high doses. Data on the long-term safety of cannabis treatments are limited. Until risk-benefit profiles are better characterized, doctors in states where cannabis therapy is legal should recommend it for peripheral neuropathy only after careful consideration.

PMID: 30526755 DOI: 10.3949/ccjm.85a.17115

Free full text

Publication type

LinkOut - more resources

Opioid AB 1417 #1 Page 1 of 2 1-23-19

PubMed

Format: Abstract

Full text links

PMC FREE Full text

Cannabis Cannabinoid Res. 2017 Jun 1;2(1):160-166. doi: 10.1089/can.2017.0012. eCollection...

Cannabis as a Substitute for Opioid-Based Pain Medication: Patient Self-Report.

Reiman A[1], Welty M[2], Solomon P[3].

Author information

Abstract

Introduction: Prescription drug overdoses are the leading cause of accidental death in the United States. Alternatives to opioids for the treatment of pain are necessary to address this issue. Cannabis can be an effective treatment for pain, greatly reduces the chance of dependence, and eliminates the risk of fatal overdose compared to opioid-based medications. Medical cannabis patients report that cannabis is just as effective, if not more, than opioid-based medications for pain. **Materials and Methods:** The current study examined the use of cannabis as a substitute for opioid-based pain medication by collecting survey data from 2897 medical cannabis patients. **Discussion**: Thirty-four percent of the sample reported using opioid-based pain medication in the past 6 months. Respondents overwhelmingly reported that cannabis provided relief on par with their other medications, but without the unwanted side effects. Ninety-seven percent of the sample "strongly agreed/agreed" that they are able to decrease the amount of opiates they consume when they also use cannabis, and 81% "strongly agreed/agreed" that taking cannabis by itself was more effective at treating their condition than taking cannabis with opioids. Results were similar for those using cannabis with nonopioid-based pain medications. **Conclusion:** Future research should track clinical outcomes where cannabis is offered as a viable substitute for pain treatment and examine the outcomes of using cannabis as a medication assisted treatment for opioid dependence.

KEYWORDS: cannabis; harm reduction; opiates; opioids; pain; substitution

PMID: 28861516 PMCID: PMC5569620 DOI: 10.1089/can.2017.0012

Free PMC Article

HB 1417 page 63
Migraine #1 1-23-19

PubMed

Format: Abstract

Full text links

Headache. 2018 Jul;58(7):1139-1186. doi: 10.1111/head.13345.

Medicinal Properties of Cannabinoids, Terpenes, and Flavonoids in Cannabis, and Benefits in Migraine, Headache, and Pain: An Update on Current Evidence and Cannabis Science.

Baron EP[1].

Author information

Abstract

BACKGROUND: Comprehensive literature reviews of historical perspectives and evidence supporting cannabis/cannabinoids in the treatment of pain, including migraine and headache, with associated neurobiological mechanisms of pain modulation have been well described. Most of the existing literature reports on the cannabinoids Δ^9-tetrahydrocannabinol (THC) and cannabidiol (CBD), or cannabis in general. There are many cannabis strains that vary widely in the composition of cannabinoids, terpenes, flavonoids, and other compounds. These components work synergistically to produce wide variations in benefits, side effects, and strain characteristics. Knowledge of the individual medicinal properties of the cannabinoids, terpenes, and flavonoids is necessary to cross-breed strains to obtain optimal standardized synergistic compositions. This will enable targeting individual symptoms and/or diseases, including migraine, headache, and pain.

OBJECTIVE: Review the medical literature for the use of cannabis/cannabinoids in the treatment of migraine, headache, facial pain, and other chronic pain syndromes, and for supporting evidence of a potential role in combatting the opioid epidemic. Review the medical literature involving major and minor cannabinoids, primary and secondary terpenes, and flavonoids that underlie the synergistic entourage effects of cannabis. Summarize the individual medicinal benefits of these substances, including analgesic and anti-inflammatory properties.

CONCLUSION: There is accumulating evidence for various therapeutic benefits of cannabis/cannabinoids, especially in the treatment of pain, which may also apply to the treatment of migraine and headache. There is also supporting evidence that cannabis may assist in opioid detoxification and weaning, thus making it a potential weapon in battling the

opioid epidemic. Cannabis science is a rapidly evolving medical sector and industry with increasingly regulated production standards. Further research is anticipated to optimize breeding of strain-specific synergistic ratios of cannabinoids, terpenes, and other phytochemicals for predictable user effects, characteristics, and improved symptom and disease-targeted therapies.

KEYWORDS: CBD; THC; cannabidiol; cannabinoids; cannabis; flavonoids; headache; marijuana; migraine; terpenes; Δ9-tetrahydrocannabinol

PMID: 30152161 DOI: 10.1111/head.13345

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Arthritis HB 1417 Page 65 #1 1-23-19

PubMed

Format: Abstract

Full text links

Pain. 2017 Dec;158(12):2442-2451. doi: 10.1097/j.pain.0000000000001052.

Wolters Kluwer | PMC FREE Full text

Attenuation of early phase inflammation by cannabidiol prevents pain and nerve damage in rat osteoarthritis.

Philpott HT[1], O'Brien M, McDougall JJ.

Author information

Abstract

Osteoarthritis (OA) is a multifactorial joint disease, which includes joint degeneration, intermittent inflammation, and peripheral neuropathy. Cannabidiol (CBD) is a noneuphoria producing constituent of cannabis that has the potential to relieve pain. The aim of this study was to determine whether CBD is anti-nociceptive in OA, and whether inhibition of inflammation by CBD could prevent the development of OA pain and joint neuropathy. Osteoarthritis was induced in male Wistar rats (150-175 g) by intra-articular injection of sodium monoiodoacetate (MIA; 3 mg). On day 14 (end-stage OA), joint afferent mechanosensitivity was assessed using in vivo electrophysiology, whereas pain behaviour was measured by von Frey hair algesiometry and dynamic incapacitance. To investigate acute joint inflammation, blood flow and leukocyte trafficking were measured on day 1 after MIA. Joint nerve myelination was calculated by G-ratio analysis. The therapeutic and prophylactic effects of peripheral CBD (100-300 μg) were assessed. In end-stage OA, CBD dose-dependently decreased joint afferent firing rate, and increased withdrawal threshold and weight bearing ($P < 0.0001$; $n = 8$). Acute, transient joint inflammation was reduced by local CBD treatment ($P < 0.0001$; $n = 6$). Prophylactic administration of CBD prevented the development of MIA-induced joint pain at later time points ($P < 0.0001$; $n = 8$), and was also found to be neuroprotective ($P < 0.05$; $n = 6$-8). The data presented here indicate that local administration of CBD blocked OA pain. Prophylactic CBD treatment prevented the later development of pain and nerve damage in these OA joints. These findings suggest that CBD may be a safe, useful therapeutic for treating OA joint neuropathic pain.

PMID: 28885454 PMCID: PMC5690292 DOI: 10.1097/j.pain.0000000000001052

[Indexed for MEDLINE] **Free PMC Article**

#1 HB1417 Page 66
Cancer/Inflammation 1-23-19

PubMed

Format: Abstract

Full text links

Biomed Res Int. 2018 Dec 4;2018:1691428. doi: 10.1155/2018/1691428. eCollection 2018.

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Cannabis sativa L. and Nonpsychoactive Cannabinoids: Their Chemistry and Role against Oxidative Stress, Inflammation, and Cancer.

Pellati F[1], Borgonetti V[2], Brighenti V[1], Biagi M[2], Benvenuti S[1], Corsi L[1].

Author information

Abstract

In the last decades, a lot of attention has been paid to the compounds present in medicinal *Cannabis sativa* L., such as Δ^9-tetrahydrocannabinol (Δ^9-THC) and cannabidiol (CBD), and their effects on inflammation and cancer-related pain. The National Cancer Institute (NCI) currently recognizes medicinal *C. sativa* as an effective treatment for providing relief in a number of symptoms associated with cancer, including pain, loss of appetite, nausea and vomiting, and anxiety. Several studies have described CBD as a multitarget molecule, acting as an adaptogen, and as a modulator, in different ways, depending on the type and location of disequilibrium both in the brain and in the body, mainly interacting with specific receptor proteins CB_1 and CB_2. CBD is present in both medicinal and fibre-type *C. sativa* plants, but, unlike Δ^9-THC, it is completely nonpsychoactive. Fibre-type *C. sativa* (hemp) differs from medicinal *C. sativa*, since it contains only few levels of Δ^9-THC and high levels of CBD and related nonpsychoactive compounds. In recent years, a number of preclinical researches have been focused on the role of CBD as an anticancer molecule, suggesting CBD (and CBD-like molecules present in the hemp extract) as a possible candidate for future clinical trials. CBD has been found to possess antioxidant activity in many studies, thus suggesting a possible role in the prevention of both neurodegenerative and cardiovascular diseases. In animal models, CBD has been shown to inhibit the progression of several cancer types. Moreover, it has been found that coadministration of CBD and Δ^9-THC, followed by radiation therapy, causes an increase of autophagy and apoptosis in cancer cells. In addition, CBD is able to inhibit cell proliferation and to increase apoptosis in different types of cancer models. These activities seem to involve also alternative pathways, such as the interactions with TRPV and GRP55 receptor complexes. Moreover, the finding that the acidic precursor of CBD (cannabidiolic acid, CBDA) is able to inhibit the migration of breast cancer cells and to downregulate the proto-oncogene c-fos and the

cyclooxygenase-2 (COX-2) highlights the possibility that CBDA might act on a common pathway of inflammation and cancer mechanisms, which might be responsible for its anticancer activity. In the light of all these findings, in this review we explore the effects and the molecular mechanisms of CBD on inflammation and cancer processes, highlighting also the role of minor cannabinoids and noncannabinoids constituents of Δ^9-THC deprived hemp.

PMID: 30627539 PMCID: PMC6304621 DOI: 10.1155/2018/1691428

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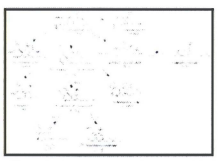
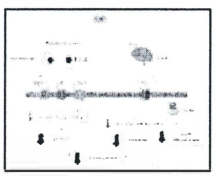
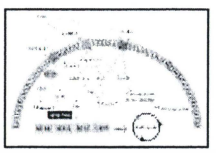

Publication type

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HB1417 #2

19.0429.02001
Title.

Prepared by the Legislative Council staff for
Representative Vetter
January 21, 2019

1-23-19

PROPOSED AMENDMENTS TO HOUSE BILL NO. 1417

Page 1, line 3, after the third comma insert "30,"

Page 4, after line 7, insert:

"**SECTION 4. AMENDMENT.** Subsection 30 of section 19-24.1-01 of the North Dakota Century Code is amended and reenacted as follows:

30. "Pediatric medical marijuana" means a medical marijuana product containing cannabidiol ~~which may not contain a maximum concentration or amount of tetrahydrocannabinol of more than six percent~~."

Renumber accordingly

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North Dakota House Human Services Committee

January 23th 2019

Chairman Robin Weisz / Vice Chairwoman Karen Rohr and members of the Committee, my name is Steven James Peterson of The Committee for Compassionate Care of North Dakota.

The Committee for Compassionate Care is a patient advocacy group seeking to enable fair and reasonable access to medical marijuana in the state of North Dakota.

I am in support of House Bill 1417

- These changes reflect the volume of cannabis needed by certain patients undergoing treatment like chemotherapy that can cause extreme nausea and vomiting
- The inclusion of the opioid addiction issues here is of critical importance in the current reality of North Dakota and the nation
- This bill is a proper step in the right direction for the realization of a "working" North Dakota Medical Marijuana program.

I am available for any questions about this bill.

Steven James Peterson, District 44 Fargo North Dakota

701-936-4362 Steven@ravenrisingllc.com

HB 1417
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1-23-19

Representatives and Senators,

I am a disabled veteran that served in 7.5 years Active duty in the Navy, and I wouldn't change a thing. During my service I deployed three times on-board the USS Iwo Jima, at an intelligence agency, and in Afghanistan. My service has left me disabled, I have suffered from chronic pain in my back and knees, PTSD, and other conditions. I have tried numerous pharmaceuticals including Benzodiazepines, S. S. R. I.s, Opiates, sleeping drugs, and other pharmaceuticals all prescribed to me by the VA or when I was on active duty. Some of the pharmaceuticals I was prescribed have dangerous side effects, from psychotic episodes to literally suicidal thoughts or suicide. After my service while living in Colorado I discovered, with the help of a few great people, the great benefits to medical cannabis. While in Colorado, I was able to stop taking the dangerous drugs the VA was prescribing with the help of cannabis and deal with my issues rather than just burying them under pills.

Cannabis allowed me to have healthy emotion again, move on, and love rather than live in a shell. With the help of cannabis since my service, I have earned a Mechanical Engineering degree, started a wonderful career, learned a lot about myself, and fell in love with a beautiful woman. All of this would not be possible without medical cannabis.

As a Veteran who receives care at the VA and chairs ND Veterans for safe access to cannabis, our providers (the VA) are not supposed to speak to Veterans about possible uses of medical cannabis. We have to many Veterans that this safe alternative can provide, much needed relief. We ask for a safe alternative to prescriptions. Many of us got involved in Measure 3 because it would get us access to quality safe medicine quickly.

We need your help! I am asking for expanding medical access and making it easier to get the medicine that will greatly help a lot of us. We are asking for safe and cost-effective access to this medicine. We also ask that the amount of concentrate allowed is comparable to the flower. By this we ask for 14 grams of concentrate a month, because concentrates are typically a 4:1 ratio when compared to flower. This means that it takes four grams of flower to make one gram of concentrate, we want a person that chooses concentrates instead of flower to have access to the same benefit. We also ask to eliminate the requirement of a pattern of care (the VA doctors are not allowed to even discuss cannabis). This Bill does a lot to get patients the access that they desperately need.

Sincerely,

Christopher Howell
US Navy 2005-2013
OEF 2011—2012
ND Vets For safe access- Chair
chowell1985.ch@gmail.com
Fargo,ND

#5 HB1417
1-23-19

Committee Chair and Members,

Hello, my name is Jody Vetter. I have lived in North Dakota my entire life. My family homesteaded here in 1905. I Live in Bismarck. My husband and I have owned and operated a small business since 2003. I have a qualifying medical condition. For my own benefit and curiosity I completed a certification program on the physiology and health of THC and CBD from Alternate Medical Health, as well as a course in the core knowledge of the endocannabinoid system from The Medical Cannabis Institute.

I am in favor of House Bill NO. 1417. I believe it should receive a DO PASS recommendation.

This bill is critical for patients to gain access to medical cannabis. Many patients have doctors that are unable or unwilling to certify them for medical cannabis. Physicians cannot state professionally that a person would benefit from cannabis nor recommend any ingestion forms or methods. They can only certify you have a condition listed without fear of federal involvement.

Based on my study of THC and CBD it is warranted to have endometriosis, interstitial cystitis, neuropathy, opioid use disorder, opioid withdrawal, migraine, rheumatoid arthritis and ehlers-danlos syndrome added to the list of conditions. All of these conditions are treatable with cannabis. Dr. Peter Grinspoon, a contributing editor for Harvard Health Publishing, the media and publishing division of Harvard Medical School, also confirms this in a Harvard Health Blog, January 15th 2018.

I believe cancer patients may require more medicine then the standard patient. It is important they get the respect and compassion they deserve and need with the struggle they face just to survive.

Finally, It is important to also have a written certification form patients can physically take to their doctors to fill out. Coming to your appointment with a paper to fill out seems to be less work for the physician.

Thank you for your time and consideration. I can be reached at 701-400-8078 or jodylvetter@hotmail.com.

Sincerely,
Jody Vetter

Pogel

HB1417
#6
1-23-19

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House Bill 1417
Human Services
January 23, 2019

Good morning Chairman Weisz and members of the Human Services Committee. My name is Jason Wahl, Director of the Division of Medical Marijuana within the Department of Health. I am here to oppose certain sections and provide information on House Bill 1417 related to proposed changes to language within the Medical Marijuana chapter of state law.

The Committee has already heard testimony to a number of changes included within House Bill 1417. Sections 2, 5, 6, 7, and 8 of this bill are the same or very similar to the changes proposed in House Bill 1283 regarding a bona fide provider-patient relationship and a written certification. We have worked with Representative Kathy Skroch, legislative council, and legal regarding House Bill 1283 and are supportive of the changes with that bill. As a result, we would oppose the changes to the bona fide provider-patient relationship and written certification as House Bill 1417 is currently written.

Section 3 of House Bill 1417 would make changes to the list of debilitating medical conditions. Previously, testimony was heard on House Bill 1272 that contained changes to the list of debilitating medical conditions as well. Only one condition (Ehlers-Danlos syndrome) is included in both bills. Chapter 171 of the 2017 Session Laws required the Department of Health to conduct a study relating to debilitating medical conditions (results of the review are included in the Medical Marijuana Program Annual Report, Fiscal Year 2018 available for viewing at www.ndhealth.gov/mm). Neuropathy

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1-23-19

related conditions was one of the top ten conditions we identified not being specifically listed in North Dakota law.

House Bill 1417 would make a change in the allowable amount to be purchased and possessed by a qualifying patient with cancer. The purchasing amount in a 30-day period would more than double, going from 2.5 ounces to 6 ounces. Also, the bill would provide for a 2.5 times higher possession limit (going from 3 ounces to 7.5 ounces). In addition, the bill would eliminate the requirement for a health care provider to authorize the use of dried leaves or flowers. This, in turn, would eliminate the requirement for a registry identification card to include whether the qualifying patient is authorized for dried leaves or flowers.

The Department of Health did submit a fiscal note regarding implementation of House Bill 1417. There are three areas of the bill that would require changes to the information technology system. This includes changes to the written certification form, the addition of an enhanced amount of dried leaves or flowers, and the removal of the additional authorization for the use of dried leaves or flowers. The estimated cost to change these items in the system is approximately $30,000.

This concludes my testimony. I am happy to answer any questions you may have.

#7
HB1417
1-23-19

I am not a drinker, not a smoker, I have never used cannabis, I am dependent on opioids, and I am a small business owner who refuses to apply for disability. I qualify for Medical Marijuana; but our state government has made it near impossible to obtain. The doctors in our great state that are willing to certify people find it near impossible to sign due to implications from their employers if they do sign it since it is still illegal federally.

My story is just one of millions across this great country. I have a liver disease and had 7 of 9 benign tumors removed in 2012 which caused nerve damage and chronic pain. I was given high levels of dilaudid. After 9 months they finally switched me to fentanyl in 2012. I am so dependent on fentanyl that the 3-day patch can not last 3-days and the doctors had to switch me to changing the patch every 48-hours instead. This contradicts the instructions on the box from the manufacture saying it is to be used for 72-hours (3-days). I was also placed on prn hydrocodone (another opioid) to help if I have daily pain a normal person would take Tylenol for but for me would be a placebo. Then we round it all off with medication for my severe anxiety and depression which do not mix well with opioids; but I need them anyway.

Everyday on the news I hear of people dying from fentanyl overdose. I have to carry narcan with me. I had to show my kids how to use it on me because of the legal-for-me drug I am on that my children are told are bad in school. I have to face the worst of 2 evils: be on opioids so my nerve damage doesn't cause heart palpitations while continuing to damage my liver OR save my liver and not be on opioids causing damage to my heart. Medical Marijuana would give me a third option. I would be able to control the pain and not further damage my organs. Instead of taking 5 prescriptions, I would be taking something God put on this earth in its natural form instead of developed in a lab.

I have been waiting since 2016 for MM to be put in place and nothing... 2+ years more damage to my body in the catch 22 I live daily. 2+ years of seeing the way my teenage looks when he sees me on the necessary evil he knows I could easily OD on. That look he gives me knowing someday he may have to save mom. I am so tired of continually waiting for them to making MM harder for people to obtain. I am tired of being high on opioids 24/7... think about that. I am HIGH ON OPIOIDS 24/7 and I am still ALLOWED to drive? (Which I do not do if I can help it and wait for my husband to drive me). People who were opposed Measure 3 this last election (legalize recreational marijuana in North Dakota) were worried about people being high driving... they do not realize that the person driving next to them is probably already high on opioids! They were so worried about me being addicted to a "gateway drug". I am already legally addicted to the gateway drug of heroine by fentanyl prescription dependency.

Our state voted to pass medical marijuana in 2016 elections and it was made it unattainable for a majority of North Dakotans. Some of these people have since passed away without being able to die with dignity and enjoy their family on their last days. But we can change it. We can be there for those still with us today struggling to have access to medical treatment our state legalized.

REFORM. Help doctors feel safe to certify conditions without fear. Help us keep our gun rights. Give back our rights back to grow so we can keep it affordable and have access for those of us not living near a dispensary and do not drive. Help expand the list of qualifying conditions.

There are so many of us that are tired dancing with the opioid devil among many other conditions. I pray all the people that are lobbying against reform today never are put in a place like I am or that many other people are in right now. But when they are; they will see why we are fighting so hard for reform.

Sincerely,

Tamara Bartz

District 31

#8
HB 1417
1-23-19

NDMA
Est.1887 NORTH DAKOTA MEDICAL ASSOCIATION

House Human Services Committee
HB 1417
January 23, 2019

Good morning Chairman Weisz and Committee Members. I am Courtney Koebele and I serve as executive director of the North Dakota Medical Association. The North Dakota Medical Association is the professional membership organization for North Dakota physicians, residents, and medical students.

NDMA is supportive of the changes contained in HB 1417 on page 4 at line 20 and page 5 at line 14, both of which remove the language that "in the health care provider's professional opinion the patient is likely to receive therapeutic or palliative benefit from the medical use of marijuana to treat or alleviate the patient's condition." We believe this may allow more providers to feel comfortable recommending medical marijuana.

As a suggestion, NDMA does support including Physician Assistants as providers who may certify. Physician assistants (PAs) undergo rigorous medical training and must graduate from an accredited PA program in order to take the national certifying exam to be licensed. Like physicians and nurse practitioners, PAs must complete extensive continuing medical education throughout their careers.

PAs are licensed healthcare providers that practice medicine to include the diagnosis and treatment of medical conditions, ordering of diagnostic studies, and have prescriptive privileges for medications. Physician assistants also are primary care providers like physicians and advanced practice registered nurses. Adding this type of primary care provider to the list of providers would increase access for those patients.

Thank you for your time today. I would be happy to answer any questions.

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#1 Pg. 1

From: Vetter, Steve M. smvetter@nd.gov
Subject: HB 1417
Date: Mar 5, 2019 at 8:31:06 AM
To: Vetter, Steve M. smvetter@nd.gov

HB 1417

Madame Chairwoman Lee and members of the Human Services committee, my name is Steve Vetter, I represent district 18, which is a small chunk of South Grand Forks, downtown, half of North Grand Forks and a small rural area extending to the Grand Forks Air Force Base.

HB 1417 after amended does a couple of things. It raises the amount of dried leaves available for cancer patients. It also has similar language found in HB1283 which allows doctors to state the debilitating condition rather to be required to give a recommendation. That discussion will be had when hearing HB 1283.

I think it is important for the committee to understand the History of the bills that are here before you: 1283/1519 & 1417 The best ideas were taken from each of the bills...

There are studies and life examples that show the effectiveness of medical cannabis on treating cancer. Because of that, the useable amount should be raised to accommodate cancer patients who require a larger dosage.
"For millennia, Cannabis sativa has been used in folk medicine to alleviate pain, depression, amenorrhea, inflammation, epilepsy, and numerous other medical conditions." However, with cancer patients cannabinoids are well known to exert palliative effects, their best established use is to treat chemotherapy-induced nausea and vomiting, but also applied for pain alleviation, appetite stimulation to maintain the patient's weight and to boost the mood of the patient. If you really think about it, it makes sense. When fighting cancer, the healthier the lifestyle of the patient, the better chance of survival. If a person is depressed, does not eat and is sick all the time, their chances are slimmer than the patient who eats without vomiting and keeps a positive attitude has a

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#1 pg. 2

better chance of survival.

I would like to offer the Emma amendment to allow for physicians to grant minors extraordinary form and THC usage. This was part of the original HB1519. I'm not sure why the committee left this part out. I am assuming that portion was taken out because A) the committee was so overwhelmed with cannabis bills and B) the committee did not receive enough information.
The Emma amendment is named after 2 year old Emma Basting who was diagnosed with Neuroblastoma, a rare pediatric cancer in December 13, 2017. A MRI identified a tumor wrapping around her spine. She was flighted to Minneapolis to begin emergent chemotherapy. After 2 weeks the tumor continued to grow, compressing her spine causing spinal cord atrophy from the t2-t7 and paralyzing her from the chest down.

The treatment regimen the Basting family is following is a trial through Children's Oncology Group for High Risk Neuroblastoma, the same protocol followed by most of the country. Emma has faced 6 rounds of chemo, 3 major surgeries, a stem cell transplant, 20 rounds of proton beam radiation and she starts her sixth and final round of immunotherapy in the next few weeks. This his completes her frontline treatment protocol.
Neuroblastoma is a tricky disease as the goal is to maintain remission as there is no set protocol for relapse and the prognosis for relapse is extremely poor. Unfortunately, half the kids that get to remission, relapse. There are some options for maintenance treatment following immunotherapy to help make the cancer remain inactive:

1. DFMO experimental trial- ODC inhibitor taken by mouth for 2 years. This would require frequent travel outside of ND because no ND hospital is enrolled in this trial

2. Neuroblastoma vaccine trial- antigens taken to cause the body's immune system to make antibodies to fight the antigens which attracts white blood cells to kill neuroblastoma. This would require frequent travel to New York for 1 year.
3. Medical Cannabis- early testing has shown both the THC and CBD can play a role in killing neuroblastoma cells. It is available in over 30 states but not ND.

So, the Emma amendment allows for similar treatments that are done in the majority of states. There are several other cases where the minor restrictions in ND law does not allow for proper cannabis treatment for minors. For example, an older teen that is weaning off of pharms could use 2000 mg of THC within 4 days. Or because of ND law a minor patient would not be allowed dried flower form even if it was the recommended form for the type of condition.

Since the bill needs a 2/3 vote to pass anyway. I don't see why an emergency amendment should not be added to the bill.

Madame Chairwoman and Senators, today is a big day, your committee will have the opportunity help thousands of ND patients to get access to life saving medicine. I would ask you to please consider the Emma amendment, emergency amendment and to give HB1417 a Do Pass recommendation. Thank you. I will stand for questions.

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#1 pg 4

Vetter, Steve M.

From: Aaron Basting <aaron.basting@gmail.com>
Sent: Thursday, February 28, 2019 8:43 AM
To: Vetter, Steve M.
Subject: Re: Medical Marijuana Question from Pediatric Oncology Patient

Steve,

Here is a written statement from Brandi and I. We won't be able to testify in Bismarck as we need to be home with Emma as she finished her cancer treatments over the next couple weeks. I hope they can accept our written statement as testimony or that we could possibly testify remotely via skype or facetime?

Dear ND Senate committee,

On December 13, 2017 our two year old daughter Emma was diagnosed with Neuroblastoma, a rare pediatric cancer. Immediately following an MRI in Grand Forks that identified a tumor wrapping her thoracic spine she was life flighted to Minneapolis to begin emergent chemotherapy, which was started this same evening. Within two weeks Emma's tumor continued to grow, compressing her spine, causing spinal cord atrophy from t2-t7, and paralyzing her permanently from the chest down. At this point her cancer was reclassified to High Risk Neuroblastoma, which carries one of the worst outlooks of all pediatric cancers and an extensive treatment regimen.

The treatment regimen we are following is a trial through the Children's Oncology Group for High Risk Neuroblastoma – this same protocol for High Risk Neuroblastoma is followed by most of the country. Since diagnosis Emma has now faced six rounds of chemo, three major surgeries, a stem cell transplant, 20 rounds of proton beam radiation, and she starts her sixth and final round of immunotherapy in early March. Immunotherapy will end mid-March and her frontline treatment protocol will be finished.

Unfortunately, the team at U of M cannot label Emma as NED (no evidence of disease). She has residual disease remaining on her spine that cannot safely be removed or biopsied. When Emma's treatments are finished, she will have scans every three months, and we will hope and pray that the tumor does not grow further.

Neuroblastoma is a tricky disease as the goal is to maintain remission as there is no set protocol for relapse and the prognosis for relapse is extremely poor. Unfortunately, half the kids that get into remission relapse. With that said there is some options for maintenance treatment following immunotherapy to help make sure the cancer remains inactive...

1. DFMO experimental trial – ODC inhibitor taken by mouth for up to two years with a modified diet. Requires frequent travel outside of ND as there's no hospital in ND enrolled in this trial.

2. Neuroblastoma vaccine trial at Memorial Sloan Kettering – antigens taken to cause body's immune system to make antibodies which in turn fight the antigens, which help attract white blood cells to kill neuroblastoma – Requires frequent travel to New York City for up to a year.
3. Medical marijuana – early testing has shown both the THC and CBD in marijuana can play a role in killing neuroblastoma cells. Currently available in 33 states.

[redacted]mma has now spent most of the past 15 months in the hospital receiving cancer treatments. During this time she had a baby brother introduced to this world, but she has had limited time spent with him because she's been receiving her treatments and isolated from other people including family due to the fear of spreading germs while her immune system is compromised. Emma has spent so much time in the hospital that the hospital life is really all that she knows. Her childhood was robbed from her and we want to give Emma a chance to live a regular life so we hope to avoid enrolling her in either the DFMO or vaccine trials. This leaves us with a couple options - either do nothing or pursue medical marijuana.

We have enrolled Emma into ND's medical marijuana program but were disappointed to find limits placed on the dosage for pediatric patients, specifically on the THC concentration allowed – 6%. Who came up with this number and what was the thought process behind this decision?

This 6% rule will basically limit pediatric patients including Emma to CBD and restricts their access to THC in our home state of North Dakota. While at the same time we could move 10 miles east of our home into Minnesota and she would have access to both THC and CBD. It's extremely concerning that kids in other states have access to these therapies but Emma would remain restricted from this therapy simply for being a resident of ND. Being a resident of ND also disqualifies Emma from enrolling in other state's medical marijuana programs while there for other treatments.

[redacted]ve understand some are concerned with the long-term effects of THC on brain development for youth. The problem we have with this idea is that since marijuana is not FDA approved, it's effect on the pediatric brain has been studied very little, if at all. For those that share this concern, we question where you stand on the toxicities of treatment for pediatric cancer.

Emma has faced a number of toxic treatments in order to save her life. Multiple rounds of chemotherapy have caused Emma to no longer eat or drink by mouth, they've caused neuro-toxicities adding to her paralysis, they've caused permanent hearing loss, they've ruined her chances of having kids of her own, and have also caused unknown damage to her brian, heart, liver, pancreas, kidneys, and lungs. Her surgeries proved to be life threatening but she chose to fight on. Her stem cell transplant also ended up being life threatening, causing the worst case of mucositis possible as her GI tract was shed from her mouth to her colon, and all of her skin was burned off from the chemo received pre-transplant. During proton beam Emma took ten times her life maximum exposure not allowing any sunlight to her tumor site safe for the remainder of her life. During immunotherapy Emma's veins and arteries lost all the blood they were carrying causing her blood pressures to reach dangerously low levels. And since Emma has counted on continuous tube feeds for her nutrition, she is now dependent on them, and if she gets ill and can't tolerate them, she becomes hypoglycemic requiring hospitalization. If we could do it all over again, we wouldn't change a thing. The benefits of the treatments have outweighed the risks. We went through all of this to save Emma's life.

[redacted]ne of the side effects that Emma is facing as I write this is chemo induced nausea and she is actively taking a prescribed medication called dronabinol. We get this medication filled by our local pharmacy and it is covered by Emma's insurance

plan. For those that don't know, dronabinol is man-made/synthetic THC. It is quite ironic that Emma has access to a synthetic form of THC but does not have access to natural THC because pediatric medical marijuana in ND will limit her to 6%.

While we are happy there is finally a medical marijuana program available in ND, we do not agree that the state should limit the dosage amounts of either CBD or THC for pediatric patients. This decision should be made by the pharmacist and doctor in conjunction with the patient's family, just like every other medication or therapy currently is. Emma's treatment options including medical marijuana should not be restricted just because she lives in North Dakota she should have the same access to these therapies regardless of where she lives. Because of this we are asking you to eliminate the pediatric medical marijuana rule and put this decision back into the provider's and parent's hands

Thank you for your time and for taking our written statement as testimony so we can be home and with Emma during her final round of immunotherapy.

Aaron and Brandi Basting

On Mon, Feb 25, 2019 at 9:50 PM Vetter, Steve M. <smvetter@nd.gov> wrote:

> Sure, that will work. I will try to get it added.

Steve Vetter

ND House of Representatives

600 E Boulevard Ave

Bismarck, ND 58505

District 18

612-770-8689

From: Aaron Basting <aaron.basting@gmail.com>
Sent: Monday, February 25, 2019 9:34 PM
To: Vetter, Steve M. <smvetter@nd.gov>
Subject: Re: Medical Marijuana Question from Pediatric Oncology Patient

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Both compounds have antitumourigenic activity *in vitro* and impeded the growth of tumour xenografts *in vivo*. Of the two cannabinoids tested, CBD was the more active. Treatment with CBD reduced the viability and invasiveness of treated tumour cells *in vitro* and induced apoptosis (as demonstrated by morphology changes, sub-G1 cell accumulation, and annexin V assay). Moreover, CBD elicited an increase in activated caspase 3 in treated cells and tumour xenografts.

Conclusions

Our results demonstrate the antitumourigenic action of CBD on NBL cells. Because CBD is a nonpsychoactive cannabinoid that appears to be devoid of side effects, our results support its exploitation as an effective anticancer drug in the management of NBL.

Keywords: Neuroblastoma, cannabidiol, Δ^9-tetrahydrocannabinol, apoptosis, tumour xenograft models, non-psychoactive cannabinoids

INTRODUCTION

Neuroblastoma (NBL) is the most frequent extracranial solid tumour in childhood. It accounts for approximately 8% of childhood cancers and is characterized by variable clinical behaviour, reflecting molecular differences in the tumour[1]. Using current risk stratification criteria, approximately 40% of NBL tumours are classified as high-risk. Treatment for children with high-risk NBL involves multimodality therapy, including chemotherapy, autologous stem-cell transplantation, surgery, radiation therapy, and immunotherapy using differentiation therapy. Despite that aggressive approach, children with NBL have very poor outcomes, and the survivors experience serious side effects related to treatment toxicity[2]. Hence, the need for new and less-toxic therapeutic strategies to treat the disease is urgent.

For millennia, *Cannabis sativa* has been used in folk medicine to alleviate pain, depression, amenorrhea, inflammation, epilepsy, and numerous other medical conditions[3]. In cancer patients specifically, cannabinoids are well known to exert palliative effects; their best-established use is the inhibition of chemotherapy-induced nausea and vomiting, but they are also applied for pain alleviation, appetite stimulation, and attenuation of wasting[4].

Recently, increasing evidence suggests that Δ^9-tetrahydrocannabinol (THC) and cannabidiol (CBD), major components of *Cannabis sativa,* and synthetic cannabinoids and the endocannabinoid anandamide have antitumour activity[5,6]. Many adult cancer types (lung cancer, glioma, thyroid cancer, lymphoma, skin cancer, pancreatic cancer, uterine cancer, and breast and prostate carcinoma) have been reported to be sensitive to the antiproliferative action of cannabinoids in a wide variety of experimental models, including cancer cell lines in culture, xenograft mouse models, and genetically engineered mice[6].

Cannabinoids act chiefly by activating the specific cannabinoid receptors CB1 and CB2[6]. However, it is now well-established that these molecules also have effects that are CB receptor–independent; other receptors, such as vanilloid receptor 1[7] and the peroxisome proliferator–activated receptors[8], could be responsible for their action.

The mechanisms involved in the antitumour effects of cannabinoids include proliferation inhibition and growth arrest[9], induction of apoptosis[10,11], stimulation of autophagy[12,13], angiogenesis inhibition[14], and anti-metastatic effects[15–17]. However, the antitumourigenic mechanism of action of CBD is as yet unknown[18].

At the molecular level, cannabinoids have been shown to trigger changes in various signalling pathways, including Akt/mammalian target of rapamycin complex 1[19], ERK, upregulation of stress-associated transcription factor p8[19,20], downregulation of matrix metalloproteinase 2[21], and vascular endothelial growth factor signalling[22]. Nevertheless, studies exploring the putative antitumourigenic properties of cannabinoids in pediatric tumours are still limited, and the molecular mechanisms underlying the antitumourigenic effect are poorly understood. Recently published data demonstrated the antitumourigenic activity of cannabinoids—mainly THC and synthetic cannabinoids—on alveolar rhabdomyosarcoma and osteosarcoma by inducing apoptosis[23] and triggering the endoplasmic reticular stress and autophagy process[24].

Our study aimed to characterize both the *in vitro* and *in vivo* effects of cannabinoids on another pediatric tumour, NBL, and to unravel the mechanism responsible for those effects. Given our positive results, we suggest that non-THC cannabinoids such as CBD might provide a basis for the development of novel therapeutic strategies in high-risk NBL, without the typical psychotropic effects of THC and without the strong side effects associated with chemotherapeutic agents.

METHODS

Cannabinoids

Δ^9-Tetrahydrocannabinol was supplied by Prof. Raphael Mechoulam, Institute for Drug Research, Medical Faculty, The Hebrew University, Ein Kerem Campus, Jerusalem, Israel. Cannabidiol was supplied by THC Pharm GmbH, Frankfurt, Germany.

Cell Cultures

The human NBL cell lines SK-N-SH[25] and IMR-32[26] were purchased from ATCC (Manassas, VA, U.S.A.) and the European Collection of Authenticated Cell Cultures (Salisbury, U.K.) respectively. The NUB-6[27] and LAN-1 cell lines were kindly provided by Dr. Shifra Ash, Schneider Children's Medical Center of Israel[28].

SK-N-SH cells were cultured in Eagle minimum essential medium (ATCC), supplemented with 10% fetal bovine serum (FBS) and 100 U/mL penicillin–streptomycin (Gibco, Paisley, U.K.). IMR-32 cells were cultured in Eagle basal medium (Sigma–Aldrich, St. Louis, MO, U.S.A.) supplemented with 2 mmol/L glutamine, 1% non-essential amino acids, 10% FBS, and 100 U/mL penicillin–streptomycin. LAN-1 and NUB-6 cells were cultured in RPMI-1640 (Gibco) supplemented with 10% FBS and 100 U/mL penicillin– streptomycin. All the cell lines were cultured at 37°C in a humidified atmosphere containing 5% CO_2.

MTT Test

An MTT assay (Biological Industries, Kibbutz Beit-Haemek, Israel) was used to evaluate the effect of CBD and THC on NBL cell viability. SK-N-SH, LAN-1, IMR-32, and NUB-6 cells (5×10^3 cells/mL) were plated (200 µL) in triplicate in flat-bottom 96-well plates in the appropriate medium. The cells were allowed to adhere to the plate surface overnight and were then cultured with increasing doses of THC or CBD (0–50 µg/mL) for 24, 48, and 72 hours. Cell viability was then determined by MTT assay, which measures the reduction of MTT to formazan by the mitochondria of viable cells[29]. Formazan was measured spectrophotometrically by absorption at 560 nm in a PowerWaveX plate reader (BioTek, Winooski, VT, U.S.A.). All experiments were repeated at least 3 times. Cell morphologies were assessed daily by light microscopy.

Microscopy Analysis

One day before treatment, SK-N-SH cells were plated (1×10^6 cells per 9-cm plate). After 48 hours of incubation with CBD (10 μg/mL), cell morphology changes were assessed by light microscopy (Olympus CKX41: Olympus, Tokyo, Japan).

Cell-Cycle Analysis

One day before THC or CBD treatment, SK-N-SH cells were plated (1×10^6 cells per 9-cm plate). After 24, 48, and 72 hours of treatment, the cells were washed in phosphate-buffered saline (PBS: Biological Industries), detached using a solution of 0.1% trypsin (Biological Industries), and spun at 1100 rpm. The resulting pellet was resuspended in 250 μL cold PBS, and the cells were fixed overnight with 5 mL cold 75% ethanol (Sigma–Aldrich) and PBS at −20°C. The pellet was then washed twice with cold PBS (followed by centrifugation at 1100 rpm for 7 minutes). Distribution of the cells in G1, S, and G2/M phases of the cell cycle were monitored after nuclei had been stained with 50 μg/mL propidium iodide (Sigma–Aldrich) containing 125 U/mL protease-free RNase (Sigma–Aldrich) in 0.5% Triton (Bio-Lab, Jerusalem, Israel) and had been PBS-buffered for 30 minutes in the dark. The cells were analyzed using an Epics XL-MCL flow cytometer and the FlowJo software application (Beckman Coulter, Brea, CA, U.S.A.).

Apoptotic Cell Death

Annexin V Assay One day before CBD treatment (7.5 μg/mL and 10 μg/mL for 24 hours and 48 hours), cells were plated (1×10^6 cells per 9-cm plate). Cells treated with 1–10 μmol/L staurosporine (Sigma –Aldrich) for 24 hours served as a positive control; untreated cells served as a negative control. Treated cells, untreated cells, and positive control cells were harvested, and after the annexin V assay [human recombinant annexin V (APC conjugate, catalogue no. ALX-209–252: Enzo Life Sciences, Ann Arbor, MI, U.S.A.); annexin V binding buffer, no. 556454 (BD Pharmingen, San Diego, CA, U.S.A.); and 7-aminoactinomycin D, no. 559925 (BD Pharmingen)] were analyzed using an Epics XL-MCL flow cytometer and the FlowJo software application.

Caspase Assay One day before CBD treatment (7.5 μg/mL and 10 μg/mL for 24 hours), cells were plated (1×10^6 cells per 9-cm plate). Cells were harvested, and proteins were extracted with radioimmunoprecipitation assay buffer (Sigma–Aldrich). Protein concentrations were calibrated using the BCA Protein Assay Reagent Kit (Pierce, Rockford, IL, U.S.A.).

Samples were separated on 12% SDS-PAGE (Bio-Rad, Rishon LeZion, Israel) and transferred onto nitro filters (Schleicher and Schuell Bioscience, Dassel, Germany). The blots were reacted using caspase 3 (8G10) rabbit monoclonal antibody (Cell Signalling Technology, Danvers, MA, U.S.A.) as the primary antibody. The secondary antibody, horseradish peroxidase conjugated goat anti-rabbit antibody (Jackson ImmunoResearch Laboratories, Farmington, CT, U.S.A.), was detected by chemiluminescence. Signals were detected using an ECL Kit (Amersham Pharmacia, Little Chalfont, U.K.) and visualized by exposure to radiography film.

Invasion Assay

Tumour cell invasion was assayed in Transwell chambers (Transwell 3422: Corning, Corning, NY, U.S.A.) pre-coated with Cultrex Basement Membrane Extract (Trevigen, Gaithersburg, MD, U.S.A.). Membrane filters were placed in 24-well tissue-culture plates according to manufacturer guidelines. After 24 hours of treatment with CBD (15 μg/mL and 20 μg/mL), cells were harvested, and 2×10^5 cells suspended in 200 μL serum-free medium were added to the upper surface of each chamber. The bottom of the chamber was filled with 750 μL medium with 10% FBS. After 24 hours in which cells were

allowed to migrate to the underside of the membrane, the invaded cells were fixed with paraformaldehyde (Electron Microscopy Sciences, Hatfield, PA, U.S.A.) and stained with crystal violet (Sigma–Aldrich).

In Vivo Studies

All experiments involving mice were approved and performed according to the guidelines issued by the Sheba Medical Center Research Committee for the Care and Use of Laboratory Animals (permit no. 803/12).

To study the *in vivo* antitumour activity of cannabinoids, NBL tumours were induced in nonobese diabetic immunodeficient (NOD/SCID) mice by subcutaneous injection. Briefly, 1×10^7 SK-N-SH cells suspended in 100 µL serum-free medium and Cultrex (1:1) were injected subcutaneously into the rear flank of 5- to 8-week-old NOD/ SCID mice. Mice were maintained in a pathogen-free environment and monitored weekly for tumour growth. Secondary tumours were detected by palpation and were measured with external callipers. Volume was calculated as $(\text{width})^2 \times (\text{length}) \times 0.52$. When tumours had reached an average size of 400 mm^3, the mice were randomly assigned to treatment and control groups (each $n = 12$). They were then injected intraperitoneally for 14 days with THC (20 mg/kg daily), CBD (20 mg/kg daily), or vehicle (ethanol) or were left untreated. At the end of the treatment period, the mice were euthanized, and the tumours were excised and processed for further analyses.

Histology Formalin-fixed tissues were dehydrated, embedded in paraffin, and sectioned at 4 µm. The slides were warmed to 60°C for 1 hour and then processed by a fully automated protocol. Immunostainings were calibrated on a Benchmark XT staining module (Ventana Medical Systems, Tucson, AZ, U.S.A.). Briefly, after sections had been dewaxed and rehydrated, a CC1 Standard Benchmark XT pre-treatment for antigen retrieval (Ventana Medical Systems) was selected for active caspase 3. Active caspase 3 antibody (Epitomics, Burlingame, CA, U.S.A.) was diluted 1:10 with Antibody Diluent (Ventana Medical Systems) and incubated for 1 hour at 37°C. Detection was performed using an ultraView detection kit (Ventana Medical Systems) and counterstained with hematoxylin (Ventana Medical Systems). After the run on the automated stainer was completed, the slides were dehydrated in 70% ethanol, 95% ethanol, and 100% ethanol (10 s each). Before cover-slipping, the sections were cleared in xylene (10 s) and mounted with Entellan (EMD Millipore, Billerica, MA, U.S.A.). The stained sections were reviewed under light microscopy and analyzed by a pathologist.

Statistical Analysis

Unless otherwise specified, results are shown as means or medians ± standard deviation. A Kruskal–Wallis test, followed by a post hoc Mann–Whitney test, was used to evaluate significant differences in the viability of cell lines, the growth rate of xenografts, and the counts of positive cleaved caspase 3 cells for the various treatment groups. A p value less than 0.05 was considered statistically significant. All analyses were performed using the IBM SPSS Statistics software application (version 21: IBM, Armonk, NY, U.S.A.).

RESULTS

Viability of NBL Cell Lines *In Vitro*

We used an MTT assay to assess the effect of THC and CBD on the viability of the SK-N-SH, NUB-6, IMR-32, and LAN-1 NBL cell lines [Figure 1(A)]. *In vitro,* after 24 hours of treatment, CBD and THC had already effectively reduced the viability of NBL cell lines in a dose- (0–50 µg/mL) and time-dependent manner, with CBD having the better effect. Better response to treatment was observed in the

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SK-N-SH and NUB-6 cell lines, as demonstrated by a 50% reduction in cell viability at lower CBD or THC concentrations (5 μg/mL and 15 μg/mL for SK-N-SH and NUB-6 respectively, compared with >20 μg/mL for IMR-32 and LAN-1). The same trend was found, and even enhanced, after treatment with THC and CBD for 48 hours [Figure 1(A)]. More importantly, the response after treatment of SK-N-SH cells with CBD (10 μg/mL) was better than the response after treatment with the same concentration of THC [Figure 1(B), $p = 0.0004$ for 24 and 48 hours of treatment].

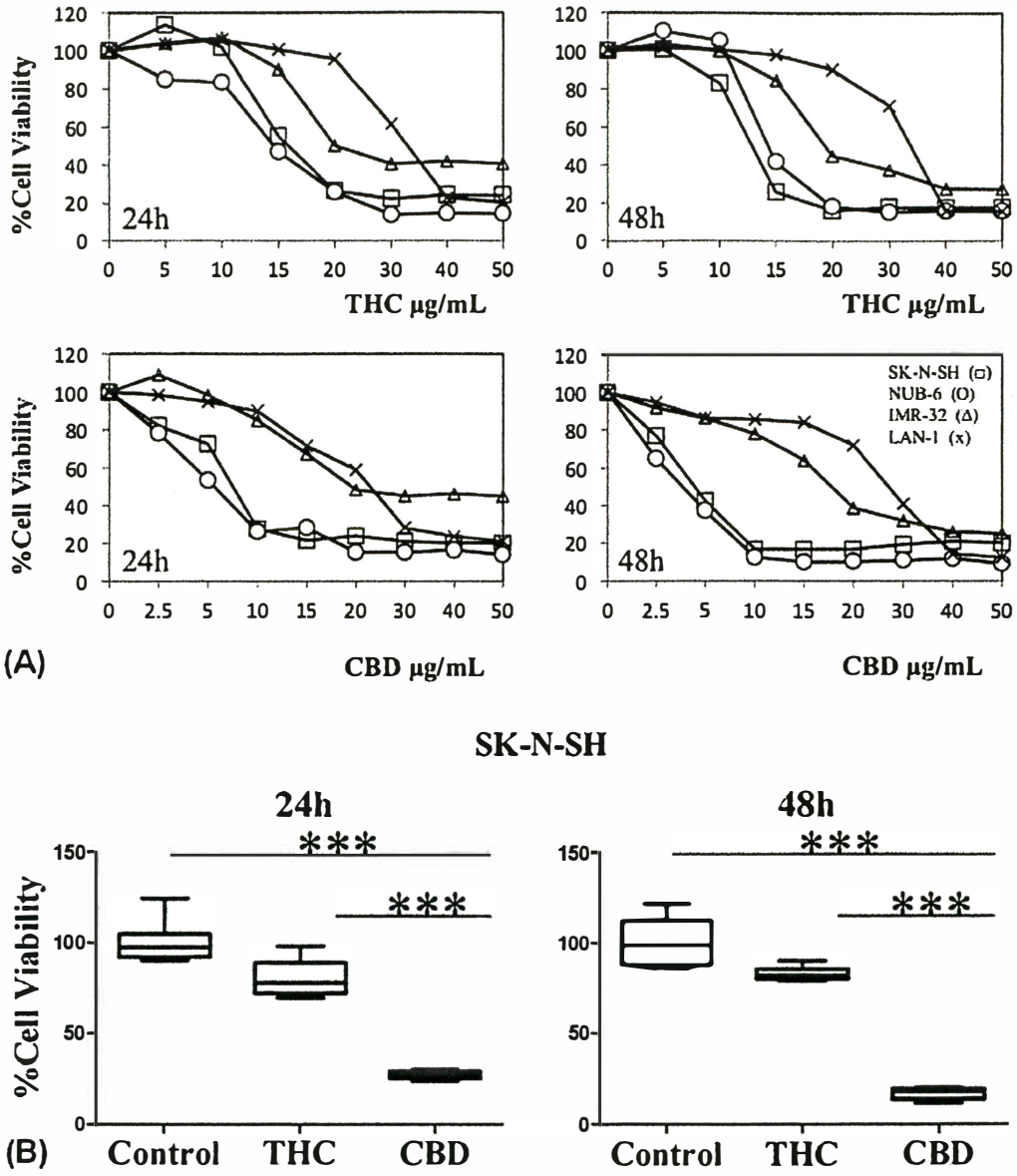

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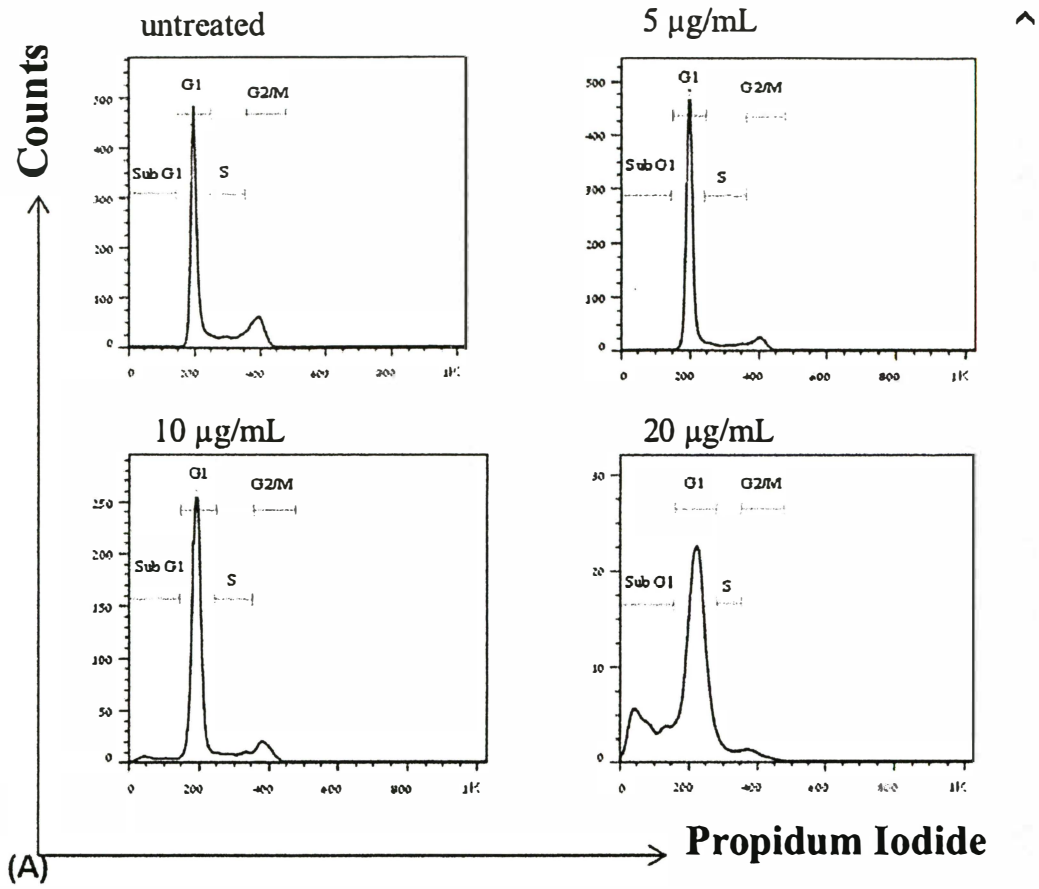
FIGURE 1

Δ^9-Tetrahydrocannabinol (THC) and cannabidiol (CBD) reduce viability of neuroblastoma (NBL) cell lines *in vitro,* with CBD having a better effect. (A) Cell lines SK-N-SH (open squares), NUB-6 (open circles), IMR-32 (open triangles), and LAN-1 (crosses) were incubated with increasing concentrations (0–50 µg/mL) of THC or CBD for 24 hours and 48 hours. Viability was measured by MTT assay. (B) Mean ± standard deviation of SK-N-SH cell viability after incubation with 10 µg/mL THC or CBD for 24 and 48 hours. *** Denotes a significant change relative to control ($p = 0.0004$). Data are expressed as a percentage of the vehicle control and are the mean of pooled results from experiments performed in triplicate.

The foregoing data indicate that anti-NBL activity is better with CBD than with THC in all NBL cell lines tested. Because cell lines showed varying sensitivity to CBD, we chose the most sensitive SK-N-SH cell line to confirm the antiproliferative effect of CBD in further *in vitro* and *in vivo* experiments.

Cell-Cycle Analysis

We next studied the effect of 48 hours of treatment with increasing doses of CBD (5–20 μg/mL) on cell-cycle progression [Figure 2(A)]. During treatment with CBD (5 μg/mL), the percentage of SK-N-SH cells sequestered in the G1 compartment rose to 82.4% from 65.8% in untreated control cells [Figure 2(B)]. Accordingly, the percentages of cells in G2 and S phase were found to be decreased, indicating that those cell populations had undergone G1 phase arrest (similar results were obtained when NUB-6 cells were treated with 10 μg/mL CBD; data not shown). Furthermore, an accumulation of SK-N-SH cells in sub-G1 phase [Figure 2(B)] was detected when that line was incubated with 10 μg/mL CBD (4.27%) and 20 μg/mL CBD (25.3%), indicating the possibility that treatment with CBD induced apoptosis in a dose-dependent manner.



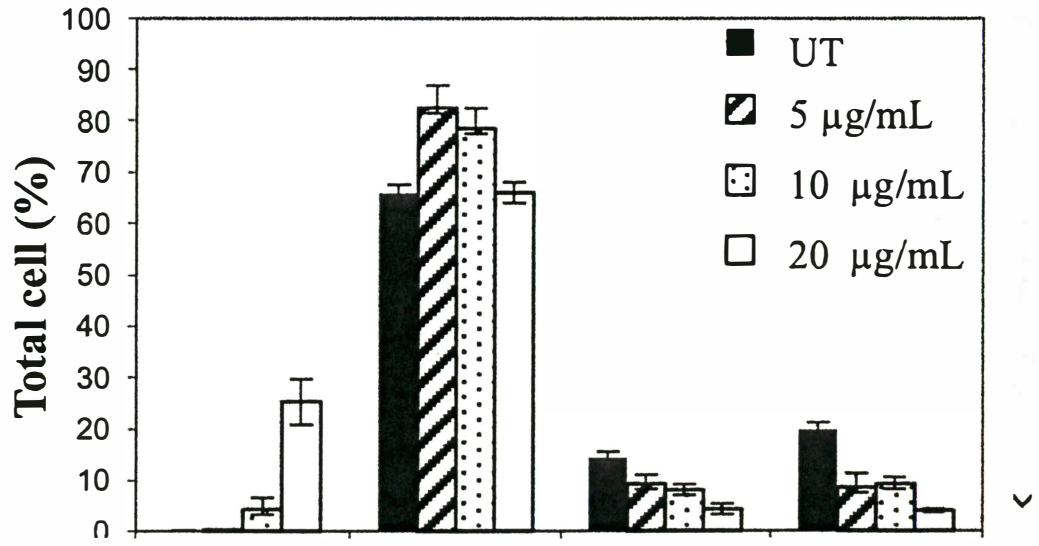


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FIGURE 2

Alteration of SK-N-SH cell cycle progression induced by cannabidiol (CBD). (A) Cell cycle analysis in untreated SK-N-SH cells and in cells treated with increasing concentrations of CBD for 48 hours. (B) Change in cell accumulation percentages during cell cycle progression after incubation with CBD for 48 hours. UT = untreated.

Apoptotic Cell Death

To verify our hypothesis that the reduction in NBL cell viability associated with CBD treatment was indeed attributable to apoptotic cell death, we first examined morphology changes after CBD treatment. Microscopic analysis showed that treatment with 10 μg/mL CBD affected cell morphology; the number of cells that had lost their normal shape, becoming rounded and swollen, and that floated in the medium increased [Figure 3(A)]. Those results confirmed that CBD treatment might induce the appearance of typical features of apoptosis.

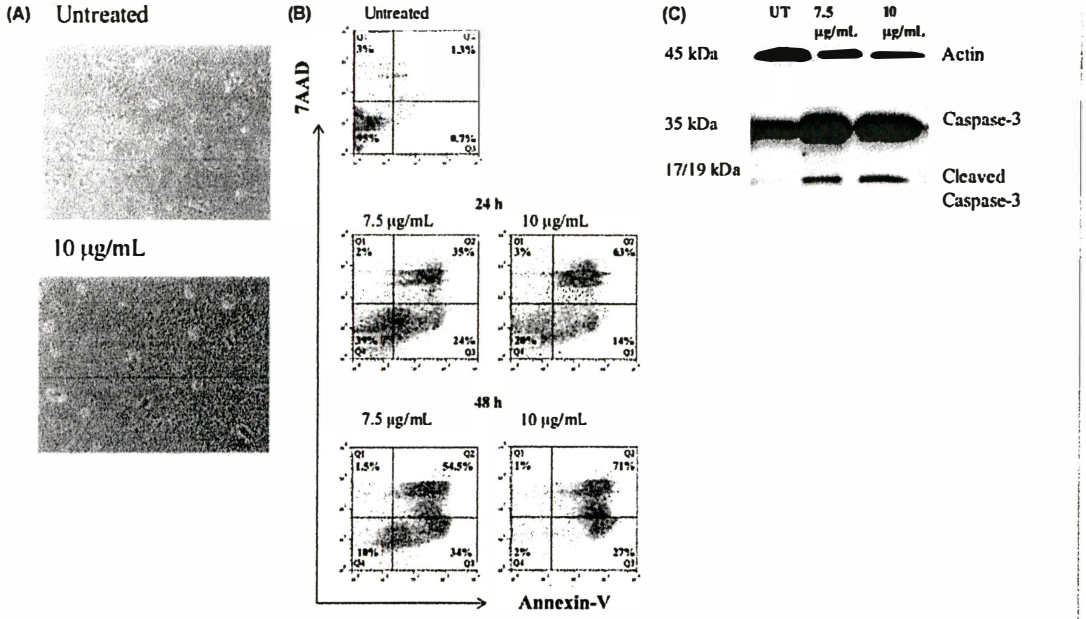

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FIGURE 3

Apoptotic effects of cannabidiol (CBD) on SK-N-SH cells. (A) Changes in SK-N-SH cell morphology: untreated cells compared with cells treated with 10 μg/mL CBD for 48 hours. (B) Apoptotic effects of CBD on SK-N-SH cells analyzed by annexin-V assay. Cells were treated with CBD in a dose- and time-dependent manner (7.5 μg/mL, 10 μg/mL; 24 hours, 48 hours) and were stained with annexin-V and 7-amino actinomycin D (7AAD). Q1 = percentage of dead cells; Q2 = percentage of cells in late apoptosis; Q3 = percentage of cells in early apoptosis; Q4 = percentage of live cells. (C) Apoptotic effects of CBD on SK-N-SH cells analyzed by caspase-3 assay. Cells were treated with increasing doses of CBD (7.5 μg/mL, 10 μg/mL) for 24 hours.

Next, we used an annexin V assay to measure the percentage of cells undergoing apoptosis after CBD treatment [Figure 3(B)]. Staurosporine-treated cells were used as a positive control. Treatment of SK-N-SH cells with CBD (7.5 μg/mL and 10 μg/mL) for 24 hours and for 48 hours resulted in an increase in the early apoptotic cell population (annexin V–positive and 7-aminoactinomycin D–negative) in a time-dependent manner. A dose- and time-dependent increase in the late apoptotic cell population was also demonstrated (annexin V–positive and 7-aminoactinomycin D–positive). Early apoptosis was demonstrated in 24% of cells incubated for 24 hours with CBD (7.5 μg/mL), but in only 0.7% of

untreated cells. As Figure 3 shows, the proportion of late apoptotic cells increased to 63% from 35% after 24 hours of treatment with increasing concentrations of CBD (10 μg/mL and 7.5 μg/mL respectively)

Finally, to further confirm the apoptotic effects of CBD on SK-N-SH cells, we measured apoptosis by caspase 3 assay [Figure 3(C)]. After 24 hours of treatment with increasing doses of CBD (7.5 μg/mL and 10 μg/mL), a dose-dependent cleavage of caspase 3 was found as evaluated by the appearance of activated p17 and p19 fragments on Western blot analysis.

Altogether, the foregoing results confirm that treatment with CBD induces apoptosis in the SK-N-SH NBL cell line.

Cell Invasiveness

As shown in Figure 4, cell invasion in Transwell chambers was dramatically decreased for SK-N-SH NBL cells treated for 24 hours with CBD (15 μg/mL and 20 μg/mL) than for untreated cells.

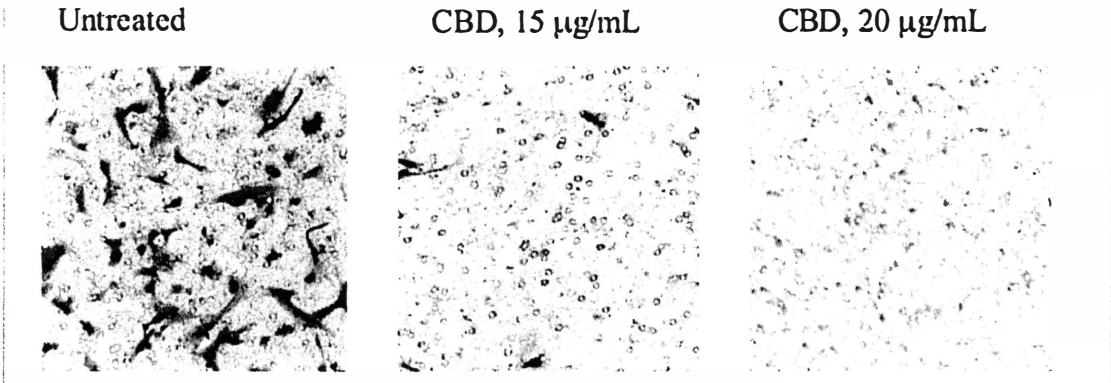

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FIGURE 4

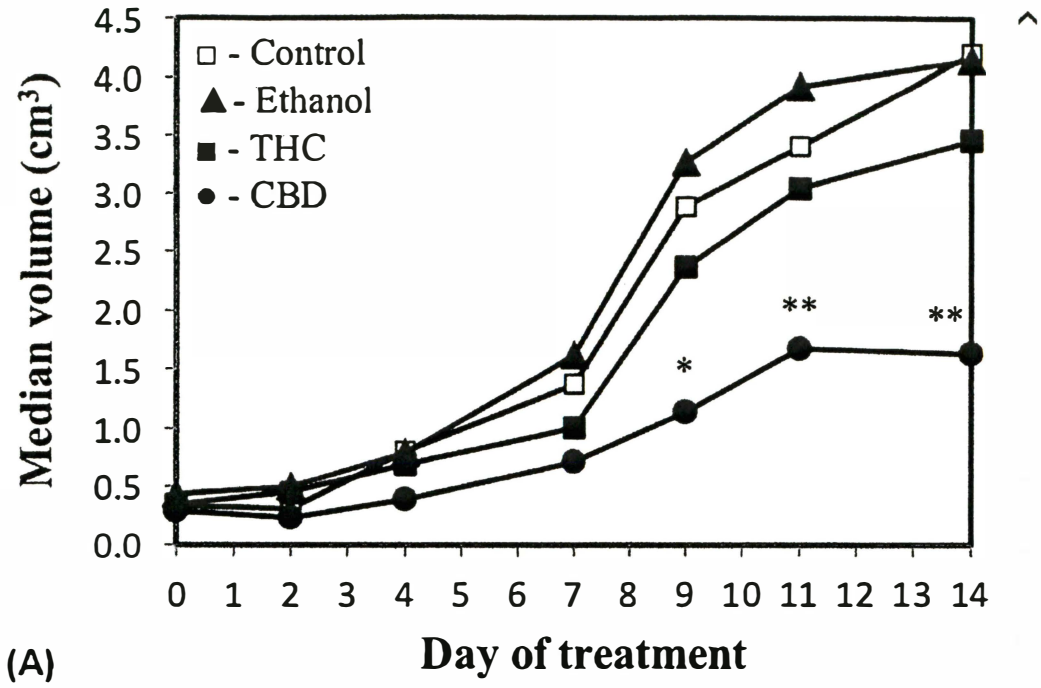
Anti-invasiveness effect of cannabidiol (CBD) on SK-N-SH cells. The invasion assays were performed using cell cultures (2×10^5 cells/well) treated with CBD (15 μg/mL, 20 μg/mL) for 24 hours; results were compared with those for untreated cells (2×10^5 cells/well). For each well (treated or untreated cells), 10 fields were examined by light microscopy.

Tumour Growth Rate in Mouse Xenograft Model

Because tumour regression in an animal xenograft model represents an important endpoint of clinical relevance, we evaluated the ability of cannabinoids to reduce NBL tumour growth *in vivo*. Tumour xenografts were first generated by subcutaneous injection of SK-N-SH cells into NOD/SCID mice. The mice were then treated with daily intraperitoneal injections of 20 mg/kg THC, 20 mg/kg CBD, or ethanol vehicle (control), or were left untreated for 14 days.

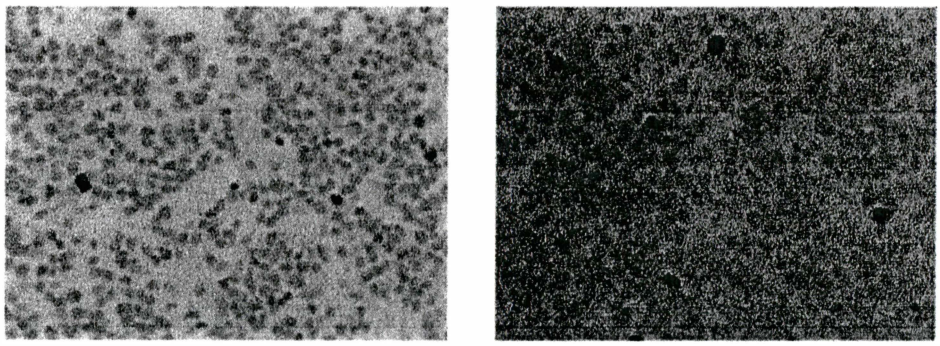
Tumour growth was significantly reduced in THC- and CBD-treated mice than in the vehicle-treated or untreated mice [Figure 5(A)]. Interestingly, response to treatment was observed to be better in the group treated with CBD than in the group treated with THC: Median xenograft volume at the end of treatment

was 2.31 cm^3 in the CBD-treated group compared with 4.28 cm^3 in the untreated group ($p = 0.029$) and 4.31 cm^3 in the vehicle-treated group ($p = 0.036$). In the THC-treated group, median volume was 3.46 cm^3, which was significant only compared with the untreated group ($p = 0.039$).



(A)

(B)



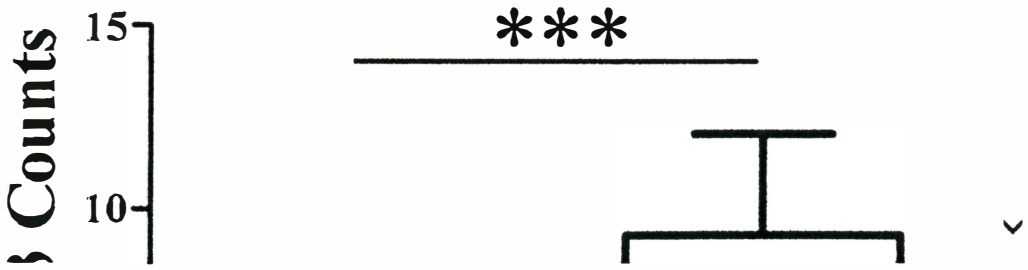


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FIGURE 5

Cannabidiol (CBD) suppresses tumour growth in a mouse xenograft model and increases cleaved caspase-3 staining in treated xenografts. (A) Growth rate of SK-N-SH cell–derived tumour xenografts treated for 14 days with intraperitoneal injections of ethanol-vehicle (n = 12, closed triangles), 20 mg/kg Δ^9-tetrahydrocannabinol (n = 12, closed squares), 20 mg/kg CBD (n = 12, closed circles) and untreated

controls (n = 12, open squares). Data represents tumour volume during 14 days of treatment. [a] $p < 0.05$ and [b] $p < 0.01$ for CBD compared with ethanol treatment (Mann–Whitney U-test). (B) Activated caspase-3 immunostaining in SK-NS-H cell–derived tumour xenografts treated with CBD 20 mg/kg or ethanol vehicle for 14 days. (C) Counts of cleaved caspase-3 immunoreactive cells in 18×10 lens fields from xenografts of CBD- and ethanol-treated mice. [a] $p < 0.0001$ compared with ethanol.

To further define the *in vivo* effect of CBD treatment with respect to apoptosis induction, we analyzed tissue obtained from tumour xenografts. Tumours were excised after the last day of treatment, and paraffin-embedded sections were analyzed immunohistochemically with the apoptosis indicator cleaved caspase 3. Cells positive for cleaved caspase 3 were detected with significantly greater frequency in sections of xenografts from CBD-treated mice [Figure 5(B)] than in sections from ethanol-treated mice [$p < 0.001$, Figure 5(C)].

To summarize, THC and CBD both suppressed the SKN-SH tumour xenograft growth rate, with CBD treatment demonstrating a better effect. Moreover, the better efficacy of CBD and its effect on the induction of activated caspase 3 are consistent with the results obtained *in vitro*.

DISCUSSION

In recent years, interest in the role of cannabinoids, mainly THC, in cancer therapy has been renewed because of the ability of these molecules to limit tumour cell proliferation and to induce selective cell death[5,6,30]. The response to treatment with cannabinoids has been investigated and demonstrated in a wide variety of adult tumours[30]; however, the effect has been studied in only a few pediatric tumours[23,24]. We therefore investigated the role of cannabinoids in a pediatric tumour, NBL, which is the most frequent extracranial solid tumour of childhood and which still carries a very poor prognosis[1].

We focused only on the major compounds in cannabis, THC and CBD. The results obtained in the *in vitro* studies can be summarized as follows:

- Both molecules—and CBD in particular—reduced the viability of NBL cells.
- The effect of CBD seemed to be mediated by apoptotic cell death, as demonstrated by morphology changes, accumulation of sub-G1 cells, annexin V assay, and increased expression of cleaved caspase 3.
- The invasiveness of NBL cells was also reduced with CBD treatment.

Based on that first set of results, we studied the effect of CBD and THC on xenograft tumours generated in NOD/SCID mice from SK-N-SH cells that had already demonstrated the greatest sensitivity to the effect of those molecules. In accord with the findings from the *in vitro* experiments, THC and CBD both reduced the xenograft growth rate, with CBD showing a superior effect.

Our *in vitro* data suggesting that CBD inhibits the proliferation of, and induces apoptosis in, NBL cells—together with its remarkable effect on NBL xenografts—are, to the best of our knowledge, the first to show an antitumour effect of CBD on NBL cells. Moreover, the results obtained in our study indicate that, of the two cannabinoids tested, CBD was more effective on the SK-N-SH cell line and on xenografts than was the more-studied THC. Those results accord with recently emerging data showing an effect of CBD on other tumours such as glioblastoma and breast, lung, prostate, and colon cancer[18,31–34].

Δ^9-Tetrahydrocannabinol, the second most abundant cannabinoid in *Cannabis sativa,* has been shown to induce apoptosis and to inhibit tumour cell viability and invasiveness in various tumours[34–38], as our study also demonstrated. Recently, CBD was also reported to enhance the production of reactive oxygen species in cancer cells[39], to downregulate the metastatic factor Id1, and to upregulate the pro-differentiation factor Id2[16,40].

The mechanism by which CBD produces the observed effects has not yet been completely clarified, but seems to be independent of the CB1 and CB2 receptors. Various studies have demonstrated that CBD acts as an agonist for vanilloid receptor 1 and for the TRPV2, TRPA1, PPARG, and 5-HT1A receptors, and as an antagonist for the TRPM8 and GPR55 receptors[41]. However, CBD's antitumourigenic molecular mechanisms of action have not been studied in NBL. A hint can be found in several reports showing that various NBL cell lines express the foregoing receptors shown to be involved in the action of CBD[42–44]. Several studies have demonstrated that activation of those receptors with agonists other than CBD mediates cell death in a variety of NBL cell lines, including SK-N-SH[45,46], the CBD-responsive cell line in our study.

As a potential therapeutic agent, CBD could have many advantages, especially compared with psychoactive THC. Because most—if not all—of the psychoactive effects of cannabinoids are produced by activation of the central CB1 receptors[47], CBD, which has been shown to act independently of CB1, is devoid of psychoactive effects[48] and can serve as a more suitable treatment, especially in children. Additionally, it shares the palliative properties and low toxicity profile described for other cannabinoids, has none of the strong side effects associated with chemotherapeutic agents[10,18,49], and might have synergistic activity with well-established antineoplastic substances[10,50].

The most widely used route of cannabinoid administration is smoking—an unattractive clinical option, particularly in children. Our work indicates that systemic (intraperitoneal) administration of CBD effectively reduces tumour growth, and use in a clinical setting can therefore be based on other routes of administration, such as in oral or oromucosal treatments.

CONCLUSIONS

Our findings about the activity of CBD in NBL support and extend previous findings about the antitumour activities of CBD in other tumours and suggest that cannabis extracts enriched in CBD and not in THC could be suitable for the development of novel non-psychotropic therapeutic strategies in NBL. Use of CBD either as single agent or in combination with existing compounds and chemotherapy agents is a possibility. Combination therapy might improve the antitumourigenic effects of other treatments and allow for a reduction in the chemotherapy dose, minimizing toxicity and long-term sequelae. Future studies are needed to highlight the pathways involved in the antitumourigenic effects of CBD in NBL as demonstrated in the present work.

CONFLICT OF INTEREST DISCLOSURES

We have read and understood *Current Oncology*'s policy on disclosing conflicts of interest, and we declare that we have none.

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Cannabinoids in Pediatrics

Christopher T. Campbell, PharmD,✉ Marjorie Shaw Phillips, MS, and Kalen Manasco, PharmD

✉Corresponding author.
Department of Pharmacotherapy and Translational Research (CTC, KM), University of Florida College of Pharmacy, Gainesville, Florida; Department of Pharmacy (CTC, KM), University of Florida Health Shands Children's Hospital, Gainesville, Florida; Department of Pharmacy (MSP), Augusta University Medical Center, Augusta, Georgia; Department of Clinical and Administrative Pharmacy (MSP), University of Georgia College of Pharmacy, Augusta, Georgia
Correspondence Christopher T. Campbell, PharmD; campbellc@cop.ufl.edu

Abstract

Despite its controversial nature, the use of medical marijuana and cannabis-derived medicinal products grows more popular with each passing year. As of November 2016, over 40 states have passed legislation regarding the use of either medical marijuana or cannabidiol products. Many providers have started encountering patients experimenting with cannabis products for a wide range of conditions. While the debate continues regarding these agents for both medicinal and recreational use in the general population, special consideration needs to be made for pediatric use. This review will deliver the history of marijuana use and legislation in the United States in addition to the currently available medical literature to equip pediatric health care providers with resources to provide patients and their parents the best recommendation for safe and appropriate use of cannabis-containing compounds.

Keywords: CBD, cannabidiol; cannabis; epilepsy; pediatrics; pharmacy

Introduction

Over the past several years, medical marijuana use has become a controversial topic not only within the medical community but also at state and national legislative levels. Although marijuana and its derivatives are currently Schedule 1 substances per the federal Controlled Substances Act (CSA), many states have relaxed their legislation to allow use. More recently, the use of cannabidiol (CBD) products in pediatrics has sparked additional debate, and pediatric providers have started encountering patients experimenting with these products in their daily practice, necessitating an understanding of the history and available medical literature on this topic.

Many of the misconceptions regarding medical marijuana in the pediatric population stem from negative connotations associated with the term *marijuana* owing to its psychoactive effects. Therefore, it is important to define the various terms associated with products that are currently being used by the public as well as by pediatric researchers. *Cannabis* is a general term that refers to the 3 species of hemp plants (*Cannabis sativa*, *Cannabis indica*, *Cannabis ruderalis*).[1] *Marijuana* is a term that describes the dried leaves, flowers, stems, and seeds from the hemp plant that are often smoked for recreational and medicinal use. Marijuana contains various different chemicals called *cannabinoids*.

Cannabinoids are the chemicals found within cannabis that interact with specific receptors, namely, cannabinoid (CB) receptors, within the body. The over 60 types of cannabinoids currently identified differ by the degree to which they are psychoactive.[2] While delta-9-tetrahydrocannabinol (THC), the cannabinoid most commonly associated with marijuana as a drug of abuse, is psychoactive, other cannabinoids including *CBD* are not. THC has been linked to the development of schizophrenia, and a contributor to neurodevelopment deficits in adolescents.[3,4] Different marijuana strains will have varying amounts of both THC and CBD, and thus the concentrations and ratios of these different cannabinoids within a product, especially for pediatric use, has been a subject of interest not only for medical professionals but also for state legislators as well.

History and Regulation

Dating back as far as 2000 BC, hemp plants had been used for various medicinal and industrial purposes. In 1851, the United States Pharmacopeia (USP) classified marijuana as a legitimate medical compound and many physicians supported its use for conditions such as epilepsy, chronic migraines, and pain.[5] Reports of Victorian-era neurologists using Indian hemp to treat epilepsy were also promising.[6] However, when phenobarbital and phenytoin came to the market in the early 1900s, the use of marijuana-based products declined.

In the 1930s, political propaganda sought to associate marijuana use, specifically by minority and low-income populations, with psychosis, addiction, and violent crime. Many believe this was influenced by several prominent businessmen in competing synthetic fiber industries in attempts to reduce the size of the growing hemp industry.[5] Marijuana soon became labeled as a drug of abuse and to discourage its use, Congress passed the Marijuana Tax Act of 1937 placing a heavy tax on cannabis and hemp use for both medicinal and industrial purposes. Despite opposition from the American Medical Association (AMA) and physicians who believed in the medical efficacy of marijuana, by 1941, all cannabis preparations were removed from the USP and National Formulary.

In the 1960s and early 1970s, marijuana soon became associated with recreational use by anti-establishment groups further adding to the stigma associated with its usage. By 1970, the CSA labeled cannabis as a Schedule 1 substance. This relatively short era of recreational marijuana use has influenced how the public perceives the drug. Since that time, there have been repeated unsuccessful attempts to reconsider its Schedule 1 status to allow for easier investigation.[5] The AMA and the American Academy of Pediatrics (AAP) have reaffirmed their opposition to the legalization of medical and recreational marijuana use outside of any US Food and Drug Administration (FDA) regulatory process.[7]

The AAP also supports further research into the indications and correct dosage for cannabinoids in addition to developing policy around how to verify purity and formulations.[8] In the meantime, the AAP has suggested good practices to follow when considering the use of marijuana, recreationally or medically (Table 1).

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Table 1.

Recommendations from the American Academy of Pediatrics[8]

Research and development should be conducted of pharmaceutical cannabinoids. The AAP recommends changing marijuana from a DEA Schedule 1 to a DEA Schedule 2 to facilitate this research.
The federal and state governments should establish robust health surveillance regarding the impact of marijuana, particularly on children and adolescents.
In states that have legalized marijuana for recreational use, the AAP strongly recommends strict enforcement of rules and regulations that limit access, marketing, and advertising to youth.
Where marijuana is sold legally, either for medicinal or recreational purposes, it should be contained in child-proof packaging to prevent accidental ingestion.
The AAP discourages adults from using marijuana in the presence of children because of the influence of role modeling by adults on child and adolescent behavior.

AAP, American Academy of Pediatrics; DEA, Drug Enforcement Administration

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To date, however, 8 states and the District of Columbia have passed legislation to legalize recreational marijuana use, with an additional 20 states allowing for some form of medical cannabis. Fourteen nonmedical marijuana states have specific legislation regarding CBD (Figure).[9–11] The changing legislative and regulatory landscape has significantly impacted the use of cannabinoid products in this country. Discussion about the safe and efficacious use of these products in a responsible way that protects vulnerable populations, including pediatrics, is necessary.

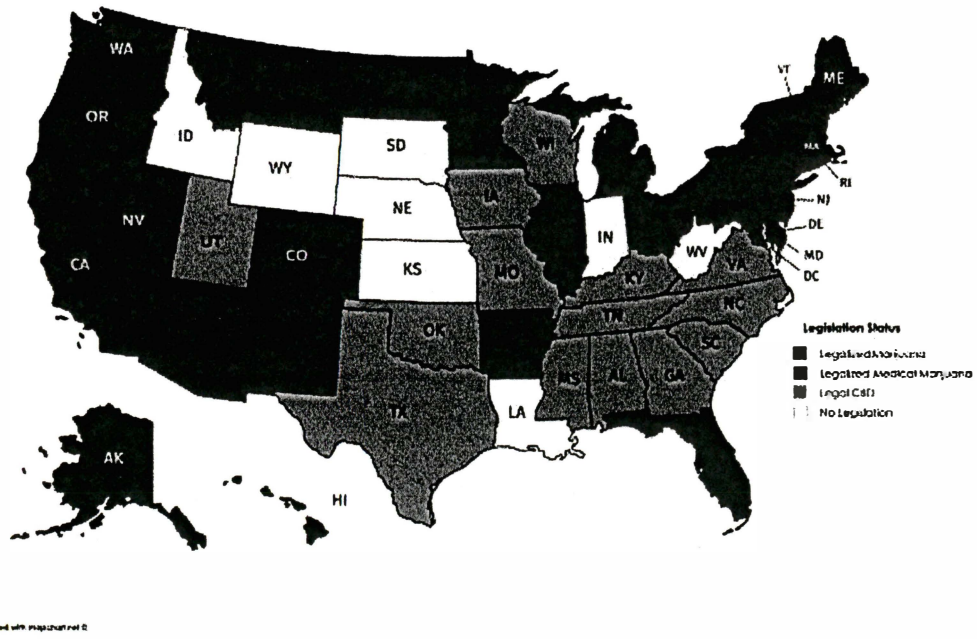

Figure.

2017 Legislative status of marijuana in the United States

Pharmacology

Similar to endogenous opioids, a human's central nervous system is impregnated with cannabinoid receptors and endocannabinoids. In the early 1990s, 2 receptors were discovered, cannabinoid type 1 (CB1) and cannabinoid type 2 (CB2). Both CB1 and CB2 are G-coupled protein receptors located presynaptically and control the release of neurotransmitters at both inhibitory and excitatory synapses. CB1 is mostly expressed on presynaptic peripheral and central nerve terminals and is believed to be responsible for psychologic effects on pleasure, memory, thought, concentration, sensory and time perceptions, and coordinated movement. CB2 receptors, concentrated in peripheral tissues and immune cells, may play an anti-inflammatory and immunosuppressive role. In addition to directing the release of various neurotransmitters, this receptor regulates the release of certain cytokines. Innervation of both these receptors results in both physiological (tachycardia, hypertension, dry mouth and throat) as well as psychological (elation, euphoria, heightened perception, irritability, poor coordination and balance) effects.[3,5]

Additionally, endocannabinoids N-arachidonoylethanolamine (anandamide) and 2-arachidonoylglycerol, both arachidonic acid derivatives, bind with CB1 and CB2. While the function of these endogenous ligands is not fully understood, their action may be attributed as antiemetic, antianalgesic, and anti-inflammatory. Endocannabinoids can also play a role in excitation of the neuronal networks, thus having effect on the quality of a seizure. Previous studies have documented deficiencies in endocannabinoids in temporal lobe epilepsy patients as well as a rise in anandamide concentrations post seizures in mice, suggesting an antiseizure activity profile.[6]

The 2 most studied exogenous cannabinoids include THC and CBD. THC is a partial agonist at both CB1 and CB2 receptors and achieves its psychoactive properties likely through modulation of gamma-aminobutyric acid (GABA) and glutamine. THC seems to possess antiseizure activity but may be a proconvulsant in certain species.[12] CBD, however, does not appear to bind to either CB1 or CB2 but does possess neuroprotective and anti-inflammatory effects.[5] Several possible mechanisms of CBD have been proposed: inhibition of cyclooxygenase and lipoxygenase, inverse agonism at CB1/CB2 receptors, and enhancement of anandamide.[3] It is proposed that CBD may be effective in epilepsy through modulation of the endocannabinoid system. CBD halts the degradation of the endocannabinoid anandamide, which may have a role in inhibiting seizures. Additionally, research demonstrates that CBD may play a role with the regulation of T-type calcium channels and nuclear peroxisome proliferator-activated receptor-γ, both of which have been implicated in seizure activity.[12] Because CBD is one of the most abundant cannabinoids within cannabis resin and its mechanism is still unclear, there is peaked interest in the possible clinical indications that it could treat including epilepsy, pain, and inflammatory disorders.

Several other synthetic forms of cannabinoids have been available for use in some countries, including dronabinol, nabilone, and nabiximols (Table 2). These products are being used to treat nausea and vomiting associated with chemotherapy, anorexia and weight loss in patients with acquired immune deficiency syndrome (AIDS), and relief of spasticity and neuropathic pain associated with multiple sclerosis (MS).[13–16] Epidiolex (GW Pharmaceuticals, Cambridge, United Kingdom) is a CBD product currently in clinical trials.[17]

Table 2.

Synthetic Cannabinoid Products

Drug	Component(s)	FDA Approval Status	Indication
Dronabinol[13,15]	Synthetic THC	FDA approved – 1985	Treatment of nausea and vomiting associated with cancer chemotherapy in patients who have failed to respond adequately to conventional antiemetic therapy. Treatment of anorexia associated with weight loss in patients with acquired immune deficiency syndrome.
Nabilone[14,15]	Synthetic dimethylheptyl analogue of THC	FDA approved – 1985	Treatment of nausea and vomiting associated with cancer chemotherapy in patients who have failed to respond adequately to conventional antiemetic therapy.
Nabiximols[15,16]	Tetranabinex (plant-derived THC) and Nabidiolex (plant-derived CBD)	Not FDA approved Available in 16 countries outside of the United States	Symptomatic relief of spasticity in adults with multiple sclerosis who have not responded adequately to other therapy and who demonstrate meaningful improvement during an initial trial of therapy. Symptomatic relief of neuropathic pain in patients with multiple sclerosis. Intractable cancer pain.
Epidiolex[17]	Plant-derived CBD	Undergoing clinical trials	Clinical trials ongoing for epilepsy conditions.

CBD, connabidiol; FDA, US Food and Drug Administration; THC, delta-9-tetrahydrocannabinol

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Pharmacokinetics

Historically, patients and recreational users have inhaled or vaporized marijuana, resulting in a quick onset and higher peak concentrations. Owing to first-pass metabolism, the enteral route decreases the bioavailability of THC to from 5% to 20% and CBD to from 6% to 19% and increases the time to onset.[3,18,19] Differences in absorption between various age groups, populations, and individual people make it difficult to recommend a one-size-fits-all dosage strategy. Interpatient variability may affect which blood concentrations will be effective, and tolerance is known to occur owing to downregulation of CB1 receptors.[3,18]

Both THC and CBD are highly lipophilic with long half-lives, 30 hours versus 9 to 32 hours, respectively.[3,18,20] CBD is also highly protein bound and is both metabolized by and a potent inhibitor of the CYP450 enzymes (2C19, 3A4), potentially causing significant medication interactions.[3,18,20,21] While CYP inducers such as phenytoin and carbamezapine may decrease CBD concentrations, CBD is known to increase concentrations of clobazam, an antiepileptic drug approved by the FDA in 2011 for the treatment of Lennox-Gastaut syndrome (LGS). CBD inhibits CYP3A4 and CYP2C19, preventing the degradation of clobazam and its active metabolite, N-desmethylclobazam. In an expanded access trial, patients with concomitant clobazam and CBD use had increases in clobazam concentrations of > 60% and N-desmethylclobazam, of 500%.[22] At this time it is not clear what other drug interactions may exist and what dosage manipulations may be necessary.

Clinical Data

The debate about the use of cannabinoid products in pediatric patients has persisted owing to the lack of well-developed and published randomized controlled trials. There has been a wide variety of mostly case series and international studies for adult indications, such as chronic pain, MS, headache, and various neuropsychiatric disorders, which are beyond the scope of this review but have been reviewed elsewhere.[20] The pediatric literature lacks the same breadth owing to public stigma and restrictions on investigational use. This has resulted in retrospective and parentally reported data in epilepsy and behavioral conditions. Despite the overall lack of published data on CBD in pediatric patients, most of the literature is devoted to its use in epilepsy. Current large prospective trials are underway for different epilepsy indications, and recent animal studies researching use in perinatal brain injury and neuroblastoma may open new avenues to consider cannabinoids for pediatrics.

Epilepsy. The data in pediatric epilepsy have been surrounding the use of CBD products as well as unregulated THC/CBD products from private dispensaries. A Cochrane review[23] was conducted in 2012 to assess the safety and efficacy of cannabinoid use in patients with epilepsy. The authors included blinded and unblinded randomized controlled trials. Only 4 studies met their criteria, including 1 abstract and 1 letter to the editor (Table 3). All 4 trials were of low quality with small sample sizes and variations in product, dose, frequency, and duration.[24–27] The authors summarized the finding that a CBD dose of 200 mg to 300 mg daily was safely administered over a short period. The only reasonable conclusion made was that the efficacy of CBD use could not be confirmed, but the rate of adverse reactions in each of the studies was low over a short period.

Table 3.

Included Studies in Cochrane Review[23]

Study	Patient Demographics	Intervention	Results
Mechoulam et al[24]	Nine patients with uncontrolled temporal lobe epilepsy who had failed multiple medications	Group 1: CBD 200 mg daily (n = 4) Group 2: Placebo daily (n = 5) Duration: 3 mo	Group 1: 2 patients seizure free entire 3 mo; 1 patient with partial improvement (not defined) Group 2: no patient improvement
Cunha et al[25]	Fifteen patients (11 female) with temporal lobe irritative activity; ages: 14–49 yr	Group 1: CBD 200–300 mg daily (n = 8) Group 2: Placebo (n = 7) Duration: 18 wk	Group 1: 50% (n = 4) of patients had complete improvement Group 2: 14% (n = 1) of patients with complete improvement
Ames[26]*	Twelve institutionalized, mentally retarded patients with frequent seizures	Group 1: CBD 300 mg daily for 1 wk, then CBD 200 mg daily (n = ?) Group 2: Placebo (n = ?)	No statistically significant difference in seizure frequency between the 2 groups
Trembly[27]†	Twelve patients with incompletely controlled epilepsy	Each patient served as his or her own control on the following schedule: • 3 mo of normal outpatient epileptics • 6 mo of placebo • 6 mo of CBD 100 mg 3 times a day	"No discernable effect" on MMPI, Beck depression inventory, trail-making test, and finger-tapping test

CBD, cannabidiol; MMPI, Minnesota Multiphasic Personality Inventory
* Letter to the Editor only available, resulting in incomplete data
† Abstract only available, resulting in incomplete data and lack of results

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The American Academy of Neurology conducted a systematic review in 2014 which included 34 studies that used medical marijuana to treat MS, epilepsy, and movement disorders.[28] The authors included 2 studies to assess the role of cannabinoids in decreasing seizure frequency.[25,26] Of note, these

studies were also evaluated in the Cochrane review. The authors[28] concluded that "data are insufficient to support or refute the efficacy of cannabinoids for reducing seizure frequency" and thus, there is not sufficient evidence to advise patients to use cannabinoid products in epilepsy.

Despite this, parents and patients are making the decision to use these products for 3 reasons according to Cilio et al:[12] 1) prominent Internet and nation media attention; 2) reports of cases of children successfully treated with CBD products; and 3) the belief that treatments derived from natural products are safer or more effective.[12] National attention has been on those patients who have moved to states where CBD use is legal and researchers have sought to gather data from parental observations. It is important to note that the following studies are based on parental perceptions and thus we cannot draw definitive conclusions.

The most famous case was presented on a CNN special, "Weed."[29] Charlotte is a little girl from Colorado who was diagnosed with Dravet syndrome at the age of 3 months. She suffered from frequent status epilepticus. Charlotte failed multiple medications, and at 5 years of age, she had significant cognitive delay and required help with all of her activities of daily living.[30] Her parents sought out a group in Colorado that created an oral, liquid, high-concentration CBD-to-THC strain of cannabis. Once her parents started giving her this strain, dubbed "Charlotte's Web", within 3 months Charlotte had a > 90% reduction in her seizure frequency and by month 20, Charlotte was able to perform most of her daily activities independently with only 2 to 3 nocturnal tonic-clonic seizures per month. Stories like Charlotte's have prompted parents across the country in similar situations to move their families across the country to gain access to these products.

In a retrospective chart review of 75 children and adolescents younger than 18 years who were given oral cannabis extracts for treatment of their epilepsy, 57% of parents reported some improvement in seizure frequency with 33% reporting a >50% reduction in seizures.[31] Dosage information was not reported and parents used various formulations and concentrations of CBD and THC. Parents also described improvements in behavior and alertness (33%), language (11%), and motor skills (11%). Major adverse effects noted were somnolence (12%) and gastrointestinal symptoms (11%).

Investigators at Stanford University administered a survey to 150 parents on Facebook to identify parentally reported effects of CBD on their child's seizures.[32] Of 19 respondents aged 2 to 16 years, 18 had treatment-resistant epilepsy for more than 3 years before CBD use. Based on parental response, 84% reported a reduction in child's seizure frequency with 50% having a greater than 80% reduction in seizure frequency. Twelve of these 19 patients were also able to be weaned from another antiepileptic drug. In addition, parents reported overall better mood, increased alertness, and better sleep. Parents reported oral CBD dosages of 0.5 mg/kg/day to 28.6 mg/kg/day and THC of 0 to 0.8 mg/kg/day.

In a similar Facebook survey administered by researchers at the University of California, Los Angeles, the authors[33] similarly reported an 85% reduction in seizure frequency among 117 respondents, with an average age of 6 years. Most patients (86%) conveyed that changes in frequency occurred within 14 days. As with previous surveys, dosage and formulations were varied but based on parental report of formulation used. Overall, most parents (83.5%) reported using an oral CBD product with at least a 15:1 ratio of CBD to THC. Of the 40% of respondents who provided dosages, the median weight-based dose of CBD was 4.3 mg/kg/day given in 2 to 3 oral doses. As mentioned above, these surveys should be evaluated carefully given the inability to verify dose, formulation, and response. The conclusion that can be made is that there is a rather strong positive parental perception regarding the efficacy of cannabinoids, specifically CBD.

Most orphan drug designations for CBD are for pediatric seizure disorders (Table 4).[34] A search of ClinicalTrials.gov in November 2016 identified 4 completed Phase 2 and Phase 3 protocols for pediatric seizure disorders, as well as 14 ongoing treatment trials, including intermediate-size expanded

access protocols (up to 50 patients each). Published findings from open-label use of CBD for treatment-resistant epilepsy under an expanded-access program at 11 epilepsy centers in the United States suggest that CBD might reduce seizure frequency and might have an adequate safety profile in children and young adults with this condition.[35] Congressional testimony in June 2015 indicated that 20 intermediate-size expanded access Investigational New Drug Applications had been authorized to treat approximately 420 children with 1 CBD product; most of these are not listed on ClinicalTrials.gov.[36]

Table 4.

Orphan Drug Designations for Cannabidiol in the Treatment of Pediatric Conditions[34] *

Orphan Designation	Designation Date	Sponsor
Dravet syndrome'	November 14, 2013	GW Pharma Ltd
Lennox-Gastaut syndrome	February 24, 2014	GW Pharma Ltd
Lennox-Gastaut syndrome	June 23, 2014	Insys Development Company Inc
Dravet syndrome'	July 1, 2014	Insys Development Company Inc
Pediatric schizophrenia (pediatrics is defined as 0 through 16 years of age)	November 17, 2014	Insys Development Company Inc
Neonatal hypoxic ischemic encephalopathy'	April 22, 2015	GW Pharma Ltd
Infantile spasms	July 23, 2015	Insys Development Company Inc
Fragile X syndrome	February 23, 2016	Zynerba Pharmaceuticals Inc
Tuberous sclerosis complex	April 19, 2016	GW Pharma Ltd
Infantile spasms	June 13, 2016	GW Research Ltd

FDA, US Food and Drug Administration

* None of these products are FDA approved for orphan or any other indication

' Granted FDA Fast Track designation for this indication. A drug development program with Fast Track designation is afforded greater access to the FDA for the purpose of expediting the drug's development, review, and potential approval to get important new drugs to the patients earlier

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After announcing positive results from 2 pivotal randomized, double-blind, Phase 3 trials for the treatment of seizures related to LGS, and a third for seizures associated with Dravet syndrome in 2016, GW Pharmaceuticals expects to submit a single New Drug Application for both indications to the FDA in the first half of 2017 for its proprietary pharmaceutical-grade CBD product Epidiolex.[37] In the second LGS study, patients randomized to the investigational product 20 mg/kg/day (n = 76) or 10 mg/kg/day (n = 73) added to their current antiepileptic treatment, experienced a median reduction in monthly drop seizures of 42% and 37%, compared to 17% in the placebo group (n = 73); a difference that was statistically and clinically significant ($p = 0.0047$ and $p = 0.0016$, respectively).[37] These data confirmed the results of the first LGS trial in which 86 patients receiving Epidiolex 20 mg/kg/day achieved a 44% mean reduction in monthly drop seizures as compared to 22% for 85 patients in the placebo group ($p = 0.0135$).[38] Patients with Dravet syndrome receiving the GW Pharmaceuticals' CBD in addition to their baseline antiepileptic regimen (n = 61) achieved the primary endpoint of a significant reduction in convulsive seizures ($p = 0.01$, median reduction of 39%) assessed over the 14-week treatment period as compared with the addition of placebo (n = 59).[39] Insys Therapeutics (Phoenix, AZ) has reported that their synthetic pharmaceutical CBD in a nonalcoholic, medium-chain triglyceride-based formulation was generally well tolerated in a completed Phase 1/Phase 2 safety and pharmacokinetic study in 61 pediatric patients with treatment-resistant epilepsy at total daily doses up to 40 mg/kg.[40]

Behavioral Conditions. Cannabinoids and CBD use in this patient population is a growing interest on social media sites. While the data for these indications are limited to case reports using dronabinol, some of the benefits of CBD on behavior and motor skills reported in the aforementioned retrospective studies in epilepsy may be transferable to this population as well. A 6-year-old patient with early infant autism received enteral dronabinol drops titrated up to 3.62 mg/day. He had improvements in hyperactivity, irritability, lethargy, stereotype, and speech.[41] In a published abstract, Kruger et al[42] report on the effect of dronabinol use in treating self-injurious behavior in 10 mentally retarded adolescents. The dronabinol dose ranged from 2.5 mg twice daily to 5 mg 4 times a day. Seven of the 10 patients had significant improvement in their self-injurious behavior that lasted through the follow-up at 6 months. Two of the 10 patients experienced agitation and the drug was discontinued. An Israeli single-center, double-blind, placebo-controlled cross-over trial of CBD and THC in a 20:1 mixture for behavioral problems in children with autistic spectrum disorder is scheduled to start in January 2017.[43]

Perinatal Brain Injury. Perinatal brain injury can be induced by neonatal asphyxia, stroke-induced focal ischemia, and neonatal hypoxia-ischemic encephalopathy, among other things. These conditions lead to long-lasting functional impairment due to neuroinflammation, apoptotic-necrotic cell death, and brain lesions.[44] Several adjunctive medication therapies in addition to hypothermia, include magnesium sulfate and minocycline which may play a role in modulating neuroinflammation and apoptosis. The endocannabinoid system responds early to neuronal damage, working to prevent glutamate excitotoxicity and regulate the inflammatory response. While there are no current human studies, results from mice and pig models demonstrate that CBD can reduce the density of necrotic neurons and modulate cytokine release.[45,46]

Neuroblastoma. Most recently, researchers have reported on the use of CBD in both *in vitro* and *in vivo* animal studies of neuroblastoma (NBL), a common childhood cancer.[47] Investigators are proposing that antitumor activity is achieved by action at vanilloid and peroxisome proliferator-activated receptors. *In vitro*, they found that both CBD and THC reduced the viability of NBL cells in a dose- and time-dependent manner. When comparing the two, CBD had a significantly better response in reducing viability of NBL cells than THC. They next treated mice with daily intraperitoneal injections of THC, CBD, or ethanol, or gave no treatment. Tumor growth in both the THC and CBD groups was significantly reduced.

What's The Harm?

Worldwide, marijuana is the most commonly abused illegal substance and adolescent daily use is on the rise.[18] Adolescents perceive that marijuana use is not as much of a risk owing to legalization and decriminalization, leading to its use both recreationally and to self-treat anxiety and other psychiatric conditions. Unfortunately, the neurocognitive and behavioral effects of marijuana use in pediatric patients, including its effects on psychological dysfunction, amotivation syndrome, and carcinogenic risk, have been widely reported.[4,21]

Evolving legislation and the increased use of cannabinoid products outside of investigational studies have also impacted our health care delivery and emergency resources. The state of Colorado has been on the forefront of the medicinal and recreational use of cannabis debate. Wang et al[48] reported the occurrences of pediatric emergency department visits associated with marijuana exposure before and after changes in drug enforcement in 2009. A total of 1378 patients younger than 12 years were evaluated for unintentional ingestions from January 1, 2005, to December 31, 2011. Before 2009, no patients (0/790; 0%) sought care at this emergency department for accidental marijuana ingestions as compared with 14 patients (14/588; 2.4%) after 2009 ($p < 0.001$). Patients ranged in age from 8 months to 12 years and presented with symptoms of lethargy, ataxia, and respiratory insufficiency. While the dosages were not reported, 7 patients ingested a marijuana edible. Eight of the 14 patients were

admitted to the hospital with 2 admissions to the pediatric intensive care unit. Prior to diagnosis, these 14 patients received routine testing such as urinalyses, complete blood counts, and complete metabolic panels. Some of these patients also received more invasive testing including computed tomography, activated charcoal, lumbar punctures, and intravenous antibiotics. All of these contribute to higher hospital and emergency room costs, increased lengths of stay, and potential harm to the patients.

In addition to increased emergency room visits, from 2005 to 2011, the call volume at Poison Control Centers for pediatric marijuana exposures had increased by 30.3% in states where marijuana has been decriminalized as compared to a steady rate in states that have not adopted marijuana decriminalization legislation.[49] While marijuana and CBD products are becoming more available, these products remain in DEA (Drug Enforcement Administration) Schedule 1 status and are therefore not regulated in manufacturing, packaging, and labeling outside of clinical trials. As seen in the Colorado case study, 50% of the unintentional ingestions were secondary to an edible, which children can easily mistake for food if not supervised by parents. None of these products are required to have safety packaging to prevent accidental ingestion by children. In addition, no warning labels or verification of product ingredients is required, leaving the medical community caught between providing safe medical care and allowing patient autonomy. As mentioned previously, the AAP has published recommendations to limit the access of marijuana to children.

Pharmacist's Role

In 2007, amidst medical marijuana legalization in several states, Seamon et al[21] identified that pharmacists needed to be attentive to the legislative changes going on at the state and federal levels. Pharmacists are uniquely poised to understand the medicinal chemistry as well as the practical implications associated with decriminalization and legalization. Pharmacists can continue to educate both medical professionals and lay people about the differences among cannabinoids, and help to remove the stigma around appropriate and legal use of CBD products. At the same time, medical professionals need to remember the documented deleterious effects of acute marijuana intoxication on neurocognitive development and psychiatric issues.

Many health care facilities are working through processes that address patient use of these medications. Because use of cannabis products outside of approved clinical trials is not legal under federal law, thus not permitted under Centers for Medicare & Medicaid Services (CMS) Conditions of Participation, there are significant challenges in managing hospitalized patients. Whatever the state and situation, pharmacists need to be aware of the external factors associated with allowing a patient to use CBD in an inpatient setting.

Pharmacists are also poised to participate in the design and evaluation of current and future research in this area. The importance of drug interactions between CBD and other antiepileptics remains uncertain both for the efficacy and safety of CBD products. The difference in concentrations, dosages, and formulations of various products sold at private dispensaries is not standardized or regulated. Differences in state legislation on allowable concentrations and amounts can be confusing for patients and their families, and pharmacists can help to provide that information. Various organizations have been helpful in updating and summarizing this information.[9]

Conclusions

Cannabis and its ingredients have had a fascinating history over the past 4000 years, but lack of published data precludes fully recommending its use for medicinal purposes in pediatrics. Further study is underway and will add to our knowledge of the efficacy and safety of CBD in pediatrics. Long-term

studies to assess neurocognitive development with CBD will need to be assessed as well. As pharmacists, it is our duty to provide our patients and their parents with the most accurate, safe, and legally appropriate advice.

Abbreviations

AAP	American Academy of Pediatrics
AIDS	acquired immune deficiency syndrome
AMA	American Medical Association
CB	cannabinoid
CB1	cannabinoid type 1 receptor
CB2	cannabinoid type 2 receptor
CBD	cannabidiol
CMS	Centers for Medicare & Medicaid Services
CNN	Cable News Network
CSA	Controlled Substances Act
DEA	Drug Enforcement Administration
FDA	US Food and Drug Administration
GABA	gamma-aminobutyric acid
LGS	Lennox-Gastaut syndrome
MS	multiple sclerosis
NBL	neuroblastoma
THC	delta-9-tetrahydrocannabinol
USP	United States Pharmacopeia

Footnotes

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Articles from The Journal of Pediatric Pharmacology and Therapeutics : JPPT are provided here courtesy of **Pediatric Pharmacology Advocacy Group**

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#1 Pg. 40

PubMed

Format: Abstract

Full text links

Curr Oncol. 2016 Mar;23(2):S15-22. doi: 10.3747/co.23.2893. Epub 201

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In vitro and in vivo efficacy of non-psychoactive cannabidiol in neuroblastoma.

Fisher T[1], Golan H[2], Schiby G[3], PriChen S[4], Smoum R[5], Moshe I[1], Peshes-Yaloz N[6], Castiel A[6], Waldman D[2], Gallily R[7], Mechoulam R[5], Toren A[8].

Author information

Abstract

BACKGROUND: Neuroblastoma (nbl) is one of the most common solid cancers in children. Prognosis in advanced nbl is still poor despite aggressive multimodality therapy. Furthermore, survivors experience severe long-term multi-organ sequelae. Hence, the identification of new therapeutic strategies is of utmost importance. Cannabinoids and their derivatives have been used for years in folk medicine and later in the field of palliative care. Recently, they were found to show pharmacologic activity in cancer, including cytostatic, apoptotic, and antiangiogenic effects.

METHODS: We investigated, in vitro and in vivo, the anti-nbl effect of the most active compounds in Cannabis, Δ(9)-tetrahydrocannabinol (thc) and cannabidiol (cbd). We set out to experimentally determine the effects of those compounds on viability, invasiveness, cell cycle distribution, and programmed cell death in human nbl SK-N-SH cells.

RESULTS: Both compounds have antitumourigenic activity in vitro and impeded the growth of tumour xenografts in vivo. Of the two cannabinoids tested, cbd was the more active. Treatment with cbd reduced the viability and invasiveness of treated tumour cells in vitro and induced apoptosis (as demonstrated by morphology changes, sub-G1 cell accumulation, and annexin V assay). Moreover, cbd elicited an increase in activated caspase 3 in treated cells and tumour xenografts.

CONCLUSIONS: Our results demonstrate the antitumourigenic action of cbd on nbl cells. Because cbd is a nonpsychoactive cannabinoid that appears to be devoid of side effects, our results support its exploitation as an effective anticancer drug in the management of nbl.

KEYWORDS: Neuroblastoma; apoptosis; cannabidiol; non-psychoactive cannabinoids; tumour xenograft models; Δ9-tetrahydrocannabinol

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#1 pg. 41

PubMed

Format: Abstract

Full text links

Epilepsia. 2019 Jan;60(1):6-19. doi: 10.1111/epi.14608. Epub 2018 Dec 4.

Cannabis-based products for pediatric epilepsy: A systematic review.

Elliott J[1,2], DeJean D[3], Clifford T[1,4], Coyle D[1], Potter BK[1], Skidmore B[5], Alexander C[6], Repetski AE[6], Shukla V[2], McCoy B[7,8], Wells GA[1,2].

Author information

Abstract

OBJECTIVE: To assess the benefits and harms of cannabis-based products for pediatric epilepsy.

METHODS: We identified in this living systematic review randomized controlled trials (RCTs) and nonrandomized studies (NRSs) involving children with epilepsy treated with cannabis-based products. We searched MEDLINE, Embase, PsycINFO, Cochrane Library, and gray literature (April 25, 2018). The primary outcome was seizure freedom; secondary outcomes were seizure frequency (total, ≥50% reduction), quality of life, sleep, status epilepticus, death, gastrointestinal adverse events, and visits to the emergency room. Data were pooled by random-effects meta-analysis. Risk of bias was assessed for each study, and GRADE was used to assess the quality of evidence for each outcome.

RESULTS: Four RCTs and 19 NRSs were included, primarily involving cannabidiol. All RCTs were at low risk of bias, whereas all NRSs were at high risk. Among RCTs, there was no statistically significant difference between cannabidiol and placebo in seizure freedom (relative risk [RR] = 6.77, 95% confidence interval [CI] = 0.36-128.38; 1 RCT), quality of life (mean difference = 0.6, 95% CI = -2.6 to 3.9; 3 RCTs), sleep disruption (mean difference = -0.3, 95% CI = -0.8 to 0.2; 3 RCTs), or vomiting (RR = 1.00, 95% CI = 0.51-1.96; 4 RCTs). There was a statistically significant reduction in the median frequency of monthly seizures with cannabidiol compared with placebo (-19.8%, 95% CI = -27.0% to -12.6%; 3 RCTs) and an increase in the number of participants with at least a 50% reduction in seizures (RR = 1.76, 95% CI = 1.07-2.88; 1 RCT) and diarrhea (RR = 2.25, 95% CI = 1.38-3.68; 3 RCTs). Death and status epilepticus were infrequently reported.

SIGNIFICANCE: Evidence from high-quality RCTs suggests that cannabidiol probably reduces seizures among children with drug-resistant epilepsy (moderate certainty). At this

time, the evidence base is primarily limited to cannabidiol, and these findings should not be extended to all cannabis-based products.

KEYWORDS: cannabidiol; cannabis; efficacy; pediatric drug-resistant epilepsy; safety; seizure; systematic review

PMID: 30515765 DOI: 10.1111/epi.14608

Secondary source ID

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19.0429.03001
Title.

Prepared by the Legislative Council staff for
Representative Vetter
March 4, 2019

PROPOSED AMENDMENTS TO ENGROSSED HOUSE BILL NO. 1417

Page 1, line 1, after "2" insert ", 38,"

Page 1, line 1, remove the third "and"

Page 1, line 2, after "19-24.1-03" insert ", subsection 5 of section 19-24.1-05, subsection 4 of section 19-24.1-20, and subdivision a of subsection 4 of section 19-24.1-21"

Page 1, line 3, after "marijuana" insert "and usable marijuana for minors under the medical marijuana program"

Page 2, after line 12, insert:

"**SECTION 2. AMENDMENT.** Subsection 38 of section 19-24.1-01 of the North Dakota Century Code is amended and reenacted as follows:

38. "Usable marijuana" means a medical marijuana product or the dried leaves or flowers of the plant of the genus cannabis in a combustible delivery form. However, the term does not include the dried leaves or flowers unless authorized through a written certification and does not include a cannabinoid edible product. In the case of a registered qualifying patient who is a minor, "usable marijuana" is limited to pediatric medical marijuana unless another form of usable marijuana is expressly authorized through a written certification."

Page 2, line 22, after the period insert "A health care provider may expressly authorize a minor to use a form of usable marijuana which is not limited to pediatric medical marijuana."

Page 4, after line 4, insert:

"**SECTION 5. AMENDMENT.** Subsection 5 of section 19-24.1-05 of the North Dakota Century Code is amended and reenacted as follows:

5. The department may not issue a registry identification card to a qualifying patient who is a minor unless:

   a. The department receives documentation the minor's health care provider has explained to the parent or legal guardian with responsibility for health care decisions for the minor the potential risks and benefits of the use of pediatric medical marijuana or other form of authorized usable marijuana to treat or alleviate the debilitating medical condition; and

   b. The department receives documentation the parent or legal guardian with responsibility for health care decisions for the minor consents in writing to:

      (1) Allow the minor's use of pediatric medical marijuana or other form of authorized usable marijuana to treat or alleviate the debilitating medical condition;

(2) Serve as the minor's designated caregiver or identifies a registered designated caregiver to act as the minor's designated caregiver;

(3) Control the acquisition of usable marijuana and control the dosage and frequency of the use of usable marijuana by the minor; and

(4) If serving as the minor's designated caregiver, prevent the minor from accessing the usable marijuana by storing the usable marijuana in an enclosed, locked facility.

SECTION 6. AMENDMENT. Subsection 4 of section 19-24.1-20 of the North Dakota Century Code is amended and reenacted as follows:

4. In addition to any other penalty applicable in law, except as otherwise expressly authorized in a written certification, a dispensary or a dispensary agent is guilty of a class B felony for intentionally selling or otherwise transferring usable marijuana, in a form other than pediatric medical marijuana, to a registered designated caregiver, for use by a registered qualifying patient who is a minor. A person convicted under this subsection may not continue to be affiliated with a compassion center and is disqualified from further participation under this chapter.

SECTION 7. AMENDMENT. Subdivision a of subsection 4 of section 19-24.1-21 of the North Dakota Century Code is amended and reenacted as follows:

a. May not dispense usable marijuana to a person other than a registered qualifying patient or a registered qualifying patient's registered designated caregiver. If a registered qualifying patient is a minor:

(1) The dispensary or agent of the dispensary may not dispense usable marijuana to a minor; and

(2) ~~The~~Except as otherwise expressly authorized in a written certification, the usable marijuana dispensed to the minor's designated caregiver must be in the form of pediatric medical marijuana."

Renumber accordingly

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#2 pg.1

North Dakota Senate Human Services Committee

March 4th 2019

Madam Chairwoman Lee and members of the Committee, my name is Steven James Peterson of The Committee for Compassionate Care of North Dakota.

The Committee for Compassionate Care is a patient advocacy group seeking to enable fair and reasonable access to medical marijuana in the state of North Dakota.

My role is to provide access to subject matter experts nationally that can address all of these questions and concerns the committee members may have.

I am in support of House Bill 1417

- My main concern is to clear up the language so that it isn't misunderstood by accident that currently sounds like only cancer patients can receive cannabis flower.
- These changes reflect the volume of cannabis needed by cancer patients undergoing treatment like chemotherapy that can cause extreme nausea and vomiting which we completely support.
- This bill is a proper step in the right direction for the realization of a "working" North Dakota Medical Marijuana program.

I am available for any questions about this bill.

Steven James Peterson, District 44 Fargo North Dakota

701-936-4362 Steven@ravenrisingllc.com

Re. HB 1417 Amendment (proposed)

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#3 pg. 1

Nate is 23 years old and has been a medical cannabis patient since June 2015. He started taking medication for his behaviors when he was around 5 years old and taking much larger and stronger psychotropic meds when he was around 13. I am convinced all of those medications also killed his pancreas and he has had both Types 1 & 2 diabetes for about 10 years now. Seven months after starting cannabis, he no longer needed any of those psychotropic pharmaceuticals. He went from 18 pills a day to zero. ZERO! His insulin needs have also decreased since starting cannabis.

He's safe. We're safe. We're all living happily together under the same roof and enjoying life. He even started going to a day program again, where he enjoys spending time with his peers.

We have adjusted Nate's cannabis intake up and down over the years. We have found many variables that have determined how much he would likely need. While Nate was weaning from all of the pharmaceuticals under a doctor's guidance, he needed his cannabis intake increased over time to not only compensate for the medications, but to help mitigate withdrawal symptoms as well. His doses of THC were quite large for a period of time – up to 200mg/day on good days and 400mg/day on not so good days - but have since greatly decreased. He now takes one 50mg+/- capsule that I make out of ground and decarboxylated plant matter each morning.

Jenni Mai, CA
262.770.6571

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#4 pg. 1

To whom it may concern

My name is Sandy Christianson and I have an 11yr old son that is currently a medical marijuana patient in the state of California. My son was born with medically refractory epilepsy and has suffered with seizures his entire life. In addition to his seizures he also exhibits major behavioral issues caused from mild autism as well as side effects from the many medications he takes.

Before beginning our journey with cannabis and after trying every seizure and anti-psychotic drug we were offered, my son was still suffering from 100s of seizures a night as well as extremely aggressive behaviors. He would go to school and threaten other children, hit and kick them, he has thrown desks and chairs in class. He would elope and try to run away from the school on multiple occasions. Tried to use pencils to hurt either himself or others. He was destructive at home and has ripped doors off hinges, put holes in walls, destroyed many household electronics including televisions, cell phones, computers etc..He was aggressive and violent towards myself, his younger sister, had attacked his 75 year old grandmother as well as his father. He was miserable and angry 90% of the time. We had also tried to have my son admitted to a hospital multiple times with no success. We were told to take him home and either deal with the behaviors or sedate him. None of which we were successful with.

After much desperation and hitting a dead end we applied for a recommendation and turned to cannabis to help my son. At first we tried using CBD oil. We tried multiple brands, doses, full spectrum, isolates etc....Unfortunately for my son it had the opposite effect. He had a major increase in seizure activity and experienced some of the worst Grand Mal seizures I have ever seen him have. In addition it worsened his behaviors and he became extremely manic.

Next we turned to using THC. Immediately we saw relief with his seizures as well as his behaviors. Over the next several months we trialed several different strains of THC. The more we increased the THC, the more seizure control we saw and the better his behavior became. Eventually we landed at 250mgs of THC a day and have been using this dose for over 8 months successfully. This 250mg dose of THC helps to control 99% of his seizures and eliminates almost all of his destructive and aggressive behaviors. Any lower of a dose lacks seizure and behavioral control. Currently My son is able to attend school regularly in a mainstreamed class with support. He has wonderful behavior most days (he's a prepubescent boy and we have some hiccups on occasion which is to be expected- he's still human) and is an extremely well liked child among his peers. He is able to participate in daily activities and be a kid!! My son and daughter have a great relationship, are friends and we have many moments to be grateful for as a family.

We are extremely thankful for the results we have seen using high doses of THC. If we were limited in the amount of THC or cannabis that we could use daily my son would've never found the relief he needed. We may have even given up on cannabis altogether and he would be either locked in an institution or possibly dead because of the amount of seizures he was having.

What we have learned from using cannabis and speaking with multiple families on a daily basis is that everyone has differing needs. Some need very low doses of cannabis while others are requiring high doses of cannabis. For those that need higher doses and have a greater deficiency in their Endocannabinoid system, it would be cruel to limit them to less than sufficient amounts to help their individual condition. It would be similar to not allowing a diabetic to have the proper amount of insulin, or treating a cancer patient with just half of the needed therapy to help them survive. THC is medicine that is helping my child survive. It would be devastating if he was limited to anything less than what is needed to help him thrive.

Sandy Christianson

Riverside, California

562-833-0654

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3/5/19
#5 pg. 1

Tanya Stueve
Williston
701.651.7432

My son Trent is nine and has epilepsy. In the past his seizures have lasted up to two hours. Presently we're controlling them with a ketogenic diet. But sometimes epileptics revert. But sometimes epileptics revert due to the onset of hormones when they reach puberty.

When and if this happens to Trent, we want access to a full range of medical options including amounts of THC which are effective, not necessarily predetermined by a one size fits all minor restriction.

Parents deserve as many tools as possible to treat their ill children.
Please seriously considered adding the presented amendment to HB 1417.

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#6 pg. 1

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House Bill 1417
Human Services Committee
March 5, 2019

Good morning Madam Chair Lee and members of the Human Services Committee. My name is Jason Wahl, Director of the Division of Medical Marijuana within the Department of Health. I am here to support and provide information on House Bill 1417 related to proposed changes to language within the Medical Marijuana chapter of state law. It should be noted several additional changes are necessary to the Medical Marijuana chapter not currently addressed by the bill.

House Bill 1417 would make a change in the amount allowed to be purchased and possessed by a qualifying patient with cancer. The purchasing amount in a 30-day period would more than double, going from 2.5 ounces to 6 ounces. Also, the bill would provide for a 2.5 times higher possession limit (going from 3 ounces to 7.5 ounces).

Similar to House Bill 1283, this bill would modify the definition of written certification by removing language currently requiring a health care provider to state in their professional opinion the patient is likely to receive therapeutic or palliative benefit from the medical use of marijuana. We do support this change to the law as we have heard from the medical community that this requirement may be limiting the number of health care providers willing to complete a written certification.

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#6 pg. 2

The bill would also eliminate the requirement for a health care provider to authorize the use of dried leaves or flowers. This, in turn, would eliminate the requirement for a registry identification card to include whether the qualifying patient is authorized for dried leaves or flowers.

With the current language in House Bill 1417, there are other sections under NDCC Chapter 19-24.1 that require amendments to provide consistency. For example, NDCC Section 19-24.1-01, Subsection 38 references authorization of dried leaves or flowers and is not amended under House Bill 1417. This would cause a conflict in statute. We would be willing to work with our legal counsel or Legislative Council to introduce an amendment to the bill to address all necessary revisions for the Committee's consideration.

With the dispensing of marijuana products recently to registered qualifying patients, we have identified an additional potential change to state law that we would like to bring to the Committee's attention. Under the program, a qualifying patient may not purchase more than 2,000 milligrams of THC in medical marijuana products in a 30-day period. The Committee may want to consider revising this amount as this amount appears low. For example, the concentrates available when the dispensary opened were disposable vaporizing pens and shatter. A vaporizing pen had 453 milligrams of THC while shatter had 356 milligrams of THC. An individual would only been allowed to purchase four vaporizing pens under the 2,000 milligram limit. As a contrast, under the state of Ohio's program, which began

dispensing the middle of January 2019, a patient may collectively purchase within a 90-day period:

- 26,550 milligrams of THC in patches, locations, creams, or ointments;
- 9,900 milligrams of THC in oils, tinctures, or capsules; and
- 53,100 milligrams of THC in oil for vaporization.

The Department of Health did submit a fiscal note regarding implementation of House Bill 1417. There are three areas of the bill that would require changes to the information technology system. This includes changes to the written certification form, the addition of an enhanced amount of dried leaves or flowers, and the removal of the additional authorization for the use of dried leaves or flowers. The estimated cost to change these items in the system is approximately $30,000. The Department of Health would use funds derived from fees to pay for the costs associated with the changes. The fees are deposited into a special fund and appropriated through a continuing appropriation.

This concludes my testimony. I am happy to answer any questions you may have.

Prepared by the Legislative Council staff for
Senator J. Lee
March 7, 2019

PROPOSED AMENDMENTS TO ENGROSSED HOUSE BILL NO. 1417

Page 1, line 1, after "2" insert ", 38,"

Page 1, line 1, remove the third "and"

Page 1, line 2, after "19-24.1-03" insert ", subdivision a of subsection 5 of section 19-24.1-05, subsection 7 of section 19-24.1-10, section 19-24.1-11, subsection 4 of section 19-24.1-21, and subsection 10 of section 19-24.1-32"

Page 1, line 3, after "marijuana" insert "; and to declare an emergency"

Page 2, after line 12, insert:

"**SECTION 2. AMENDMENT.** Subsection 38 of section 19-24.1-01 of the North Dakota Century Code is amended and reenacted as follows:

38. "Usable marijuana" means a medical marijuana product or the dried leaves or flowers of the plant of the genus cannabis in a combustible delivery form. However, the term does not include ~~the dried leaves or flowers unless authorized through a written certification and does not include~~ a cannabinoid edible product. In the case of a registered qualifying patient who is a minor, "usable marijuana" is limited to pediatric medical marijuana."

Page 4, after line 4, insert:

"**SECTION 5. AMENDMENT.** Subdivision a of subsection 5 of section 19-24.1-05 of the North Dakota Century Code is amended and reenacted as follows:

a. The department receives documentation the minor's health care provider has explained to the parent or legal guardian with responsibility for health care decisions for the minor the potential risks ~~and benefits~~ of the use of pediatric medical marijuana ~~to treat or alleviate the debilitating medical condition~~; and

SECTION 6. AMENDMENT. Subsection 7 of section 19-24.1-10 of the North Dakota Century Code is amended and reenacted as follows:

7. A registered qualifying patient's certifying health care provider shall notify the department in writing if the health care provider's registered qualifying patient no longer has a debilitating medical condition ~~or if the~~. The health care provider ~~no longer believes the patient will receive therapeutic or palliative benefit from the medical use of marijuana~~may notify the department if a bona fide provider-patient relationship ceases to exist. The qualifying patient's registry identification card becomes void immediately upon the health care provider's notification of the department and the registered qualifying patient shall dispose of any usable marijuana in the cardholder's possession within fifteen calendar days, in accordance with rules adopted under this chapter.

SECTION 7. AMENDMENT. Section 19-24.1-11 of the North Dakota Century Code is amended and reenacted as follows:

19-24.1-11. Registry identification cards.

1. The contents of a registry identification card must include:

   a. The name of the cardholder;

   b. A designation as to whether the cardholder is a qualifying patient, designated caregiver, or compassion center agent;

   c. A designation as to whether a qualifying patient is a minor;

   d. A designation as to whether a qualifying patient or a designated caregiver's qualifying patient is authorized to use ~~the~~an enhanced amount of dried leaves or flowers of the plant of the genus cannabis to treat or alleviate the patient's debilitating medical condition of cancer;

   e. The date of issuance and expiration date;

   f. A random ten-digit alphanumeric identification number containing at least four numbers and at least four letters which is unique to the cardholder;

   g. If the cardholder is a designated caregiver, the random identification number of the qualifying patient the designated caregiver is authorized to assist;

   h. A photograph of the cardholder; and

   i. The phone number or website address at which the card can be verified.

2. Except as otherwise provided in this section or rule adopted under this chapter, a registry identification card expiration date must be one year after the date of issuance.

3. If a health care provider ~~states in the written~~limits certification ~~that the qualifying patient would benefit from the medical use of marijuana~~ until a specified date, less than one year, the registry identification card expires on that date.

SECTION 8. AMENDMENT. Subsection 4 of section 19-24.1-21 of the North Dakota Century Code is amended and reenacted as follows:

4. A dispensary or agent of the dispensary may not dispense usable marijuana unless the dispensary first uses the verification system to confirm the registered qualifying patient or registered designated caregiver identification card is valid. A dispensary or agent of the dispensary:

   a. May not dispense usable marijuana to a person other than a registered qualifying patient or a registered qualifying patient's registered designated caregiver. If a registered qualifying patient is a minor:

PROPOSED AMENDMENTS TO ENGROSSED HOUSE BILL NO. 1417

Page 2, line 12, replace "two" with "four"

Mar. 9, 2019

Dear North Dakota Human Services Committee,

My name is Heidi Curtis, I am the mother of a young girl named Charly Curtis. Our family lives in Indiana where it is still illegal on all levels to possess any form of marijuana. My daughter Charly passed away on Feb 7th, 2019 due to a seizure in her sleep. Charly was only 6 years old.

Charly had a condition called Dup15q syndrome. This diagnosis gave her a high probability of autism, sensory processing disorder, ADHD and epilepsy. Charly suffered from all of the above. Her first seizure happened before she was 3. Those first seizures were small absent or drop seizures and were controlled quite easily with Keppra. Charly was thriving in her life! She was learning how to say 3 word phrases, she knew all her colors, shapes, numbers and the entire alphabet by site. Things were going fairly well for her. Her behaviors left much to be desired at times. She was still prone to meltdowns over everyday things like the vacuum cleaner or being told no to taking 6 baths a day. She was generally a very happy girl with mounds of energy.

This all changed in the blink of an eye on January 13th, 2019. That day Charly had the first of many grand mal seizures. I was in the hospital recovering from an extensive back surgery. Her father David was with her on that Sunday afternoon. He contacted her neurologist the next day and had to beg for an appointment. She was scheduled to be seen on January 22nd. In that short time waiting for that appointment Charly had a few more grand mal seizures and they were getting stronger and longer every time. At the appointment we discovered that what we thought was a sensory behavior or a tick was actually small drop seizures. She was having these episodes up to 50 times a day on top of the grand mal seizures. The neurologist diagnosed her with Lennox Gastout Syndrome. He prescribed Onfi , a Diastat suppository for emergencies and an order for Epidiolex. Epidiolex is the only med approved in Indiana for uncontrolled seizures. This medication required quite a bit of paperwork and we were told it would take at least 3 weeks to receive. We were told to call back that Friday January 25th with an update on how the Onfi was working.

On January 25th we attempted to reach the neurologist 7 times. Charly was still having seizures at an increasing rate. Adding the Onfi seemed to have made them worse instead of better. She was having multiple seizures a day. As a last resort we reached out to friends who had just returned from Colorado for a THC brownie. As I mentioned earlier, THC and marijuana are not legal in IN! We were well aware of the risk we were getting ready to take. We decided to give some of this brownie to Charly to see if it would help her seizures like it has so many others in the nation. After talking with a few parents who were legally giving their child THC products and had great success with it we knew we had to try something. The doctors office still had not called back by that evening, knowing that we were breaking the law and could lose our children if anyone knew, we gave Charly a small piece off of that brownie.

The brownie had a very quick effect! The girl that had not been herself for almost 2 weeks was happy, bouncing and chatty again! She did not have another seizure that evening. We followed up Saturday and Sunday 2x a day doing the same. Just a small piece of that brownie had stopped her seizures in their tracks! We decided on Monday to see if it really was the brownie making the

difference and didn't give her any of it. We only gave her her normal medications, including the newly added Onfi. She didn't even make it to lunch time before she had already had 2 grand mal seizures. We gave her an afternoon dose of that brownie and the rest of the evening was again uneventful. At this point we had finally reached the neurologist and were advised to wean her back off the Onfi and wait for the Epidiolex. A follow up appointment was scheduled for February 1st. Again we reached out to friends who had returned from Colorado and this time we were able to get a THC/CBD oil blend. We started using that instead of the brownie so we could gauge the actual doing of THC. We used the oil from January 29th onward. She was having a few break through seizures at this point but we were hesitant to give her too much. Without the guidance of someone at a dispensory we were throwing darts in the dark. We returned to the doctor on February 1st, we told the doctor at that appointment that we had been giving her THC drops. The only response we got was "you do what you have to do".

On the morning of February 6th we received a call from the neurologist telling us they had forgotten to have us sign a paper for the Epidiolex. The order had not even left their office yet!! We looked to see how much THC we had left, and knew that we only had enough for a few more days at best. There was no way the Epidiolex would make it in time. At that time David called Michigan to see what we would have to do to get into a dispensory there and purchase the drops ourselves. We were told it would take having a Michigan ID and seeing a doctor in Michigan before we could get a medical marijuana card. We had no other option at this point than for Dave to drive to Colorado and get more. The idea of running out and the seizures coming back full force was terrifying. So he left that afternoon at 2pm and drove straight through to Colorado.

The morning of February 7thy I went to get Charly out of bed. She was unresponsive, cold and her lips were already blue. We called 911 and rushed her to the ER. Charly was pronounced dead at 8:55am. I told the doctors we had been giving her THC and that started a DCS investigation and due to her young age another investigation to rule out foul play. It has taken a month of tests and meetings with DCS before we were finally cleared. I am still waiting on her death certificate a month later. The coroner finally gave her cause of death this week. "Complications of seizure disorder Lennox Gastout Syndrome".

Other parents we have talked to are giving their children much higher doses than we were giving Charly. Honestly I don't feel that we were giving her nearly enough. For example another child with the same disorders as Charly is taking 160mg of THC in a day! Some children require more, some less but they are in states where it is legal and they are able to freely find the right dose without fear of persecution.

I have recently learned that the maximum allowed amount for a person under age 19 in ND is 2000 mg a month. That breaks down to approximately 64.5 mg a day in a 31 day month. Most people are dosing every 8 hours. (I should have been doing a bedtime dose in hindsite) That would only be 20 mg 3x a day. Honestly that is not enough to control most seizures let alone touch the behaviors that result from autism.

Sincerely,
Heidi Curtis

Charly Curtis 10/14/2012–2/7/2019

New Castle, IN

Medical Events Timeline

2012: Diagnosed with chromosomal abnormality which resulted in autism, ADHD and epilepsy

2014: Began suffering mild drop seizures, controlled with Keppra

1/13/2019: First of many grand mal seizures

1/22/2019: Diagnosed with Lennox Gastaut Syndrome (rare, severe epilepsy), prescribed Onfi and Diastat. Started paperwork to obtain Epidiolex, pharmaceutical derived from natural cannabis. Epidiolex would not be available for at least 3 weeks, due to bureaucracy.

1/25/2019: Curtises call neurologist 7 times, to no avail. Charly's seizures had increased. Parents give Charly small cannabis brownie piece that friends bought at a Colorado dispensary. Seizures ceased. Charly's activity/mood/speech improved.

1/26-27: Charly given small cannabis brownie pieces. No seizures.

1/28/2019: Curtises test efficacy of the cannabis. They do NOT give Charly any brownie. Charly suffers 2 grand mal seizures before noon. Charly given brownie piece. Seizures stop.

Neurologist advises Curtises to wean Charly off Onfi.

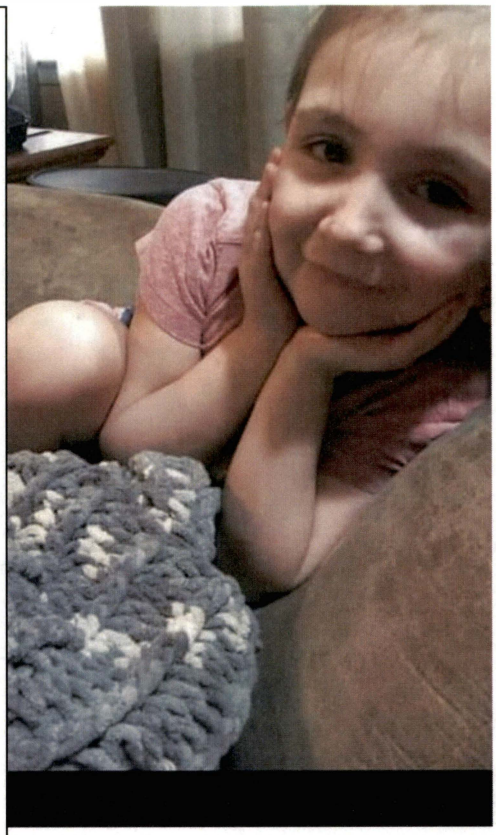
1/29/2019: Curtises started dosing Charly with THC/CBD oil blend (Colorado dispensary) from friends. Charly suffers a few breakthrough seizures. Activity/mood/speech remain satisfactory. Curtises do not increase cannabis dose, fearing legal implications and **OIL SUPPLY DEPLETION.**

2/1/2019: Curtises tell doctor Charly is ingesting cannabis oil. Doctor responds, "You do what you have to do."

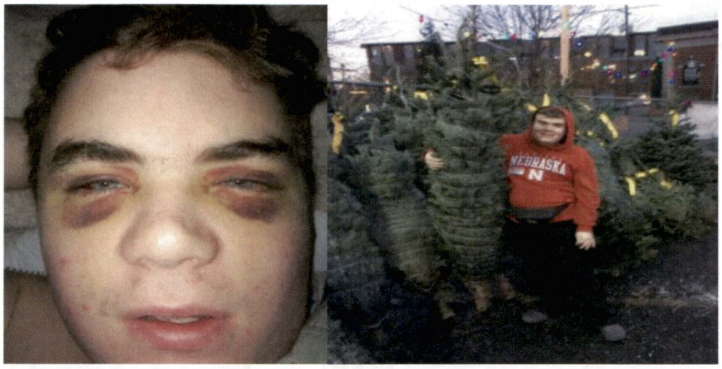
2/6/2019: Neurologist informs Curtises that one signature on Epidiolex paperwork was missed. 3-week waiting period would restart after signature obtained.

Curtises explore acquiring legal medical marijuana in Michigan. Deem it to be too time consuming. **Curtises determine remaining oil will run out within a few days.** Dave Curtis, dad, starts 16 hr drive to Denver to buy same cannabis oil.

2/7/2019: Charly found unresponsive in bed. Pronounced dead at 8:55 am. Cause of death—complications of seizure disorder. Father, Dave Curtis, had arrived in Denver 45 minutes prior to receiving the call.

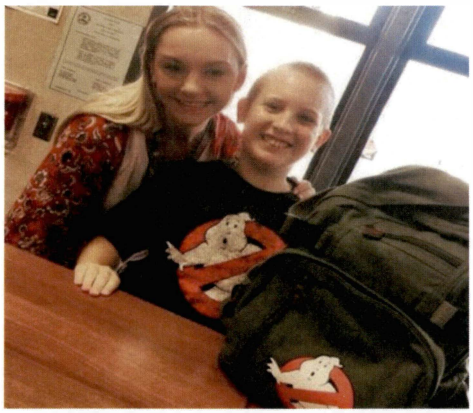


Legal Medical Cannabis Patients Requiring More Than 2,000 mg THC Per Month

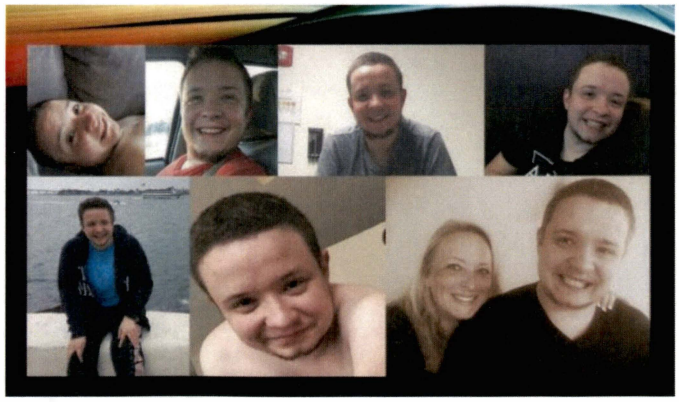


Alex Irvin, Portland, Oregon
Autism, Tourette's
Thrives on 120-240 mg THC daily, plus vaporizing in emergencies.
Monthly THC Need: 3600 to 7200 mg
Contact: Jill Irvin
503.805.3105
Jillirvin@wpa4a.org

Alex after an emergency room visit for uncontrollable self injury. Right, Alex, on cannabis, volunteering at Christmastime.



Boy Christianson, 11 (mother wants his first name private)
Riverside, CA
Autism, epilepsy
Needs 250 mg daily to control seizures
Monthly THC Need: 7500 mg
Contact: Sandy Christianson
562.833.0654



Nate Mai, 23, Canyon Lake, CA
Autism
Needed 200-400 mg THC daily to control aggression/pharmaceutical withdrawals during med weaning
Monthly THC Need During Weaning: At least 6,000 mg THC
Current Monthly THC Need: 1500 mg
Note that in order to get to this lower THC need Nate required higher dosages during med weaning
Contact: Jenni Mai
info@wpa4a.org

Mar. 11, 2019

Senate Human Services Committee:

I wish to address the technical aspects of the impossibility of a cannabis overdose.

Cannabinoid receptors are effectively absent in the brainstem cardiorespiratory centers (Glass, Faull, & Dragunow, 1997). This is believed to preclude the possibility of a fatal overdose from cannabinoid intake.

Cannabinoid receptors, unlike opioid receptors, are not located in the brainstem areas controlling respiration.

The University of New Mexico released a study indicating that the symptom relief generated by THC dwarfs that of CBD. THC doesn't simply make a patient high.

I encourage the legislature to increase North Dakota's monthly purchase limit to 5,000 mg THC. Patients who require higher THC amounts are very ill and thus most deserving of adequate medication.

Please refer to the email entitled “Cannabis Research”, sent to you by Alexa Johnson, for a study that questions the supposed link between cannabis and schizophrenia.

Gail Pederson, SPRN in Holistic Nursing HN-BC
Valley City, ND
Bewellhealingarts@gmail.com