MMP053

New Jersey Department of Health
Medicinal Marijuana Program
PO 360
Trenton, NJ 08625-0360

MEDICINAL MARIJUANA PETITION
(N.J.A.C. 8:64-5.1 et seq.)

INSTRUCTIONS

This petition form is to be used only for requesting approval of an additional medical condition or treatment thereof as a "debilitating medical condition" pursuant to the New Jersey Compassionate Use Medical Marijuana Act, N.J.S.A. 24:6I-3. Only one condition or treatment may be identified per petition form. For additional conditions or treatments, a separate petition form must be submitted.

NOTE: This Petition form tracks the requirements of N.J.A.C. 8:64-5.3. Note that if a petition does not contain all information required by N.J.A.C. 8:64-5.3, the Department will deny the petition and return it to petitioner without further review. For that reason the Department strongly encourages use of the Petition form.

*This completed petition **must** be postmarked **August 1 through August 31, 2016** and sent by **certified mail** to:*

New Jersey Department of Health
Office of Commissioner - Medicinal Marijuana Program
Attention: Michele Stark
369 South Warren Street
Trenton, NJ 08608

Please complete each section of this petition. If there are any supportive documents attached to this petition, you should reference those documents in the text of the petition. If you need additional space for any item, please use a separate piece of paper, number the item accordingly, and attach it to the petition.

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1. **Petitioner Information**

Name: [redacted]
Street Addr
City, State,
Telephone
Email Addre

2. **Identify the medical condition or treatment thereof proposed. Please be specific. Do not submit broad categories (such as "mental illness").**

Alzheimer's

3. **Do you wish to address the Medical Marijuana Review Panel regarding your petition?**

- ☑ Yes, in Person
- ☑ Yes, by Telephone
- ☐ No

4. **Do you request that your personally identifiable information or health information remain confidential?**

- ☐ Yes
- ☑ No

If you answer "Yes" to Question 4, your name, address, phone number, and email, as well as any medical or health information specific to you, will be redacted from the petition before forwarding to the panel for review.

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CHIEF OF STAFF

5. Describe the extent to which the condition is generally accepted by the medical community and other experts as a valid, existing medical condition.

Alzheimer's is defined as a progressive, degenerative disorder that attacks the brain's nerve cells or neurons, resulting in loss of memory, thinking and language skills, and behavioral changes.

6. If one or more treatments of the condition, rather than the condition itself, are alleged to be the cause of the patient's suffering, describe the extent to which the treatments causing suffering are generally accepted by the medical community and other experts as valid treatments for the condition.

All of the conventionally perscribed medications for Alzheimer's have severe adverse side effects including but not limited to ... SEE ATTACHED PAGES

7. Describe the extent to which the condition itself and/or the treatments thereof cause severe suffering, such as severe and/or chronic pain, severe nausea and/or vomiting or otherwise severely impair the patient's ability to carry on activities of daily living.

SEE ATTACHED PAGES

8. Describe the availability of conventional medical therapies other than those that cause suffering to alleviate suffering caused by the condition and/or the treatment thereof.

SEE ATTACHED PAGES

9. Describe the extent to which evidence that is generally accepted among the medical community and other experts supports a finding that the use of marijuana alleviates suffering caused by the condition and/or the treatment thereof. *[Note: You may attach articles published in peer-reviewed scientific journals reporting the results of research on the effects of marijuana on the medical condition or treatment of the condition and supporting why the medical condition should be added to the list of debilitating medical conditions.]*

SEE ATTACHED PAGES

10. Attach letters of support from physicians or other licensed health care professionals knowledgeable about the condition. List below the number of letters attached and identify the authors.

I certify, under penalty of perjury, that I am 18 years of age or older; that the information provided in this petition is true and accurate to the best of my knowledge; and that the attached documents are authentic.

Signature of Petitioner [redacted]	Date 8/31/16

6.
ARICEPT --
GENERIC NAME(S): DONEPEZIL
COMMON SERIOUS SIDE
EFFECTS :

- loss of appetite
- muscle cramps
- nausea
- trouble in sleeping
- unusual tiredness or weakness
- vomiting
- diarrhea

LESS COMMON SERIOUS SIDE EFFECTS:

- Abnormal dreams
- constipation
- dizziness
- drowsiness
- fainting
- frequent urination
- headache
- joint pain, stiffness, or swelling
- mental depression
- pain
- unusual bleeding or bruising
- weight loss

RARE SERIOUS SIDE EFFECTS:

- Black, tarry stools
- bloating
- bloody or cloudy urine
- blurred vision
- burning, prickling, or tingling sensations
- cataract
- chills
- clumsiness or unsteadiness
- confusion
- cough
- decreased urination
- difficult or painful urination
- dryness of mouth
- eye irritation
- fever
- flushing of skin
- frequent urge to urinate
- high or low blood pressure
- hives

- hot flashes
- increase in sexual desire or performance
- increased heart rate and breathing
- increased sweating
- increased urge to urinate during the night
- irregular heartbeat
- itching
- loss of bladder control
- loss of bowel control
- mood or mental changes, including abnormal crying, aggression, agitation, delusions, irritability, nervousness, or restlessness
- nasal congestion
- pain in chest, upper stomach, or throat
- problems with speech
- runny nose
- severe thirst
- shortness of breath
- sneezing
- sore throat
- sunken eyes
- tightness in chest
- tremor
- troubled breathing
- wheezing
- wrinkled skin

SITE - http://www.ncbi.nlm.nih.gov/pubmedhealth/PMH0045791/#!po=79.1667

RAZADYNE --
GENERIC NAME(S): GALANTAMINE HBR
LESS COMMON SERIOUS SIDE EFFECTS:

- Chest pain or discomfort
- lightheadedness, dizziness, or fainting
- shakiness in the legs, arms, hands, or feet
- shortness of breath
- slow or irregular heartbeat
- unusual tiredness

RARE SERIOUS SIDE EFFECTS:

- Blurred vision
- confusion
- decreased urination
- dizziness, faintness, or lightheadedness when getting up suddenly from lying or sitting position
- dry mouth
- fainting
- fast, irregular, pounding, or racing heartbeat or pulse
- feeling of warmth
- rapid breathing
- redness of the face, neck, arms, and occasionally, upper chest

- sunken eyes
- sweating
- thirst
- troubled breathing
- unusual tiredness or weakness
- wrinkled skin

COMMON SIDE EFFECTS:

- Decreased appetite and weight loss

LESS COMMON SIDE EFFECTS:

- Acid or sour stomach
- belching
- discouragement
- feeling sad or empty
- general feeling of discomfort or illness
- heartburn
- increased sweating
- indigestion
- irritability
- lack of appetite
- lack or loss of strength
- loss of interest or pleasure
- muscle spasms
- stomach discomfort, upset, or pain
- trouble concentrating
- trouble sleeping
- unusual drowsiness, dullness, tiredness, weakness, or feeling of sluggishness

RARE SIDE EFFECTS:

- Burning, crawling, itching, numbness, prickling, "pins and needles", or tingling feelings
- change in taste
- dry heaves
- loss of taste
- unusually deep sleep
- unusually long duration of sleep

SITE --http://www.ncbi.nlm.nih.gov/pubmedhealth/PMH0045514/#DDIC601639.side_effects_section

NAMENDA/NAMENDA XR --
GENERIC NAME(S): MEMANTINE
Side effects include but are not limited to:
Common Serious Side Effects -

- Bloating or swelling of the face, arms, hands, lower legs, or feet
- blurred vision
- dizziness
- headache
- nervousness
- pounding in the ears
- rapid weight gain
- slow or fast heartbeat

- tingling of the hands or feet
- unusual weight gain or loss

Common non serious side effects -

- Confusion
- Less Common Non Serious Side Effects-
- Anxiety
- back pain
- bladder pain
- bloody or cloudy urine
- change in walking and balance
- chills
- clumsiness or unsteadiness
- cough producing mucus
- coughing
- diarrhea
- difficult, burning, or painful urination
- difficulty with breathing
- difficulty with moving
- discouragement
- dry mouth
- fear
- feeling sad or empty
- fever
- frequent urge to urinate
- general feeling of discomfort or illness
- hyperventilation
- insomnia
- irritability
- joint pain
- loss of appetite
- loss of bladder control
- loss of interest or pleasure
- lower back or side pain
- muscle pain or stiffness
- nausea
- nervousness
- pain
- pain in the joints
- restlessness
- seeing, hearing, or feeling things that are not there
- shortness of breath
- sleepiness or unusual drowsiness
- sore throat
- tightness in the chest
- tiredness
- trouble with concentrating
- trouble with sleeping

- unusual tiredness or weakness
- vomiting
- wheezing

SITE --http://www.ncbi.nlm.nih.gov/pubmedhealth/PMH0044632/#DDIC601519.side_effects_section
EXELON --
GENERIC NAME(S): RIVASTIGMINE
COMMON SERIOUS SIDE EFFECTS:

- Bladder pain
- bloody or cloudy urine
- difficult, burning, or painful urination
- frequent urge to urinate
- lower back or side pain

LESS COMMON SERIOI SIDE EFFECTS:

- Blurred vision
- dizziness
- headache
- loss of bladder control
- nervousness
- pounding in the ears
- slow or fast heartbeat

RARE SERIOUS SIDE EFFECTS:

- Abdominal or stomach pain or tenderness
- arm, back, or jaw pain
- chest pain, tightness, heaviness, or discomfort
- confusion
- convulsions
- decreased urine
- difficult or troubled breathing
- dilated neck veins
- extreme fatigue
- false beliefs that cannot be changed by facts
- irregular breathing
- irregular heartbeat
- loss of consciousness
- rapid breathing
- seizures
- severe nausea or vomiting
- shortness of breath
- sunken eyes
- swelling of the face, fingers, feet, or lower legs
- wrinkled skin

COMMON SIDE EFFECTS THAT USUALLY DISPATE:

- Diarrhea
- discouragement
- fear
- feeling of constant movement of self or surroundings
- feeling sad or empty

- irritability
- lack of appetite
- lack or loss of strength
- loss of interest or pleasure
- nausea
- redness at the application site
- sensation of spinning
- stomach pain
- tiredness
- trouble concentrating
- trouble sleeping
- upper stomach pain
- vomiting
- weight loss

RARE COMMON SUDE EFFECTS THAT DISIPATE

- Blindness
- burning, stinging, or pain at application site
- continuing ringing or buzzing or other unexplained noise in the ears
- decreased vision
- difficulty with moving
- eye pain
- hearing loss
- itchy skin
- muscle pain or stiffness
- pain in the joints
- pale skin
- tearing
- troubled breathing with exertion
- unusual bleeding or bruising

SITE-- http://www.ncbi.nlm.nih.gov/pubmedhealth/PMH0045504/#DDIC602493.side_effects_section

7.
Alzheimer's causes severe suffering and severely and ultimately completely impairs the patients ability to carry on actives of daily living in the following ways --
Cognition: mental decline, difficulty thinking and understanding, confusion in the evening hours, delusion, disorientation, forgetfulness, making things up, mental confusion, difficulty concentrating, inability to create new memories, inability to do simple math, or inability to recognize common things
Behavioral: aggression, agitation, difficulty with self care, irritability, meaningless repetition of own words, personality changes, lack of restraint, or wandering and getting lost
Mood: anger, apathy, general discontent, loneliness, or mood swings
Psychological: depression, hallucination, or paranoia
Whole body: loss of appetite or restlessness
Also common: inability to combine muscle movements or jumbled speech
As the disease progresses patients eventually loose the ability to drink, eat, have control over their bladder and bowels, walk. Also, patients may loose the abilities to communicate properly or even speak.
In the earlier stages of the disease the patient is more lucid and aware of their deteriorating condition. They are aware of the changes happening to them and will be extremely upset by what's happening to them and how their life is slipping away. Their living with the condition itself causes severe mental suffering on a daily basis.
In the later stages of the disease more physical deterioration happens in the form of loosing the ability to eat and drink, bathe themselves, loose control of their blader and bowels, walk, etc. The later stages cause serious suffering in the form of physical suffering. Loosing the ability to eat causes weight loss which increases the risk of pressure sore which are both severely and chronicly painful. Loosing the ability to walk also greatly increases the risk of developing pressure sores. Also people in the later stages will have a much greater cognitive and mental decline which will include loosing the ability to recognize their caregivers, and where they are. Being in that state will cause great mental suffering. Mid to late stage Alzheimer's also severely impairs a patient's ability to carry on activities of daily living.
All of the drugs approved for treating Alzheimer's come with serious and debilitating side effects as listed above in question 6.

8. Their is no conventional medical therapy to alleviate suffering that does not also cause suffering in and of itself. All of the conventionally prescribed medications have many adverse side effects that cause the patient to suffer and while certain other medications can be prescribed to help alleviate the side effects; those medications also can cause their own side effects.

9.
The use of medicinal marijuana is a scientifically, medically, and anecdotally proven treatment for the prevention of Alzheimer's; as well as a treatment of current symptoms and it greatly aids in helping prevent, for as long as possible, new symptoms and the progression of severity in current symptoms.
Alzheimer's is as stated above a progressive, degenerative disorder that attacks the brain's nerve cells, or neurons, resulting in loss of memory, thinking and language skills, and behavioral changes.
The cause of Alzheimer's is believed to be the formation of a plaque in the brain that leads to nerve cell death. Beta-amyloid is a protein that clumps together in the brain to form the toxic plaque. The plaque disrupts communication between neurons in the brain. This disruption leads to Alzheimer's symptoms.
Increased beta-a production also leads to an increase expression of pro-inflammatory proteins in nerve cells. That causes inflammation and eventual nerve cell death itself.
The inflammation in the brain is a major component of the damage caused by Alzheimer's.
The human brain contains nerve cells that contain receptors that are activated by lipid molecules called endocannabinoids. Endocannabinoids aid nerve cell signaling. The same signaling that is disrupted by the beta-amyloid plaque.
Marijuana contains tetrahydrocannabinol; also known as THC. THC is similar to endocannabinoids and it activities that same receptors therefore aiding in nerve cell signaling. THC aiding in nerve cell signaling reduces and can halt Alzheimer's symptoms for sometime. THC aiding in the nerve cell signaling can also prevent Alzheimer's symptoms and Alzheimer's itself from developing.
Marijuana is undeniably beneficial in treating the symptoms of Alzheimer's.
Significant reduction in behavioral and psychological symptoms of dementia has been widely reported with patients that have used medical marijuana.
Marijuana helps to reduce agitation and aggression in Alzheimer's patients. aggression is one of the many emotional behaviors regulated by cannabinoid receptors – specifically CB1 receptors. Marijuana activates the CB1 receptors therefore regulating emotional behaviors including but not limited to reducing agitation and aggression.
Medical marijuana helps reduce the delusions that Alzheimer's patients experience. Alzheimer's patients will sometimes experience delusions in the mid to late stages of Alzheimer's because symptoms like memory loss can cause confusion such as being unable to recognize faces or places or remember certain objects or names. That confusion can cause some people to experience delusions while they try to figure things out.
Medical marijuana is beneficial in reducing Alzheimer's symptoms. Marijuana is an extremely potent anti-inflammatory. The use of marijuana is guaranteed to result in a lessened degree of widespread inflammation throughout the body and brain. Lowering or stopping brain inflammation can both halt and prevent all of Alzheimer's symptoms including but not limited to delusions, confusion, memory loss.
THC has been found to impede the enzyme that is responsible for the collection of beta-amyloid plaque in a manner "considerably superior" to all approved Alzheimer's drugs.

http://www.ncbi.nlm.nih.gov/m/pubmed/25024327/
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http://healthland.time.com/2012/10/29/how-cannabinoids-may-slow-brain-aging/
http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2562334/
http://m.jneurosci.org/content/25/8/1904.abstract
http://www.israelnationalnews.com/News/News.aspx/125564
http://pubs.acs.org/doi/abs/10.1021/mp060066m
http://www.salk.edu/news-release/cannabinoids-remove-plaque-forming-alzheimers-proteins-from-brain-cells/
http://m.pnas.org/content/95/14/8268.full
http://www.ncbi.nlm.nih.gov/m/pubmed/16521031/
http://www.cnn.com/2016/07/25/health/alzheimers-medical-marijuana/

PubMed Search term

↓ Full text

The potential therapeutic effects of THC on Alzheimer's disease.

Cao C, et al. J Alzheimers Dis. 2014.

Authors

Cao C[1], Li Y[2], Liu H[1], Bai G[2], Mayl J[3], Lin X[1], Sutherland K[4], Nabar N[5], Cai J[2].

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Citation

J Alzheimers Dis. 2014;42(3):973-84. doi: 10.3233/JAD-140093.

Abstract

The purpose of this study was to investigate the potential therapeutic qualities of Δ9-tetrahydrocannabinol (THC) with respect to slowing or halting the hallmark characteristics of Alzheimer's disease. N2a-variant amyloid-β protein precursor (AβPP) cells were incubated with THC and assayed for amyloid-β (Aβ) levels at the 6-, 24-, and 48-hour time marks. THC was also tested for synergy with caffeine, in respect to the reduction of the Aβ level in N2a/AβPPswe cells. THC was also tested to determine if multiple treatments were beneficial. The MTT assay was performed to test the toxicity of THC. Thioflavin T assays and western blots were performed to test the direct anti-Aβ aggregation significance of THC. Lastly,

Similar articles

Antibodies against beta-amyloid reduce Abeta oligomers, glycogen synthase kinase-3beta activation and tau phosphorylation in vivo and in vitro.
Ma QL, et al. J Neurosci Res. 2006.

Curcumin mediates presenilin-1 activity to reduce β-amyloid production in a model of Alzheimer's Disease.
Xiong Z, et al. Pharmacol Rep. 2011.

Berberine ameliorates β-amyloid pathology, gliosis, and cognitive impairment in an Alzheimer's disease transgenic mouse model.
Durairajan SS, et al. Neurobiol Aging. 2012.

GSK-3 is essential in the pathogenesis of Alzheimer's disease.
Review article
Takashima A, et al. J Alzheimers Dis. 2006.

Formaldehyde as a trigger for protein aggregation and potential target for mitigation of age-related, progressive cognitive impairment.
Review article
Su T, et al. Curr Top Med Chem. 2016.

See all

THC was tested to determine its effects on glycogen synthase kinase-3β (GSK-3β) and related signaling pathways. From the results, we have discovered THC to be effective at lowering Aβ levels in N2a/AβPPswe cells at extremely low concentrations in a dose-dependent manner. However, no additive effect was found by combining caffeine and THC together. We did discover that THC directly interacts with Aβ peptide, thereby inhibiting aggregation. Furthermore, THC was effective at lowering both total GSK-3β levels and phosphorylated GSK-3β in a dose-dependent manner at low concentrations. At the treatment concentrations, no toxicity was observed and the CB1 receptor was not significantly upregulated. Additionally, low doses of THC can enhance mitochondria function and does not inhibit melatonin's enhancement of mitochondria function. These sets of data strongly suggest that THC could be a potential therapeutic treatment option for Alzheimer's disease through multiple functions and pathways.

PMID: 25024327 [PubMed - indexed for MEDLINE]

Full text

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Mol Pharm. Author manuscript; available in PMC 2008 Oct 6.
Published in final edited form as:
Mol Pharm. 2006; 3(6): 773–777.
doi: 10.1021/mp060066m

PMCID: PMC2562334
NIHMSID: NIHMS61230

A Molecular Link Between the Active Component of Marijuana and Alzheimer's Disease Pathology

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Abstract

Go to:

Alzheimer's disease is the leading cause of dementia among the elderly, and with the ever-increasing size of this population, cases of Alzheimer's disease are expected to triple over the next 50 years. Consequently, the development of treatments that slow or halt the disease progression have become imperative to both improve the quality of life for patients as well as reduce the health care costs attributable to Alzheimer's disease. Here, we demonstrate that the active component of marijuana, Δ^9-tetrahydrocannabinol (THC), competitively inhibits the enzyme acetylcholinesterase (AChE) as well as prevents AChE-induced amyloid β-peptide (Aβ) aggregation, the key pathological marker of Alzheimer's disease. Computational modeling of the THC-AChE interaction revealed that THC binds in the peripheral anionic site of AChE, the critical region involved in amyloidgenesis. Compared to currently approved drugs prescribed for the treatment of Alzheimer's disease, THC is a considerably superior inhibitor of Aβ aggregation, and this study provides a previously unrecognized molecular mechanism through which cannabinoid molecules may directly impact the progression of this debilitating disease.

Keywords: Cannabinoids, Alzheimer's disease, Acetylcholinesterase

Introduction

Go to:

Since the characterization of the *Cannabis sativa*-produced cannabinoid, Δ^9-tetrahydrocannabinol (THC) (Figure 1), in the 1960's,[1] this natural product has been widely explored as an anti-emetic, anti-convulsive, anti-inflammatory, and analgesic.[2] In these contexts, efficacy results from THC binding to the family of cannabinoid receptors found primarily on central and peripheral neurons (CB1) or immune cells (CB2).[3] More recently, a link between the endocannabinoid system and Alzheimer's disease has been discovered[4] which has

provided a new therapeutic target for the treatment of patients suffering from Alzheimer's disease.[5] New targets for this debilitating disease are critical as Alzheimer's disease afflicts over 20 million people worldwide, with the number of diagnosed cases continuing to rise at an exponential rate.[6,7] These studies have demonstrated the ability of cannabinoids to provide neuroprotection against β-amyloid peptide (Aβ) toxicity.[8-10] Yet, it is important to note that in these reports, cannabinoids serve as signaling molecules which regulate downstream events implicated in Alzheimer's disease pathology and are not directly implicated as effecting Aβ at a molecular level.

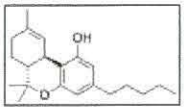


Figure 1
Chemical structure of Δ^9-tetrahydrocannabinol (THC).

One of the primary neuropathological hallmarks of Alzheimer's disease is deposition of Aβ into amyloid plaques in areas of the brain important for memory and cognition.[11] Over the last two decades, the etiology of Alzheimer's disease has been elucidated through extensive biochemical and neurobiological studies, leading to an assortment of possible therapeutic strategies including prevention of downstream neurotoxic events, interference with Aβ metabolism, and reduction of damage from oxidative stress and inflammation.[12] The impairment of the cholinergic system is the most dramatic of the neurotransmitter systems affected by Alzheimer's disease and as a result, has been thoroughly investigated. Currently, there are four FDA-approved drugs that treat the symptoms of Alzheimer's disease by inhibiting the active site of acetylcholinesterase (AChE), the enzyme responsible for the degradation of acetylcholine, thereby raising the levels of neurotransmitter in the synaptic cleft.[13] In addition, AChE has been shown to play a further role in Alzheimer's disease by acting as a molecular chaperone, accelerating the formation of amyloid fibrils in the brain and forming stable complexes with Aβ at a region known as the peripheral anionic binding site (PAS).[14,15] Evidence supporting this theory was provided by studies demonstrating that the PAS ligand, propidium, is able to prevent amyloid acceleration *in vitro*, whereas active-site inhibitors had no effect.[16] Due to the association between the AChE PAS and Alzheimer's disease, a number of studies have focused on blocking this allosteric site.[17] Recently, we reported a combined computational and experimental approach to identify compounds containing rigid, aromatic scaffolds hypothesized to disrupt protein-protein interactions.[18-20] Similarly, THC is highly lipophilic in nature and possesses a fused tricyclic structure. Thus, we hypothesized that this terpenoid also could bind to the allosteric PAS of AChE with concomitant prevention of AChE-promoted Aβ aggregation.

Experimental Section

Go to:

Docking procedures

THC was docked to the mouse AChE structure (PDB ID code 1J07) using AutoDock 3.0.5.[21] Twenty docking runs (100 million energy evaluations each) were run with a 26.25 Å × 18.75 Å × 26.25 Å grid box with 0.375 Å grid spacing. This grid box was designed to include regions of both the catalytic site and the peripheral anionic site. Otherwise, standard docking settings were used for the AutoDock calculations, as previously detailed.[18]

Acetylcholinesterase inhibition studies

All assays were performed using a Cary 50 Bio UV-visible spectrophotometer using an 18-cell changer, and conducted at 37 °C, using a Cary PCB 150 Water Peltier System. Solutions of acetylthiocholine iodide (ATCh iodide) and 5,5′-dithio-bis-(2-nitrobenzoic) acid (DTNB) were prepared according to the method of Ellman, *et al.*[22] Stock solutions of acetylcholinesterase from *E. electricus* were prepared by dissolving commercially available enzyme in 1% gelatin. Prior to use, an aliquot of the gelatin solution was diluted 1:200 in water. For the assay, the solution was diluted until enzyme activity between 0.10-0.13 AU/min at 500 μM ACTh iodide was

obtained. Compounds were prepared as solutions in methanol.

Assays were performed by mixing AChE, THC, and 340 μM DTNB in 100 mM phosphate buffer, pH 8.0, containing 5% methanol. Solutions were incubated at 37 °C for five minutes before the reaction was initiated by the addition of ATCh iodide (75 – 300 μM). The increase of absorbance at 412 nm was monitored for 2 to 5 min. All assays were run in triplicate. Initial rates were determined by subtracting the average observed initial rate from the non-enzymatic reaction.

Linear regression analysis of reciprocal plots of $1/v_o$ versus 1/[S] for four THC concentrations was performed using Microsoft Excel software. The slope$_{1/v}$ was plotted against [I] to give K_i values. Propagation of error was performed to determine the error, ΔK_i.

For studies to determine the mutual exclusivity of THC and propidium iodide, experiments were performed identically to simple THC inhibition studies with a fixed concentration of ACTh iodide (125 μM), and varied concentrations of propidium iodide (0-25 μM) and THC (0-15 μM).

AChE-induced β-amyloid peptide aggregation in the presence of AChE ligands

The aggregation of the β-amyloid peptide was measured using the thioflavin T-based fluorometric assay as described by LeVine[23] and Bartolini.[16] Assays were measured using a SpectraMAX Gemini fluorescence plate reader with SOFTmax PRO 2.6.1 software. $A\beta_{1-40}$ stock solutions were prepared in DMSO and HuAChE stocks prepared in distilled water. All stock solutions of Aβ and HuAChE were used immediately after preparation.

In a 96-well plate, triplicate samples of a 20 μL solution of 23 nM of Aβ, 2.30 μM HuAChE and various concentrations of THC in 0.215 M sodium phosphate buffer, pH 8.0 were prepared. These solutions were incubated at room temperature along with triplicate solutions of Aβ alone, Aβ and AChE, and Aβ plus THC at various concentrations. After 48 h, a 2 μL aliquot was removed from each well, placed in a black-walled, clear-bottomed 96-well plate, and diluted with 50 mM glycine-NaOH buffer, pH 8.5, containing 1.5 μM thioflavin T to a total volume of 200 μL. After incubating for 5 min, the fluorescence was measured using λ_{exc} = 466 nm and λ_{em} = 490 nm with excitation and emission slits of 2 nm. The fluorescence emission spectrum was recorded between 450 and 600 nm, with excitation at 446 nm.

The fluorescence intensities were averaged, and the average background fluorescence of buffer only, or buffer and THC, was subtracted. The corrected fluorescence values were plotted with their standard deviation. The equation, $F_i/F_o \times 100\%$, where F_i is the fluorescence of AChE, Aβ, and THC, and F_o is the fluorescence of AChE and Aβ, was used to quantify the extent to which each compound inhibits Aβ aggregation. The student's t-test function of Microsoft Excel was used to determine p values and assess statistical significance between reactions.

Control experiments containing AChE, THC, and thioflavin T or AChE and thioflavin T alone were also performed to ensure that any observed fluorescence decrease was not attributable to the molecular rotor properties of thioflavin T upon binding to AChE. For these reactions, all concentrations were identical to those used in the described Aβ aggregation assays (*vide supra*).

Results and Discussion

Go to:

THC binding to AChE initially was modeled *in silico* using AutoDock 3.0.5.[21] Twenty docking runs with 100 million energy evaluations each were performed with a 26.25 Å × 18.75 Å × 26.25 Å grid box with 0.375 Å grid spacing, which included regions of both the catalytic site and the PAS. Examination of the docking results revealed that THC was predicted to bind to AChE with comparable affinity to the best reported PAS binders, with the primary binding interaction observed between the ABC fused ring of the THC scaffold and the Trp86 indole side chain of AChE (Figure 2). Further interactions were also evident between THC and the backbone carbonyls

of Phe123 and Ser125. Encouraged by these results, we tested the ability of THC to inhibit AChE catalytic activity. Steady-state kinetic analysis of THC inhibition revealed that THC competitively inhibits AChE (K_i = 10.2 μM) (Figure 3A). This level of inhibition is relatively modest, yet it is important to note that inhibition of acetylcholine cleavage is not a prerequisite for effective reduction of Aβ aggregation; indeed, most PAS binders are moderate AChE inhibitors displaying either non-competitive or mixed-type inhibition.[16] While THC shows competitive inhibition relative to the substrate, this does not necessitate a direct interaction between THC and the AChE active site. In fact, given the proximity of the PAS to the protein channel leading to the catalytic triad active site, it is possible to block substrate entry into the active site while bound to the PAS, thus preventing the formation of an ESI complex.[18,24] In order to test this hypothesis, additional kinetic experiments were performed to determine the mutual exclusivity of THC and propidium, a well characterized purely noncompetitive AChE inhibitor and PAS binder. Dixon plots of v^{-1} versus propidium concentration at varying concentrations of THC returned a series of parallel lines, indicating that THC and propidium cannot bind simultaneously to AChE (Figure 3B). Thus, these studies verify our docking results and demonstrate that THC and propidium are mutually exclusive PAS inhibitors. Additionally, recent reports have suggested that the selectivity of a given inhibitor for AChE over butyrylcholinesterase (BuChE) can be correlated with the ability of a compound to block AChE-accelerated Aβ aggregation.[25,26] Kinetic examination of BuChE inhibition revealed a slight reduction in enzymatic activity at high concentrations of THC ($IC_{50} \geq 100$ μM); however, these experiments were limited by the poor solubility of THC in aqueous solution.

Figure 2

Predicted binding mode of THC (gray) to AChE (orange ribbon). The catalytic triad residues of AChE (green) and water molecules included in the docking calculations (light blue spheres) are shown.

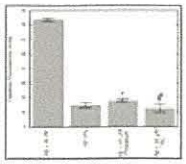
Figure 3

(A) Kinetic analysis of AChE inhibition by 0.0 (●), 6.25 (▲), 12.5 (◆), and 25.0 μM (■) THC. Steady state kinetic analysis was performed using acetylthiocholine (75-300 μM) and Ellman's reagent (340 μ ...

The activity of THC towards the inhibition of Aβ aggregation was then investigated using a thioflavin T (ThT)-based fluorometric assay to stain putative Aβ fibrils.[23] Using this assay, we found that THC is an effective inhibitor of the amyloidogenic effect of AChE (Figure 4). In fact, at a concentration of 50 μM, propidium does not fully prevent AChE-induced aggregation (p = 0.03, student's T-test), while THC completely blocks the AChE effect on Aβ aggregation, with significantly greater inhibition than propidium (p = 0.04, student's T-test), one of the most effective aggregation inhibitors reported to date.[16] However, the observed decrease in fluorescence could also be rationalized as a result of a competition between THC and ThT for the same site on AChE. It has been shown that ThT also can bind to the PAS and that this binding leads to an increase in fluorescence. Presumably, this phenomenon results from ThT serving as a molecular rotor in which fluorescence quantum yield is sensitive to the intrinsic rotational relaxation; thus, when molecular rotation is slowed by protein binding, the quantum yield of the molecule can increase dramatically.[27,28] In order to ensure that the observed fluorescence decrease was due to fibril inhibition, control experiments were performed using AChE, THC, and ThT. Reactions containing AChE and ThT alone showed the same fluorescence output as those containing AChE, THC, and ThT, providing convincing evidence that any observed reduction in fluorescence can be attributed to fewer Aβ fibrils.

Figure 4

Inhibition of AChE-induced Aβ aggregation by THC and propidium (* $p < 0.05$ versus Aβ



only; # $p < 0.05$ versus Aβ + propidium).

Conclusion

Go to:

We have demonstrated that THC competitively inhibits AChE, and furthermore, binds to the AChE PAS and diminishes Aβ aggregation. In contrast to previous studies aimed at utilizing cannabinoids in Alzheimer's disease therapy,[8-10] our results provide a mechanism whereby the THC molecule can directly impact Alzheimer's disease pathology. We note that while THC provides an interesting Alzheimer's disease drug lead, it is a psychoactive compound with strong affinity for endogenous cannabinoid receptors. It is noteworthy that THC is a considerably more effective inhibitor of AChE-induced Aβ deposition than the approved drugs for Alzheimer's disease treatment, donepezil and tacrine, which reduced Aβ aggregation by only 22% and 7%, respectively, at twice the concentration used in our studies.[7] Therefore, AChE inhibitors such as THC and its analogues may provide an improved therapeutic for Alzheimer's disease, augmenting acetylcholine levels by preventing neurotransmitter degradation and reducing Aβ aggregation, thereby simultaneously treating both the symptoms and progression of Alzheimer's disease.

Acknowledgments

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This work was supported by the Skaggs Institute for Chemical Biology and a NIH Kirschstein National Research Service Award to L.M.E.

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Br J Pharmacol. 2007 Nov; 152(5): 655–662. PMCID: PMC2190031
Published online 2007 Sep 10. doi: 10.1038/sj.bjp.0707446

Alzheimer's disease; taking the edge off with cannabinoids?

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Received 2007 May 29; Revised 2007 Jul 3; Accepted 2007 Aug 8.

Abstract

Go to:

Alzheimer's disease is an age-related neurodegenerative condition associated with cognitive decline. The pathological hallmarks of the disease are the deposition of β-amyloid protein and hyperphosphorylation of tau, which evoke neuronal cell death and impair inter-neuronal communication. The disease is also associated with neuroinflammation, excitotoxicity and oxidative stress. In recent years the proclivity of cannabinoids to exert a neuroprotective influence has received substantial interest as a means to mitigate the symptoms of neurodegenerative conditions. In brains obtained from Alzheimer's patients alterations in components of the cannabinoid system have been reported, suggesting that the cannabinoid system either contributes to, or is altered by, the pathophysiology of the disease. Certain cannabinoids can protect neurons from the deleterious effects of β-amyloid and are capable of reducing tau phosphorylation. The propensity of cannabinoids to reduce β-amyloid-evoked oxidative stress and neurodegeneration, whilst stimulating neurotrophin expression neurogenesis, are interesting properties that may be beneficial in the treatment of Alzheimer's disease. Δ^9-tetrahydrocannabinol can also inhibit acetylcholinesterase activity and limit amyloidogenesis which may improve cholinergic transmission and delay disease progression. Targeting cannabinoid receptors on microglia may reduce the neuroinflammation that is a feature of Alzheimer's disease, without causing psychoactive effects. Thus, cannabinoids offer a multi-faceted approach for the treatment of Alzheimer's disease by providing neuroprotection and reducing neuroinflammation, whilst simultaneously supporting the brain's intrinsic repair mechanisms by augmenting neurotrophin expression and enhancing neurogenesis. The evidence supporting a potential role for the cannabinoid system as a therapeutic target for the treatment of Alzheimer's disease will be reviewed herewith.

Keywords: Alzheimer's disease, cannabinoid, CB_1 receptor, CB_2 receptor, β-amyloid, neurodegeneration

Pathophysiology of Alzheimer's disease

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Alzheimer's disease (AD) is a chronic debilitating neurodegenerative condition that is associated with progressive cognitive decline and profound neuronal loss, and estimated to affect 10% of people over the age of 65 years and 25% of people over the age of 80 years (Herbert *et al.*, 2003). Western society is developing an increasingly aged population and this demographic shift is associated with a rise in the prevalence of age-related illnesses such as AD. The United Nations population projections estimate that 370 million people will be older than 80 years by 2050 and the associated increase in patients with AD will pose a substantial socio-economic burden. While a small

proportion of AD cases have a genetic basis, the majority of cases are sporadic with unknown aetiology. A consistent feature of the AD brain is the presence of senile plaques composed of pathogenic extracellular deposits of β-amyloid (Aβ), a 1–42 amino acid peptide derived from aberrant processing of the transmembrane amyloid precursor protein (Walsh and Selkoe, 2007). Aβ fragments are proposed to play a central role in the genesis of the disease by evoking neuronal cell death (Boland and Campbell, 2003). The senile plaques are located within various brain regions but the hippocampus, cerebral cortex and amygdala are particularly vulnerable and plaques begin to form in these regions early in the disease process resulting in memory loss and behavioural changes (Ogomori *et al.*, 1989). A second pathological hallmark of the disease is the hyperphosphorylation of the microtubule-associated protein, tau, resulting in formation of the intracellular neurofibrillary tangles that impair inter-neuronal communication (Mi and Johnson, 2006). AD is also associated with neuroinflammatory events and oxidative stress that are likely to exacerbate the disease process. Epidemiological studies support an involvement of inflammatory mechanisms in AD since patients using non-steroidal anti-inflammatory drugs for a 2-year period have a 60–80% reduction in the risk for the disease, while long-term non-steroidal anti-inflammatory drug treatment attenuates disease onset and reduces the severity of symptoms (Rich *et al.*, 1995). Microglia are the Principal immune cells in the brain and in the AD brain they surround the senile plaques, possibly recruited to the plaque region in an attempt to clear the Aβ burden by phagocytosis (Wilkinson and Landreth, 2006). In AD, the Aβ deposition exceeds the phagocytic ability of the microglia and the persistent presence of activated microglia at the plaque results in a prolonged release of proinflammatory cytokines such as interleukin-1β (Bayer *et al.*, 1999; Heneka and O'Banion, 2007) which mediate local inflammation and have the proclivity to increase the processing of amyloid precursor protein to generate more Aβ fragments (Heneka and O'Banion, 2007), as well as having a direct neurotoxic influence (Vereker *et al.*, 2000). The association of activated microglia at the periphery of the senile plaque contributes to the generation of reactive oxygen species that mediate the oxidative damage found in the brains of patients with AD (Wilkinson and Landreth, 2006). Thus, inflammation and oxidative stress play a critical role in the disease process and anti-inflammatory and antioxidant strategies are likely to have enormous therapeutic potential for AD patients. Other factors that are thought to contribute to the pathophysiology of AD include dysregulation of intracellular calcium homeostasis and excitotoxicity (LaFerla, 2002). Cholinergic neurones are particularly vulnerable in AD and current therapeutics include acetylcholinesterase (AChE) inhibitors that aim to enhance acetylcholine (ACh) availability. However, such drugs are only suitable for the mild cognitive impairment that occurs early in the disease and no treatments are currently available to reverse the progression of the disease.

Cannabinoid system in the brain

Go to:

The discovery of an endogenous cannabinoid (CB)-signalling system in the brain has prompted much research into understanding how this system regulates physiological and pathological events within the central nervous system. The endocannabinoid molecules, 2-arachidonoyl glycerol and anandamide, interact with the G-protein-coupled cannabinoid receptors, CB_1 and CB_2. These receptors are also activated by phytocannabinoids, such as Δ^9-tetrahydrocannabinol (Δ^9-THC), isolated from the *Cannabis sativa* plant. The action of endocannabinoids at their receptors is terminated by enzymatic degradation of the endocannabinoids, or by membrane transport (Piomelli, 2003). Early reports indicating a potential role for the cannabinoid system in the management of AD are based on the finding that Dronabinol, an oil-based solution of Δ^9-THC, improves the disturbed behaviour and stimulates appetite in AD patients (Volicer *et al.*, 1997), and alleviates nocturnal agitation in severely demented patients (Walther *et al.*, 2006). More recently, an increasing body of evidence has accumulated to suggest antioxidant, anti-inflammatory and neuroprotective roles of the cannabinoid system (Jackson *et al.*, 2005). Such properties may be harnessed to circumvent the neurodegenerative process and offer more effective approaches to treat AD (Pazos *et al.*, 2004). In this review the recent experimental evidence that highlights the potential of the cannabinoid system to alleviate some of the pathology and cognitive decline associated with AD will be discussed.

The cannabinoid system in the AD brain

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The CB_1 receptor is abundant within the brain and associated with the cortex, hippocampus, cerebellum and basal ganglia (Herkenham *et al.*, 1991). CB_1 receptors in the hippocampus contribute to the effect of cannabinoids on learning and memory (Riedel and Davies, 2005); cognitive processes, which are disrupted early in the course of AD. CB_2 receptors have a more limited expression in the central nervous system, being largely confined to neurones within the brainstem (Van Sickle *et al.*, 2005), cerebellum (Ashton *et al.*, 2006) and microglia (Nunez *et al.*, 2004). Post-mortem studies of AD brains have detected increased expression of CB_1 and CB_2 receptors on microglia within the senile plaque, while CB_1 expression is reduced in neurones more remote from the plaque (Ramirez *et al.*, 2005). Also, cannabinoid receptors in the AD brain are nitrosylated, and this may contribute to the impaired coupling of these receptors to downstream effector signalling molecules (Ramirez *et al.*, 2005). Other studies have failed to establish a link between changes in CB_1 receptors in the AD brain and the specific pathological events that take place in this illness (Westlake *et al.* 1994), and report no changes in expression of CB_1 receptors in the vicinity of the senile plaque (Benito *et al.*, 2003). However, the endocannabinoid metabolizing enzyme, fatty acid amide hydrolase, is upregulated in the senile plaque (Benito *et al.*, 2003), and may contribute to the increase in expression of anandamide metabolites, such as arachidonic acid, in the vicinity of the senile plaque. Such a pathway may be involved in increasing the production of prostaglandins and related pro-inflammatory molecules that are pertinent to the inflammatory process of AD. The association of fatty acid amide hydrolase with astrocytes within the senile plaque may participate in the astrocytic events that culminate in the reactive gliosis that is observed in regions rich in Aβ deposits (Wyss-Coray, 2006).

Cannabinoids mediate neuroprotection

Go to:

Neuronal damage can increase the production of endocannabinoids (Stella *et al.*, 1997; Marsicano *et al.*, 2003), and cells lacking CB_1 receptors are more vulnerable to damage (Marsicano *et al.*, 2003). Those studies indicate that neural cannabinoid tone influences neuronal survival and suggest that augmentation of the cannabinoid system may offer protection against the deleterious consequences of pathogenic molecules such as Aβ. Recently, Aβ has been demonstrated to induce hippocampal degeneration, gliosis and cognitive decline, with a concomitant increase in the production of the endocannabinoid, 2-arachidonoyl glycerol, and this may reflect an attempt of the endocannabinoid system to provide neuroprotection from Aβ-induced damage (Van Der Stelt *et al.*, 2006). Furthermore, in that study, when endocannabinoid uptake was inhibited by VDM-11, the Aβ-induced neurotoxicity and memory impairment were reversed, although this was dependent upon early administration of the reuptake inhibitor. Those findings suggest that robust and early pharmacological enhancement of brain endocannabinoid levels may protect against the deleterious consequences of Aβ. Other endocannabinoids, such as anandamide and noladin ether, have been found to reduce Aβ neurotoxicity *in vitro* via activation of the CB_1 receptor and engagement the extracellular-regulated kinase pathway (Milton, 2002). Thus, endocannabinoids can reverse the negative consequences of exposure to Aβ, and such findings suggest that drugs designed to augment endocannabinoid tone, including inhibitors of membrane uptake and fatty acid amide hydrolase inhibitors, may have potential in the treatment of AD. However, the study by Van Der Stelt *et al.* (2006) cautions that the timing of endocannabinoid upregulation by pharmacological intervention in relation to the time-course of development of the disease pathology is crucial, since administration of VDM-11 later in the pathological cascade actually worsens memory retention in rodents. Also, the physiological role of the cannabinoid system in mnemonic processes should not be underestimated. In the hippocampus CB_1 receptor activation is negatively associated with the performance of rodents in memory tasks (Castellano *et al.*, 2003), possibly via a reduction in hippocampal ACh levels (Gifford *et al.*, 2000), while the CB_1 antagonist, SR141716A improves performance in memory tasks (Wolff and Leander, 2003). Furthermore, the impairment in memory evoked by Aβ in rodents is reversed by SR141716A (Mazzola *et al.*, 2003), suggesting that CB_1 receptor blockade may be beneficial in reversing the

amnesia associated with AD. However, given the evidence for a neuroprotective role of the CB_1 receptor (Marsicano *et al.*, 2003; Alger, 2006), CB_1 antagonists pose the risk of exacerbating the neurodegenerative component of the disease, which may negate the beneficial effects of such drugs on amnesia.

Cannabinoids and excitotoxicity

Go to:

The dysregulation of intracellular Ca^{2+} homeostasis (Smith *et al.*, 2005) and excessive activation of the *N*-methyl D-aspartate (NMDA) subtype of glutamate receptor, leading to excitotoxicity, are features of the AD brain (Sonkusare *et al.*, 2005). All of the clinical mutations in the presenilin genes (PS1/PS2) that have been linked with the inherited form of AD disrupt calcium signalling (Smith *et al.*, 2005), which may contribute to subsequent neurodegeneration and memory impairments (Rose and Konnerth, 2001). Also, Aβ can itself directly increase voltage-dependent Ca^{2+} channel activity (MacManus *et al.*, 2000), as well as forming Ca^{2+}-permeable pores in lipid bilayers (Arispe *et al.*, 1993), to increase intracellular Ca^{2+} concentration as part of the pathogenic mechanism. Aβ also reduces glutamate uptake by astrocytes and increases the activation of glutamate receptors to evoke excitotoxicity (Sonkusare *et al.*, 2005). Thus, strategies that reduce Ca^{2+} influx and limit excitotoxicity may confer neuroprotection in AD. The non-competitive NMDA receptor antagonist, memantine (Namenda, Ebixa) is used in the treatment of moderate to severe AD (Cosman *et al.*, 2007), and its beneficial properties are based on an ability to inhibit pathological, but not physiological, functions of NMDA receptors, as well as antioxidant action and a propensity to increase production of brain-derived neurotrophic factor in the brain (Sonkusare *et al.*, 2005). Manipulation of the cannabinoid system has several consequences that mirror those observed with memantine. Thus, the protective effects of some cannabinoids are related to the direct regulation of the NMDA receptor, since the non-psychotropic cannabinoid, HU-211, acts as a stereoselective inhibitor of the NMDA receptor and protects rat forebrain cultures (Nadler *et al.*, 1993) and cortical neuronal cultures (Eshhar *et al.*, 1993) from NMDA-induced neurotoxicity. Furthermore, activation of the CB_1 receptor protects mouse spinal neurons (Abood *et al.*, 2001) and cultured hippocampal neurones (Shen and Thayer, 1998) from excitotoxicity, possibly through inhibition of presynaptic Ca^{2+} entry (Mackie and Hille, 1992; Twitchell *et al.*, 1997) and the subsequent suppression of excessive glutamatergic synaptic activity (Shen and Thayer, 1998; Takahashi and Castillo, 2006). CB_1 receptor agonists also inhibit glutamate release, which may contribute to a reduction in excitotoxicity (Wang, 2003). The evidence for a Ca^{2+}-dependent synthesis of anandamide and 2-arachidonoyl glycerol (Di Marzo *et al.*, 1994; Stella *et al.*, 1997) would suggest that endocannabinoids are generated in response to an intracellular Ca^{2+} load in an attempt to provide feedback inhibition of excitotoxicity. In this regard it is notable that endocannabinoid upregulation is a feature of a number of neurotoxic paradigms that are associated with elevated intracellular Ca^{2+} concentration (Hansen *et al.*, 2001). Alternative mechanisms that are pivotal to cannabinoid-mediated protection include inhibition of $[Ca^{2+}]_i$ by reducing calcium release from ryanodine-sensitive stores (Zhuang *et al.*, 2005), inhibition of protein kinase A and reduced nitric oxide generation (Kim *et al.*, 2006). Like memantine, cannabinoids are also capable of increasing brain-derived neurotrophic factor to confer protection against excitotoxicity (Khaspekov *et al.*, 2004). In non-neuronal cells, the induction of nerve growth factor is also facilitated by cannabinoids, acting through the PI3K/PKB pathway (Sanchez *et al.*, 2003), and activation of the CB_1 receptor by the endocannabinoid, 2-arachidonoyl glycerol, can also couple to an axonal growth response, whereas CB_1 receptor antagonists inhibit axonal growth (Williams *et al.*, 2003). Thus, dampening excessive glutamatergic transmission and excitotoxicity, coupled with neurotrophic actions, may represent interesting actions of cannabinoids that could be exploited for the treatment of AD.

Cannabidiol prevents Aβ-mediated neurotoxicity

Go to:

Cannabidiol (CBD) is the principal non-psychoactive component of *Cannabis sativa*, with potent antioxidant properties that offer neuroprotection against glutamate toxicity (Hampson *et al.*, 1998). In differentiated PC12

cells exposed to Aβ, CBD reduces the induction of inducible nitric oxide synthase (iNOS), nitric oxide production and activation of the stress-activated protein kinase p38 and the inflammatory transcription factor, nuclear factor-κB (Esposito *et al.*, 2006a), providing evidence for a CBD-mediated downregulation of the inflammatory signalling events associated with exposure to Aβ. As well, CBD reduces Aβ-induced neuronal cell death by virtue of its ability to scavenge reactive oxygen species and reduce lipid peroxidation; antioxidant properties that occur independently of the CB_1 receptor (Iuvone *et al.*, 2004). CBD also reverses tau hyperphosphorylation, a key hallmark of AD, by reducing phosphorylation of glycogen synthase kinase-3β, a tau protein kinase responsible for the tau hyperphosphorylation in AD (Esposito *et al.*, 2006b). Moreover, since glycogen synthase kinase-3β also evokes amyloid precursor protein processing to increase Aβ production (Phiel *et al.*, 2003), the CBD-mediated inhibition of glycogen synthase kinase-3β is likely to be effective in reducing the amyloid burden. Thus, from such *in vitro* studies one can speculate that CBD may be therapeutically beneficial in AD, since it can prevent the deleterious effects of Aβ and ameliorate several features of AD pathology, including tau hyperphosphorylation, oxidative stress, neuroinflammation and apoptosis. Whether such actions of CBD are retained in the AD brain remains to be established, and experiments to test the effect of CBD in the various transgenic animal models of AD are eagerly awaited. In the meantime, reports that CBD is effective as an antioxidant and neuroprotectant in an animal model of Parkinson's disease (Lastres-Becker *et al.*, 2005), and orally effective in a rat model of chronic inflammation (Costa *et al.*, 2007), lend support to its potential therapeutic value in AD. There are a number of advantages of CBD as a therapeutic agent for AD; it is devoid of psychoactive activity and since CB receptors are nitrosylated in the AD brain, a feature that may hinder CB receptors coupling to their downstream effectors (Ramirez *et al.*, 2005), a therapy that does not depend on signalling through CB receptors may have a distinct advantage. Sativex is a cannabinoid-based oromucosal spray, containing CBD and THC, that is devoid of tolerance or withdrawal symptoms (Perez, 2006). This therapy is already available for the treatment of neuropathic pain and multiple sclerosis and may be exploited in the future for the treatment of AD.

CB_2 receptors and neuroinflammation

Go to:

The CB_2 receptor is largely confined to glial cells in the brain (Nunez *et al.*, 2004), although some studies have reported CB_2 receptors in neuronal populations within the brainstem and cerebellum (van Sickle *et al.*, 2005; Ashton *et al.*, 2006). CB_2 receptors have been implicated in the control of neural survival (Fernandez-Ruiz *et al.*, 2007) and mediate neuroprotection through their anti-inflammatory actions (Ehrhart *et al.*, 2005). CB_2 receptors are upregulated in activated microglia and astrocytes, and this upregulation is proposed to control the local production of proinflammatory mediators such as interleukin-1β, reactive oxygen species and prostaglandins. In the AD brain and in animal models of AD-like pathology, CB_2 receptors are upregulated within the active microglia present in those brain regions where senile plaques are abundant (Benito *et al.*, 2003; Ramirez *et al.*, 2005). The upregulation of CB_2 in such pathological situations may be an attempt to reduce neuroinflammation since CB_2 receptor activation *in vitro* reduces the microglial production of pro-inflammatory molecules (Facchinetti *et al.*, 2003). Such control in the production of inflammatory mediators may be due to a direct impact on activity of transcription factors, such as nuclear factor κB (Panikashvili *et al.*, 2005; Esposito *et al.*, 2006a). Thus, the neuroprotective mechanisms of cannabinoids are likely to include a downregulation in activity of the transcription factors that are pertinent to induction of the pro-inflammatory cytokines that serve as key players in neurodegenerative disease, while also stimulating the production of anti-inflammatory species such as IL-1ra (Molina-Holgado *et al.*, 2003). The manipulation of such inflammatory pathways may be exploited for the treatment of AD. In support of this contention, Ramirez *et al.* (2005) have demonstrated that in rats treated with Aβ, the induction of AD-like pathology and cognitive impairment, is reversed by the CB_1/CB_2 agonist, WIN,55212–22 and the CB_2-selective agonist, JWH-133. Since the CB_2 receptor was only associated with activated microglia located within the plaque, those authors have suggested that the CB_2 receptor may be a promising target for AD by virtue of its ability to serve as a brake for the neuroinflammatory cascade that is a

feature of AD. CB_2 agonists offer the advantage of being devoid of psychoactivity, although it is important to recognize that they may have other side effects such as immune suppression (Pertwee, 2005), which would be undesirable in an elderly population.

Cannabinoids and neurogenesis in the adult brain

Go to:

Another exciting mechanism that could account for the ability of cannabinoids to confer neuroprotection may be related to their regulation of neurogenesis. Adult neurogenesis can occur in the dentate gyrus of the hippocampus and the subventricular zone (Grote and Hannan, 2007), resulting in the presence of newly generated neurones. In several mouse models of AD neurogenesis is reduced (Dong *et al.*, 2004), although it should be noted that in the post-mortem AD brain, neurogenesis is increased (Jin *et al.*, 2004). Factors that enhance neurogenesis, such as dietary restriction and upregulation of brain-derived neurotrophic factor, enhance neurogenesis and improve the memory performance in animal models of AD (Lee *et al.*, 2000). Thus, targeting adult neurogenesis is receiving interest as a means to mitigate the symptoms of AD. In this regard it is notable that the cannabinoid system also regulates neurogenesis (Galve-Roperh *et al.*, 2007). Adult neurogenesis is defective in mice lacking CB_1 receptors (Jin *et al.*, 2004), and the synthetic cannabinoid, WIN55212-2, stimulates adult neurogenesis by opposing the antineurogenic effect of nitric oxide (Kim *et al.*, 2006). Also, the CB_1 agonist HU-210 has anxiolytic and antidepressant effects, which may be a functional consequence of enhanced neurogenesis (Jiang *et al.*, 2005). CB_2 receptor activation also stimulates neural progenitor proliferation *in vitro* and *in vivo* (Palazuelos *et al.*, 2006), and targeting neurogenesis via the CB_2 receptor would avoid undesired psychoactive side effects. Thus, the neuroprotective effects of cannabinoids may involve short-term adaptation to neuronal stress, such as limiting excitotoxicity, as well as longer-term adaptations, such as enhancing neurogenesis. It remains to be established whether or not the beneficial effects of cannabinoids on memory, neuroinflammation and neurodegeneration in animal models of AD are due to a functional consequence of an enhancement in neurogenesis.

Targeting acetylcholinesterase with cannabinoids

Go to:

Currently there are four approved drugs (tacrine, Cognex; donepezil, Aricept; rivastigmine, Exelon; galantamine, Reminyl) that are used to alleviate the symptoms of early stage AD by inhibiting the active site of AChE, thus increasing the levels of ACh at the synaptic cleft and enhancing cholinergic transmission. In addition, AChE accelerates that assembly of Aβ peptides into fibrillar species by forming complexes with Aβ via the peripheral anionic site on AChE (Inestrosa *et al.*, 1996), an interaction that increases the neurotoxicity of the Aβ fibrils (Alvarez *et al.*, 1998). Thus, AChE inhibitors offer a two-pronged attack for the treatment of AD by virtue of their ability to enhance ACh availability, as well as reduce amyloidogenesis and subsequent neurotoxicity. A recent study has demonstrated that Δ^9-THC competitively inhibits AChE and prevents the AChE-induced aggregation of Aβ by virtue of Δ^9-THC binding to the peripheral anionic site on AChE (Eubanks *et al.*, 2006). Compared with tacrine and donepezil, Δ^9-THC was found to be more robust inhibitor of Aβ aggregation, suggesting that Δ^9-THC and its analogues warrant further investigation as AChE inhibitors for use in the treatment of AD.

Do cannabinoids have a role for the treatment of other neurodegenerative conditions?

Go to:

It is also worth considering how the aforementioned properties of cannabinoids may be beneficial in ameliorating the symptoms of other diseases in which neuroinflammation, oxidative stress and neurodegeneration are key features, such as multiple sclerosis and Parkinson's disease. Benito *et al.* (2007) have reported that components of the cannabinoid system are upregulated in multiple sclerosis (MS) plaques, suggesting that endocannabinoids either have a role in the pathogenesis of MS or may be upregulated as a consequence of the pathology. MS is associated with excitotoxicity (Pitt *et al.*, 2000; Smith *et al.*, 2000) and neuroinflammation (Ziemssen, 2005), and

these represent features of the disease that cannabinoids may be able to circumvent. In encephalomyelitis virus-induced demyelinating disease, an animal model of MS, the mixed cannabinoid agonist HU210 reduces axonal damage and improves motor function as a consequence of a concomitant activation of the CB_1 receptor in neurones and CB_2 in astrocytes (Docagne *et al.*, 2007). Other studies in animal models of MS have demonstrated a role for the CB_2 receptor in enhancing T-cell apoptosis (Sanchez *et al.*, 2006) and suppressing microglial activation (Ehrhart *et al.*, 2005), while the CB_1 receptor is associated with neuroprotection (Pryce and Baker, 2007). Such neuroprotective and antioxidant properties of cannabinoids also underlie their ability to reverse the motor deficits in animal models of Parkinson's disease (Lastres-Becker *et al.*, 2005; Garcia-Arencibia *et al.*, 2007), and lend support of a potential role for cannabinoid-based therapies to mitigate the symptoms of a range of neurodegenerative conditions.

Conclusion

Go to:

Alzheimer's disease is a devastating illness for which there is no cure. Current AD drugs, which serve as AChE inhibitors, have a number of unpleasant side effects such as hepatotoxicity and gastrointestinal disturbances. While the NMDA receptor antagonist, memantine, can modify the disease, it cannot reverse the process of neurodegeneration. Manipulation of the cannabinoid pathway offers a novel pharmacological approach for the treatment of AD that may be more efficacious than current treatment regimes. Cannabinoids can reduce the oxidative stress, neuroinflammation and apoptosis that is evoked by Aβ, while promoting the brain's intrinsic repair mechanisms. Certain cannabinoids, such as Δ^9-THC, may also increase ACh availability and reduce amyloidogenesis, although potential psychoactive side effects may hinder its clinical usefulness. Cannabinoids clearly offer a multifaceted approach for the treatment of AD and future studies should focus on examining the efficacy of cannabinoids in the array of animal models that exhibit AD-like pathology and cognitive decline. Targeting the CB_2 receptor to reduce neuroinflammation while stimulating neurogenesis is likely to be of particular interest, given the reduced risk of psychoactive activity and the close association of the CB_2 receptor with the senile plaque, thus limiting drug effects to the region of pathology and sparing the potential for widespread effects on normal neurophysiological processes. In conclusion, manipulation of the cannabinoid system offers the potential to upregulate neuroprotective mechanisms while dampening neuroinflammation. Whether these properties will be beneficial in the treatment of AD in the future is an exciting topic that undoubtedly warrants further investigation (Figure 1).

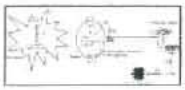

Figure 1

Potential sites of action of the cannabinoid system for the treatment of AD. Activation of the CB_2 receptor reduces the formation of reactive oxygen species (ROS) and the release of interleukin-1β from microglia, thus exerting an anti-inflammatory ...

Abbreviations

Go to:

Aβ	β-amyloid
AD	Alzheimer's disease
CB	cannabinoid
CBD	cannabidiol

NMDA *N*-methyl D-aspartate

Δ^9-THC Δ^9-tetrahydrocannabinol

Notes

Go to:

Conflict of interest

The authors state no conflict of interest.

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