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ABSTRACT

The present invention relates to the use of cannabidiol for use in the prevention or treatment of neurodegenerative conditions. Preferably the neurodegenerative condition to be prevented or treated is prion disease.

BACKGROUND TO THE INVENTION

Neurodegenerative conditions can be described as the progressive damage or death of neurones. Neurones are nerve cells in the brain whose primary function is to assist in the memory process. The damage or death of neurones leads to a gradual deterioration of the functions controlled by the affected part of the nervous system.

Prion diseases are a large group of related neurodegenerative conditions. These diseases affect both animals and humans and have long incubation periods but all typically rapidly progress once clinical symptoms begin.

All prion diseases are fatal, with no effective form of treatment currently available.

Prion diseases are a unique type of disease in that they can be inherited; they can occur sporadically; or they can be infectious. The infectious agent in the prion disease is composed almost entirely of an abnormal
conformation of a host-encoded glycoprotein called the
prion protein.

A prion can be defined as "small proteinaceous infectious
particles that resist inactivation by procedures that
modify nucleic acids".

The first type of prion disease to be described was
scrapie, which is a disease that affects sheep and has
been recognised for over 250 years. The disease symptoms
are hyperexcitability, itching, and ataxia, which leads
on to paralysis and death. The transmission of this
disease was first demonstrated in 1943 when a population
of Scottish sheep was accidentally inoculated against a
common virus using a formalin extract of lymphoid tissue
from an animal with scrapie.

There are two possible methods of transmission in sheep:
infection of pasture with placental tissue carrying the
agent followed by ingestion, or direct sheep-lamb
transmission by acquired infection.

Prion diseases are often called spongiform
encephalopathies because of the post mortem appearance of
the brain with large vacuoles in the cortex and
cerebellum.

Most mammalian species are susceptible to prion diseases,
specific examples include: Scrapie, which affects sheep,-
TME (transmissible mink encephalopathy), which affects
mink; CWD (chronic wasting disease), which affects
muledeer and elk; and BSE (bovine spongiform
encephalopathy), which affects cows.
Humans are also susceptible to several different prion diseases: these include: CJD (Creutzfeld-Jacob Disease); GSS (Gerstmann-Straussler-Scheinker) syndrome; FFI: (Fatal Familial Insomnia); Kuru and Alpers Syndrome.

These original classifications were based on a clinical evaluation of a patient's family history, symptoms and are still widely used, however more recent and accurate molecular diagnosis of the disease is gradually taking the place of this classification.

The incidence of sporadic CJD is about 1 per million per year. GSS occurs at about 2% of the rate of CJD. It is estimated that 1 in 10,000 people are infected with CJD at the time of death. These figures are likely to be underestimates since prion diseases may be misdiagnosed as other neurological disorders.

In humans prion diseases are characterised by loss of motor control; dementia; paralysis wasting and eventually death; typically following pneumonia.

Visible end results at post-mortem are non-inflammatory lesions, vacuoles, amyloid protein deposits and astrogliosis.

GSS is distinct from CJD, it occurs typically in people aged between 30 to 50, and is characterised by cerebellar ataxia and concomitant motor problems, dementia is less common and the disease course lasts several years before death.

CJD typically occurs in people aged between 40 and 60,
and cerebral involvement makes dementia a more common symptom. Additionally the patient seldom survives a year.

FFI pathology is characterised by severe selective atrophy of the thalamus and Alpers syndrome is the name given to prion diseases in infants.

Humans can be infected by prions in two ways:

1. Acquired infection. Here the prion enters the body either from the diet or following medical procedures such as surgery, growth hormone injections, and corneal transplants). In acquired infection an infectious agent implicated.

2. Genetic. Apparent hereditary mendelian transmission where it is an autosomal and dominant trait. Where the prion disease is genetic, it is not *prima facie* consistent with an infectious agent.

Prion diseases are both infectious and hereditary diseases. In addition, they can also be sporadic, in that there are cases in which there is no known risk factor although it seems likely that infection was acquired in one of the two ways listed above.

Scrapie in sheep, bovine spongiform encephalopathy in cattle and Creutzfeldt- Jakob disease (CJD) in humans belong to a group of fatal neurodegenerative disorders called Transmissible Spongiform Encephalopathies (TSEs) or prion diseases.
Prion diseases in both humans and other mammals are invariably fatal and there is currently no proven treatment. The urgent need to find effective anti-prion therapies has been strengthened by the emergence of variant CJD (vCJD) caused by contaminated beef consumption and the fact that vCJD can be transmitted via blood transfusion.

A critical event in TSE pathogenesis is the conversion of the normal protease-sensitive host prion protein (PrPsen) to an aggregated and protease-resistant form, (PrPres). Both PrP isoforms are required for infection and pathogenesis. Although PrPres has been recovered in various tissues such as spleen, tonsils and muscles, tissue damage is most severe in the central nervous system (CNS) of the prion affected host. Intra-neuronal vacuolization, severe neuronal cell death, microglia activation and astrogliosis are the main hallmarks of TSEs.

In affected brains, the amount and location of PrPres deposits are clearly linked to histopathological lesions. Thus, the presence of PrPres is considered indicative of TSE disease. One possible approach to TSE therapy is the inhibition of PrPres formation in the CNS.

There are a number of potential treatments in development or under consideration. Nevertheless, no compounds have been identified that have a therapeutic benefit after infection has reached the CNS. One possibility is that the blood brain barrier (BBB) restricts the access of many potential anti-TSE inhibitors.
Clearly there is a significant requirement for an efficacious agent that is able to prevent or treat neurodegenerative conditions such as prion disease.

Cannabinoids are a group of chemicals known to activate cannabinoid receptors in cells. These chemicals, which are found in cannabis plants, are also produced endogenously in humans and other animals, these are termed endocannabinoids. Synthetic cannabinoids are chemicals with similar structures to plant cannabinoids or endocannabinoids.

Cannabinoids possess the characteristics of being cyclic molecules exhibiting particular properties such as the ability to easily cross the BBB, weak toxicity and few side effects. Thus making them of interest as potential anti-prion drugs.

Plant cannabinoids can also be isolated such that they are "essentially pure" compounds. These isolated cannabinoids are essentially free of the other naturally occurring compounds, such as, other minor cannabinoids and molecules such as terpenes.

Essentially pure compounds have a degree of purity up to at least 95% by total weight. Some essentially pure cannabinoids (whether synthetic or isolated) have been suggested to be neuroprotective agents, either by direct antagonism of the NMDA receptor or by reducing the influx of calcium ions into the cell by another means such as binding with cannabinoid receptors.

Surprisingly, the applicants have found that the administration of the cannabinoid cannabidiol (CBD) is
efficacious in the prevention and treatment of neurodegenerative conditions. In particular it has been found that cannabidiol is useful in the prevention and treatment of prion diseases in humans and other mammals.

Cannabidiol (CBD) is a non-psychoactive component of *cannabis sativa*. CBD was found to inhibit the formation of PrPres in cells and exhibited neuroprotective activity against PrPres-induced neurotoxicity.

Moreover, CBD increased survival times and inhibited PrPres accumulation in the brains of scrapie-infected mice.

**SUMMARY OF INVENTION**

According to the first aspect of the present invention there is provided the use of cannabidiol (CBD) in the manufacture of a pharmaceutical formulation or a veterinary preparation for use in the prevention or treatment of a transmissible neurodegenerative disorder.

A transmissible neurodegenerative disorder is one that can be defined by being capable of transmission by either an infectious agent or by familial means.

Preferably the transmissible neurodegenerative disorder is a prion disease.

References to CBD, particularly with regard to therapeutic use, will be understood to also encompass pharmaceutically acceptable salts of the cannabinoid. The term "pharmaceutically acceptable salts" refers to salts
or esters prepared from pharmaceutically acceptable non-toxic bases or acids, including inorganic bases or acids and organic bases or acids, as would be well known to persons skilled in the art. Many suitable inorganic and organic bases are known in the art.

The scope of the invention also extends to derivatives of CBD that retain the desired activity of the prevention or treatment of neurodegenerative conditions. Derivatives that retain substantially the same activity as the starting material, or more preferably exhibit improved activity, may be produced according to standard principles of medicinal chemistry, which are well known in the art. Such derivatives may exhibit a lesser degree of activity than the starting material, so long as they retain sufficient activity to be therapeutically effective. Derivatives may exhibit improvements in other properties that are desirable in pharmaceutically active agents such as, for example, improved solubility, reduced toxicity, enhanced uptake, etc. Known derivatives of CBD include CBDA, CBD-dimethyl heptyl, cannabidivarin (CBDV), and abnormal CBD.

Preferably the CBD is an extract from at least one cannabis plant.

More preferably the CBD extract from at least one cannabis plant is a botanical drug substance.

Preferably the CBD extract from at least one cannabis plant is produced by extraction with supercritical or subcritical CO₂.
Alternatively the CBD extract from at least one cannabis plant is produced by contacting plant material with a heated gas at a temperature which is greater than 100°C, sufficient to volatilise one or more of the cannabinoids in the plant material to form a vapour, and condensing the vapour to form an extract.

Preferably the CBD extract from at least one cannabis plant comprises all the naturally occurring cannabinoids in the plant.

Alternatively the CBD is in a substantially pure or isolated form.

A "substantially pure" preparation of cannabinoid is defined as a preparation having a chromatographic purity (of the desired cannabinoid) of greater than 90%, more preferably greater than 95%, more preferably greater than 96%, more preferably greater than 97%, more preferably greater than 98%, more preferably greater than 99% and most preferably greater than 99.5%, as determined by area normalisation of an HPLC profile.

Preferably the substantially pure CBD used in the invention is substantially free of any other naturally occurring or synthetic cannabinoids, including cannabinoids that occur naturally in cannabis plants. In this context "substantially free" can be taken to mean that no cannabinoids other than CBD are detectable by HPLC.

Substantially pure CBD can be prepared from a botanical drug substance. A technique has been established by the
applicant and is described in their granted United Kingdom patent, GB2393182.

In another aspect of the present invention the CBD is in a synthetic form.

Preferably the CBD is formulated as a pharmaceutical composition further comprising one or more pharmaceutically acceptable carriers, excipients or diluents.

The invention also encompasses pharmaceutical compositions comprising CBD, or pharmaceutically acceptable salts or derivatives thereof, formulated into pharmaceutical dosage forms, together with suitable pharmaceutically acceptable carriers, such as diluents, fillers, salts, buffers, stabilizers, solubilizers, etc.

The dosage form may contain other pharmaceutically acceptable excipients for modifying conditions such as pH, osmolarity, taste, viscosity, sterility, lipophilicity, solubility etc. The choice of diluents, carriers or excipients will depend on the desired dosage form, which may in turn be dependent on the intended route of administration to a patient.

Suitable dosage forms include, but are not limited to, solid dosage forms, for example tablets, capsules, powders, dispersible granules, cachets and suppositories, including sustained release and delayed release formulations. Powders and tablets will generally comprise from about 5% to about 70% active ingredient. Suitable solid carriers and excipients are generally known in the art and include, e.g. magnesium carbonate, magnesium stearate, talc, sugar, lactose, etc. Tablets, powders,
cachets and capsules are all suitable dosage forms for oral administration.

Liquid dosage forms include solutions, suspensions and emulsions. Liquid form preparations may be administered by intravenous, intracerebral, intraperitoneal, parenteral or intramuscular injection or infusion. Sterile injectable formulations may comprise a sterile solution or suspension of the active agent in a non-toxic, pharmaceutically acceptable diluent or solvent. Liquid dosage forms also include solutions or sprays for intranasal, buccal or sublingual administration. Aerosol preparations suitable for inhalation may include solutions and solids in powder form, which may be combined with a pharmaceutically acceptable carrier, such as an inert compressed gas.

Also encompassed are dosage forms for transdermal administration, including creams, lotions, aerosols and/or emulsions. These dosage forms may be included in transdermal patches of the matrix or reservoir type, which are generally known in the art.

Pharmaceutical preparations may be conveniently prepared in unit dosage form, according to standard procedures of pharmaceutical formulation. The quantity of active compound per unit dose may be varied according to the nature of the active compound and the intended dosage regime. Generally this will be within the range of from 0.1mg to 5000mg.

According to a second aspect of the present invention there is provided a method for the treatment or prevention of a transmissible neurodegenerative disorder.
In yet a further aspect of the invention there is provided cannabidiol (CBD) for use in the prevention or treatment of a transmissible neurodegenerative disorder.

The term "neurodegenerative condition" is used to describe different groups of conditions and diseases. These groups include but are not limited to: neurodegenerative diseases, ischemic diseases, brain injury or damage and age-related or autoimmune neural degeneration.

Neurodegenerative diseases arise when degeneration of the neural pathway occurs as a result of a specific disease. Prion disease is an example of a neurodegenerative disease.

The preferred embodiments of the first aspect of the current invention as described above apply mutatis mutandis to all further aspects also described.

Certain aspects of this invention are further described, by way of example only.

SPECIFIC DESCRIPTION

Prion diseases are transmissible neurodegenerative disorders characterized by the accumulation in the central nervous system of the protease-resistant prion protein (PrPres), a structurally misfolded isoform of its physiological counterpart PrPsen.
Both neuropathogenesis and prion infectivity are related to PrPres formation. Cannabidiol (CBD) was tested in its ability to inhibit PrPres accumulation in both mouse and sheep scrapie-infected cells, along with other structurally related cannabinoid analogs.

The test articles were also tested by in their ability to limited cerebral accumulation of PrPres and their effect on the survival time of infected mice.

In addition, the neurotoxic effects of PrPres and affected PrPres induced microglial cell migration was tested.

Example 1:

Test articles: Drugs were dissolved at ICT$^2$ M in ethanol and stored at -20°C until used.

Cell cultures: Nil microglia were grown in RPMI1640 containing 10% FCS and P/S. Epithelial cells (Rov9) chronically infected with natural sheep scrapie (Rov9sc+) were grown in DMEM supplemented with 10% FCS, P/S and 1μg/ml doxycycline. Neuroblastoma cells chronically infected with the murine Chandler strain (N2asc+) were grown in Opti-MEM supplemented with 10% FCS, P/S and 1μg/ml G418. PrPres-cured neuroblastoma cells (N2asc-) were obtained by treatment with Congo red (1μg/ml) for several passages.

Assay for PrP-res accumulation from N2asc+ and Rov9sc+ cultures: N2asc+ and Rov9sc+ cells were seeded at 10% confluent density in the appropriate medium and treated with the indicated concentration of drugs for four days
Control experiments were performed in the presence of ethanol alone, the amount of ethanol being fixed to 1% in all conditions. Cultures were split every four days at a 1:4 dilution and incubated in the presence of the drug for the indicated number of passages. Confluent cultures were homogenized in lysis buffer (50mM Tris-HCl pH 7.4 containing 150mM NaCl, 0.5% Triton X-100, 0.5% sodium deoxycholate, 5mM EDTA), and then centrifuged at 3,000xg for 5min. For detection of PrPsen, one-tenth of a post-nuclear supernatant was mixed with the denaturing loading buffer. For detection of PrPres, lysates were digested with 20µg of proteinase K (PK) /mg of total protein for 30min at 37°C before centrifugation at 20,000xg for 90min. Pellets were resuspended in denaturing loading buffer, boiled and loaded onto a 12% polyacrylamide gel. Mouse and sheep PrPres were assayed with the SAF83 or SAF70 monoclonal antibodies, respectively. Blots were developed using an enhanced chemoluminescence system (ECL, Amersham) with a LAS3000 detector (Fuji). To correct for any loading artifact, blots with non-PK digested proteins were re-probed with the anti-Erk antibody.

Densitometry analyses were performed with a "National Institutes of Health" IMAGE software on the PK digested immuno-positive band corresponding to the glycosylated PrP form and results expressed as a percentage of control levels.

In vivo CBD treatments: Tga20 mice, which overexpress murine PrP, and C57BL/6 mice were intraperitoneally (ip) infected with 100µl of a 2% homogenate prepared from the brains of terminally ill 139A scrapie-infected C57BL/6 mice. As a negative control, Prnp0/0 mice were infected
under the same experimental conditions. Mice were treated ip three times a week for the indicated period of time with 200µl of 20 or 60mg/kg CBD diluted 1:1:2 in an ethanol: cremophor: NaCl 0.9% mixture. A control group of scrapie-infected animals was treated only with the vehicle mixture. In order to evaluate the toxicity of CBD, scrapie-free mice were treated with the higher CBD dose (i.e. 60mg/kg) three times a week for twelve weeks.

As indicated, treatments began on the day of scrapie inoculation (t=0) or 30 and 120 days post-inoculation (dpi). Animals were monitored every two days and the onset of clinical scrapie was defined when mice showed at least three of the following signs: ataxia, kyphosis, generalized tremor, swaying gait, and tail stiffness.

Metabolic labeling of PrPsen-. Mouse PrP expressing the epitope to the monoclonal antibody 3F4 but without the glycoprophosphatidyl- inositol membrane [Mo3F4 (GPINEG)] was expressed in mouse fibroblast cells and a single cell clone expressing high levels of Mo3F4 (GPINEG) PrP, Mo3F4 (GPINEG)-F3, was derived. Mo3F4 (GPINEG)-F3 cells were metabolically labeled with ImCi of Tran3 5S methionine/cysteine (Perkin Elmer) and Mo3F4 (GPINEG) PrP-sen immunoprecipitated with the 3F4 antibody.

Cell-free conversion assay: Enriched PrPres isolated from the brains of mice infected with the Chandler scrapie strain was partially unfolded in 2.5M guanidine hydrochloride for 1 hour at 37°C. For each conversion reaction, 100ng of unfolded PrPres was mixed with 10 000 cpm (~1ng) of radiolabeled and immunoprecipitated Mo3F4 (GPINEG) PrPsen. Test articles freshly diluted in ethanol were added to the conversion reaction to the
indicated final concentration. Because ethanol alone can inhibit PrPres formation, the final amount of ethanol for all reactions, including the "no inhibitor" and "no PrPres" controls, was kept constant at 2.5% of the total reaction volume. This amount of ethanol has a minimal effect on PrPres formation.

Reactions with and without inhibitor were incubated in reaction buffer (0.75M guanidine hydrochloride, 1.25% sarkosyl, 5mM cetyl pyridinium chloride, 50mM sodium citrate buffer pH 6.0) for 2 days at 37°C. One tenth of the reaction was methanol precipitated and used to assay the amount of total radiolabeled PrP. In order to determine the amount of radiolabeled PrPres formed, the remainder of the reaction was digested with 12µg/ml PK for 1 hr at 37°C. Proteolysis was stopped by the addition of 10mM Pefabloc™ and 400ng of bovine thyroglobulin and proteins were methanol precipitated. Radiolabeled products were analyzed by SDS-PAGE and quantified using the Storm Phosphor Imager system.

Flow cytometry analysis: N2a cells were cultured in the presence of 5µM CBD or with ethanol alone then trypsinated and resuspended in PBS pH 7.4 supplemented with 0.1% BSA and 0.1% NaN3. Alternatively, cells were fixed in Lyse/Fix Buffer (BD Biosciences) then permeabilized in Perm Buffer III (BD Biosciences) according manufacturer recommendations. Intact or permeabilized cells were incubated with anti-PrP SAF83 antibody (1/200) in PBS-0.1% BSA for 30 min at room temperature and then incubated with Alexa488-conjugated secondary antibody (1/400). Cells were gated according to size and scatter to eliminate dead cells and debris from
analysis. Experiments were repeated three times for consistency.

Confocal laser microscopy: N2a cells were cultured for four passages in the presence of 5µM CBD or with ethanol alone then transferred to glass coverslips. For cell surface PrPsen detection, coverslips were washed twice with cold PBS and fixed in paraformaldehyde 2% for 10 min at room. After 20 minutes in PBS-5% BSA, each coverslip was incubated for 30 minutes in PBS-5% BSA containing the anti-PrP antibody SAF83 (1/200). Where indicated, the incubation with primary antibodies was done on permeabilized cells, i.e., in PBS-5%BSA supplemented with 0.1% Triton X-100. Cells were rinsed three times in PBS and then incubated with the appropriate conjugated secondary antibodies (1/200) in PBS-5% BSA. Coverslips were mounted on glass slides containing 1μg/ml diamidino-4',6-phenylindol-2 dichlorhydrate (DAPI) to stain nuclei. Cells were observed under a laser scanning confocal microscope (SP5) equipped with a DMIRBE inverted microscope and an argon-krypton laser. Images were acquired as single transcellular optical sections and averaged over at least 4 scans/frame.

Scrapie and scrapie-free homogenates preparation: N2asc+ homogenates (hgtsc+), used as a source of PrPres, were obtained in detergent-free conditions. The preparation was up to 75% PrPres with a final concentration of about 20pg/µl. N2asc- homogenates (hgtsc-) from uninfected cells are PrPres-free and were used as negative controls.

Neurotoxicity assay on primary cultures of neurons: Cortical neurons from embryonic day-14 mice were
prepared. Cells were plated at a density of 5 x 10^4 cells/well in 96-well tissue-plastic dishes. Neurons were grown in Neurobasal medium supplemented with B27 and 10µM cytosine β-D-arabinofuranoside to prevent glial growth. Cultures used after 6-8 days of differentiation were 95% neurons. Cultures were incubated with the indicated concentration of drug or ethanol alone before the addition of 5 or 10µl of hgtsc- or hgtsc+ (1 and 2ng/ml PrPres, respectively). After overnight incubation, microglia were added to neurons in the ratio 1:10. Neuronal viability was determined using the MTS method.

**Microglia migration assay:** Neurons (5 x 10^5 cells/well) plated in 24-wells were incubated with PBS, hgtsc-, or hgtsc+ (0.2ng/ml PrPres) in the absence or presence of CBD for 24h at 37°C. Nil microglia were added to the top of a Boyden's chamber (5 x 10^4 cells/200 µl) and allowed to migrate through polyester filters for 6h. Cultures were then fixed with 3% paraformaldehyde, stained with Crystal violet and the cells counted (5 random fields/filter) under an inverted microscope.

**RESULTS**

**Effects of cannabinoid derivatives on PrPres accumulation in mouse and sheep scrapie-infected cells**

A series of cannabinoid derivatives were screened for their ability to prevent PrPres accumulation in scrapie-infected cells.

The cannabinoid derivatives belonged to three different groups: endocannabinoids (AEA, AG), natural components of
cannabis sativa (THC, CBD) and synthetic non-metabolized molecules (MAFE, RMA).

Two well-established scrapie-infected cell models were used, namely neuroblastoma cells chronically infected with the Chandler murine strain of scrapie (N2asc+) and epithelial cells infected with natural sheep scrapie (Rov9sc+).

N2asc+ and Rov9sc+ cells were treated continuously with 5µM of each tested drug over four passages of the cells.

In N2asc+ and Rovsc+ cells, the level of PrPres accumulation was drastically reduced in the presence of CBD as estimated by western blot analysis.

In both scrapie-infected cell types, RMA induced a slight decrease of the PrPres content compared to the untreated control while AEA, AG and MAFE had no effect.

To assay the effect of CBD on PrPres accumulation over time, Chandler-infected N2asc+ were treated with 5µM CBD for five consecutive passages and the PrPres level was analyzed for each passage. From the second passage, the amount of PrPres was significantly reduced in cells treated with 5 µM CBD. Over the course of successive passages, the PrPres amount decreased progressively until it was barely detectable by the fifth passage.

The reduction of PrPres accumulation was dependent on both the number of passages of treated cells and the CBD concentration.
Similar effects of CBD were observed on N2asc+ infected with another murine scrapie strain named 22L.

In summary, CBD is a unique cannabinoid derivative able to strongly prevent PrPres formation, regardless of scrapie strain, in both mouse and sheep scrapie-infected cells.

Cerebral accumulation of PrPres and survival time of scrapie-infected mice

The in vivo PrPres accumulation was also tested. This was investigated by inoculating mice with the 139A murine scrapie strain followed by treatment with 20mg/kg of CBD.

Forty days post-infection mice were sacrificed and western blot analysis was performed on brain and spleen, these are the crucial organs involved in TSE pathology, in order to detect and quantify the amount of PrPres.

As expected, no PrP immunoreactivity was detected in the brain or spleen of infected Prnp0/0 mice, as they fail to accumulate PrPres and are known to be resistant to prion infection.

In the brains of CBD-treated mice, PrPres was barely detectable whereas substantial amounts of PrPres were present in the brain of pre-symptomatic untreated control mice.

Measurement of the amount of PrPsen in brain lysates revealed no significant change between control and CBD-treated mice suggesting that CBD did not effect the level of PrPsen expression in vivo.
Surprisingly, no significant difference in PrPres accumulation was observed in spleen homogenates from control and CBD-treated animals.

Quantification of the western blot data confirmed that the amount of cerebral PrPres accumulation was significantly different in CBD-treated animals when compared to control animals (p<0.05, Mann-Whitney test) whereas no significant difference was detected in the spleen.

To determine whether CBD could affect prion disease in vivo, we infected wild-type mice by ip inoculation with a high dose of 139A murine scrapie strain. This strain was chosen because its incubation period after peripheral inoculation is shorter and less variable than other strains. Starting on the day of infection (0dpi) and continuing three times a week over a four week period, mice were treated with 20mg/kg of CBD. Treatment with CBD significantly increased the survival time of the infected wild-type mice compared to the vehicle treated group (p=0.02, Mann-Whitney test).

Treatment over a longer period of time (up to 12 weeks) or treatment with a higher dose of CBD (60mg/kg over 4 weeks) also led to a significant increase in the survival time of infected mice (p=0.003 and 0.0003 respectively).

To determine if CBD also inhibited prion disease progression during the later stages of infection, we began treatment of infected mice at either 30 or 120dpi. When 20mg/kg of CBD-treatment was initiated at 30dpi, the
survival time of mice was significantly increased compared to the vehicle-treated group (p=0.03). However, no significant difference was observed when CBD-treatment was started at 120 dpi.

Overall, CBD delayed prion disease onset in scrapie-infected mice in a time and concentration dependent manner.

Due to their high level of PrP expression, Tga20 mice have shorter incubation times than wild-type mice and thus were used as a rapid assay for testing inhibition of scrapie disease. As above, Tga20 mice were infected ip with the 139A scrapie strain and treated with 60mg/kg CBD ip three times a week over four weeks. When treatment was applied starting the day of infection, CBD significantly delayed both the appearance of clinical signs of the disease (delay 5.7 days; p=0.021) and death (delay 5.4 days; p=0.022) when compared to the vehicle-treated group.

When CBD-treatment started at 30 dpi (approximately one-third of the incubation time), no significant delay in the progression of the disease was observed. During the time course of these experiments, no significant side effects were seen in non-infected, CBD-treated mice.

In summary, the data demonstrate that, when applied on the day of infection or as late as a month post-infection, CBD slows down PrPres accumulation in the brains of prion-infected mice and delays the onset of terminal prion disease.
PrPres accumulation in vitro

Scrapie-infected mice treated with CBD had significantly-lower levels of PrPres accumulation in the brain while scrapie infected cells exposed to CBD had significantly-decreased levels of PrPres.

These data suggested that CBD could exert its anti-scrapie effect in vivo either by destabilizing preexisting PrPres aggregates or by preventing PrPres formation. We first tested the capacity of cannabinoids to destabilize PrPres aggregates in vitro by assaying the change in sedimentation properties of PrPres in the presence of cannabinoids.

PK-digested homogenates prepared from N2asc+ were incubated with 10µM of each compound for two days. At the end of the incubation time, the homogenates were assayed for PrPres by western blotting. No difference in the amount of PrPres was observed in cannabinoid-treated N2asc+ homogenates versus untreated homogenates.

Next, the ability of CBD to directly inhibit PrPres formation was tested in a cell-free assay. AG, CBD and THC were added in increasing concentrations to a reaction mixture containing PrPres derived from Chandler scrapie-infected mice and 35SMo3F4 (GPINEG) PrPsen, a protein known to efficiently convert to PrPres in vitro. The amount of 35S-Mo3F4 (GPINEG) PrPres generated was then determined. The addition of up to 50µM of CBD did not appear to significantly decrease the amount of 35S-Mo3F4 (GPINEG) PrPres formed when compared either to reactions with no inhibitor added or to reactions containing AG and THC which have no anti-scrapie
properties. These results suggest that, mechanistically, CBD does not decrease PrPres formation via direct interactions with either PrPsen or PrPres.

**Expression level and sub-cellular location of PrPsen**

It is well established that the relative level of PrPres production correlates with the level of PrPsen expression. Thus, the level of PrPsen in N2a cells was analysed.

The cells were treated with AG, CBD or THC over four passages. None of the tested cannabinoids, even CBD, was able to modify PrPsen expression level.

To examine the possibility that CBD could change the subcellular distribution of PrPsen and thus effect PrPres formation, cell surface and intracellular PrPsen was observed on unpermeabilized and permeabilized N2a cells respectively using both fluorescence-activated cell sorting (FACS) and confocal microscopy techniques. Cells treated with CBD for two and four passages expressed the same amount of surface-bound and intracellular PrPsen than AG-treated cells.

Confocal microscopy observation confirmed that CBD treatment had no significant influence on the plasma membrane expression of PrPsen compared to AG or vehicle alone.

As well, no differences in PrPsen-positive intracellular compartments could be detected in the presence of CBD.
Thus, CBD appears to have little or no influence on the expression level and cell trafficking of PrPsen.

**Neurotoxicity and microglial cell migration induced by PrPres**

Since some cannabinoids have been shown to exert neuroprotective effects following various experimental brain injuries, it was of interest to test the neuroprotective properties of CBD against PrPres-induced neuronal cell death in primary neuronal cell culture.

Neurons exposed to hgtsc+ for two days were clearly damaged as reflected by the disappearance of normal cell bodies and the presence of fragmented neurites when compared to neurons exposed to the PrPres-free N2asc-homogenate (hgtsc-).

The addition of CBD appeared to decrease PrPres-induced neurotoxicity since the number and morphology of the neurons was similar to that observed in cells exposed to hgtsc-. Neuronal viability was also monitored by measuring the reduction of mitochondrial activity using the MTS assay. CBD treatment resulted in a concentration dependent increase in the number of viable neurons for both concentrations of hgtsc+ tested.

Since CBD has been shown to regulate microglia migration it was of interest to determine whether or not CBD could affect this PrPres-induced chemotactic mechanism. The migration of Nil microglia toward a chamber containing neurons incubated with PBS, hgtsc- or hgtsc+ was monitored in the presence of increasing concentrations of CBD. Hgtsc+ exposed neurons induced an increase in the
number of migrating microglia compared to hgtsc- and PBS-
exposed neurons.

Importantly, no effect on the basal migration rate of microglial cells was observed with CBD alone, indicating that CBD by itself does not have in vitro chemotactic properties. Thus, CBD was able to impair PrPres-induced microglial cell migration in a concentration-dependent manner.

CONCLUSION

These data generated by the series of experiments described in the above example provides clear evidence that cannabidiol (CBD) may protect neurons against the multiple molecular and cellular factors involved in the different steps of the neurodegenerative process, which take place during prion infection. When combined with its ability to target the brain and its lack of toxic side effects, CBD may represent a promising new anti-neurodegenerative or new anti-prion drug.
Claims:

1. Use of cannabidiol (CBD) in the manufacture of a pharmaceutical formulation or veterinary preparation for use in the prevention or treatment of a transmissible neurodegenerative disorder.

2. Use as claimed in claim 1, wherein the transmissible neurodegenerative disorder is a prion disease.

3. Use as claimed in claim 1 or 2, wherein the CBD is an extract from at least one cannabis plant.

4. Use as claimed in claim 3, wherein the extract from at least one cannabis plant comprises all the naturally occurring cannabinoids in the plant.

5. Use as claimed in any of the preceding claims, wherein the CBD is a botanical drug substance.

6. Use as claimed in claim 1, wherein the CBD is in a substantially pure form.

7. Use as claimed in claim 1, wherein the CBD is in an isolated form.

8. Use as claimed in claim 1, wherein the CBD is in a synthetic form.

9. Use as claimed in any of the preceding claims, wherein the CBD is formulated as a pharmaceutical composition further comprising one or more pharmaceutically acceptable carriers, excipients or diluents.
10. Use as claimed in any of the preceding claims, wherein the CBD is in a unit dosage form.

11. Use as claimed in claim 10, wherein the amount of CBD per unit dose is within the range of from 0.1mg to 500mg.

12. A method for the treatment or prevention of a transmissible neurodegenerative disorder, which comprises administering to a subject in need thereof a therapeutically effective amount of CBD.

13. Cannabidiol (CBD) for use in the prevention or treatment of a transmissible neurodegenerative disorder.

14. Cannabidiol for use as claimed in claim 13, wherein the transmissible neurodegenerative disorder is a prion disease.

15. Cannabidiol for use as claimed in claim 13 or 14, wherein the CBD is an extract from at least one cannabis plant.

16. Cannabidiol for use as claimed in claim 15, wherein the extract from at least one cannabis plant comprises all the naturally occurring cannabinoids in the plant.

17. Cannabidiol for use as claimed in one of claims 13 to 16, wherein the CBD is a botanical drug substance.
18. Cannabidiol for use as claimed in claim 13, wherein the CBD is in a substantially pure form.

19. Cannabidiol for use as claimed in claim 13, wherein the CBD is in an isolated form.

20. Cannabidiol for use as claimed in claim 13, wherein the CBD is in a synthetic form.

21. Cannabidiol for use as claimed in any of one claims 13 to 20, wherein the CBD is formulated as a pharmaceutical composition further comprising one or more pharmaceutically acceptable carriers, excipients or diluents.

22. Cannabidiol for use as claimed in any one of claims 13 to 21, wherein the CBD is in a unit dosage form.

23. Cannabidiol for use as claimed in claim 22, wherein the amount of CBD per unit dose is within the range of from 0.1mg to 5000mg.
According to International Patent Classification (IPC) into both national classification and IPC

B. FIELDS SEARCHED

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, EMBASE, BIOSIS, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category*</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
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<td>P,X</td>
<td>WO 2007/083098 A (GW PHARMA LTD [GB]; GUY GEOFFREY [GB]; PLATT BETTINA [GB]) 26 July 2007 (2007-07-26) claims 1,3,12,13</td>
<td>1-23</td>
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Further documents are listed in the continuation of Box C. See patent family annex.

Date of the actual completion of the international search: 15 October 2008

Date of mailing of the international search report: 28/10/2008

Name and mailing address of the ISA/Authorized officer:

European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel.: (+31-70) 340-2040 Fax: (+31-70) 340-3016

Peri's Antol, Berta
<table>
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<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
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### INTERNATIONAL SEARCH REPORT

**Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [x] Claims Nos.:
   - because they relate to subject matter not required to be searched by this Authority, namely:
     - Although claim 13 is directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
   - because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
   - because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [x] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest**

- [ ] The additional search fees were accompanied by the applicants protest ana, where applicable, the payment of a protest fee.
- [ ] The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- [ ] No protest accompanied the payment of additional search fees.
<table>
<thead>
<tr>
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<td>GB 2434312 A</td>
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