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FRONTIER

Cannabinoids: Do they have the potential to treat the symptoms of multiple sclerosis?

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Abstract

This article reviews the role of cannabinoids in inhibiting neurodegeneration in models of multiple sclerosis (MS). MS is a chronic, debilitating disease of the central nervous system (CNS), induced by autoimmunity-driven inflammation that leads to demyelination and thus disconnection of the normal transmission of nerve impulses. Despite the use of an array of immune modulating drugs that restore blood brain barrier function, disability continues in patients concomitant with the loss of axons in the spinal cord. MS patients therefore suffer neuropathic pain, spasticity and tremor. Anecdotal evidence suggests that MS patients using cannabis, though illegal, achieve symptomatic relief from neuropathic pain and spasticity associated with MS. The discovery of the endogenous cannabinoid (endocannabinoid) system that naturally exists in the body and which responds to cannabinoids to exert their effects has aided research into the therapeutic utility of cannabinoids. The endocannabinoid system consists of two G-protein coupled receptors cannabinoid receptor type-1 (CB_1) and CB_2. CB_1 is mainly expressed in the CNS and CB_2 is predominantly found in leukocytes, while an increasing number of potential ligands and endocannabinoid degradation molecules are being isolated. Several studies have highlighted the involvement of this system in regulating neurotransmission and its ability to prevent excessive neurotransmitter release, consistent with a capacity to provide symptomatic relief. In summary, antagonism of the CB_1 receptor pathway contributes to neuronal damage in chronic relapsing experimental allergic encephalomyelitis (EAE) and suppresses tremor and spasticity. The addition of exogenous CB_1 agonists derived from cannabis also afforded significant neuroprotection from the consequences of inflammatory CNS disease in EAE and experimental allergic uveitis models. Although clear neuroprotective benefits of cannabinoids have been demonstrated, the unwanted psychotropic effects need to be addressed. However, manipulating the endogenous cannabinoid system may be one way of eliciting beneficial effects without some or all of the unwanted side effects.

Key words: Multiple sclerosis; Axonal damage; Neurodegeneration; Neuroprotection

Core tip: Multiple sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system and causes disability, neuropathic pain, spasticity and tremor in affected patients. Although illegal, users of cannabis report relief from pain and spasticity, probably due to the endogenous cannabinoid system that exists. Cannabinoid receptor type-1 (CB_1)-deficient mice accrue greater levels of neurodegeneration and poorly tolerate inflammatory and excitotoxic insults after immune attack in a model of MS, experimental allergic encephalomyelitis. Treatment of animals affected by experimental allergic uveitis (EAU) with CB_1 agonists also provided significant neuroprotection from the con-

sequences of EAU, suggesting that cannabinoids may slow down neurodegeneration in MS.

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BIOGRAPHY

Dr Zubair Ahmed received his PhD from University College London, London, United Kingdom. He completed his first postdoctoral training period at the Institute of Neurology in London before moving to the University of Birmingham to continue his second postdoctoral training period. In 2007, he was awarded an Research Councils United Kingdom Academic Fellowship, the remit of which was to develop an independently funded research group before being promoted to Lecturer in 2011. He is currently a Senior Lecturer in Neuroscience. His research interests covers numerous aspects of the molecular biology of central nervous system (CNS) axon regeneration and degeneration and his most notable contribution is to the understanding of the role of caspase-2 in apoptosis of retinal and dorsal root ganglion neurons, the contribution of an endogenous mechanism for receptor shedding, the identification of growth factors capable of promoting retinal ganglion cell survival and axon regeneration, identification of alternative neuronal receptors capable of interacting with key molecules in blocking CNS axon regeneration and the observations that cannabinoids inhibit neurodegeneration in models of multiple sclerosis (MS). His curriculum vitae lists over 50 peer-reviewed publications, 2 book chapters and numerous presentations at national and international meetings.

INTRODUCTION

MS is an inflammatory demyelinating disease of the CNS and results in disruption to the normal transmission of nerve impulses due to lesions in the CNS[1,2]. Despite immune modulating drugs that reduce blood brain barrier dysfunction, disability often continues in patients and suggests that neurodegenerative changes are key to the progression of disease[3-5]. MS patients thus display spasticity, neuropathic pain associated with neuroinflammation, excitotoxicity and chronic neurodegeneration. It is also established that axonal/neuronal loss, which occurs early in the disease process, is an important contributor of permanent disability and is often associated with active inflammation. Disability only becomes evident when normal compensatory mechanisms are exhausted, while demyelinated axons are left vulnerable to further damage by electrical activity[6,7]. Therefore identifying neuroprotective strategies are a key goal in the fight against MS.

Although the *Cannabis sativa* plant has been used for centuries as a medicinal preparation to relieve the symptoms of inflammatory and neuropathic disorders, its use is illegal[8]. However, anecdotal accounts from MS patients indicated that cannabis might offer symptomatic relief of pain and spasticity associated with MS[9]. Plant-derived "cannabinoids" have provided important insights into the biology of cannabis and have led to a multitude of clinical trials using cannabinoids to control pain and spasticity in MS patients[10]. The main active ingredient of *Cannabis sativa* was defined in 1964 as (-)-trans-delta-9-tetrahydrocannabinol [Δ^9-THC or dronabinol (international non-proprietary name)][11]. Δ^9-THC is not only responsible for the majority of the pharmacological actions of cannabis but also its psychoactive effects. Numerous other cannabinoids and phytochemicals also exist in the cannabis plant including cannabidiol (CBD) which is non-psychoactive and is not a cannabinoid receptor agonist[12].

CLINICAL TRIALS OF CANNABIS IN MS

Although many patients self-medicated with cannabis, there were few clinical studies that demonstrated reliable clinical evidence of the benefits of cannabis use in MS. Some of the first few studies on the effect of Δ^9-THC were not encouraging since small sample sizes were used and Δ^9-THC had no effect on objective measures, despite patients reporting subjective improvements in the symptoms of MS[13]. For example, the first systematic, placebo-controlled trial with Δ^9-THC and a *Cannabis sativa* plant extract, given to 16 MS patients with severe spasticity, showed no effect of the cannabinoids on spasticity[14]. A much larger trial involving 660 MS patients receiving Δ^9-THC or natural Cannabis oil or a placebo, the mean reported effect on spasticity was not significantly different between control and treatment groups[15]. However, patient-reported spasticity was reduced, agreeing with earlier studies that showed subjective improvements in spasticity related to MS[14,15]. However, in a 12-mo follow up study of 657 patients, Δ^9-THC was reported to have a significant effect on the objective measures of spasticity[16]. Several later studies have also shown variable results depending on cannabis preparation, dosing regime and patient numbers, throwing into doubt the use of clinical measures of spasticity.

At present, the large number of clinical trials in MS have not clarified whether cannabis is beneficial in MS but have thrown up questions about the rating of "spasticity", route of delivery, source and dosing regime[10]. Despite these reservations, self-medicated use of cannabis continues. In a postal questionnaire involving the responses received from 110 MS patients in the South of England, 43% confirmed their use of cannabis with 68% of these patients specifically using cannabis to relieve the symptoms of MS[17]. Patients affirmed that their main reason for choosing to self-medicate with cannabis was due to pain and spasms, while a small proportion used cannabis for sleep related problems[17]. In a further study, over 90% of a cohort of 112 MS patients based in the United Kingdom and United States declared that

self-medication with cannabis improved nocturnal pain, spasms and muscular pain[18].

A recent study showed that of the 572 MS patients enrolled in a clinical study, 272 (47.6%) responded to Sativex, an oral-mucosal spray that contains Δ^9-THC and CBD, treatment within 4 wk with a response that was defined as > 20% decrease in spasticity[19]. A second phase of this study demonstrated that the cannabis extracts significantly reduced spasticity and the frequency of spasms while improving sleep quality over an extended 12-wk period, compared to placebo controls[19]. This has led to approval of the use of Sativex in the treatment of MS-related spasticity. These studies demonstrate the potential beneficial roles of cannabis use in MS patients, although in general its supply and use remains illegal.

ENDOCANNABINOID SYSTEM

The discovery of the endogenous cannabinoid (endocannabinoid) system that naturally exists in the body and responds to cannabinoids to exert their effects has aided research into the therapeutic utility of cannabinoids. This has fuelled the search for alternative modes of delivery into the human body, rather than the traditional method of "smoking". The endocannabinoid system consists of at least two families of lipid signalling molecules (the N-acyl ethanolamines and the monoacyl-glycerols), multiple enzymes in the biosynthesis and degradation of these lipids, as well as two G-protein coupled receptors [cannabinoid receptor type-1 (CB_1) and CB_2] (reviewed by[10]). CB_1 is mainly expressed in the CNS while CB_2 is predominantly found in leukocytes, while an increasing number of potential ligands and endocannabinoid degradation molecules are being isolated[20]. Several studies have highlighted the involvement of this system in regulating neurotransmission and its ability to prevent excessive neurotransmitter release, consistent with a capacity to provide symptomatic relief in MS[21,22].

Endocannabinoids are produced on demand and are retrogradely transported across the postsynaptic membrane to engage with CB_1 receptors, suppressing neurotransmitter release. The pharmacology of endocannabinoids is rapidly evolving with the discovery of new cannabinoid mimetics and novel CB receptor interactions that include orphan receptors and other CB receptors being proposed[23-26]. All these receptor functions are either sensitive to, or are regulated by CB receptors and culminate in neuropathic pain and inflammation, suggesting that novel drug targets based around these proteins might be useful in treating neuroinflammation and neuropathic pain in different neurological conditions. In addition, activation of the CB receptor inhibits adenylate cyclase that then reduces the levels of the second messenger cyclic adenosine monophosphate (cAMP), thus regulating cellular mechanisms such as cell fate[27]. CB receptor activation also inhibits voltage-dependent Ca^{2+} channels and activates inwardly rectifying K^+ channels, a process that underlies CB-induced depression of excitatory neurotransmission[28,29]. Thus, the CB system is a potentially useful target for exploitation in neurodegenerative diseases such as MS.

AXONAL DAMAGE IN MS

Although MS is defined as an inflammatory demyelinating disease of the CNS, axonal loss is also a key feature of the disease. In MS patients, analysis of their spinal cord lesions suggested that the permanent loss in function was not primarily due to demyelination but due to axonal loss[30]. Axonal loss is generally associated with inflammatory macrophage infiltration but is variable in MS lesions and can be severe in certain cases. For example, axonal density is reduced by 60%-70% in actively demyelinating lesions and is characterised by the presence of axonal spheroids, endbulbs, or focal accumulation of proteins[31-33]. Although shadow plaques appear after injury that is consistent with an attempt to remyelinate axons, ongoing axonal injury is present[34]. This suggests that during the early stages of remyelination axons are more susceptible to damage, a process that is related to the patterns of Na^+ channels at the widened surfaces of the nodes of Ranvier[35]. Axonal injury is also present in normal appearing white matter[36,37] and may occur as a result of secondary Wallerian degeneration[38]. However, this is not the only mechanism of axonal damage since two distinct patterns are observed: one that takes place within demyelinated lesions and correlates with lesional activity, while the other is diffuse axonal injury that is associated with inflammation and can additionally affect non-demyelinated nerve fibres[33].

Axonal injury in MS is also selective to the size of axon fibres. Small calibre axons are more prone to injury compared to thick axons and may relate to thin axons requiring a higher energy demand in terms of their critical mass of mitochondria[39]. Like MS, widespread axonal damage is also seen in experimental allergic encephalomyelitis (EAE)[34,40]. However, experimental models of MS reveal different mechanisms of axonal injury and there is currently no agreement for which mechanism is relevant to MS patients. Axonal injury mechanisms may involve T-lymphocytes and antibodies as part of the adaptive immune response as well as components of the innate immune system, driven by macrophages and microglia. For example, axonal injury can be driven by an antigen-specific cytotoxic T cell response, induced in neurons and glia by the expression of pro-inflammatory cytokines, leading to neuronal death and axonal transection[41-43].

Another mechanism of axonal damage relies on the production of auto-antibodies against cell surface molecules in neurons and axons. A subset of MS patients were described that mounted an antibody response against neurofascin, which is expressed on axons and oligodendrocyte processes at the nodes of Ranvier[44]. Systemic injection of neurofascin during EAE exacerbates the clinical symptoms of the disease with severe levels of axonal injury within lesions[44]. These observations suggest that auto-antibodies can directly mediate axonal damage. In MS lesions, axon damage is closely linked to

the presence of macrophages and microglia that are in intimate contact with axons[3,5] and are known to produce a number of cytotoxic molecules including reactive oxygen and nitric oxide intermediates[33]. The expression of these molecules, especially inducible nitric oxide synthase from macrophages, correlates with areas of axonal injury in acute EAE[45]. In summary, axonal damage is a feature of MS and EAE and correlates with functional deficits. EAE models demonstrate that axonal damage occurs through a variety of mechanisms. However, recent reports have highlighted the role of mitochondrial injury and subsequent energy failure, induced by oxygen and nitric oxide free radicals as a possible mechanism of axonal injury. Understanding of the pathway to axon injury will aid in the discovery of new molecules for therapeutic intervention.

CANNABINOIDS IN MS

There is an abundance of evidence suggesting that MS patients gain symptomatic relief from cannabis extracts[46]. For example, Sativex, an oral-mucosal spray containing Δ^9-THC and CBD is anti-spasmodic and analgesic in MS patients[47], while neuropathic pain associated with MS is relieved by dronabinol, an oral preparation of Δ^9-THC analog[15]. Meta-analysis has also revealed that CB-based preparations are superior in the treatment of MS-related neuropathic pain than the placebo, confirming their beneficial effects in symptomatic relief[48]. This is consistent with the animal model of MS, EAE where treatment with exogenous cannabinoids controlled spasticity in chronic relapsing EAE models[49].

Modulation of the endocannabinoid system is also apparent in MS and EAE such that brain levels of CB receptors are downregulated in EAE while plasma levels of endocannabinoids are increased in MS patients[50,51]. Synthetic CB, HU-211, reduced the clinical severity of acute EAE in female Lewis rats as well as reducing inflammatory cell infiltration into the CNS[52], while the WIN55, 212-2, a CB_1 and CB_2 receptor agonist reduces T cell differentiation and hence reduces EAE severity in Theiler's murine encephalomyelitis virus-induced demyelinating disease, a mouse model of chronic-progressive MS[53], suggesting a key involvement of the CB receptors in the pathogenesis of EAE. Induction of EAE in CB_1 receptor-deficient mice causes rapid and progressively more neurodegeneration than in wild-type counterparts[54].

In our highly cited study on the role of cannabinoids in EAE, reported that the cannabinoid system was neuroprotective during EAE since CB_1-deficient mice poorly tolerated inflammatory and excitotoxic insults and showed significant accumulation of neurodegeneration after EAE[55]. We induced chronic relapsing EAE (CREAE) in wild type ABH, CB_1 gene (*Cnr1*)-deficient and congenic ABH. *Cnr1*$^{+/+}$ mice with mouse spinal cord homogenate emulsified in complete Freund's adjuvant on days 0 and 7 and monitored clinical disease progression over time. Mice developed characteristic paralytic disease episodes followed by remission with an increasing amount of residual deficit[49,56]. Whilst disease induction in both CB_1-deficient and CB_1 wild-type mice were similar, CB_1-deficient mice exhibited significantly higher levels of residual deficit. This deficit was quantitated in an open-field activity chamber and confirmed that CB_1-deficient mice displayed significantly more immobility and paresis than wild type mice, accumulating significantly more axonal damage after relapses. CB_1-deificent mice also developed spasticity after only a single attack, which is not seen in wild type mice until after three to four relapses of disease[49].

Numerous mechanisms cause neuronal death and axonal damage in EAE, including the influx of toxic ions such as Ca^{2+} and caspase-3-mediated apoptosis[57]. CB_1-deficient mice demonstrated significantly lower levels of active caspase-3 during acute EAE compared with wild type mice, while caspase-3 was detected in dying axons, consistent with that observed in MS[3]. These results suggested that the elevated neurodegeneration in CB_1-deficient mice may be due to caspase-3-mediated apoptosis and axonal damage and hence agonism of the CB_1 receptor pathway is neuroprotective and may control neurological symptoms such as tremor and spasticity[49].

We also investigated whether glutamate toxicity can be regulated by cannabinoids[55]. Glutamate excitotoxity causes neuronal damage in both MS and EAE. For example, the glutamate antagonist, amantadine, reduces the relapse rate in MS patients[58], while elevated glutamate levels have been observed in cerebrospinal fluid from MS patients[59]. In EAE, the enhanced levels of glutamate agonists may result in aberrant astrocyte function, since activated astrocytes normally regulate glutamate levels through enzymes such as glutamate dehydrogenase and glutamine synthetase, both of which are down-regulated during EAE[60,61]. The amount of CNS glutamate is also affected by abnormal changes in neuronal and glial glutamate transporters, all of which raise the levels of glutamate in the CNS during EAE and ultimately lead to the synthesis of mediators responsible for neuronal dysfunction[59,62-64]. We reported that after *in vitro* stimulation of N-methyl-D-aspartic (NMDA) receptors in cerebellar granule cells, there was significantly more neuronal Ca^{2+} influx in CB_1-deficient mice than in wild type controls suggesting that the cannabinoid receptors may tonically regulate Ca^{2+} influx. The NMDA receptor antagonist MK-801 took longer to reduce Ca^{2+} back to basal levels in CB_1-deficient mice than in congenic controls while CB_1 agonism with CP55, 940 inhibited NMDA-induced Ca^{2+} influx in wild type mice but had no effect on CB_1-deficient mice. These results suggest that Ca^{2+} is not only dysregulated in the absence of CB_1 receptors but that postsynaptic control of NMDA-receptor activation is also compromised. These *in vitro* results were confirmed by *in vivo* injections of kainic acid, a specific agonist of the kainate receptor that mimics the effects of glutamate, since injection of kainic acid in CB_1-deficient mice induced seizures and mortality within 10 min, while no effect of kainic acid was observed in wild type or congenic wild type control mice despite using 50-fold higher doses.

Therefore, CB1-receptors clearly regulate ionotropic glutamate receptor activity, leading to enhanced susceptibility to excitotoxic damage in CB_1-deficient mice.

Furthermore, we reported that CB_1 agonists protected mice against the consequences of CNS inflammation in models of experimental allergic uveitis (EAU)[55]. EAU is an inflammatory disease of the eye and after sensitization with for example, interphotoreceptor retinal binding peptide in B10. RIII mice, the neuroretina is completely destroyed within 14-16 d[65]. CB_1 receptor agonism with R(+)-WIN-55,212-2 and Δ^9-THC both significantly inhibited photoreceptor damage without affecting inflammatory infiltrates suggesting that agonism of CB_1 is neuroprotective. Taken together, the results of our study demonstrated that cannabinoids protect against the neurodegenerative events in models of MS and EAU.

CANNABINOID-1 RECEPTOR-MEDIATED NEUROPROTECTION IN MODELS OF MS

A feature of MS is neuronal loss, and in MS patients, loss of spinal cord axons correlate with neurological disability together with reduced N-acetyl aspartate levels in chronic MS patients[32]. Although axonal loss occurs early during the progression of MS[66], once a threshold of 15%-35% axonal loss in mice has been reached, permanent disability results[67,68]. In CB_1-deficient mice, significant axonal loss is evident after a single acute attack of EAE, suggesting that the mere presence of CB_1 is itself neuroprotective[55]. In EAE and MS, the presence of axonal damage correlates with inflammation that produces a range of neurotoxic agents such as glutamate, cytokines as well as creating oxidative stress in the environment of the CNS and damaging the blood-brain barrier[3,5,52,69-72].

Cannabinoids, however, can protect against acute hypoxia, excitotoxicity, oxidative and traumatic insults both *in vitro* and *in vivo*[73-76]. Cannabinoids can also inhibit both pre- and post-synaptic glutamate induced calcium responses and thus inhibit neurotoxicity[21,55,76], an effect that we showed to be CB_1-dependent[55]. In accord with our observations, CB_1-deficient mice were more susceptible to NMDA and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)/kainite glutamate receptor excitotoxicity[55], however, Δ^9-THC and CBD protected against NMDA-, AMPA- and kainite-agonist-induced cell death[77,78]. Delta-9-THC also protected retinal neurons from death induced by peroxynitrite-mediated NMDA-induced toxicity[79]. These observations demonstrate a clear neuroprotective role of cannabinoids in MS.

CANNABINOID-2-MEDIATED EFFECTS IN MODELS OF MS

Although much of the focus in MS is devoted to CB_1 receptor pharmacology, the recognition that CB_2 receptors possess immunomodulatory properties has led to an increased focus on CB_2 receptors as potential therapeutic targets. Unlike CB_1 receptors, activation of the CB_2 receptor is not psychoactive and therefore targeting CB_2 receptors with selective agonists is a promising therapeutic avenue that is immunomodulatory without being psychoactive. For example, early indications came from experiments that showed that the administration of WIN55212-2, which functions as a CB_1 and CB_2 receptor agonist, attenuated the progression of EAE in C57BL/6 mice immunized with myelin oligodendrocyte-derived glycoprotein35-55 (MOG35-55)[80]. Furthermore, a selective antagonist of the CB_1 did not modulate the protective effect of WIN55212-2 but a selective antagonist of the CB_2 receptor blocked the effects of WIN55212-2. This led to the suggestion that the protective effects of WIN55212-2 was mediated through the CB_2 receptor[80]. However, later studies showed that the CB_1 receptor might play a neuroprotective role in the latter stages of EAE[49,54].

Other highly selective CB_2 receptor agonists such as O-1966 are also useful in the fight against MS since they do not produce psychoactive effects, determined by its low affinity to CB_1 receptors[81]. Administration of O-1966 in a chronic (C57BL/6/MOG), relapsing-remitting and an adoptive transfer model attenuated disease progression and improved motor function[82]. In addition, encephalitogenic T cells derived from CB_2-deficient mice were shown to be more aggressive in terms of CNS infiltration and increased severity of EAE, despite displaying similar levels of proliferation, apoptosis and cytokine production in the spleen as wild-type T cells[83]. Moreover, treatment of mice with CB_2 receptor agonists attenuate white cell trafficking across the blood-brain barrier while dendritic cells differentiated in the presence of selective CB_2 receptor agonist and inhibited T cell proliferation and shifted cytokine responses from inflammatory to anti-inflammatory molecules[82]. Recently, it has been shown that high concentrations of IFN-γ disrupts $P2X_7$ purinergic receptor signalling and thus inhibit the neuroprotective effects of endogenous cannabinoids[84]. Therefore, inhibiting IFN-γ by exogenous CB_2 agonists represents an obvious therapy to prevent the disruption of $P2X_7$ signalling.

VALUE OF ANIMAL MODELS IN THE STUDY OF MULTIPLE SCLEROSIS

The validity of EAE as a model of MS is a topic of active debate with some researchers contenting that it is unsuitable due to its inability to mimic some of the pathological, immunologic and chronic features of MS. For example, EAE is usually monophasic whereas MS displays chronic relapsing features. Histological and magnetic resonance imaging data demonstrate axonal and cortical damage in MS but not in some models of EAE[85]. The extravasation of red blood cells into the CNS of swiss jim lambert mice and Lewis rats with EAE are not typical of MS[86,87], while encephalitogenic regions associated with myelin basic protein (MBP) or proteolipid protein normally activate more $CD4^+$ than $CD8^+$ T cells

but in inflammatory MS, $CD8^+$ T cell predominate[88-90]. However, there are many other immunological differences between mouse and human MS and these must be overcome if greater levels of success in drug development for human MS are to be achieved[91].

Several other points are worth considering in terms of the use of EAE as an animal model: (1) EAE provides little insight into the progression of MS in terms of the small amounts of demyelinated axons in EAE compared to MS while mice with EAE rarely exhibit ongoing functional deterioration that MS patients often display[92,93]; (2) The use of C57BL/6 mice in EAE studies limits the investigation of the mechanism of relapsing-remitting forms that more commonly affect MS patients (Ransohoff *et al*[93], 2002); (3) Treatment with factors that exert neurobiological effects also impact on immune and inflammatory cells and thus making results difficult to interpret (Ransohoff *et al*[93], 2002); and (4) EAE is generally generated using antigens that affect $CD4^+$ T cells while $CD8^+$ T cells that predominate in MS lesions are overlooked[92,94]. Likewise, the role of B cells is largely neglected despite recent data that demonstrate their importance in the pathogenesis of MS[95,96].

In summary, EAE has a long history in the fight against MS, however, its predictive value for treatment efficacy is poor. Nevertheless, it is widely used as a first-line animal model of MS and has provided mechanistic insights into the neuroinflammatory aspects of MS.

LIMITATIONS OF ANIMAL MODELS OF MS

One of the biggest limitations on the use of EAE as a model of MS is the fact that disease has to be induced with complete Freund's adjuvant and heat-inactivated *Mycobacterium tuberculosis* rather than mimicking a spontaneous disease like MS. This leaves very little room for disease pathways and fails to represent the complexity of disease inducing mechanisms in MS. Demyelination, a feature of MS is also not obvious in all EAE models while the time course for disease manifestation may be days, EAE more closely resembles post-infectious acute demyelinating events[97]. In contrast, MS develops over years with patients presenting with more protracted epitope spreading than that observed in EAE mice[98]. There are also many other immunological differences between EAE and MS that need consideration in the development of potential therapies for MS.

Therapeutic developments from EAE have translated poorly to human MS. For example, only a few molecules that showed efficacy in EAE have been successful in MS trials. One of these molecules is Glatiramer acetate, a synthetic amino acid copolymer originally designed to mimic encephalitogenic MBP, but instead suppresses EAE by other mechanisms and reduced MS relapses by 30%[99,100]. However, the efficacy of Glatiramer acetate has been questioned by a systematic Cochrane review which calls into question the use of Glatiramer acetate in MS[101]. Tysabri (Natalizumab) and Gilenya (fingolimod) are the only two other drugs that have been licence for use in human MS. Natalizumab binds to the α4 subunit of α4β1 and α4β7 integrins and blocks binding to their endothelial receptors (VCAP-1 and mucosal addressin-cell adhesion molecule 1, thereby attenuating inflammation and ongoing inflammation[102,103]. Fingolimod is a sphingosine-1-phosphate-receptor modulator that prevents egress of lymphocytes from lymph nodes and significantly improved relapse rates compared to placebo controls[104]. At present therefore, it remains unclear why pre-clinical EAE studies predict treatment efficacy in human MS so poorly, however, EAE is still an important first-line model system in the development of new treatments for MS.

BETTER ANIMAL MODELS OF MULTIPLE SCLEROSIS

To make greater progress, better animals models of MS need to be considered when testing new drugs. One fact that to be borne in mind is the fact that MS is a heterogeneous disease in terms of genetics, environmental effects, disease course, pathological treatments and treatment responsiveness[105]. Currently the majority of experiments are performed in inbred strains while clinically, the molecular mechanisms that determine the efficacy during prolonged follow-up of hundreds of patients are far more complex. Thus, it is likely that genetic differences account for some of the inter-patient differences in clinical efficacy of various drugs. Improved animal models may take into account the therapeutic effect of drugs in more than one animal model, treatments to be instigated after disease onset, long-term disease periods, use spontaneous disease models and incorporate human risk. The use of partially humanized mouse models to address the genetic and disease variability are a step in the right direction towards developing better therapeutics for MS[106].

SUMMARY AND FUTURE PROSPECTS

Studies have shown that antagonism of the CB_1 receptor pathway contributes to neuronal damage in CREAE and the relative worsening of tremor and spasticity. The addition of exogenous CB_1 agonists derived from cannabis afforded significant neuroprotection from the consequences of inflammatory CNS disease in EAE and EAU models. Although clear neuroprotective benefits of cannabinoids have been demonstrated, the unwanted psychotropic effects need to be addressed. The adverse psychotropic effects, its role in appetite, pain and cognition together with the observation that the CB_1 receptor is downregulated in some neurodegenerative diseases limits the usefulness of cannabinoids in the treatment of MS. Alternative treatments may be more useful and includes the development of drugs based on CBD, the non-psychoactive part of cannabis that possess anti-inflammatory and anti-oxidant properties. Furthermore,

the CB_2 receptor is being recognised as a potential target since it regulates neuroinflammation and neurogenesis while being non-psychoactive.

In summary, the endocannabinoid system exerts multiple actions and may be useful in the development of therapies to treat neurodegenerative diseases. However, the biggest challenge remains, namely the development of drugs that lack the adverse psychoactive side effects.

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