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INVITED REVIEW

Can cannabinoids be a potential therapeutic tool in amyotrophic lateral sclerosis?

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Abstract

Amyotrophic lateral sclerosis (ALS) is the most common degenerative disease of the motor neuron system. Over the last years, a growing interest was aimed to discovery new innovative and safer therapeutic approaches in the ALS treatment. In this context, the bioactive compounds of Cannabis sativa have shown antioxidant, anti-inflammatory and neuroprotective effects in preclinical models of central nervous system disease. However, most of the studies proving the ability of cannabinoids in delay disease progression and prolong survival in ALS were performed in animal model, whereas the few clinical trials that investigated cannabinoids-based medicines were focused only on the alleviation of ALS-related symptoms, not on the control of disease progression. The aim of this report was to provide a short but important overview of evidences that are useful to better characterize the efficacy as well as the molecular pathways modulated by cannabinoids.

Key Words: *amyotrophic lateral sclerosis; cannabinoids; symptomatic ALS treatment; experimental ALS model; clinical trials; mechanisms of neuroprotection*

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Amyotrophic Lateral Sclerosis (ALS)

Amyotrophic lateral sclerosis (ALS) is the most common degenerative disease of the motor neuron system. The incidence is about 1–3 cases per 100,000 population per year. In Italy it is estimated that at least 3,500 patients and 1,000 new cases per year (http://www.osservatoriomalattierare.it/sla). ALS is characterized by relentless progression of muscle wasting and weakness until death ensues typically due to respiratory muscle failure. Generally, ALS patients present a number of clinical symptoms, including weakness, spasticity, cachexia, dysarthria and drooling, and pain secondary to immobility, among others (Zarei et al., 2015).

The most abundant forms of ALS are sporadic (90%), but the disease may be also familiar (10%), associated with mutations in the superoxide dismutase-1 gene (*SOD-1*), that encodes for a key antioxidant enzyme, and also in TAR-DNA binding protein-43 (TDP-43) and FUS (fused in sarcoma) which encode proteins involved in pre-mRNA splicing, transport and stability (Hardiman et al., 2011). Recently, mutation in non-coding hexanucleotide repeat sequence (GGGGCC) in the *C9orf72* gene was considered as the most common genetic cause of ALS (Matamala et al., 2016). The exact function of this protein remains undefined; however, it seems to play a major role in cellular trafficking, mainly in neurons (Williams et al., 2013). The *C9orf72* mutation was found also in frontotemporal dementia (FTD) patients (Farg et al., 2014). Since 20% of ALS patients develops dementia with a frontotemporal phenotype, this mutation may explain the link between familial FTD and ALS (Farg et al., 2014).

Although the pathogenic mechanisms that underlie ALS are yet unknown, it is believed that ALS could have a multifactorial etiology, where environmental factors can greatly contribute to pathology triggering. Moreover, several mechanisms including mitochondrial dysfunction, protein aggregation, oxidative stress, excessive glutamate activity, inflammation and apoptosis are involved in ALS pathogenesis leading to motor neuron cell death in the brain and spinal cord (Zarei et al., 2015).

To date, the only therapy available for ALS is the glutamate-antagonist riluzole that was able to inhibit the presynaptic release of glutamate, most likely by blockade of voltage-gated sodium channels. However, riluzole has limited therapeutic efficacy and also it is able to moderately prolong patient survival (Miller et al., 2007). Therefore, new innovative and safer therapeutic approaches are urgently needed, at least aimed at delaying the neurodegenerative processes of the ongoing disease.

Over the last years, a growing interest has been focused to cannabinoids, the bioactive compounds of *Cannabis sativa*, for their antioxidant, anti-inflammatory and anti-excitotoxic effects exhibited in preclinical models of central nervous system disease (Croxford, 2003). Here, we provided an overview of the potential usefulness of cannabinoid agents in the management of ALS.

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Overview on Cannabinoids

The Cannabis plant, also known as marijuana, contains over 500 natural compounds and about 70 of these are classified as cannabinoids (Fischedick et al., 2009). The discovery of $^{\Delta 9}$-tetrahydrocannabinol (THC) as the major psychoactive principle in Cannabis, as well as the identification of numerous non-psychoactive cannabinoids such as cannabidiol (CBD), cannabigerol (CBG), cannabinol (CBN), cannabichromene (CBC), $^{\Delta 9}$-tetrahydrocannabivarin ($^{\Delta 9}$-THCV) and cannabidivarin (CBDV), has led to a significant growth in research aimed at understanding the therapeutic effects of these compounds.

Cannabinoids exert many of their activities by binding cannabinoid (CB) receptors. To date, two types of receptors have been identified to have different tissue distribution and mechanisms of signaling. CB_1 receptors are expressed mainly on neurons and glial cells in various parts of the brain, CB2 receptors are found predominantly in the cells of immune system. Both CB_1 and CB_2 receptors belong to the family of G-protein coupled receptors (GPCRs) that, after cannabinoid agonist binding and signaling, exert an inhibitory effect on adenylate cyclaseactivity, activation of mitogen-activated protein kinase, regulation of calcium and potassium channels, and other signal transduction pathways (Munro et al., 1993). Moreover, there is increasing evidence supporting the existence of additional cannabinoid receptors (no-CB_1 and no-CB_2) in both central and peripheral system, identified in CB_1 and CB_2-knockout mice, involving intracellular pathways that play a key role in neuronal physiology. This kind of receptors includes transient receptor potential vanilloid type 1 (TRPV1), G protein-coupled receptor 55 (GPR55), G protein-coupled receptor 18 (GPR18), G protein-coupled receptor 119 (GPR119) and 5-hydroxytryptamine receptor subtype 1A (5-HT1A) (Pertwee et al., 2010). $^{\Delta 9}$-THC, of which is well-known psychotropic effects, is believed to perform the majority of itsactions in the CNS binding CB_1 and CB_2 receptors. Non-psychotrophic phytocannabinoids exert multiple pharmacological effects *via* CB_1/CB_2 receptors as well as no-CB_1 and no-CB_2 receptors (Pertwee et al., 2010).

Overall, recent studies showed that cannabinoids inhibit the release of pro-inflammatory cytokines and chemokine in neurological preclinical models suppressing in this way the inflammatory response (Velayudhan et al., 2014). They show also a potent action in inhibiting oxidative and nitrosative stress, modulating the expression of inducibile nitric oxide synthase and reducing the production of reactive oxygen species (ROS) (Velayudhan et al., 2014). Moreover, cannabinoids were found to exert anti-glutamatergic action by inhibiting glutamate release and enhancing the effect of the inhibitory neurotransmitter gamma-aminobutyric acid (GABA) (Croxford, 2003). Just about all these properties exhibited by these compounds, have prompted researchers to investigate their potential therapeutic effects in ALS, providing interesting results.

Neuroprotective Effects of Cannabinoids in Experimental Model of ALS

Recent *in vivo* studies support that cannabinoids may be

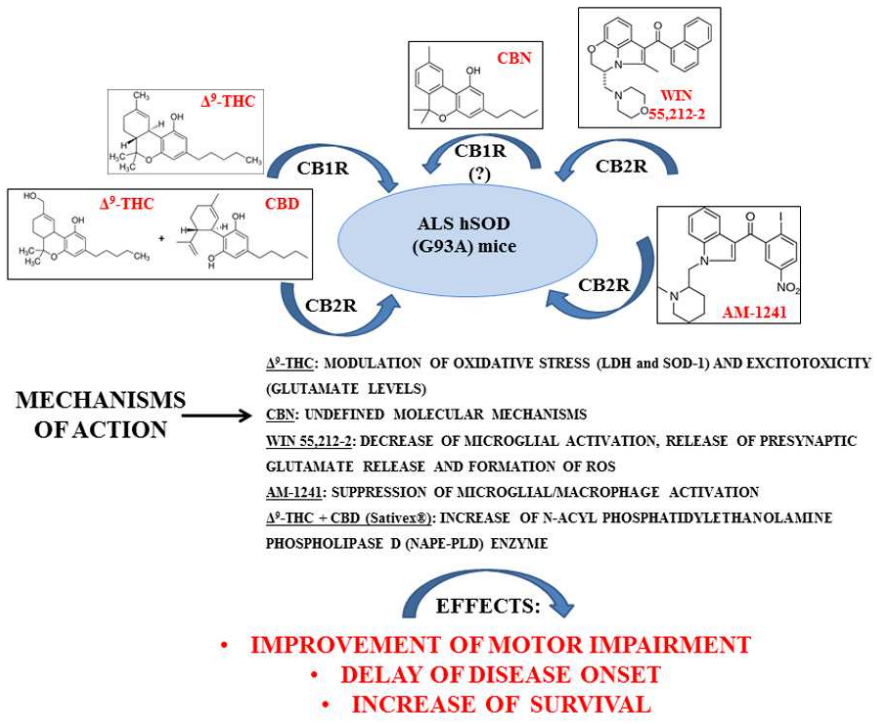

Figure 1 Schematic illustration of the neuroprotective mechanisms of action of cannabinoids into ALS hSOD(G93A) mice.
$^{\Delta 9}$-THC: $^{\Delta 9}$ tetrahydrocannabinol; ALS: amyotrophic lateral sclerosis; CBD: cannabidiol; CB_1R: cannabinoid 1 receptor; CBN: cannabinol.

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beneficial as neuroprotective agents in ALS. The most commonly used murine model for human ALS is the hSOD (G93A) transgenic mouse, which is genetically engineered to develop clinical symptoms similar to those observed in humans with ALS.

Treatment with $^{\Delta 9}$-THC in ALS hSOD(G93A) mice, either before or after signs onset, improves motor impairment and increases survival by 5% probably *via* its anti-glutamatergic and anti-oxidant activity (Raman et al., 2004). Moreover, it was demonstrated that $^{\Delta 9}$-THC attenuates oxidative stress in ALS hSOD(G93A) mouse spinal cord primary cultures, that were exposed to the oxidant tert-butyl hydroperoxide (TBH) in the presence of $^{\Delta 9}$-THC and SR141716A, the CB_1 receptor antagonist, as assessed by lactate dehydrogenase (LDH) and SOD-1 release. Specifically, the antioxidant effect of $^{\Delta 9}$-THC was not CB_1-receptor mediated; since the CB_1 receptor antagonist SR141716A did not diminish the antioxidant effect (Raman et al., 2004). $^{\Delta 9}$-THC was found also to protect against excitotoxicity produced by kainic acid in primary neuronal cultures, obtained from ALS hSOD(G93A) mouse spinal cord, by activation of CB_1 receptor. In this case, the neuroprotective effect was blocked with the CB_1 receptor antagonist, SR141716A, indicating a receptor-mediated effect (Raman et al., 2004). Therefore, treatment with cannabinoids may reduce elevated glutamate levels observed during ALS by modulating excitotoxicity events.

Moreover, treatment with cannabinol (CBN), a non-psychotropic cannabinoid, through its residual affinity to CB_1 receptors, is able to delay significantly disease onset in ALS hSOD(G93A) mice subcutaneously implanted with osmotic mini-pumps. However, the molecular mechanisms remain undefined. On the contrary, survival was not affected (Weydt et al., 2005).

Likewise, a significant delay in disease progression was found when CB_1/CB_2 receptor agonist WIN 55,212-2 was intraperitoneally administered to ALS hSOD(G93A) mice beginning after onset of motor impairment and tremor (at 90 days old), however, survival was not extended (Bilsland et al., 2006). Genetic ablation of the fatty acid amide hydrolase (FAAH) enzyme, which results in raised levels of the endocannabinoid anandamide, prevented the appearance of disease signs in 90-day-old to ALS hSOD(G93A) mice. However, elevation of cannabinoid levels with either WIN55, 212-2 or FAAH ablation had no effect on life span. On the contrary, CB_1 deletion had no effects on disease onset in ALS hSOD(G93A) mice, but extend lifespan by 15 days, a 13% increase in survival. Therefore, the beneficial effects exhibited by cannabinoids may be mediated by non-CB_1 receptors, but presumably by CB_2 ones. Moreover, the neuroprotective effects of cannabinoids were ascribed to a decrease of microglial activation, presynaptic glutamate release and formation of ROS (Bilsland et al., 2006).

Also, it was demonstrated that mRNA, receptor binding and function of CB_2, but not CB_1, receptors are dramatically and selectively up-regulated in the spinal cords of ALS hSOD(G93A) mice in a temporal pattern paralleling disease progression (Shoemaker et al., 2007). It was found that daily intraperitoneal administration of the selective CB_2 agonist, AM-1241, initiated after disease onset in ALS hSOD(G93A) mice, delayed motor impairment and increased survival by 56%. The beneficial effects of cannabinoids could potentially be mediated *via* CB_2 receptor-mediated suppression of microglial/macrophage activation in the spinal cords of symptomatic G93A mice and that CB_2 receptors are selectively up-regulated in spinal cords as a compensatory, protective measure (Shoemaker et al., 2007).

Few years ago, the neuroprotective effects of a mixture of two extracts in approximately a 1:1 ratio (2.7 mg of $^{\Delta 9}$-THC and 2.5 mg of CBD) commercially known as Sativex® were investigated by using ALS hSOD(G93A) transgenic mice (Moreno-Martet et al., 2014). Sativex® was found to be effective in delaying ALS progression in the early stages of disease and in animal survival, although the efficacy was decreased during progression of disease. Also, it has been demonstrated that changes occur in endocannabinoid signaling, particularly a marked up-regulation of CB_2 receptors in SOD(G93A) transgenic mice together with an increase of N-acyl phosphatidylethanolamine phospholipase D (NAPE-PLD) enzyme, which is responsible for the generation of anandamide (N-arachidonoylethanolamine), the ligand of cannabinoid and vanilloid receptors (Moreno-Martet et al., 2014). Therefore, the efficacy of cannabinoids in slowing ALS progression, in extending life expectancy and in reducing the overall gravity of the disease is mainly due to activation of CB_2 receptors. More specifically, it was widely demonstrated that drugs activating CB_2 receptors, expressed predominantly in immune cells and non-neuronal tissues, successfully improve the symptoms of several inflammatory diseases (Walter and Stella, 2004). However, further studies are necessary to assess the neuroprotective effects of cannabinoids that target CB_2 receptors. Molecular mechanisms underlying cannabinoids-driven neuroprotective effects in ALS hSOD(G93A) mice model are illustrated in **Figure 1**.

Potential Therapeutic Effects of Cannabinoids in Human ALS

The cannabinoid system seems to be involved in the pathogenesis of ALS. Spinal cord from ALS patients demonstrate motor neurons damages marked by CB_2-positive microglia/macrophages. Moreover, a recent study analyzing activated microglia from spinal cord in human ALS patients demonstrated a CB_2 receptors increase. So all these data show how editing CB_2-mediated processes could change ALS progression and how much the endocannabinoid system is potentially involved in reducing neuro-inflammation, excitotoxicity, and oxidative cell damage (Yiangou et al., 2006). The possibility that cannabinoids may provide therapeutic effects in ALS has been also investigated at the clinical level. However, the small number of people with ALS that reported using *Cannabis* and the few studies performed on human ALS, makes difficult the interpretation of the achieved results. Nevertheless, it is believed that *Cannabis* could be useful in the symptomatic treatment of ALS.

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According to a single observational study of patients with ALS only the 10% who admitted consuming *Cannabis*, revealed moderate relief of several symptoms, including appetite loss, depression, pain, and drooling was found (Carter and Rosen, 2001; Amtmann et al., 2004).

In addition, spasticity is also major problem for ALS patients, which reported that *Cannabis* can subjectively improve spasticity (Amtmann et al., 2004). Moreover, a randomized, double-blind crossover study investigating the safety and tolerability of $^{\Delta 9}$-THC in ALS patients revealed that oral $^{\Delta 9}$-THC administration was well tolerated, but a non-significant attenuation of cramp frequency and intensity were found. Other studies confirmed the same results, demonstrating that *Cannabis* is remarkably safe with realistically no possibility of overdose.

There are no clinical studies so far that have tried to prove the potential of cannabinoids as disease-modifying therapies as widely supported by experimental studies, so this hypothesis remains a major challenge for future research.

Conclusion

In light of the above findings, there is a valid rationale to propose the use of cannabinoid compounds in the pharmacological management of ALS patients. Cannabinoids indeed are able to delay ALS progression and prolong survival. However, most of the studies that investigated the neuroprotective potential of these compounds in ALS were performed in animal model, whereas the few clinical trials that investigated cannabinoids-based medicines were focused only on the alleviation of ALS-related symptoms, not on the control of disease progression. This remains the major challenge for the future and it may be facilitate by the recent approval of the first cannabinoid-based drug (Sativex®) available for clinical use. In the last years, a growing interest is focused on the combination drug approach with existing medications in order to maximize the therapeutic efficacy and minimize the adverse effects commonly observed with conventional therapies. We strongly hope to have provided a short but important overview of evidences that are useful to better characterize the efficacy as well as the molecular pathways modulated by cannabinoids. We hope that our studies could be an alert to encourage the scientific community to further studies to confirm the therapeutic use of cannabinoids in this devastating disease.

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Conflicts of interest: *None declared.*

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