

SHORT REPORT

Cannabinol delays symptom onset in SOD1 (G93A) transgenic mice without affecting survival

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Abstract

Therapeutic options for amyotrophic lateral sclerosis (ALS), the most common adult-onset motor neuron disorder, remain limited. Emerging evidence from clinical studies and transgenic mouse models of ALS suggests that cannabinoids, the bioactive ingredients of marijuana (*Cannabis sativa*) might have some therapeutic benefit in this disease. However, Δ^9 -tetrahydrocannabinol (Δ^9 -THC), the predominant cannabinoid in marijuana, induces mind-altering effects and is partially addictive, compromising its clinical usefulness. We therefore tested whether cannabitol (CBN), a non-psychoactive cannabinoid, influences disease progression and survival in the SOD1 (G93A) mouse model of ALS. CBN was delivered via subcutaneously implanted osmotic mini-pumps (5 mg/kg/day) over a period of up to 12 weeks. We found that this treatment significantly delays disease onset by more than two weeks while survival was not affected. Further research is necessary to determine whether non-psychoactive cannabinoids might be useful in ameliorating symptoms in ALS.

Key words: *Motor neuron disease, cannabinoids, therapy, mouse model*

Introduction

Amyotrophic lateral sclerosis (ALS) is a devastating adult-onset neurodegenerative disease characterized by the progressive loss of motor neurons from the spinal cord, brainstem and motor cortex (1). The vast majority of cases are sporadic and their etiology unknown. Oxidative stress, excitotoxicity, neuro-inflammation and disrupted trophic support are some of the factors implicated in ALS pathogenesis (2). The disease is always fatal, typically within five years, and therapeutic options are limited to symptom management and riluzole, an anti-glutamergic compound that modestly slows disease progression in humans (3).

Cannabinoids are potentially beneficial in ALS, for they inhibit excitatory transmission and immune responses and affect muscle tone (4,5). A recent drug screening study using an *in vitro* model of Huntington's disease has shown that four major plant derived cannabinoids: Δ^9 -tetrahydrocannabinol (Δ^9 -THC), Δ^8 -tetrahydrocannabinol (Δ^8 -THC), cannabitol (CBN) and cannabidiol (CBD), have a strong cell survival promoting effect (6). The

mechanism underlying this protective effect is not understood, but interestingly the pharmacological characteristics suggest that the protection is likely to be receptor-independent and not related to anti-oxidant properties (6). The therapeutic potential for cannabinoids in ALS is already being explored clinically. A phase II clinical study of Δ^9 -THC in ALS has shown some benefit in relieving symptoms such as spasticity and insomnia (7). Also, in a recent survey ALS patients reported symptom relief from recreational use of marijuana (8). Raman et al. reported recently that daily injections of 5–10 mg/kg Δ^9 -tetrahydrocannabinol (Δ^9 -THC), the major psychoactive cannabinoid, can modestly but significantly extend survival in an ALS mouse model, possibly via its anti-glutamergic activity (9). Also, we reported that certain endocannabinoids, the endogenous ligands of CB-receptors, are elevated early during ALS pathogenesis in the SOD1 (G93A) transgenic mouse model, suggesting that they participate in a defense response (10).

It should be emphasized that cannabinoids possess several pharmacological properties that favor their

potential use as therapeutic agents for neurological diseases (4): they have a remarkably low toxicity and are lipophilic, allowing them to readily cross the blood-brain barrier. Finally, many of the metabolites retain the biological activity of the original compounds, enhancing and prolonging the biological effects.

Unfortunately, the beneficial properties are accompanied by psychotropic effects, which compromise clinical application. To circumvent these unwanted effects, we tested whether non-psychotropic cannabinoids, such as cannabitol (CBN) can delay disease progression and extend survival in the G93A SOD1 mouse model of ALS.

Material and methods

Transgenic animals

Eighteen age-matched male Tg(SOD1-G93A)2Gur (11) mice were obtained from Jackson Laboratories (Bar Harbor, MA; as a generous gift from Project ALS) and at 42 days (6 weeks) of age were assigned randomly to either the treatment or placebo group.

Drug delivery

Mini-osmotic pumps (ALZET2004) were loaded with 17.5 mg/ml CBN (Sigma) dissolved in polyethylene glycol 400 (PEG 400) (Fluka) or PEG 400 alone according to manufacturer's instructions. These parameters were chosen to release 125 μ g CBN/day, equivalent to a dose of 5 mg/kg/day in a 25 g mouse. Under isoflurane inhalation anesthesia, the pumps were placed surgically into a subcutaneous pouch between the shoulders. After 28 days the pumps were replaced (up to two times) until the animals reached end-stage.

Mouse monitoring

An investigator blinded to the experimental condition assessed disease progression using a motor score and the PaGE test as described previously (12). A motor score of 4 indicated no motor deficit, 3 indicated that animals suspended by the tail exhibited hind limb tremors, 2 indicated gait abnormalities, 1 indicated the dragging of at least one hind limb and 0 indicated end-stage, i.e., when animals that were laid on their back were unable to right themselves within 30 sec. Onset was defined retrospectively as the earliest time when the mice showed symptoms (i.e., score <4) for ≥ 2 consecutive weeks. End-stage was defined as a motor score of 0 or a weight loss of >20%, whichever occurred first. All experiments and animal care were performed in accordance with the University of Washington IACUC guidelines.

Data analysis

Statistical analysis (Kaplan-Meier curves, Logrank test) was performed using Prism 3.0 (Graph Pad,

CA) and $p < 0.05$ was considered to be statistically significant.

Results

We used three behavioral parameters to monitor disease progression and survival in the cannabitol treated mice and their controls. Hind-limb tremors when suspended by the tail are typically the earliest behavioral abnormalities that can be detected (12). Figure 1A shows that the onset of these mild motor abnormalities was significantly delayed by 17 days ($p = 0.0092$) for the CBN treated group compared to vehicle treated controls. The median time of onset of functional motor difficulties as assessed with the PaGE test was 100 days for the placebo and 108 days for the CBN treated groups (Figure 1B). This difference however did not reach statistical significance ($p = 0.112$). Finally, there was no difference in mean age at which the animals from either group reached end-stage, our surrogate marker for survival (127 days, both groups) (Figure 1C).

Discussion

Our results show that CBN delays symptom onset in SOD1 (G93A) mice without affecting survival. The present study is the first to investigate the effect of a non-psychotropic cannabinoid in murine transgenic ALS and to report the use of subcutaneously implanted osmotic mini-pumps for cannabinoid delivery in this or any other neurological disease model. The dose of 5 mg/kg/day and the repeated pump replacements were well tolerated.

The discrepancy between the effect of CBN on symptom onset and survival is unusual and surprising. Several explanations could account for our observation. Possibly, CBN, through its residual affinity to CB1 receptors, acts as an anti-spastic agent and masks the earliest symptoms without affecting our surrogate marker of survival. This interpretation would suggest that CBN has therapeutic potential in symptom control for ALS rather than affecting disease progression or survival. An alternative possibility is that CBN, in addition to its beneficial effect, has considerable toxicity, which eventually leads to an accelerated disease progression and offsets the delay in symptom onset. Further studies, especially histological analysis, are needed to determine the true nature of the CBN effect on symptom and disease progression in ALS. Another line of research would be to determine if different doses and regimens of CBN have more pronounced effects and particularly whether treatment beginning after symptom onset is equally beneficial in this model.

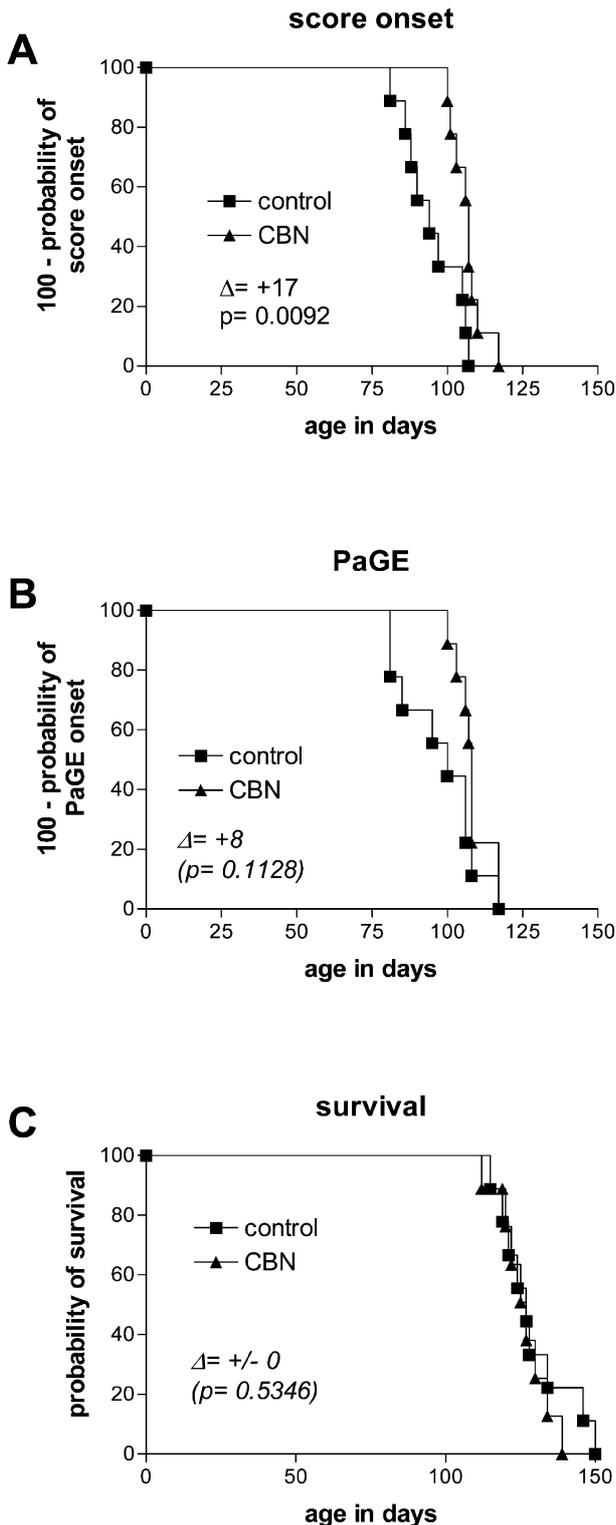


Figure 1. Cannabinol (CBN) delays symptom onset, but does not prolong survival in the SOD1 (G93A) transgenic mouse model of ALS. (A) Cumulative probability of onset of symptoms in the scoring system. (B) Functional motor deficits measured with the paw grip endurance test (PaGE). (C) Survival was not affected by the CBN treatment. Δ : Difference between treatment and control group in days. p indicates statistical significance as assessed by the Kaplan-Meier curves and Logrank test.

Conclusion

In conclusion, we found that the non-psychotropic cannabinoid CBN delays disease onset in the SOD1 model of ALS. However, this regimen did not affect survival. While it is tempting to speculate that the delay in disease onset reflects symptom-modulating activity of CBN, further research, including histological studies, is necessary to fully understand this effect.

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