Cannabidiol Treatment in a Murine Model of Systemic Lupus Erythematosus Accelerates Proteinuria Development

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ABSTRACT:

Background: Cannabidiol is a non-psychotropic component of cannabis sativa. Known to induce an immunomodulatory effect, cannabidiol's therapeutic properties have been explored in a number of autoimmune diseases. Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by its varied manifestation and systemic involvement.

Objectives: To evaluate the effect of cannabidiol on disease progression in a murine model of SLE.

Methods: NZBxW/F$_1$ mice were treated with either cannabidiol or vehicle by subcutaneous injections. Disease progression was estimated by measurements of proteinuria, histological evaluation of renal disease, levels of anti-dsDNA antibodies, and survival analysis.

Results: Mice treated with cannabidiol exhibited rapid progression of glomerular disease with significantly increased proteinuria compared to the control group. Survival analysis revealed a trend ($P = 0.058$) toward decreased survival among mice treated with cannabidiol. No statistical difference was observed in levels of anti-dsDNA antibodies.

Conclusions: Cannabidiol accelerated disease progression in a murine model of SLE. Caution is advised in cannabinoid treatment for SLE patients until further data are collected.

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KEY WORDS: cannabidiol, cannabinoids, cannabis, NZBxW/F$_1$, systemic lupus erythematosus (SLE),

S ystemic lupus erythematosus (SLE) is an autoimmune disease. Known also as "the disease of a thousand faces," lupus can manifest in numerous fashions, ranging from cutaneous involvement to renal failure and neurological symptoms, with arthritis being the most common manifestation [1,2]. As with most autoimmune diseases, a clear understanding of SLE etiopathology remains elusive. Current theories are based on the mosaic of autoimmunity, suggesting that SLE onset is precipitated by a combination of genetic factors as well as environmental factors [3]. Although research has come a long way and new horizons of therapy are continuously sought, SLE remains one of the 20 main causes of death among women aged 5 to 65 years [4]. Recently, as legalization of medicinal cannabis spreads, cannabinoids have been sought out as a new prospect of therapy for rheumatic diseases [5].

Named after the plant cannabis sativa, the endocannabinoid system is a specialized system composed from unique receptors, endocannabinoid ligands, and enzymes. The two main cannabinoid receptors are referred to as Cannabinoid Receptor 1 (CB1 receptor) and Cannabinoid Receptor 2 (CB2 receptor) [6]. CB1 receptor is highly expressed in the brain and is presumably responsible for the psychotropic effects of cannabis. Nevertheless, expression of CB1 receptor is not limited to the brain, with evidence attesting to CB1 expression through the body [5]. Contrary to CB1 receptor, CB2 receptor is mainly referred to as the peripheral receptor, with conflicting evidence regarding its expression in the brain [7]. Of special note is CB2's wide expression on immune cells, demonstrating the potential effect that cannabinoids may hold on autoimmune diseases [8]. The remaining key components of the endocannabinoids system are the endocannabinoid ligands anandamide (AEA) and 2-arachidonoylglycerol (2-AG), which act as agonists to the cannabinoid receptors. Degradation of AEA and 2-AG is mainly executed by fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL), respectively [5].

Exogenous stimulation of the endocannabinoid system is predominantly attributed to tetrahydrocannabinol

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(THC), the main constitute of cannabis sativa. Stimulating both the CB1 and CB2 receptors, tetrahydrocannabinol is the main psychoactive component in cannabis. Contrary to tetrahydrocannabinol, cannabidiol (CBD), the second most abundant constitute in cannabis, is regarded as a non-psychotropic substance [5,6]. Cannabidiol is characterized by a low affinity to CB1 and CB2 receptors and presumed to act as a negative allosteric modulator of CB1 receptor and an inverse agonist of CB2 receptor [9,10]. Despite having a low affinity to CB2 receptor, cannabidiol is known to induce an immunomodulatory effect. Animal studies have supported its therapeutic properties in a number of autoimmune diseases [8,11]. Research on cannabinoids has made marked progress in the field of autoimmunity over the past years, revealing disequilibrium of the endocannabinoid system as a new piece in the mosaic of autoimmunity [5,12].

Focusing on SLE, Navarini and colleagues [12] evaluated the levels of plasma endocannabinoids in SLE patients compared to healthy volunteers. Plasma levels of 2-AG were found to be significantly elevated in SLE patients, while no statistical difference was observed in levels of anandamide. Interestingly, 2-AG plasma levels were significantly increased in SLE patients with a lower clinical score compared to patients with a high clinical score. Moreover, examination of peripheral mononuclear blood cells (PMBC) in SLE patients revealed a positive correlation between CB2 mRNA expression and C3 and C4 plasma levels [12]. These findings suggest that manipulation of the endocannabinoid system in SLE might be therapeutic. Based on these findings, a case for cannabinoids treatment in SLE can be argued.

PATIENTS AND METHODS

ANIMALS
The study was conducted on female NZBxW/F$_1$ mice, a murine model of SLE, characterized by proteinuria, progressive immune complex glomerulonephritis, and presence of antinuclear antibodies [13]. Female NZBxW/F$_1$ mice were purchased from Harlan-Netherlands B.V. Mice were delivered at age 14 weeks, before proteinuria onset. NZBxW/F$_1$ mice were kept in a conventional animal facility with individually ventilated cages. Mice were divided into two groups and received daily treatment as follows: 25 mg/kg cannabidiol (n=10) or phosphate-buffered saline (PBS) (n=11) given subcutaneously, treatment started at week 14. The experiment was approved by Israel Ministry of Health ethics committee and conducted accordingly.

REAGENTS
Synthetic cannabidiol (100%) was provided by Prof. Mechoulam's laboratory (Hebrew University, Jerusalem, Israel). PBS was used as a vehicle.

PROTEINURIA MEASUREMENT
Proteinuria was measured every 2 weeks using Bayer Multistix dipsticks (Bayer, Fernwald, Germany). Proteinuria levels were graded according to the manufacture, ranging from undetectable protein levels to 2000 mg/dl.

HISTOLOGIC EVALUATION OF GLOMERULONEPHRITIS
Renal disease was evaluated by histological staining. Mice were sacrificed at the end of the experiment. Subsequently, kidney samples were obtained and fixated in paraffin. Following fixation, kidney sections were stained by Periodic acid–Schiff (PAS) staining. All slides were analyzed and evaluated by a licensed pathologist.

QUANTIFICATION OF CIRCULATING ANTI-dsDNA ANTIBODIES
Measurement of serum anti-dsDNA antibodies was performed every 2 weeks. Titer levels were quantified by ELISA. Mice sera were diluted and incubated in DNA coated plates. Bound antibodies were detected using peroxidase-conjugated goat anti-mouse IgG (H + L) (Jackson ImmunoResearch Laboratories, Inc., USA). Results were read using a Titertrek ELISA reader (450 nm filter/620 nm reference filter) (ThermoFisher, Massachusetts, USA).

STATISTICAL ANALYSIS
Mann–Whitney test was used to compare proteinuria and anti-dsDNA antibody levels in the two treatment groups. Survival analysis was estimated by Kaplan–Meier protocol and compared by using log-rank test. Statistical analyses were performed using IBM Statistical Package for the Social Sciences statistics software, version 25.0 for Macintosh (SPSS, IBM Corp, Armonk, NY, USA).

RESULTS

CANNABIDIOL'S EFFECT ON PROTEINURIA
Proteinuria is a key marker of disease activity in SLE murine models; hence, proteinuria was used to evaluate disease progression through time. Measurement of mice proteinuria levels commenced at 7 weeks after treatment began.

Compared to the control group, mice treated with cannabidiol had significantly increased proteinuria rates up to week 17, as depicted in Figure 1. At 18 weeks of treatment, proteinuria levels of the two groups started to converge, as at this stage most mice reached peak proteinuria levels. Hence, no statistical difference was observed after week 18. In addition, a marked increase in protein secretion was observed between weeks 13 and 15 among mice who were treated with cannabidiol.

CANNABIDIOL'S EFFECT ON HISTOLOGICAL FINDINGS
In addition to proteinuria, renal disease was also evaluated by histological staining. Kidney samples were obtained at the end

of the experiment and stained with PAS. Histological preparation of mice treated with cannabidiol, as well as mice treated with vehicle, were classified as lupus nephritis class-III.

Yet, as illustrated in Figure 2, subtle histological changes in the two groups were noticeable. Pathological findings of mice treated with cannabidiol revealed focal crescents, which were not found in the control group. These results correlate with the rapid progression of glomerular disease in the cannabidiol group.

CANNABIDIOL'S EFFECT ON SERUM ANTI-dsDNA ANTIBODIES LEVELS

Elevated titer of circulating anti-dsDNA antibodies is one of the hallmark signs of SLE disease as well NZBxW/F$_1$ mice.

To evaluate disease progression antibodies, titer levels were measured every 2 weeks, as displayed in Figure 3. Although titer levels of anti-dsDNA antibodies appeared to be higher in the control group than in the cannabidiol group at the beginning of the follow-up period, no statistical difference was observed.

SURVIVAL ANALYSIS

Despite medical advances, SLE remains a significant cause of death among young women; therefore, improved survival remains an important goal. Correlating the glomerular disease, a clear trend ($P = 0.58$) of decreased survival was observed in mice that received cannabidiol treatment, as illustrated in Figure 4.

DISCUSSION

The field of medicinal cannabis has advanced greatly over the past decade, from being labeled as a narcotic drug to extensive legalization throughout the world, including Australia, Canada, Israel, 14 countries across Europe, and a majority of states in the United States [14]. Furthermore, in some countries, products containing cannabidiol are labeled as a natural supplement and consumption requires no medical supervision [14]. Our study is the first to examine the effect of exogenous cannabinoids in the form of cannabidiol on a murine model of SLE. Based on successful previous studies conducted using cannabidiol on models of autoimmune diseases, such as rheumatoid arthritis and multiple sclerosis, we anticipate that cannabidiol will have a beneficial effect on SLE disease progression [8,11]. Surprisingly, mice treated with cannabidiol exhibited rapid progression of glomerulonephritis. Proteinuria levels of the cannabidiol group were significantly elevated compared to the control group, up to week 17 [Figure 1]. The turning point at week 18 can be ascribed to technical limitations. As mice reached the end of their life expectancy, proteinuria levels also reached their peak levels according to the measure technique. Survival analysis substantiate the previous results. Although not statistically significant, mice treated with cannabidiol exhibited a strong

Figure 1. Cannabidiol's effect on proteinuria levels in NZBxW/F1 mice. Mean proteinuria levels were calculated for each group and compared over time
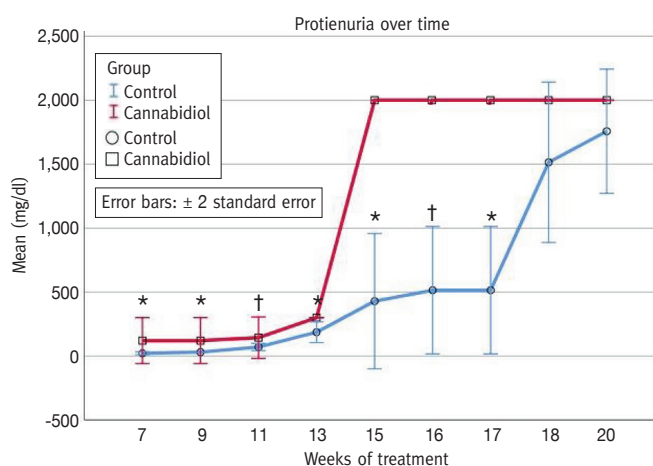

*$P \leq 0.01$
†$P \leq 0.05$

Figure 2. Histologic evaluation of renal disease in NZBxW/F$_1$ mice treated with cannabidiol vs. vehicle. **[A]** Representative kidney sample from the control group. Lupus nephritis Class III, highly active, **[B]** Representative kidney sample from the cannabidiol group. Lupus nephritis Class III, focal crescents
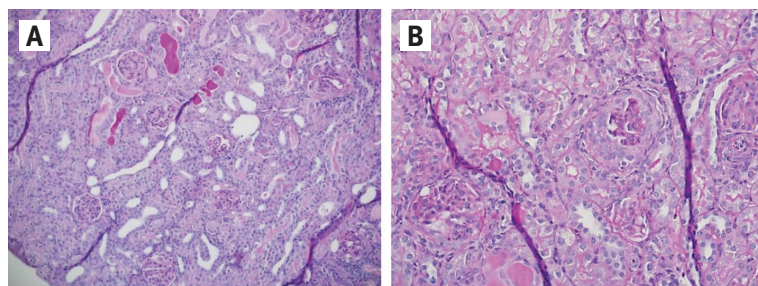

trend for decreased survival compared to control ($P = 0.058$). Contrary to the proteinuria results, no difference was observed in the anti-dsDNA results in the two groups. The conflicting results between proteinuria levels and anti-dsDNA antibody levels raise the question whether cannabidiol's deleterious effect on NZBxW/F$_1$ mice originates from its effect on disease progression or whether it has a direct effect.

Relying on previous animal studies, it appears that cannabidiol acts as a protective agent in cases of renal injury [15]. Furthermore, clinical trials conducted on cannabidiol support the assumption that cannabidiol does not influence renal function directly. Therefore, there is no foundation to assume the deleterious effect of cannabidiol on renal function is a direct one. Remarkably, a poster describing a prospective cohort study reached similar results to our study. SLE patients consuming cannabis were followed for a duration of 5 years and compared to non-cannabis-consuming SLE patients. Consistent with our

Figure 3. Cannabidiol's effect on circulating anti-dsDNA antibody levels in NZBxW/F₁ mice. Mean anti-dsDNA antibody levels were calculated for each group and compared over time. No statistical significance was observed
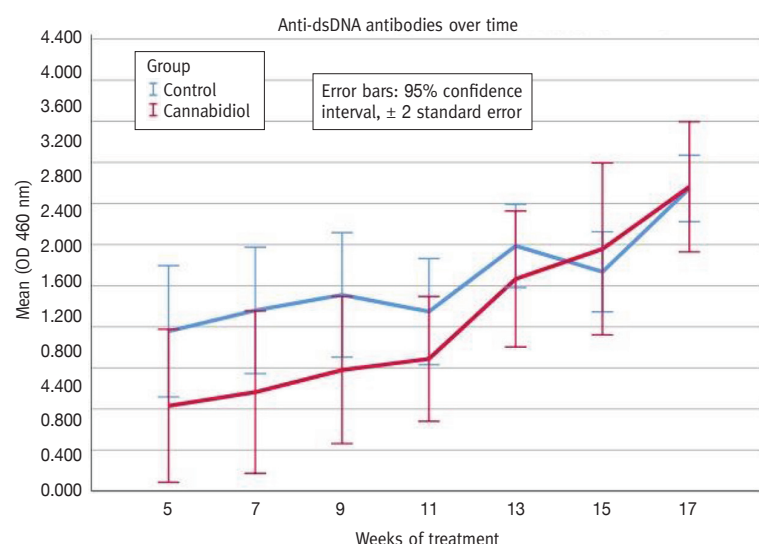
Figure 4. Cannabidiol's effect on NZBxW/F₁ mice survival. Mice treated with cannabidiol exhibited a trend for decreased survival compared to controls ($P = 0.058$)
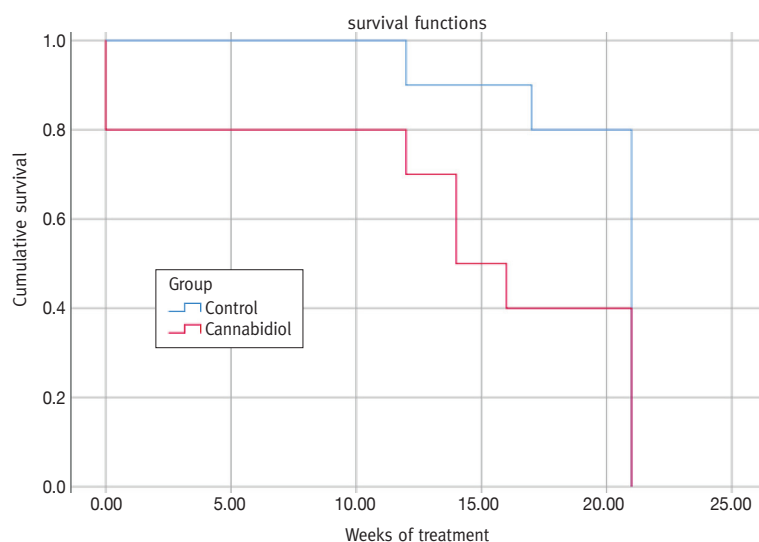
results, consumption of cannabis correlated with an increase in end-stage renal disease requiring dialysis ($P < 0.006$). Although these results complement our findings, further analysis of data revealed an association between cannabis consumption and poor compliance to medical care that could possibly explain the large proportion of end-stage renal disease in SLE cannabis consumers. In addition, parallel to our results, patients consuming cannabis had no differences in levels of anti-dsDNA antibodies compared to non-consuming patients [16]. These

findings support the assumption that cannabidiol might have an idiosyncratic effect on SLE patients, causing severe proteinuria without effecting anti-dsDNA levels. A possible explanation for the variance in cannabidiol's effect on SLE in contrast to rheumatoid arthritis or multiple sclerosis could possibly be ascribed to interleukin 10 (IL-10) and its role in SLE's disease pathogenesis. Although many refer to SLE as the architype of autoimmune diseases, SLE is distinctive in its pathway, hypothesized to be driven by T follicular helper cells [17].

While IL-10 is usually regarded as an anti-inflammatory cytokine, evidence suggests that its part in SLE pathogenesis might be different. Acting not only as an anti-inflammatory cytokine, IL-10's effect on B-cells is dichotomous, both augmenting and reducing B-cells apoptosis, depending on the B-cell state of activation [18]. In SLE, IL-10 levels appear to correlate disease activity as well as organ involvement [19,20]. Furthermore, IL-10's possible pathogenic role in SLE was demonstrated both in NZBxW/F₁ mice and in humans [21,22]. NZBxW/F₁ mice treated with anti-IL-10 antibodies displayed a decrease in disease severity and a delayed disease onset; whereas, mice receiving IL-10 had an accelerated course of disease [21]. Correspondingly, in a clinical trial conducted by Llorente and colleagues [22], anti-IL-10 treatment was shown to reduce disease activity in SLE patients. Although potentially an undesirable side-effect, it is possible that cannabidiol increased the IL-10 levels in NZBxW/F₁ mice, presumably acerbating their condition. Cannabidiol's ability to augment IL-10 levels was previously demonstrated in a murine model of colitis [23]. In addition, analysis of blood samples drawn from cannabis addicts and healthy controls revealed high IL-10 levels among cannabis consumers [24]. The link between cannabis, IL-10, and SLE outcomes can also be exemplified indirectly by the results shown by the Jalil [16] and McCarthy [19] groups. In an experiment by the McCarthy team, high IL-10 levels were found to correlate with CNS involvement in SLE patients [19]. Complementing this idea, during a 5 year follow-up period, Jalil et al. found that cannabis consumption was associate with a higher incidence of neuropsychiatric lupus [16]. Hence, it is possible that the cannabidiol treatment induced an increase in IL-10 secretion, presumably accelerating disease development.

A different hypothesis for the deleterious effect cannabidiol might have on SLE patients lies in the genetic code. A specific genetic variation in the gene coding for CB2 receptor named Q63R was previously identified in humans. Correlating with various autoimmune disease, the Q63R variation reduces CB2 receptor ability to inhibit T cell proliferation [8]. Since cannabidiol is classified as a weak inverse agonist of CB2 receptor [10], it is possible that in genetically susceptible individuals (or strains of mice) cannabidiol could be more harmful than beneficial. Thus far, no data exist regarding Q63R variation and the effect of cannabidiol treatment. A better understanding of the possible implication is warranted, not only in SLE patients

but for all autoimmune diseases. This interaction might explain the discrepancy between the successful results of cannabinoid treatment in animal model of autoimmune diseases (such as rheumatoid arthritis and diabetes [8]) in contrast to the futile results in human trials. Furthermore, based on these findings, a personalized approach for cannabidiol treatment in autoimmune diseases might be advocated until further studies are conducted. While cannabidiol treatment might be beneficial for some autoimmune patients, it is possible that in the specific subset of patients with a CB2 variation it might induce the opposite effect. Since this hypothesis is of relevance only for cannabidiol, we believe that our results do not contradict the therapeutic effect other cannabinoids might have in SLE.

Pathak et al. [25] showed the potential that endocannabinoids might hold in SLE. In contrast to our study, Pathak's group manipulated the endocannabinoid system by inhibition of FAAH, thus causing an indirect increase in anandamide levels. NZB mice treated with URB597 (a FAAH inhibitor) presented decreased levels of total IgM and anti-dsDNA antibodies compared to the control group [25]. With regard to these results, it should be noted that FAAH inhibition could possibly affect the degradation of other fatty acids.

The apparent inconsistency between our results and those of Pathak's laboratory calls for the characterization of the endocannabinoid system in SLE. In an effort to better understand cannabinoids treatment in SLE, we acknowledge that our study has numerous weaknesses, the first being the small sample size. Although the sample size does not suffice for conclusive recommendations to be made, the implications of our results could be of paramount importance. Currently, despite paucity of pre-clinical or clinical data regarding medical cannabis, there are SLE patients receiving medical cannabis. The possibility that cannabidiol might be hazardous for SLE patients is of great importance, emphasizing the necessity of evidence-based medicine.

CONCLUSIONS

Our results suggest that cannabidiol treatment might exacerbate glomerular disease in SLE. Correspondingly, a trend for decreased survival was seen in mice treated with cannabidiol. No statistical differences were observed in levels of serum anti-dsDNA antibodies. Based on our results, we believe there is a great need to better characterize cannabinoid treatment in SLE patients. A prudent approach regarding cannabinoids treatment in SLE is advised.

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