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Dexanabinol (HU-211) effect on experimental autoimmune encephalomyelitis: implications for the treatment of acute relapses of multiple sclerosis

Anat Achiron [a,*], Shmuel Miron [a], Vered Lavie [b], Raanan Margalit [c], Anat Biegon [b]

[a] *Multiple Sclerosis Center, Sheba Medical Center, 52621 Tel-Hashomer, Ramat Gan, Israel*
[b] *Pharmos, Kiryat Weizmann, Rehovot, Israel*
[c] *Department of Cell Biology, Weizmann Institute of Science, Rehovot, Israel*

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Abstract

Dexanabinol (HU-211) is a synthetic non-psychotropic cannabinoid which suppresses TNF-α production in the brain and peripheral blood. The effects of dexanabinol in rat experimental autoimmune encephalomyelitis (EAE) were studied using different doses, modes of administration and time regimes. Dexanabinol, 5 mg/kg i.v. given once after disease onset (day 10), significantly reduced maximal EAE score. Increasing the dose or treatment duration resulted in further suppression of EAE. Drug administration at earlier phases during disease induction was not effective. Histological studies supported the clinical findings demonstrating reduction in the inflammatory response in the brain and spinal cord in animals treated with dexanabinol. The results suggest that dexanabinol may provide an alternative mode of treatment for acute exacerbations of multiple sclerosis (MS). © 2000 Elsevier Science B.V. All rights reserved.

Keywords: EAE; Multiple sclerosis; TNF-α inhibition; Relapse; Animal model

1. Introduction

Dexanabinol (HU-211), a synthetic cannabinoid, is a novel substance of the cannabinoid family which lacks psychotropic activity (Mechoulam et al., 1988; Mechoulam et al., 1990) but has NMDA blocking and antioxidant properties (Feigenbaum et al., 1989; Nadler et al., 1993; Eshhar et al., 1995). The drug has been evaluated in a variety of animal models and in healthy human volunteers. This highly lipophilic molecule readily crosses the blood–brain barrier (BBB) and was found to reduce BBB breakdown, brain edema and neuronal death in several animal models of traumatic and ischemic brain injury (Biegon et al., 1993; Shohami et al., 1993; Bar Joseph et al., 1994; Belayev et al., 1995a,b; Shohami et al., 1995; Bass et al., 1996; Yoles et al., 1996). In cultured macrophages, the compound has also been shown to suppress the release of TNF-α following LPS stimulation (Burnette-Curley and Cabral, 1995). The same phenomenon was observed in vivo, whereby dexanabinol suppressed the increase in TNF-α levels in the brain following intracisternal LPS or head injury, and in the plasma following systemic LPS injection (Gallily et al., 1997; Shohami et al., 1997). Phase I clinical trials in young healthy volunteers showed that dexanabinol is safe and well tolerated when administered intravenously at doses up to and including 200 mg (Brewster et al., 1997). The above described mechanisms combined with the safe profile of the drug encouraged us to hypothesize that the use of dexanabinol could be beneficial as a novel treatment approach for neurological damage associated with acute exacerbations of multiple sclerosis (MS). The rationale for dexanabinol treatment in acute exacerbations of MS is based on its ability to penetrate the BBB and inhibit TNF-α production as well as reduce edema and free radical damage that occur during the acute inflammatory response (Raine, 1994). The present study was conducted to evaluate the effectiveness of dexanabinol treatment in the experimental autoimmune encephalomyelitis (EAE) model in the rat. EAE is an inflammatory and demyelinating paralytic disease of the central nervous system resulting from the sensitization of susceptible laboratory animals by myelin antigens or myelin basic protein (MBP) specific T-lymphocytes (Martin and McFarland,

* Corresponding author. Tel.: +972-3-5303811; fax: +972-3-5348186; e-mail: achiron@post.tau.ac.il

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1995). This animal model is used as an analogue to human MS. We have evaluated the effect of dexanabinol in the EAE model to establish its possible role in disease suppression in relation to time, dose and mode of administration.

2. Materials and methods

2.1. Animals

Female Lewis rats bred at the Weizmann Institute of Science, Rehovot, Israel, 6 to 8 weeks old, body weight 180 to 200 g, were used for the animal experiments. Animals were kept under controlled light/dark and temperature conditions and fed ad lib. Each experimental group consisted of 10 rats.

2.2. Active induction of EAE

Animals were inoculated subcutaneously in a hind footpad with an emulsion of 25 μg of guinea-pig MBP in CFA containing 40 μg of *Mycobacterium tuberculosis* (Difco, Detroit, MI) in 0.1 ml of oil.

2.3. Clinical assessment of EAE

Clinical signs of EAE appeared 10–12 days after disease induction. Degree of clinical disease was scored as follows: 0 = no signs; 1 = loss of tail tonicity; 2 = paralysis of hind limbs; 3 = paralysis of all four limbs; 4 = quadriplegic animal, in a moribund state; 5 = death induced by EAE. Disease evaluation continued till recovery (day 18–19 after induction).

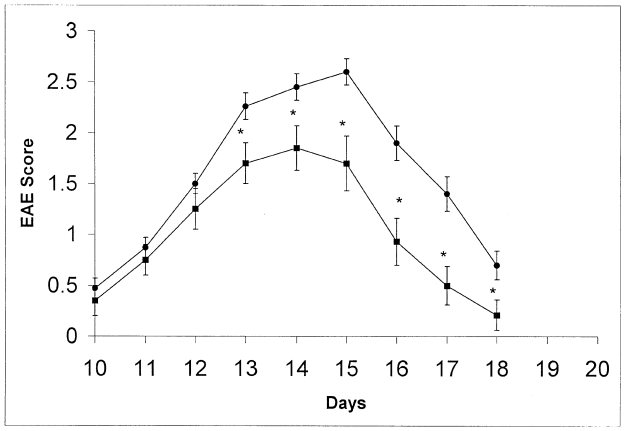


Fig. 2. Effect of two repeated doses of dexanabinol given after disease onset on EAE severity and duration. Two injections of the drug (10 mg/kg i.v.), 6 h apart, were given on the day of disease onset. $^{*}p < 0.05$, one-way ANOVA with repeated measures followed by Duncan's post hoc test. Circles represent vehicle control animals and squares represent treatment with dexanabinol 5 mg/kg.

2.4. Histology

On day 15 after disease induction (peak disease severity), three animals chosen randomly from each group were sacrificed for brain and cord histology as follows: The animals were deeply anaesthetized with Na-pentobarbiton and sacrificed by cardiac perfusion with 4% paraformaldehyde in heparinized PBS. Brains and spinal cords were removed and further fixed over night at room temperature with the same fixative. Whole brains and a cervical and lumbar segment of the spinal cord were processed for histology by dehydration with graded series of ethanols, and embedding in Paraplast (Sigma). Sections (10 μm)

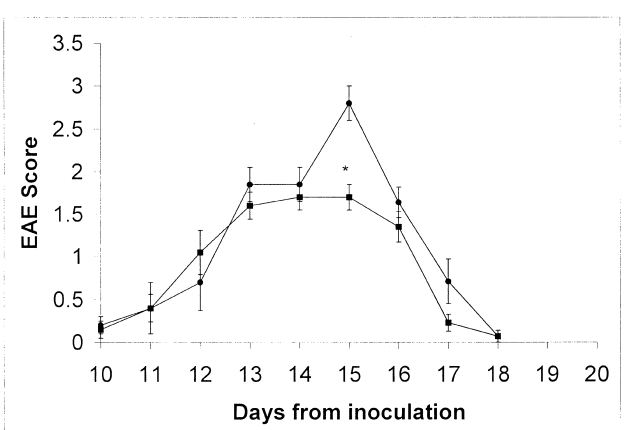


Fig. 1. Effect of a single injection of dexanabinol given on day 10 after disease induction on the course of EAE. The drug (5 mg/kg i.v.) was given once on day 10 following MBP inoculation. $^{*}P < 0.05$, one-way ANOVA with repeated measures followed by Duncan's post hoc test. Circles represent vehicle control animals and squares represent treatment with dexanabinol 5 mg/kg.

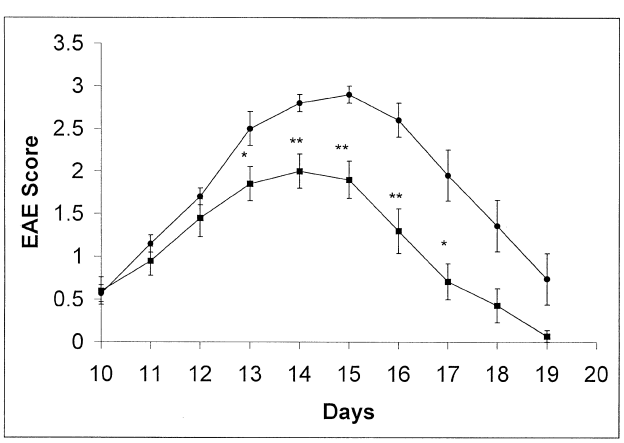
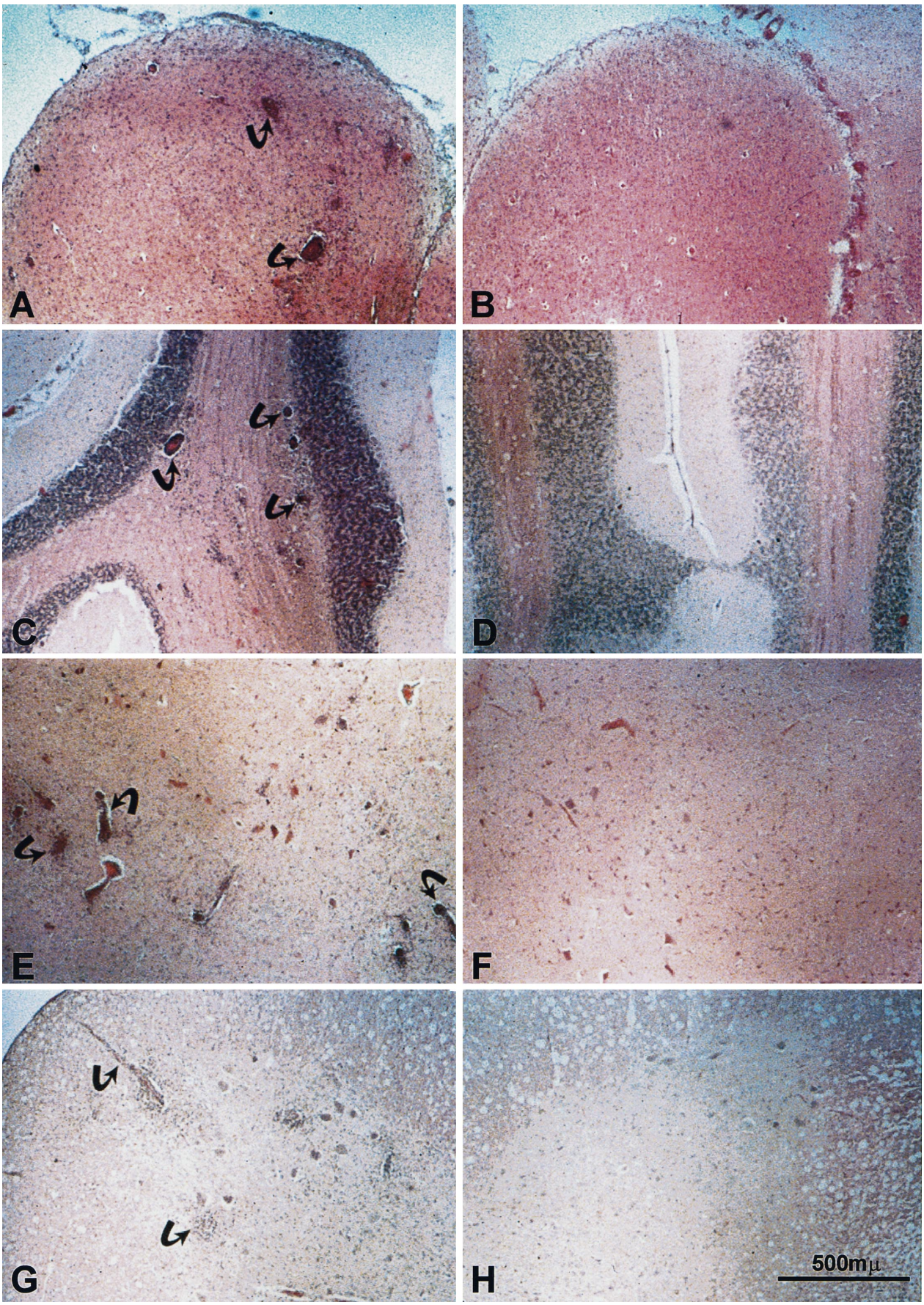


Fig. 3. Effect of three repeated doses of dexanabinol given after disease onset on EAE severity and duration. Three daily injections of the drug (5 mg/kg) were given starting on the day of disease onset. $^{*}p < 0.05$; $^{**}p < 0.01$; one-way ANOVA with repeated measures followed by Duncan's post hoc test. Circles represent vehicle control animals and squares represent treatment with dexanabinol 5 mg/kg.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were stained with hematoxylin and eosin (H&E) and examined by light microscopy. The brains were sectioned horizontally while the spinal cords were cross-sectioned. The sections were examined for the presence of infiltrating foci of white blood cells and lymphocytes. The density of infiltrating foci was examined in the brain sections at the levels of the superior and inferior colliculli (3.6–4.0 Bregma), the cerebellum at the same level and the brainstem (8.4–8.8 Bregma). In the spinal cord, infiltrating foci were examined in duplicate sections, 100 μm caudally apart, at the cervical as well as the lumbar levels.

2.5. Experimental design

2.5.1. Defining the time-regime of dexanabinol treatment during EAE development

Dexanabinol was intravenously administered as a single dose of 5 mg/kg. This dose was used as it was previously shown to reduce inflammation and provide neuroprotection in other models (e.g., Eshhar et al., 1995). The drug was given once on one of the following days: day 0 (inoculation), day 4 (early induction), day 7 (late induction), or day 10 (disease eruption-effector phase). Control groups were either untreated or treated with the dexanabinol vehicle (cosolvent ethanol/emulphore in saline) three vehicle animals per day.

2.5.2. Assessment of optimal treatment paradigm (route, frequency and dose) during the effector phase

(1) Dexanabinol was given on the first day of EAE onset (score = 1/2 or 1) either at a dose of 5 mg/kg intravenously for three successive days, or at a dose of 20 mg/kg (in oil) intraperitoneally by a single injection. Control animals were either untreated or given the appropriate vehicles (cosolvent or oil) at the same time schedule.

(2) Dexanabinol was given on the first day of EAE onset (score = 1/2 or 1) intravenously at three regimes: single injection at a dose of 10 mg/kg, two injections at a dose of 10 mg/kg each, 6 h apart, or three daily injections at a dose of 10 mg/kg each. Control animals received no treatment or the appropriate vehicle in a similar volume and time schedule.

3. Results

3.1. Dexanabinol time regime of efficacy during EAE development

Intravenous administration of dexanabinol, at a dose of 5 mg/kg on day 10 after disease induction reduced peak disease severity (measured on day 15) by 40%, $p < 0.05$ (one-way ANOVA with repeated measures followed by Duncan's post hoc test) (Fig. 1). All other treatment groups (day 0, 4 and 7) were not significantly different from control animals (data not shown). There were no deaths in the dexanabinol day 10 treatment group, compared with two deaths in the other groups.

3.2. Assessment of optimal treatment paradigm (route, frequency and dose) during the effector phase

Intraperitoneal treatment with dexanabinol 20 mg/kg by single injection on the first day of disease eruption, was slightly less effective than the intravenous treatment (30% reduction in peak disease severity, $p = 0.06$). Mortality rates were zero in all the dexanabinol treatment groups while three animals died in the control groups. The 10 mg/kg intravenously administered single dose was also effective, significantly reducing clinical score on days 15 and 16 (data not shown). Two injections of 10 mg/kg had a bigger effect than a single injection, significantly reducing clinical score on days 13 through 18 (Fig. 2). The best treatment paradigm was found to be three consecutive daily injections of dexanabinol at the dose of 5 or 10 mg/kg, starting on the day of disease eruption, which reduced peak disease severity by 50%. Statistically significant reductions in clinical score were also seen on days 13–18 (Fig. 3). The area under the curve, representing the integration of disease severity over time, was reduced by 40% ($p < 0.05$) (data not shown).

Histology: Brain and spinal cord sections of EAE animals showed infiltrating foci (brain sections 85 ± 1, spinal cord 21 ± 12) of extravasated white blood cells and lymphocytes cuffing blood vessels (Fig. 4A,C,E,G). Reduced numbers of infiltrating foci (brain sections 67 ± 18, spinal cord 18 ± 3) were demonstrated in corresponding anatomical areas of animals treated with dexanabinol (Fig. 4B,D,F,H).

4. Discussion

The clinical course of MS is characterised by acute relapses during which scattered inflammatory demyelinating lesions within the central nervous system produce varying combinations of neurological dysfunction. Treatment that has the potential to reduce the neurological sequelae of acute relapses will be of benefit to MS patients, as it will improve the natural outcome and decrease neurological disability over time. The currently used steroid treatment for acute relapses results only in short-term

Fig. 4. Infiltrating foci in brain and spinal cord sections of EAE rats stained with hematoxylin and eosin. Extravasated WBC and lymphocytes (arrows) are cuffing blood vessels. (A, B) Superior colliculus; (C, D) cerebellum; (E, F) brainstem; and (G, H) spinal cord. A, C, E and G show sections of animals with no treatment while B, D, F and H show corresponding anatomical areas of animals treated with dexanabinol.

benefit (Milligan et al., 1987), without any proven effect on neurological outcome (Frequin et al., 1994).

In the present study, we have shown that in the EAE model, dexanabinol reduced disease severity by 30%–50% when given after the onset of clinical signs, in a design that imitates an acute relapse. The experiments defining the time regime of efficacy showed the maximal effect during peak disease stage (day 15) when the drug was given at onset of clinical signs (day 10). Dexanabinol did not interfere with the early stage of EAE induction but rather suppressed the effector phase. When the drug was administered for three consecutive days compared with one single injection, severity of EAE was significantly reduced in days 13 to 18, suggesting longer suppression. Thus, i.v. treatment with dexanabinol over 3 days was more efficacious than a single i.p. or i.v. administration. Increasing the dose resulted in a small additional effect, such that the most effective dose regime to suppress acute EAE appears to be 5–10 mg/kg given intravenously for three consecutive days.

Lyman et al. (1989) evaluated the effect of delta-9 tetrahydrocanabinol given via an oral–gastric tube on the clinical course of EAE. A dosage of 5 mg/kg/day was found to be the smallest effective dose, and treatment was given at days 1, 3, 5, 7 and 9 days post-inoculation. The results of the study showed that with the exception of rats started on delta-9 tetrahydrocanabinol 9 days post inoculation, beginning of treatment at earlier time points corresponded with a delayed onset and significantly reduced clinical signs. In our study, we did not find a similar suppressive effect of dexanabinol on the disease course by early treatment protocols (days 0, 4 and 7 after disease induction). The significant reduction in disease severity was found only by late treatment (day 10), after the onset of clinical signs. Wirguin et al. (1994) tested the effect of another cannabinoid, delta 8 tetrahydrocanabinol, on EAE. They found that a dose of 40 mg/kg administered for 21 days after inoculation significantly reduced the incidence and severity of EAE. However, this effect occurred only on oral administration and not with parenteral injection. In our study, the optimal effect was seen by three consecutive injections of 5 or 10 mg/kg, and the i.v. route was superior to the i.p. route. There are several mechanisms by which dexanabinol could affect the outcome of an acute relapse. Since the treatment did not affect the disease when given during the induction phase it is conceivable that the effect was not related to T-cell activation but mainly to the increase in pro-inflammatory cytokine production that occurs at the time of relapse onset. During acute EAE, the first cytokines that appear in the central nervous system are lymphotoxin and IL-12. This coincides with the first appearance of immunohistochemically detectable inflammatory cells in the brain, shortly before onset of clinical signs (Olsson, 1995). Later, during the acute phase of the disease, IFN-γ, IL-6 and TNF-α appear, and their level of expression parallels the severity of clinical signs and degree of inflammatory cell infiltration (Philippe et al., 1993; Hautecoeur et al., 1997; Van Oosten et al., 1998). Thus, it can be assumed that dexanabinol reduced severity of EAE through TNF-α inhibition. Additionally, it is possible that dexanabinol has a direct neuroprotective effect through scavenging of free radicals as the histological results showed reduced number of necrotic foci and infiltrating cells in animals treated with dexanabinol.

In conclusion, the present study demonstrates the beneficial effect of dexanabinol in the model of EAE affecting the later stages of the immunologic cascade occurring after disease induction, and suggests its potential use as a drug for the treatment of acute exacerbations of MS.

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