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# Cannabidiol ameliorates cognitive and motor impairments in mice with bile duct ligation☆

Iddo Magen[1], Yosefa Avraham[1,*], Zvi Ackerman[2], Lia Vorobiev[1], Raphael Mechoulam[3], Elliot M. Berry[1]

[1]*Department of Human Nutrition and Metabolism, Braun's School of Public Health, Hadassah-Hebrew University Medical Faculty, 91120 Jerusalem, Israel*

[2]*Department of Internal Medicine, Hadassah Mount Scopus, Jerusalem, Israel*

[3]*Department of Medicinal Chemistry and Natural Products, Hadassah-Hebrew University Medical Faculty, Jerusalem, Israel*

***Background/Aims*: The endocannabinoid system in mice plays a role in models of human cirrhosis and hepatic encephalopathy (HE), induced by a hepatotoxin. We report now the therapeutic effects of cannabidiol (CBD), a non-psychoactive constituent of *Cannabis sativa*, on HE caused by bile duct ligation (BDL), a model of chronic liver disease.**

***Methods*: CBD (5 mg/kg; i.p.) was administered over 4 weeks to mice that had undergone BDL.**

***Results*: Cognitive function in the eight arm maze and the T-maze tests, as well as locomotor function in the open field test were impaired by the ligation and were improved by CBD. BDL raised hippocampal expression of the TNF-α-receptor 1 gene, which was reduced by CBD. However, BDL reduced expression of the brain-derived neurotrophic factor (BDNF) gene, which was increased by CBD. The effects of CBD on cognition, locomotion and on TNF-α receptor 1 expression were blocked by ZM241385, an $A_2A$ adenosine receptor antagonist. BDL lowers the expression of this receptor.**

***Conclusions*: The effects of BDL apparently result in part from down-regulation of $A_2A$ adenosine receptor. CBD reverses these effects through activation of this receptor, leading to compensation of the ligation effect.**



***Keywords*: Cognition; Activity; Inflammation; Bile duct ligation; Gene expression**

## 1. Introduction

Bile duct ligation (BDL) is a well-known model of chronic liver disease (also termed "cholestatic liver disease") in rats and, to a lesser extent, in mice. It mimics biliary liver disease in humans [1]. This model displays elevation of liver enzymes and liver fibrosis in rats [2]. Cognitive impairments are also observed [3], and motor deficits in rats are attributed to a decrease in striatal dopamine content [4]. Evidence for the role of inflammation in the BDL model comes from a study that demonstrated infiltration of peripheral TNF-α-

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* Corresponding author. Tel.: +972 2 6757547; fax: +972 2 6431105.

*E-mail address:* yosefa@md.huji.ac.il (Y. Avraham).

*Abbreviations:* HE, human cirrhosis and Hepatic Encephalopathy; CBD, cannabidiol; BDL, bile duct ligation; BDNF, brain-derived neurotrophic factor; LPS, lipopolysaccharide; ECs, endocannabinoids; CBr, cannabinoid receptors; Anandamide, arachidonoyl ethanolamide; 2-AG, 2-arachidonoylglycerol; TAA, hepatotoxin thioacetamide; $A_{2a}$Ars, adenosine receptors; i.p., intraperitoneally; Ct, threshold cycle; BSF, Binational Science Foundation.

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secreting monocytes into the brain 10 days after BDL in mice [5].

Lipopolysaccharide (LPS)-induced neuroinflammation leading to microglial activation was reported previously to be responsible for disrupted neurogenesis in the dentate gyrus region of the hippocampus [6] and for a decrease in the hippocampal level of brain-derived neurotrophic factor (BDNF) [7]. This region is known to play a crucial role in learning and memory [8].

Endocannabinoids (ECs) are a family of lipid messengers that bind to the cell surface receptors (cannabinoid receptors – CBr) also targeted by $\Delta^9$-THC, the major compound of the plant *Cannabis sativa*. Their synthesis in the CNS is triggered by an elevation of intracellular calcium levels both in neurons and in microglia [9], and they are involved in a wide range of intracellular events. Two major endocannabinoids have been identified and well characterized: arachidonoyl ethanolamide (anandamide) [10] and 2-arachidonoylglycerol (2-AG) [11]. They bind to $CB_1$ receptor on axon terminals to regulate ion channels activity and neurotransmitter release by a retrograde messenger mechanism [12]. $CB_2$ receptors exist mainly in the immune system, although recent publications have reported their existence also in the CNS, particularly in some pathological conditions [13]. The levels of 2-AG and the expression of the $CB_1$ and $CB_2$ receptors are increased in the brains of mice with acute HE, induced by the hepatotoxin thioacetamide (TAA) [14,15]. In the liver, stimulation of $CB_1$ receptors enhances fibrosis, whereas that of $CB_2$ receptors suppresses it [16,17].

Cannabidiol (CBD) is a non-psychoactive ingredient of *C. sativa* known to be a weak antagonist of $CB_1$ and $CB_2$ receptors [18]. However, many other actions have been proposed, such as adenosine reuptake inhibition and activation of 5-HT1a receptors [19]. It also has very strong anti-inflammatory activity both *in vivo*, as an anti-arthritic agent [20], and *in vitro*, manifested by inhibition of cytokine production in immune cells [21].

As cerebral inflammatory responses correlate well with cognitive and motor deficits, which are hallmarks of BDL-induced HE, we have investigated whether the anti-inflammatory CBD might reverse these deficits in BDL animals. We assumed that markers of inflammation, such as COX-2 and TNF-α receptor 1, would increase in the hippocampus after BDL, leading to cognitive and motor impairments, which would decrease after CBD administration. We also postulated that the inflammatory response would affect cognition through decrease in the hippocampal level of the neurotrophin BDNF, which is crucial for learning and memory, as was reported before [7]. As CBD is a functional agonist of adenosine inhibiting its reuptake, we also addressed the question whether its effects are mediated *via* the adenosine receptors ($A_{2a}$ARs) and whether the expression of these receptors is altered in BDL.

## 2. Materials and methods

### 2.1. Animals and surgery

Groups of eight-week-old female Sabra mice (25–30 g) were assigned at random to different groups of 10 mice per cage for use in all experiments. The mice were maintained in the animal facility (SPF unit) of the Hebrew University Hadassah Medical School, Jerusalem, and were deprived of food 12 h prior to the surgery, with free access to water. Under ketamine and xylazine anesthesia a midline laparotomy (1 cm) was performed; the common bile duct was exposed and ligated twice with 6–0 silk sutures. Sham-operated mice were laparotomized without bile duct ligation. The abdomen was closed in layers, and the animals were allowed to recover on a heating pad. All animals received humane care according to the criteria outlined in the "Guide for the Care and Use of Laboratory Animals".

### 2.2. Injections

Injections of all drugs began on the day of surgery and were performed every day for 4 weeks. CBD (5 mg/kg) in emulphore:ethanol:saline, 1:1:18, was administered to the animals intraperitoneally (i.p.). The same mixture without the drug was injected as vehicle. The dose of 5 mg/kg was chosen based on preliminary experiments which demonstrated that this dose causes the strongest effect, compared to 1 and 10 mg/kg (data not shown). ZM241385, an antagonist of $A_{2a}$AR (adenosine receptor $A_2$A) was injected i.p. at a dose of 1 mg/kg. The drug was dissolved in DMSO:saline, in a ratio of 1:19 [22].

### 2.3. Activity assessment: open field test

Activity was assessed in the open field (20 × 30 cm field divided into 12 squares of equal size) 3 weeks post-surgery as described previously [23]. Locomotor activity was recorded by counting the number of crossings by the mice at 1-min intervals. Results are presented as the mean number of crossings per minute.

### 2.4. Evaluation of cognitive function: eight arm maze test and T-maze test

The eight arm maze test was conducted 3 weeks post-surgery as described before, with minor modifications [24]. Briefly, the number of entries required for the mice to complete visits to all eight different arms of the maze was recorded each day, for five consecutive days. The average number of entries for each group in the first day (baseline performance) of experiment was set as 100%, and all raw data for the following days were transformed to percentages based on the calibration relative to the baseline.

T-maze was performed as described before [25].

### 2.5. Quantitative RT-PCR analysis of TNF-α receptor 1, BDNF, COX-2 and $A_{2a}$AR

Total hippocampal RNA was extracted using Tri reagent according to the manufacturer's instructions and reverse transcribed. RNA samples with no RT were amplified in the PCR in order to rule out the possibility of amplifying genomic DNA contamination which was present in the RNA extracted from the tissue.

Quantitative RT-PCR was carried out with Power SYBR Green PCR Master Mix (Applied Biosystems, UK), in 7900HT instrument (Applied Biosystems). Volume reaction was 15 μl and GAPDH was used as endogenous control. Threshold cycle (Ct) was determined by SDS software for each one of the samples tested, and the average Ct