Cannabinoids and Capsaicin Improve Liver Function Following Thioacetamide-Induced Acute Injury in Mice

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OBJECTIVES: We have shown the beneficial effects of cannabinoids in a murine model of hepatic encephalopathy following thioacetamide and now report their effects on the liver injury.

METHODS: Fulminant hepatic failure (FHF) was induced by administration of 200 mg/kg thioacetamide to wild-type (WT) and CB2 Knockout (KO) mice. Twenty-four hours later, mice were injected with 2-arachidonoylglycerol (CB1, CB2, and TRPV1 agonist), HU308 (CB2 agonist), SR141716 A (CB1 receptor blocker), SR141716 A + 2-AG, and SR144528 (CB2 receptor blocker), capsaicin and capsazepine (TRPV1 agonist and antagonist receptors). Mice were sacrificed 2 days after thioacetamide administration (day 3) and liver biochemistry and histopathology as well as evaluation of 2-arachidonoylglycerol levels were performed on liver tissue.

RESULTS: Liver histopathology undertaken 48 h after thioacetamide showed evidence of necrosis and inflammation. SR141716 A, HU308, and 2-arachidonoylglycerol reduced inflammation and promoted regeneration 1 day after their administration.

Liver enzymes increased after thioacetamide administration and were reversed after SR141716 A and 2-arachidonoylglycerol administered alone or combined, HU-308, but not SR144528. Thus, the beneficial effects mediated through CB2 receptors. However, CB2 KO mice still modulated liver function via the TRPV1 receptors. Capsaicin improved both liver pathology and function in WT thioacetamide-treated mice, while capsazepine impaired it.

CONCLUSIONS: The similar pattern found between the effect of cannabinoids and their antagonists on brain and liver indicated that the therapeutic effect might be directed by the improvement in both organs through CB2 receptors and/or TRPV1 receptors. Modulation of these systems may have therapeutic potential.

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INTRODUCTION

Fulminant hepatic failure (FHF) is a very serious form of liver injury caused by infectious agents, drugs, or toxins accompanied by hepatic encephalopathy (HE), and may be fatal (1, 2).

Injecting thioacetamide (TAA) to rats induces acute liver failure, with histological appearances and biochemical characteristics similar to cirrhosis in man (3–7). Hepatotoxicity of TAA is caused by the generation of free radicals and oxidative stress (8). Liver cirrhosis impairs many of the liver’s biochemical functions, one of which is the conversion of ammonia into urea. Hyperammonia affected GABA-ergic neurotransmission and subsequently, neurological function (9).

Endocannabinoids (Ecs) are neuromodulators, functionally, but not structurally, similar to THC, the psychoactive component of Cannabis. They are found in the central nervous system (CNS), immune system, gut, and many other organs, and have many physiological functions and confer neuroprotection after cerebral insults (10).

Two kinds of cannabinoid receptors, CB1 and CB2, have been characterized and cloned. CB1 receptors are mostly distributed in the brain, while CB2 receptors are found in...
immune tissues and mediate the immunomodulatory effects of Ecs (11). 2-arachidonoylglycerol (2-AG) activates both the CB1 and CB2 receptors. Synthetic antagonists for Ecs include: SR141716 A and SR144528 for the CB1 and the CB2 receptors, respectively (12, 13). Specific synthetic agonists include Noladin (CB1 receptor) and HU308 (CB2 receptor) (10). Ecs mediate the vasodilatation associated with liver cirrhosis via CB1 receptors (14), which have profibrogenic effects in the liver (15), whereas activation of CB2 receptors was antifibrogenic (16). Circulating macrophages and platelets of cirrhotic rats or humans produced anandamide and 2-AG, when infused into normal recipient rats they induced a marked hypotensive effect (17). These findings point to worsening of liver disease mediated by CB1 receptors—vasodilatation enables infiltration of metabolic toxins to the brain while fibrosis aggravates liver failure. Activation of vascular CB1 receptors located on splanchnic and hepatic vascular endothelium by Ecs is involved in the vasodilated state associated with cirrhosis (17).

Because the metabolic interrelations between the liver and the brain are responsible for the pathogenesis of the disease, this could be the result of peripheral cannabinoid receptors signaling. The two receptors have opposite effects on liver fibrosis—CB1 being profibrogenic (15) and CB2 antifibrogenic (16). Thus, the balance between the receptor systems appears to be crucial in determining the outcome of liver injury. Our previous studies have shown the neuroprotective effect of CB1 inhibition and CB2 activation in experimental HE (4). Furthermore, recent studies have described an abundance of CB2 in different sections of the brain (18).

We also studied the etiology of cerebral dysfunction in a murine model of HE. We showed that stimulation of cerebral AMP-activated protein kinase (AMPK) is a compensatory response to liver failure. This function of AMPK is regulated by Ecs that control systemic energy balance via the cannabinoid receptors. Under normal circumstances, AMPK activity is mediated by CB1 while CB2 is barely detected. However, in response to liver failure CB2 is strongly stimulated. Administration of tetrahydrocannabinol (THC) augmented AMPK activity and restored brain function in wild-type (WT) mice but not in their CB2 knockout (KO) littermates and no therapeutic effect was shown in the liver. These results suggest that HE is a disease of energy flux. CB2 signaling is a cerebral stress response mechanism and makes AMPK a promising target for its treatment by manipulation of the cannabinoid system (5).

Capsaicin (8-methyl-N-vanillyl-6-nonenamide) is the active component causing the hotness of hot peppers; it is used as an anesthetic product, and has anti-inflammatory qualities and caused apoptosis in cancer cells (19, 20). Capsaicin acts on neural cells via VR1, a nonselective cation channel, with wide distribution in the CNS, and is blocked by capsazepine (21). Di Marzo et al. raised the possibility of an interaction between Ecs and capsaicin because of structural similarities with anandamide and 2-AG (21). Golech et al. found coexpression and function of vanillloid and cannabinoid receptors in human brain endothelium (22). Several indications were found for the possible therapeutic potential of capsaicin in the treatment of hepatic failure (23).

We aim, in this work, to examine the therapeutic effect of Ecs and capsaicin on acute liver disease and consider their implications regarding disease mechanism and the development of new therapeutic modalities.

MATERIALS AND METHODS

The experimental protocol was approved by the institutional committee for the use of animals No. MD-89.52-4. Ecs were synthesized as previously reported (24). The antagonists were kindly supplied by NIDA (National Institute of Drug Abuse).

We adapted the rat model of acute liver failure induced by TAA to mice. The TAA model in mice has been extensively validated previously (3–5, 7). Liver levels of 2-AG in animals with acute liver failure were compared with those of healthy controls. The effects of cannabinoid antagonists (SR141716 A and SR144528) as well as agonists (2-AG, and HU308), capsaicin and capsazepine were studied.

Mice

Eight- to 10-wk-old female Sabra mice were assigned at random to different groups of 10 mice per cage and were used in all experiments. CB2 KO were kindly received by Prof. A. Zimmer, Germany. The food provided was Purina chow. Mice were sacrificed by decapitation. Livers were rapidly removed and kept at –70°C.

Induction of Hepatic Failure

TAA was obtained from Sigma-Aldrich and dissolved in sterile saline solution. TAA was injected by the intraperitoneal route (i.p.) as a single dose of 200 mg/kg. Twenty-four hours after injection of TAA, animals were injected (s.c) with 0.5 mL solution of 0.45% NaCl, 5% dextrose, and 0.2% KCl in order to prevent hypovolemia, hypokalemia, and hypoglycemia. The mice were intermittently exposed to infrared light in order to prevent hypothermia (4).

Administration of Cannabinoid Agonists, Antagonists, and Capsaicin

Cannabinoids and their antagonists were dissolved in a vehicle solution composed of ethanol, emulphor, and saline at a ratio of 1:1:18, respectively. 2-AG, HU308, SR141716 A, SR141716 A+2-AG, and SR144528 were injected in a dose of 5 mg/kg once only after TAA treatment based on previous experiments. Capsaicin and capsazepine were administered at a dose of 1.25 μg/kg as described by Lu et al., 2005 (25). The solutions were injected i.p. 1 day after TAA administration. Control mice were injected with vehicle.

Determination of 2-AG

Determination of 2-AG was performed 2 days after TAA administration (day 3) on liver tissue as described (26).
**GC-MS Analysis**
For quantitative analysis the samples were analyzed by GC-MS (26).

**Serum Liver Enzyme Levels**
Serum for ALT, AST, bilirubin, and ammonia measurement was obtained on day 3 in glass tubes, centrifuged, and analyzed on the day of sampling using a Kone Progress Selective Chemistry Analyzer (Kone Instruments, Espoo, Finland). All serum samples were processed in the same laboratory using the same methods and the same reference values.

**Liver Histopathology**
At necropsy, mouse livers were fixed in 10% neutral-buffered formalin. Fixed midsections of the left, median, caudate, and right liver lobe were embedded in paraffin, cut at 5 µm, and stained with hematoxylin and eosin. Liver sections were examined by a veterinary pathologist blinded to sample identity. Apoptosis/necrosis, inflammation, and regeneration were scored using a 0–4 scale. Centrilobular apoptosis/necrosis (coagulative) was graded according to the following criteria: grade 0, normal (Fig. 1A); grade 1, centrilobular apoptosis/necrosis immediately around the central vein (occupying up to one-third of acinar zone 3) in most lobules (Fig. 1B); grade 2, centrilobular necrosis involving acinar zone 3 and expanding to zone 2, occasional lobules showing bridging, occasional submassive necrosis (Fig. 1C); grade 3, bridging and submassive necrosis involving <50% of liver section (Fig. 1D); and grade 4, massive coagulative necrosis with no discernible liver tissue in >50% of section (Fig. 1E). Figure 1 demonstrates the grading system and depicts the spectrum of centrilobular apoptosis/necrosis lesions observed in this study. On the basis of size and frequency, inflammatory lesions were graded as 0, none; 1, minimal; 2, mild; 3, moderate; and 4, severe. Liver regeneration was graded based on the frequency of hepatocyte mitotic figures detected in the majority of the ×40 high-power fields examined in the preserved hepatocyte areas: 0, no mitotic figures; 1, 1–2 mitotic figures; 2, 3–4 mitotic figures; 3, 5–6 mitotic figures; and 4, >7 mitotic figures.

**Statistical Analysis**
Statistical analysis was performed using one-way analysis of variance (ANOVA), followed by post hoc paired t-test corrected for multiple comparisons. All data are expressed as mean ± SEM. The Fisher’s exact test as well as the Pearson χ² test was applied for the statistical analysis of nonparametric data for the pathological evaluations.

**RESULTS**

**Induction of FHF**
Two days after TAA induction of FHF, biochemical indices in the serum of the injected normal mice versus controls were: ALT 5,037.5 IU/mL versus 158.4 IU/mL, AST 5,103.3 IU/mL versus 108.7 IU/mL, total bilirubin, 52.8 IU/mL versus 14.0 IU/mL, and ammonia 338.0 IU/mL versus 126.4 IU/mL.

**Liver Levels of 2-AG in FHF**
Levels of 2-AG were measured in the liver of mice sacrificed 2 days after injection of TAA. There was no significant change in liver 2-AG levels. In control mice, liver 2-AG levels were 0.8 ± 0.07 nmol/g ± SEM.

**Liver Histopathology**
Normal liver histology (Fig. 1A) was seen in control animals (N = 11). In the remaining TAA-treated groups of mice, centrilobular necrosis uniformly involving the vast majority of the hepatic lobes was the cardinal histopathological feature. Centrilobular necrosis varied in severity even among mice belonging to same treatment group. In its mildest form there was individual hepatocyte necrosis and increased apoptosis, characterized by the presence of eosinophilic globules most times containing fragments of chromatin (“apoptotic” or “Councilman” bodies), immediately around the central vein occupying up to the one-third of acinar zone 3 in most lobules (Fig. 1B and E). In a number of mice, coagulative centrilobular necrosis was prominent and involved acinar zone 3 and expanded to zone 2 (Fig. 1C). Occasional lobules showed bridging or were necrotic (submassive necrosis). Other mice showed necrosis that was severe, extensive, and characterized by central-to-central bridging and submassive necrosis with hemorrhage, involving <50% of liver section (Fig. 1D). Severely affected mice had most of their liver architecture effaced showing massive coagulative necrosis and hemorrhage with no discernible liver tissue in >50% of section (Fig. 1E). Hepatocytes surrounding the necrotic areas typically showed increased multinucleation, caryomegaly, and various degrees of degeneration with occasional microvesicular steatosis. In the same areas there was also increased hepatocellular proliferation indicating an active regeneration process. Pericentral accumulation of inflammatory cells was mild when apoptosis/necrosis was not severe (Fig. 1F). The inflammatory cell component comprised primarily lymphocytes and macrophages. Occasional neutrophils were also noted. A more pronounced influx of inflammatory cells within the necrotic tissue was seen in animals with severe necrosis.

Comparison of the histopathological indices assessed for each group indicated that amelioration of TAA-induced apoptosis/necrosis reached statistical significance (P < 0.005) only with capsaicin treatment (Fig. 2, Table 1). However, inflammation was less severe in HU308 and SR141716 A-treated animals than in TAA-treated group, whereas, SR144528 treatment resulted in nonsignificant changes only. The regenerative capacity of the liver in any cannabinoid receptor agonists or antagonists-treated groups was higher than with animals to which only TAA was administered. On the contrary, capsaicin-treated animals exhibited significantly less hepatic regeneration (Table 1).
Liver histopathology depicting grades of centrilobular apoptosis/necrosis. (A) Normal mouse liver histology—score = 0. (B) Mild centrilobular necrosis/apoptosis—score = 1. (C) Centrilobular coagulative necrosis involving acinar zone 3 and partially acinar zone 2—score = 2. (D) Central-to-central (bridging) necrosis—score = 3. (E) Massive necrosis effacing liver architecture—score = 4. (F): Higher magnification of liver centrilobular area (score = 1) highlighting individual hepatocyte necrosis, presence of apoptotic bodies (arrow), pericentral inflammation and proliferating hepatocytes—regeneration (arrowheads). H–E. Bars A–E = 100 μm. Bar F = 50 μm.

Liver Cell Integrity and Viability
Liver cell integrity and viability was assessed by measuring levels of AST, ALT, GGT (not shown), bilirubin, and ammonia in the serum of normal and CB2 KO mice.

The level of liver enzymes AST, ALT, and GGT, bilirubin, and ammonia increased after TAA administration and were reversed almost to control level after administration of 2-AG, SR141716 A, 2-AG+SR141716 A, and HU308 (AST, ALT, GGT, ammonia, and bilirubin). Blocking the CB2 receptor by SR144528 did not cause any significant effect, SR144528+HU308 and SR144528 + 2-AG reversed the beneficial effect of HU308 and 2-AG on liver function except HU308+SR144528, which showed also beneficial effect on ammonia levels, however, significantly less than HU308 alone (Figs. 3–5). In CB2 KO mice the level of liver enzymes AST, ALT, bilirubin, and ammonia increased after TAA administration and were reversed after administration of SR141716 A (AST, ammonia, bilirubin), 2-AG (AST, ALT, ammonia, bilirubin), and 2-AG+SR141716 A (ammonia, bilirubin) (Figs. 6 and 7).

The level of liver enzymes AST, ALT, bilirubin, and ammonia increased after TAA administration and were reversed almost to control level after administration of capsaicin (AST, ALT, ammonia, and bilirubin) (Fig. 8A and B).

DISCUSSION
Experimental liver failure in mice induced by thioacetamide is an animal model for FHF and HE (3–7).

In our previous article (4), we studied the effects of Ecs on neurological, activity, and cognitive function in HE. Encephalopathic mice treated with SR141716 A or 2-AG or both, showed improved neurological function, activity, and cognitive function compared with untreated controls. SR141716 A showed a dose-response pattern in the
improvement of neurological function. HU308 improved neurological score via the CB2 receptors. CNS levels of 2-AG were elevated in mice with TAA-induced liver failure when compared with healthy controls.

The question is whether the improvement observed was because of a direct effect on the brain or secondarily because of improvement of liver function. We have found that 2-AG, SR141716 A, and 2-AG+SR141716 A improved liver function, thus it seems that the beneficial effect of 2-AG is not via the CB1 receptor. In order to test this assumption we blocked the CB2 receptors with SR144528 and the beneficial effect of 2-AG was reversed. Further support came from the use of HU308, a CB2 agonist. HU308 improved liver biochemistry and was blocked by the CB2 antagonist. In order to verify this, we tested CB2 KO and found to our surprise that both SR141716 A and 2-AG caused improvement in AST, ammonia, and bilirubin, and 2-AG improved ALT. Thus, it appears that in the CB2 KO, 2-AG works via the TRPV1 receptors. SR141716 A, which acts similarly, blocks the beneficial effect of 2-AG (27). In order to test this hypothesis, we used the TRPV1 receptors agonist capsaicin, and its antagonist capsazepine, which showed improvement with capsaicin on both histopathology and liver enzymes and was blocked by capsazepine.

Results obtained with CB2 KO mice showed maximal improvement with 2-AG or SR141716 A, while 2-AG+SR141716 A improved only ammonia and glucose levels (not shown), thus it seems that 2AG works via both CB2 and TRPV1 receptors, and SR141716 A on both CB1 and TRPV1 receptors and is in competition with 2-AG on the TRPV1 receptors. This explains why the treatment of 2-AG+SR141716 A is less effective. A similar compensation has been demonstrated in the CB1 KO where administration of SR141716 A to pups caused their death: however, CB1 KO survived because of alternative mechanisms (28). The similar pattern found between the effect of Ecs and their antagonists on brain and liver indicated that the therapeutic effect may be mediated by the improvement in function of both organs.

Blocking the CB2 receptor had no therapeutic effect. The beneficial effects of 2-AG on both brain and liver are different from the effect of THC described in our previous article on the brain only (5). Here, THC activated the CB2 receptors in the brain without causing any improvement in liver function. It seems that THC affects only the brain via AMPK while SR141716 A, SR141716 A+2-AG, or HU308 affect both cerebral and hepatic function. Activation of hepatic CB2 receptors limits progression of experimental liver fibrosis and during the course of chronic hepatitis daily cannabis use is an independent predictor of fibrosis progression (29). Thus, THC working as CB1 agonist on the liver had different effects than HU308 which stimulated CB2 receptors, SR141716 A blocking CB1 receptors and 2-AG which worked on both as an agonist, supports this assumption.

The discrepancy between these results may be explained by the findings of Brunet et al. who measured THC distribution in white pig tissues and showed that the fastest THC elimination was noted in liver tissue (6 h), while in brain tissue it occurred after 24 h (31). In addition, the improvement in the liver is apparently via TRPV1 receptors, which are not activated by THC.

It seems that blocking the CB1 receptor and stimulating the CB2 and TRPV1 receptors has the best therapeutic effect

Table 1. Comparison of the Frequency of Cells Showing Apoptosis/Necrosis, Inflammation, and Regeneration in the Liver

<table>
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<th>Groups Comparison</th>
<th>Apoptosis</th>
<th>Inflammation</th>
<th>Regeneration</th>
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<td>TAA vs normal</td>
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<td>Increase</td>
<td>Increase</td>
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<tr>
<td>TAA + capsaicin vs TAA</td>
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<tr>
<td>TAA + SR141716 A vs TAA</td>
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<tr>
<td>TAA + HU-308 vs TAA</td>
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<td>Decreased</td>
<td>Decreased</td>
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<tr>
<td>TAA + SR144528 vs TAA</td>
<td>NS*</td>
<td>NS*</td>
<td>Increased</td>
</tr>
<tr>
<td>TAA+2-AG vs TAA</td>
<td>NS*</td>
<td>Decreased</td>
<td>Decreased</td>
</tr>
<tr>
<td>TAA+2-AG+SR141716 A vs TAA</td>
<td>NS*</td>
<td>Decreased</td>
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* Two-sided Fisher’s exact test; † Pearson’s χ² test.
Figure 3. The effect of cannabinoid receptors agonists or antagonists on AST and ALT levels. Mice were treated with 200 mg/kg TAA. One day after TAA administration 5 mg/kg 2-AG, SR141716 A, 2-AG+ SR141716 A, HU308, SR144528, HU308 +SR144528, and 2-AG +SR144528 were injected i.p. once only after TAA treatment. Control mice were injected with vehicle. Mice were sacrificed on day 3 and liver function was evaluated. AST level was reversed by 2-AG ($P < 0.001b$), SR141716 A ($P < 0.001 c$), 2-AG+ SR141716 A ($P < 0.01 d$), and HU308 ($P < 0.001 e$). Blocking the CB2 receptor reversed the beneficial effect of HU308 ($P < 0.001 g$) and 2-AG ($P < 0.001 l$) on AST level. SR144528 did not reverse significantly AST level. ALT level was reversed by 2-AG ($P < 0.001b$), SR141716 A ($P < 0.001 c$), 2-AG+ SR141716 A ($P < 0.001 d$), and HU308 ($P < 0.001 e$). Blocking the CB2 receptor reversed the beneficial effect of HU308 ($P < 0.001 l$) and 2-AG ($P < 0.001 g$) on AST level. SR144528 did not reverse significantly ALT level.

on the liver. The treatment was very beneficial because it was administered only 1 day after TAA administration and showed a therapeutic effect on liver enzyme levels as well as on liver pathology (especially capsaicin), on day 3.

These results are in agreement with Teixeira-Clerc et al. (15, 29), who identified the CB1 as a molecular target for antifibrotic treatment in the chronic model although in our experiments, the acute model of FHF, such an antifibrotic effect could not be confirmed because the animals were sacrificed shortly after TAA.

Earlier studies have indicated that ECS acting at CB1 receptors in the hepatic vasculature may mediate the vasodilated state and hypotension that accompany advanced liver cirrhosis. Thus, CB1 blockade might prolong the life of cirrhotic individuals not only by slowing the fibrotic process, but also by improving the associated hemodynamic abnormalities (31).

In addition, our findings indicate that either cannabinoid receptor agonists or antagonists did not alter the severity of apoptosis and necrosis following TAA. It may be speculated that the beneficial effect of cannabinoids on liver function during the acute phase following TAA is not mediated via hepatic cell protection from destruction. Evidently, such a massive hepatic necrosis shortly after the toxic effect of TAA may not be compensated by cannabinoid treatment. However, whether the opposite might be evident, in the case where cannabinoid treatment would precede the TAA administration, cannot be ruled out.

Decreased inflammatory cell infiltration was noticed following HU308 and SR141716 A, as well as capsaicin.

Figure 4. The effect of cannabinoid receptors agonists or antagonists on ammonia levels. Mice were treated with 200 mg/kg TAA. One day after TAA administration 5 mg/kg 2-AG, SR141716 A, 2-AG+ SR141716 A, HU308, SR144528, HU308 +SR144528, and 2-AG +SR144528 were injected i.p. once only after TAA treatment. Control mice were injected with vehicle. Mice were sacrificed on day 3 and liver function was evaluated. Ammonia level was reversed by 2-AG ($P < 0.001b$), SR141716 A ($P < 0.001 c$), 2-AG+ SR141716 A ($P < 0.001 d$), and HU308 ($P < 0.001 e$). Blocking the CB2 receptor reversed the beneficial effect of HU308 ($P < 0.001 h$) and 2-AG ($P < 0.001 g$) on ammonia level. SR144528 did not reverse significantly ammonia level.
Cannabinoids and Capsaicin Improve Liver Function

Figure 5. The effect of cannabinoid receptors agonists or antagonists on total bilirubin levels. Mice were treated with 200 mg/kg TAA. One day after TAA administration 5 mg/kg 2-AG, SR141716 A, 2-AG + SR141716 A, HU308, SR144528, HU308 + SR144528, and 2-AG + SR144528 were injected i.p. once only after TAA treatment. Control mice were injected with vehicle. Mice were sacrificed on day 3 and liver function was evaluated. Bilirubin levels were reversed by 2-AG (P < 0.001b), SR141716 A (P < 0.001 c), 2-AG + SR141716 A (P < 0.001 d), and HU308 (P < 0.01e). Blocking the CB2 receptor reversed the beneficial effect of HU308 (P < 0.05 g) and 2-AG (P < 0.001f) on bilirubin level. SR144528 did not reverse significantly bilirubin level.

Figure 6. The effect of cannabinoid receptors agonists or antagonists on AST and ALT levels. CB2 KO mice were treated with 200 mg/kg TAA. One day after TAA administration 5 mg/kg SR141716 A, 2-AG, and SR141716 A + 2-AG were injected i.p. once only after TAA treatment. Control mice were injected with vehicle. Mice were sacrificed on day 3 and liver function was evaluated. AST levels were reversed by 2-AG (P < 0.001 c), SR141716 A (P < 0.001b), and not reversed by 2-AG + SR141716 A. ALT levels were reversed by 2-AG (P < 0.01 b) and not reversed by SR141716 A or 2-AG + SR141716 A.
Figure 7. The effect of cannabinoid receptors agonists or antagonists on ammonia and bilirubin levels. CB2 KO mice were treated with 200 mg/kg TAA. One day after TAA administration 5 mg/kg SR141716 A, 2-AG, and SR141716 A+2-AG were injected i.p. once only after TAA treatment. Control mice were injected with vehicle. Mice were sacrificed on day 3 and liver function was evaluated. Total bilirubin levels are 1/10 than were presented. Ammonia levels were reversed by 2-AG ($P < 0.01$ c), SR141716 A ($P < 0.01$ b), and 2-AG+SR141716 A ($P < 0.01$ d). Total bilirubin levels were reversed by 2-AG ($P < 0.001$ c), SR141716 A ($P < 0.001$ h) and were not reversed by 2-AG+SR141716 A.

Figure 8. The effect of capsaicin and capsazepine on AST and ALT (A) ammonia and total bilirubin (B) levels. Mice were treated with 200 mg/kg TAA. One day after TAA administration 1.25 $\mu$g/kg capsaicin and capsazepine were injected i.p. once only after TAA treatment. Control mice were injected with vehicle. Mice were sacrificed on day 3 and liver function was evaluated. Total bilirubin levels are 1/10 than were presented. AST levels were reversed by capsaicin ($P < 0.001$ b), while capsazepine reversed the beneficial effect of capsaicin ($P < 0.001$ c). ALT levels were reversed by capsaicin ($P < 0.001$ b), while capsazepine reversed the beneficial effect of capsaicin ($P < 0.001$ c). Ammonia levels were reversed by capsaicin ($P < 0.01$ b), while capsazepine showed tendency to reverse the beneficial effect of capsaicin. Total bilirubin levels were reversed by capsaicin ($P < 0.001$ c) while capsazepine reversed the beneficial effect of capsaicin ($P < 0.001$ c).
of a maximal effect by both of them. Another possible explanation is that SR141716 A reversed the activity of liver enzymes via CB1 antagonism, while 2-AG caused improvement via CB2 activation. As 2-AG acts on both receptors, the effect of SR141716 A could be counteracted by 2-AG via CB1 receptor activation, thereby reducing the overall effect of SR141716 A. Therefore, the resultant effect seen would not indicate added improvement when both compounds act together. CB2 antagonist did not show any therapeutic effect on either liver pathology or enzymes.

We conclude that the Ecs system may have an important role in the pathogenesis of FHF. The results have great importance for many patients waiting for therapeutic intervention for the disease.

In conclusion, the effect of SR141716 A, 2-AG + SR141716 A, or HU308 are because of cerebral and hepatic actions. The difference between the effect of THC and 2-AG are because of their intrinsic properties. Because SR141716 A, 2-AG, and HU308 can penetrate the blood brain barrier, their therapeutic effect is on brain and liver.

ACKNOWLEDGMENT

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STUDY HIGHLIGHTS

What Is Current Knowledge

- The therapeutic effect on liver disease (the chronic model) by blocking the CB1 cannabinoid receptor and stimulating the CB2 cannabinoid receptor.
- It is not clear whether the therapeutic effect of hepatic encephalopathy induced by fulminant hepatic failure (FHF) is mediated by the liver or on both brain and liver.

What Is New Here

- The therapeutic effect on FHF (the acute model) by using cannabinoids, capsaicin, or combined treatment. Thus, the therapeutic effect is mediated by both cannabinoid and TRPV1 receptors.
- The therapeutic effect is mediated through effects in both liver and brain.
- The proposed treatment reverses liver enzymes levels in the acute model a short time (48 h) after low-dose cannabinoids or capsaicin treatment.
- No side effects were noticed. Histological findings indicate that CB2 receptor-mediated anti-inflammatory effect may be related to the improved functional outcome of the remaining intact and regenerating hepatic cells following thioacetamide (TAA). However, in the case of capsaicin, an additional factor might be the protection of the hepatic cells.

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CONFLICT OF INTEREST

Guarantor of the article: Yosefa Avraham, Ph.D.

Specific author contributions: Yosefa Avraham, writing the manuscript, planning and design of the study, analysis and interpretation of data; Olga Zolotarev, performing the experiments; Nikolaos C. Grigoriadis, histological analysis and statistics; Theofilos Pautahidis, histological analysis and statistics; Iddo Magen, behavioral studies; Lia Vorobjiav, analysis of 2-AG levels; Andreas Zimmer, provided the CB2 KO mice; Yaron Ilan, consultant; Raphael Mechoulam, consultant; Elliot M. Berry, analysis and interpretation of the data.

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