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Short communication

Effects of repeated administration with CP-55,940, a cannabinoid CB_1 receptor agonist on the metabolism of the hepatic heme

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Abstract

Drugs metabolised by cytochrome P450 (CYP) such as analgesics may induce acute attacks in patients with hepatic porphyrias. In recent years, preclinical and clinical studies have suggested that cannabinoid pharmaceutical preparations may be potentially useful in the treatment of pain. The purpose of the study was to examine the effects of CP-55,940, a cannabinoid CB_1 receptor agonist, on the hepatic heme metabolism in mice.

To this end, hepatic activities of aminolevulinic acid synthase (ALAS), heme oxygenase (HO) and CYP levels were determined in mice treated with CP-55,940 (0.5 mg/kg/day; i.p.; 5 or 24 days).

Results showed that treatment with CP-55,940 decreased CYP concentrations by 80% and increased HO activity by 158%. However, ALAS activity also decreased by 37%, suggesting that regulatory free heme pool was not modified.

Our findings indicate that CP-55,940 and its metabolites do not behave as porphyrinogenic drugs and may potentially be safe for treating pain in patients with acute porphyrias.

Keywords: Cannabinoid CB_1 receptor agonist CP-55,940; Hepatic porphyria; Heme pathway

1. Introduction

Porphyrias are inherited disorders caused by decreased activity of the enzymes in the heme biosynthetic pathway (Anderson, Sassa, Bishop, & Desnick, 2001). Patients with acute porphyrias may develop acute attacks frequently precipitated by certain drugs

Abbreviations: ALA-S, aminolevulinate synthase; CYPs, cytochrome P-450s; GSH, gluthation; HO, heme oxygenase; PBG-D, porphobilinogen deaminase; THC, Δ9-tetrahydrocannabinol; UROD, uroporphyrinogen decarboxylase

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and other heme consuming factors. These crisis may induce life-threatening medical emergencies involving severe abdominal pain, tachycardia, nausea, vomiting, peripheral motor neuropathy and neurovisceral symptomatology. Therapeutic strategies include withdrawal of all common precipitants (drug, alcohol, fasting, infection), the administration of glucose (300–400 g/day) and in severe attacks, hematine. Supportive treatment includes monitoring for and treating complications such as hypertension, hyponatraemia and opiate analgesia (Badminton & Elder, 2002).

Cannabis has been historically used to relieve some of the symptoms associated with central nervous system disorders. In the United States and Canada, cannabinoids are prescribed as appetite stimulants and inhibitors of nausea and vomiting in patients with AIDS or those being treated chronically with cytotoxics (Grinspoon & Bakalar, 1993). Other potential uses of the cannabinoids that are currently under preclinical or clinical investigation relate to the control of pain (Croxford, 2003; Iversen & Chapman, 2002). Indeed, Δ^9-tetrahydrocannabinol (THC), the major psychoactive compound of *Cannabis sativa* and synthetic derivates of cannabinoids, exhibits antinociceptive and antihyperalgesic properties when systemically administered in different animal models of acute and inflammatory pain (Iversen & Chapman, 2002; Pertwee, 2001). Furthermore, there is evidence for an interaction between opioid and cannabinoid mechanisms (Manzanares et al., 1999). In two models of acute pain (Fuentes et al., 1999) and chronic inflammatory pain (Welch & Stevens, 1992) THC and morphine were found to act synergically, with one potentiating the antinociceptive actions of the other. Cannabinoids may produce analgesia through activation of a brainstem circuit that is also required for opiate analgesia although the pharmacology of these two mechanisms is different.

Furthermore, THC decreases the hepatic microsomal drug metabolising enzyme system 12 h after treatment without affecting ALAS activity (Watanabe, Hamajima, Narimatsu, Yamamoto, & Yoshimura, 1986), a key enzyme of heme synthesis in the liver. However, very little is known about the effects of repeated administration of cannabinoids on the activity of heme metabolism enzymes.

The purpose of this study was to examine the effects of the repeated administration of the cannabinoid CB_1 receptor agonist CP-55,940 on heme metabolism. To this end we studied hepatic morphology, cytochrome P-450 (CYP) content and activity of heme metabolism enzymes in the mouse liver.

2. Materials and methods

2.1. Animals

Male Swiss albino mice (23–29 g) obtained from Harlan (Barcelona, Spain) were maintained in a temperature- and light-controlled environment (23 ± 1 °C, light on between 8:00 a.m. and 8:00 p.m.). Food and tap water were provided ad libitum. All experiments were performed following the highest standards of human animal care in accordance with National and International Laws for the Care and Use of Laboratory Animals.

2.2. Drug and treatments

Cannabinoid CB_1 receptor agonist CP-55,940{(−)-*cis*-3-[2-hydroxy-4-(1,1,dimethylheptyl)-phenyl]-*trans*-4(-3-hydroxypropyl)cyclohexanol)} was dissolved in ethanol:cremophor:saline (1:1:18) and administered (0.5 mg/kg/day; i.p.) for 5 or 24 days. Control animals received the vehicle and were sacrificed at the same time as the treated animals. Animals were killed and their livers quickly removed, rinsed in ice-cold phosphate buffer saline and stored at −80 °C.

2.3. Biochemical determinations

Enzymatic determination of delta-aminolevulinic acid synthase (ALAS), porphobilinogen deaminase (PBGD) and heme oxygenase (HO) activities were performed as previously described (Buzaleh et al., 2004). ALA dehydratase (ALAD) activity was measured by the European Standarized method (Berlin & Schaller, 1974). Uroporphyrinogen decarboxylase (UROD) activity was determined by the method of de Verneuil, Sassa, & Kappas, 1983. Protein concentration was assessed by Bradford's method (Bradford, 1976) using a Bio-Rad Protein Assay (Bio-Rad Laboratories, München, Germany).

Porphyrin levels in the liver were evaluated using a Hitachi F-4010 fluorescence spectropho-