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The monoacylglycerol lipase inhibitor JZL184 suppresses inflammatory pain in the mouse carrageenan model

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ABSTRACT

Aim: The present study tested whether the selective monoacylglycerol lipase (MAGL) inhibitor JZL184 would reduce allodynia and paw edema in the carrageenan test.
Main methods: The anti-edematous and anti-allodynic effects of JZL184 were compared to those of PF-3845, an inhibitor of fatty acid amide hydrolase (FAAH), and diclofenac, a non-selective cyclooxygenase inhibitor. Cannabinoid receptor involvement in the anti-edematous and anti-allodynic effects of JZL184 was evaluated by administration of the respective CB_1 and CB_2 receptor antagonists rimonabant and SR144528 as well as with $CB_1(-/-)$ and $CB_2(-/-)$ mice. JZL184 (1.6, 4, 16, or 40 mg/kg) was administered for six days to assess tolerance.
Key findings: JZL184 administered before or after carrageenan significantly attenuated carrageenan-induced paw edema and mechanical allodynia. Complementary genetic and pharmacological approaches revealed that the anti-allodynic effects of JZL184 required both CB_1 and CB_2 receptors, but only CB_2 receptors mediated its anti-edematous actions. Importantly, both the anti-edematous and anti-allodynic effects underwent tolerance following repeated injections of high dose JZL184 (16 or 40 mg/kg), but repeated administration of low dose JZL184 (4 mg/kg) retained efficacy.
Significance: These results suggest that the MAGL inhibitor JZL184 reduces inflammatory nociception through the activation of both CB_1 and CB_2 receptors, with no evidence of tolerance following repeated administration of low doses.

Introduction

The endogenous cannabinoid (endocannabinoid) system consists of two G-protein-coupled cannabinoid (i.e., CB_1 and CB_2) receptors (Gerard et al., 1991; Matsuda et al., 1990), the lipid endogenous ligands N-arachidonoylethanolamine (anandamide; AEA) (Devane et al., 1992) and 2-arachidonoylglycerol (2-AG) (Mechoulam et al., 1995; Sugiura et al., 1995), and endocannabinoid biosynthetic and catabolic enzymes (Ahn et al., 2008). Whereas 2-AG binds to both cannabinoid receptors with similar affinity (Mechoulam et al., 1995), AEA possesses approximately four-fold higher affinity at CB_1 receptors than CB_2 receptors (Showalter et al., 1996). AEA and 2-AG are produced and released on demand, and are then rapidly metabolized by their respective major degradative enzymes, fatty acid amide hydrolase (FAAH) (Cravatt et al., 1996, 2001) and monoacylglycerol lipase (MAGL) (Blankman et al., 2007; Dinh, 2004). These components of the endocannabinoid system represent potential therapeutic targets to treat obesity, psychiatric disorders, neuroinflammatory diseases, cancer, pain, and inflammatory conditions (Pacher, 2006). Accordingly, a growing body of research has demonstrated that FAAH or MAGL inhibition reduces nociceptive behavior in laboratory animal models of pain.

The bulk of research examining the role of endocannabinoid catabolic enzymes in nociception has focused on FAAH (Booker et al., 2011; Chang et al., 2006; Clapper et al., 2010; Jayamanne et al., 2006; Kinsey et al., 2011; Naidu et al., 2008, 2009, 2010; Suplita et al., 2005) largely because of a greater availability of selective FAAH inhibitors than selective MAGL inhibitors. The development of JZL184, a piperidine carbamate that preferentially and irreversibly inhibits MAGL, provided the first pharmacological tool that when administered acutely increases 2-AG brain levels, without altering AEA brain levels (Long et al., 2009). Systemic administration of JZL184 reduces

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nociceptive responses in the tail withdrawal, formalin, and acetic acid stretching tests (Busquets-Garcia et al., 2011, Long et al., 2009), and chronic constriction injury (CCI) model of neuropathic pain in mice (Kinsey et al., 2009). Intraplantar injection of JZL184 produces antinociception in the formalin test (Guindon et al., 2011) and capsaicin model of nociception (Spradley et al., 2010).

Although these findings indicate that MAGL inhibition reduces nociceptive behavior in multiple preclinical pain models, the effects of JZL184 have yet to be evaluated in a prolonged model of inflammatory nociception. Thus, in the present study we tested whether JZL184 would attenuate paw edema and mechanical allodynia in the carrageenan model of inflammatory pain. For comparison, we tested the nonsteroidal anti-inflammatory diclofenac and the FAAH inhibitor PF-3845, which has been shown to possess anti-inflammatory and anti-allodynic effects in complete Freund's adjuvant (Ahn et al., 2009), LPS (Booker et al., 2011), and CCI (Kinsey et al., 2009, 2010) pain models. Because repeated JZL184 treatment or genetic deletion of MAGL results in CB_1 receptor functional tolerance (Chanda et al., 2010; Schlosburg et al., 2010), we also tested the impact of repeated administration of low and high doses of JZL184 on both dependent measures. Finally, we tested whether systemic administration of JZL184 after intraplantar carrageenan injections reverses edema and allodynia to infer whether this compound possesses efficacy to treat nociceptive behavior and edema following an inflammatory insult.

Methods

Subjects

Male C57BL/6 J mice (Jackson Laboratory, Bar Harbor, ME) as well as male and female CB_1 (−/−) and CB_2 (−/−) mice and their respective littermate controls, CB_1 (+/+) and CB_2 (+/+) mice from the Center Transgenic Colony at Virginia Commonwealth University served as subjects. CB_1 (−/−) and CB_2 (−/−) mice were backcrossed onto a C57BL/6 J background for 13 and 6 generations, respectively. The subjects weighed between 18 and 25 g, and were housed four-five mice per cage in a temperature (20–22 °C) and humidity controlled AAALAC-approved facility. Mice were given unlimited access to food and water in their home cages and were maintained on a 12/12 h light/dark cycle. The sample size for each treatment group was 6 to 10 mice/group and for knockout studies was 4 to 9 mice/group. All animal protocols were approved by the Virginia Commonwealth University Institutional Animal Care and Use Committee and were in accordance with the National Institutes of Health guide for the care and use of Laboratory animals (Institute of Laboratory and Animal Resources, 1996). After testing was completed, all mice were humanely euthanized via CO_2 asphyxia, followed by rapid cervical dislocation.

Drugs

JZL184 and PF-3845 were synthesized as described previously (Ahn et al., 2009; Long et al., 2009) by Organix, Inc. (Woburn, MA). JZL184, PF-3845, the CB_1 receptor antagonist rimonabant (SR141716, National Institute on Drug Abuse), and the CB_2 receptor antagonist SR144528 (National Institute on Drug Abuse) were dissolved in a vehicle consisting of a mixture of ethanol, alkamuls-620 (Rhone-Poulenc, Princeton, NJ), and saline in a ratio of 1:1:18. The nonselective cyclo-oxygenase (COX) inhibitor diclofenac (DIC; Tocris, Ellisville, MO) was dissolved in saline. Each drug was given via the i.p. route of administration in a volume of 10 μl/g body weight.

Carrageenan-induced paw edema

Edema was induced by giving an intraplantar injection of 0.3% carrageenan (Sigma, St Louis) in a 20 ul volume using a 30 gauge needle into the hind left paw. Paw thickness was measured with electronic digital calipers (Traceable Calipers, Friendswood, TX), prior to and 5 h following carrageenan administration, which corresponds to peak edema (Wise et al., 2008). This procedure has been used previously by our laboratory (Cravatt et al., 2004; Lichtman et al., 2004; Wise et al., 2008).

Mechanical allodynia

The mice were placed inside ventilated polycarbonate chambers on an elevated aluminum mesh table and allowed to acclimate to the apparatus for 60 min before testing. Mechanical allodynia was assessed with von Frey filaments (North Coast Medical, Morgan Hill, CA), using the "up-down" method (Chaplan et al., 1994) 5 h after carrageenan administration. The plantar surface of each hind paw was stimulated five times with each filament (0.16–6.0 g), at a frequency of approximately 2 Hz, starting with the 0.6-g filament and increasing until the mouse responded by licking and/or lifting the paw off the surface of the test apparatus. Three or more responses out of five stimulations were coded as a positive response. Once a positive response was detected, sequentially lower weight filaments were used to assess the sensory threshold for each paw.

Testing procedures

Mice were transported to the testing room, weighed, randomly assigned to the different treatment regimens, and allowed to acclimate for at least 1 h before injections. The time course for carrageenan-induced paw edema was assessed in an initial experiment. Mechanical allodynia was assessed at the 5 h time point. For consistency with our previous studies, the 5 h time point was selected to assess paw edema and mechanical allodynia. The pre-treatment times for each drug were as follows: 30 min for diclofenac (5 mg/kg), 2 h for PF-3845 (1, 3, or 10 mg/kg), and 2 h for JZL184 (1.6, 4, 16, or 40 mg/kg). In experiments assessing cannabinoid receptor mechanism of action, the CB_1 receptor antagonist rimonabant (1 mg/kg) and the CB_2 receptor antagonist SR144528 (3 mg/kg) were administered 30 min prior to JZL184 (16 mg/kg) or vehicle. It should be noted that in initial experiments, 3 mg/kg rimonabant reduced paw edema (data not shown). In contrast, 1 mg/kg rimonabant administered alone did not affect the dependent measures and was employed for the antagonism studies. Previous studies have shown that these doses of rimonabant (Lichtman et al., 1996; Lichtman and Martin, 1997, Lichtman et al., 2004) and SR144528 (Conti et al., 2002; Lichtman et al., 2004; Malan et al., 2002) block the pharmacological effects of cannabinoid receptor agonists. The anti-edematous and anti-allodynic effects of 16 mg/kg JZL184 were evaluated in CB_1 (+/+), CB_1 (−/−), CB_2 (+/+), and CB_2 (−/−) mice to further assess receptor involvement. In addition, we determined whether administration of the antagonists after carrageenan would reverse the anti-edematous and anti-allodynic effects JZL184. In this experiment, rimonabant (1 mg/kg) or SR144528 (3 mg/kg) was injected 4 h after carrageenan and edema and allodynia were measured at 5 h.

In order to assess the impact of repeated administration of JZL184 on paw edema and mechanical allodynia, the following groups of mice were tested: (Group 1) vehicle for 6 days, (Groups 2–5) vehicle for 5 days and challenged with 1.6, 4, 16 or 40 mg/kg JZL184 on day 6, and (Groups 6–9) 1.6, 4, 16 or 40 mg/kg JZL184 for 6 days. Mice were administered their respective treatments 2 h before carrageenan was injected. Edema and mechanical allodynia were then assessed 5 h later. In the final experiment, JZL184 (16 mg/kg) was administered 3 h after carrageenan to examine whether carrageenan-induced edema and allodynia would be reversed at 5 h.

Data analysis

Paw edema data are expressed as the difference in paw thickness between the 5 h and pre-injection measures. Paw withdrawal thresholds