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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 CB₁ and CB₂ receptor antagonists rimonabant and SR144528 as well as with CB₁(–/–) and CB₂(–/–) 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 CB₁ and CB₂ receptors, but only CB₂ 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 CB₁ and CB₂ receptors, with no evidence of tolerance following repeated administration of low doses.

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Appendix A. Supplementary data: Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.lfs.2012.06.020>.

Keywords

Carrageenan; Pain; Allodynia; Inflammation; 2-arachidonylethanolamine (anandamide; AEA); Monoacylglycerol lipase (MAGL); Endogenous cannabinoid; Anandamide; Fatty acid amide hydrolase; CB₁ receptor; CB₂ receptor

Introduction

The endogenous cannabinoid (endocannabinoid) system consists of two G-protein-coupled cannabinoid (i.e., CB₁ and CB₂) receptors (Gerard et al., 1991; Matsuda et al., 1990), the lipid endogenous ligands N-arachidonylethanolamine (anandamide; AEA) (Devane et al., 1992) and 2-arachidonylethanolamine (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 CB₁ receptors than CB₂ 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 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 CB₁ 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 CB₁ (-/-) and CB₂ (-/-) mice and their respective littermate controls, CB₁ (+/+) and CB₂ (+/+) mice from the Center Transgenic Colony at Virginia Commonwealth University served as subjects. CB₁ (-/-) and CB₂ (-/-) 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 CO₂ 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 CB₁ receptor antagonist rimonabant (SR141716, National Institute on Drug Abuse), and the CB₂ 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 µl 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 CB₁ receptor antagonist rimonabant (1 mg/kg) and the CB₂ 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 CB₁ (+/+), CB₁ (-/-), CB₂ (+/+), and CB₂ (-/-) 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 to the von Frey filaments in the carrageenan-injected and contralateral (i.e., control) paws at the 5 h time point were used to assess mechanical allodynia. All data are depicted as mean ± standard error of the mean (SEM). Data were analyzed using t-tests, one-way analysis of variance (ANOVA), or two-way ANOVA. Dunnett's test was used for post hoc analysis in the dose–response experiments in which the effects of each drug dose were compared to those of vehicle. The Tukey test was used for post hoc analysis to compare different treatment groups and Bonferroni planned comparisons were used to assess genotype differences. Differences were considered significant at the $p < 0.05$ level.

Results

Anti-edematous and anti-allodynic effects of JZL184 in the carrageenan model

Intraplantar administration of carrageenan induced paw edema and decreased paw withdrawal threshold over an extended time period (Supplementary Fig. 1). In contrast, the withdrawal threshold for the control paw (i.e., contralateral paw) remained constant throughout all of the studies (Table 1). As shown in Fig. 1A, JZL184 [$F(4, 33) = 24.64$, $p < 0.001$], PF-3845 [$F(3, 20) = 25.76$, $p < 0.001$], and diclofenac [$t(12) = 5.51$, $p < 0.001$] significantly attenuated carrageenan-evoked edema. Similarly, allodynia was significantly attenuated by JZL184 [$F(4, 37) = 11.95$, $p < 0.001$], PF-3845 [$F(3, 20) = 6.596$, $p < 0.05$] and

diclofenac [$t(12) = 6.03$, $p < 0.001$]. None of the treatments altered the paw withdrawal threshold in the contralateral paw (Table 1).

Cannabinoid receptors mediate the anti-allodynic and anti-edematous of JZL184

To determine whether CB₁ and CB₂ receptors mediate the anti-allodynic effects of JZL184, mice were pretreated with rimonabant (1 mg/kg), SR144528 (3 mg/kg), or vehicle 30 min prior to JZL184 (16 mg/kg) injection. As shown in Fig. 2A, rimonabant [$F(1, 20) = 10.07$, $p < 0.001$] and SR144528 [$F(1, 20) = 19.21$, $p < 0.001$] completely blocked the anti-allodynic effects of JZL184 (Fig. 2B). There was a significant interaction between JZL184 and pretreatment with the rimonabant and SR144528 on mechanical allodynia, indicating the involvement of CB₁ and CB₂ receptors. Neither rimonabant nor SR144528 significantly affected allodynia when given alone. CB₁ (–/–) [$F(1, 19) = 5.73$, $p < 0.001$; Fig. 2C] and CB₂ (–/–) [$F(1, 24) = 8.74$, $p < 0.001$; Fig. 2D] mice were resistant to the anti-allodynic effects of JZL184. In the absence of drugs, both CB₁ (–/–) and CB₂ (–/–) showed similar nociceptive behavior as the wild type controls.

The data depicted in Fig. 3, reveal that CB₂ receptors mediate the anti-edematous effects of JZL184. Whereas rimonabant was without effect [$p = 0.22$; Fig. 3A], SR144528, completely blocked the anti-edematous effects of JZL184, as revealed by a significant interaction between JZL184 and SR144528 [$F(1, 20) = 8.73$, $p < 0.001$; Fig. 3B]. Consistent with the cannabinoid receptor antagonist data, JZL184 retained its anti-edematous effects in CB₁ (–/–) mice [$p = 0.84$; Fig. 3C], but CB₂ (–/–) mice were completely resistant to the anti-edematous effects of JZL184, as indicated by a significant interaction between JZL184 and the genotype [$F(1, 21) = 59.97$, $p < 0.001$; Fig. 3D].

In the next series of experiments, we examined whether rimonabant or SR144528 injected 4 h after carrageenan would reverse the anti-edematous and anti-allodynic effects of JZL184 (16 mg/kg). The anti-edematous effects of JZL184 were not reversed by either drug [$F(5, 27) = 15.53$; Fig. 4A], but rimonabant as well as SR144528 completely reversed the anti-allodynic effects of JZL184 [$F(5, 37) = 4.05$, $p < 0.05$; Fig. 4B].

Differential tolerance following repeated administration of low dose and high dose JZL184

As genetic deletion or prolonged pharmacological inhibition of MAGL is known to produce CB₁ receptor functional tolerance (Chanda et al., 2010; Schlosburg et al, 2010), we assessed the dose–response relationship of JZL184 (1.6, 4, 16, or 40 mg/kg) after acute or repeated administration in the carrageenan assay. JZL184 dose-dependently attenuated carrageenan-induced paw edema and allodynia. As shown in Fig. 5, acute administration of 4, 16, and 40 mg/kg JZL184 significantly attenuated carrageenan-induced edema [$F(8, 65) = 11.62$, $p < 0.001$; Fig. 5A]. While the anti-edematous effects of 4 and 16 mg/kg JZL184 were maintained following repeated dosing, the anti-edematous effects of 40 mg/kg JZL184 underwent tolerance upon repeated administration. Acute administration of 4, 16, and 40 mg/kg JZL184 also significantly attenuated mechanical allodynia [$F(8, 65) = 7.953$, $p < 0.001$; Fig. 5B]. The anti-allodynic effects of 4 mg/kg JZL184 was maintained after repeated dosing; however, repeated administration of high doses of JZL184 (16 and 40 mg/kg) led to tolerance. The lowest dose of JZL184, 1.6 mg/kg, tested in this study, remained ineffective regardless of whether it was administered acutely or repeatedly. There was no edema or allodynia seen in the contralateral paw (Table 1).

JZL184 reverses carrageenan-induced anti-edema and allodynia

In the previous experiments, JZL184 administered before carrageenan decreased the development of paw edema and mechanical allodynia. In order to test whether JZL184 would reverse these responses, subjects received vehicle or JZL184 (16 mg/kg) 3 h after

carrageenan. Edema was measured just before drug administration and at 5 h and allodynia was assessed at 5 h. As shown in Fig. 6A, JZL184 produced a significant partial reversal of carrageenan-induced edema at 5 h compared with the 3 h time point [$F(1, 10) = 58.58$, $p < 0.001$]. JZL184 given after the induction of carrageenan-induced paw edema also significantly attenuated the mechanical allodynia at 5 h [Fig. 6B; $t(10) = 2.90$, $p < 0.01$]. JZL184 did not affect paw withdrawal thresholds in control paws (Table 1).

Discussion

JZL184 represents the first selective MAGL inhibitor that elevates brain 2-AG, but not anandamide, levels following acute administration (Long et al., 2009). This compound reduces nociceptive behavior in a wide range of laboratory animal models of pain, including warm water tail withdrawal, acetic acid stretching, formalin, mechanical and cold allodynia following chronic constrictive injury of the sciatic nerve, capsaicin-induced mechanical allodynia, and bone cancer tests (Busquets-Garcia et al., 2011; Guindon et al., 2011; Khasabova et al., 2011; Kinsey et al., 2009, 2010; Spradley et al., 2010). The present study increases the understanding that MAGL inhibition plays on nociception by demonstrating that JZL184 reduces carrageenan-induced paw edema and associated mechanical allodynia. These effects were similar in magnitude to those produced by the FAAH inhibitor PF-3845, as well as the nonselective COX inhibitor diclofenac. The anti-edematous effects of JZL184 were mediated through CB₂ receptors, while the anti-allodynic effects required both CB₁ receptors and CB₂ receptors. These findings are consistent with the observation that 2-AG has similar affinity for both cannabinoid receptors (Mechoulam et al., 1995). In addition, repeated administration of high dose JZL184 resulted in tolerance to its anti-allodynic and anti-edematous effects, but a low dose of JZL184 retained efficacy after repeated administration.

Initial studies investigating the in vivo consequences of inhibiting MAGL employed URB602 (Comelli et al., 2007; Desroches et al., 2008; Guindon et al., 2007a, 2007b, 2011; Guindon and Hohmann, 2008; Hohmann et al., 2005; Vandevorde et al., 2007) and *N*-arachidonyl maleimide (Burstion et al., 2008). For example, systemic or peripheral administration of URB602 suppressed formalin-induced nociception, carrageenan-induced inflammatory nociception, and partial sciatic nerve ligation-induced nociception in rats (Comelli et al., 2007, Desroches et al., 2008, Guindon et al., 2011). Although URB602 and *N*-arachidonyl maleimide inhibit MAGL in the brain, these compounds are nonselective and inhibit other serine hydrolases, including FAAH (Burstion et al., 2008; Hohmann et al., 2005; Vandevorde et al., 2007).

A key finding in the present study was that JZL184 administration 3 h after carrageenan significantly reversed the magnitude of paw edema and mechanical allodynia. Similarly, URB602 reversed carrageenan-induced paw edema and paw withdrawal latency when administered after carrageenan in mice (Comelli et al., 2007). These findings suggest that endocannabinoid catabolic inhibitors may possess therapeutic utility in treating clinical symptoms associated with inflammatory disease states.

The observations that JZL184 did not attenuate carrageenan-induced edema in SR144528-treated wild type mice and CB₂ (−/−) mice indicate a necessary role of CB₂ receptors. In contrast, complementary pharmacological and genetic approaches indicated that CB₁ receptors are expendable for the anti-edematous effects of JZL184. Likewise, the anti-edematous effect of URB602, which inhibits FAAH and MAGL with similar potency, has also been reported to be blocked by SR144528, but not by rimonabant (Comelli et al., 2007). Studies have shown that systemic and local administration of the CB₂ receptor agonist AM1241 suppressed the development of mechanical stimulation in the carrageenan model of

inflammation (Nackley et al., 2003a). This suppression was blocked by SR144528 but not by rimonabant (Nackley et al., 2003b). Previous studies have also reported that elevation of 2-AG attenuates inflammatory and immune response in vitro (Ouyang et al., 1998; Gallily et al., 2000; Facchinetti et al., 2003). Thus, inhibiting 2-AG degradation in vivo offers a strategy to augment levels of this endogenous cannabinoid to elicit anti-inflammatory effects through the stimulation of CB₂ receptors.

Whereas the anti-edematous effect of JZL184 was mediated by CB₂ receptors, its anti-allodynic effects required both CB₁ and CB₂ receptors. Similarly, the suppressive effects of JZL184 on capsaicin-induced thermal hyperalgesia and nocifensive behavior required both CB₁ and CB₂ receptors (Spradley et al., 2010). Likewise, intra-paw administration of JZL184 or URB602 produced antinociceptive effects in the formalin model, which were blocked by CB₁ and CB₂ receptor antagonists (Guindon et al., 2007a, 2011). In contrast, CB₂ receptors did not play a necessary role in the anti-allodynic effects of JZL184 in the chronic constriction injury model (Kinsey et al., 2009, 2010). Two related questions are raised by these observations. First, why do both CB₁ and CB₂ receptors play a necessarily role in the antinociceptive effects of JZL184 in some types of nociceptive assays (e.g., carrageenan, formalin, and capsaicin), but only CB₁ receptors are required in other models (e.g., warm water tail withdrawal, acetic acid stretching, CCI)? Second, what is the mechanism by which both cannabinoid receptors play a necessarily role in the anti-allodynic effects of JZL184 in the carrageenan assay? It is unlikely that the method to assess nociception accounts for these disparate findings because von Frey filaments were used to assess mechanical allodynia in both CCI and carrageenan assays. Instead, the differential cannabinoid receptor involvement may be related to the degree to which inflammatory responses contribute to the nociception as well as the concentration of the cannabinoid receptor subtypes and 2-AG at the critical sites of action. Indeed, JZL184 may be producing its actions at the site of inflammation in the paw, within the dorsal root ganglia or dorsal horn of the spinal cord, or at multiple supraspinal regions (e.g., PAG or the rostral ventromedulla). It has been shown that CB₂ receptors are upregulated in the dorsal root ganglia and paw tissue of rodents administered complete Freund's adjuvant (Hsieh et al., 2011), suggesting that CB₂ receptors in these regions could contribute to the findings reported here. For example, intraplantar carrageenan could lead to the infiltration of immune cells, such as macro-phages or neutrophils, at the site of injection, that express CB₂ receptors (Galiege et al., 1995). 2-AG activation of CB₂ receptors on infiltrating cells might synergize with the well described antinociceptive actions of CB₁ receptor stimulation on peripheral nociceptors (Agarwal et al., 2007) within the spinal cord (Yaksh, 1981; Lichtman and Martin, 1991) and within supraspinal sites of action (Lichtman et al., 1996; Martin et al., 1999). Future studies are needed to examine whether JZL184 alters inflammatory mediators (e.g, pro- and anti-inflammatory cytokines and prostaglandins, as well as infiltrating immune cells) caused by carrageenan. In order, to ascertain the relative contribution of CB₁ and CB₂ receptors in these effects, we also evaluated the ability of rimonabant and SR144528 administered 4 h after carrageenan to reverse the anti-allodynic and anti-edematous effects of JZL184. Whereas neither antagonist reversed the anti-edematous effects of JZL184, both rimonabant and SR144528 reversed the anti-allodynic effects of JZL184, indicating that these effects can be dissociated.

Another important finding in the present study was that the anti-allodynic and anti-edematous effects of JZL184 were maintained following repeated low dose administration, but the effects underwent tolerance after repeated high dose JZL184. Similarly, prolonged inactivation of MAGL via administration of high dose JZL184 (40 mg/kg) results in the loss of analgesic responses in the CCI model, cross-tolerance to exogenous cannabinoid receptor agonists (i.e., THC, and WIN55, 212-2), CB₁ receptor downregulation and desensitization in cingulate cortex, hippocampus, somatosensory cortex, and PAG (Chanda et al., 2010;

Schlosburg et al., 2010). Additionally, 2-AG elevation in MAGL ($-/-$) mice caused tonic activation and partial desensitization of CB₁ receptors (Chanda et al., 2010; Schlosburg et al., 2010; Pan et al., 2011). The findings that the anti-edematous and anti-allodynic effects of low dose JZL184 were maintained after repeated dosing are consistent with a previous report in which repeated administration of 8 mg/kg JZL184 maintained its anxiolytic-like effects under high illumination conditions in the rat elevated plus maze assay (Sciolino et al., 2011).

Conclusion

In conclusion, the present study demonstrates that the selective MAGL inhibitor, JZL184, significantly inhibits inflammatory pain, as assessed in the carrageenan assay. More specifically, JZL184 attenuated the development of paw edema and mechanical allodynia and also reversed edema and allodynia when administered after carrageenan. Complementary genetic and pharmacological approaches revealed that the anti-allodynic effects of JZL184 required both CB₁ and CB₂ receptors, whereas only CB₂ receptors had a necessary role in mediating its anti-edematous effect. We also found that the anti-allodynic and anti-edematous effects of low, but not high, doses of JZL184 do not undergo tolerance when administered repeatedly. These results indicate that the activation of CB₁ and/or CB₂ receptors by low doses of MAGL inhibitors may have beneficial effects on inflammatory pain that include the ability to prevent inflammation and reverse established inflammatory pain states.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

JZL184, PF-3845, and diclofenac partially reduced edema and allodynia in the carrageenan model. JZL184 significantly reduced carrageenan-induced paw edema (Panel A) and allodynia (Panel B). Diclofenac and PF-3845 significantly attenuated carrageenan-induced paw edema (Panel C) and allodynia (Panel D). Values represent the mean ($\pm$ SEM) mechanical paw withdrawal threshold and difference in paw thickness. * $p < 0.05$, *** $p < 0.001$ versus VEH. +++ $p < 0.001$ versus VEH. $N = 6-7$ mice/group.

Fig. 2.

The anti-allodynic effects of JZL184 require both CB₁ and CB₂ receptors. Panel A. Rimobant (SR1) blocked the anti-allodynic effects of JZL184. *** $p < 0.001$ versus VEH/VEH; ### $p < 0.001$ versus VEH/JZL184. Panel B. Similarly, SR144528 (SR2) blocked the anti-allodynic effects of JZL184. *** $p < 0.001$ versus VEH/VEH; ### $p < 0.001$ versus VEH/JZL184. Panel C. The anti-allodynic effects of JZL184 do not occur in CB₁ (-/-) mice. ** $p < 0.05$ versus (++)/VEH, ### $p < 0.001$ versus CB₁ (++)/JZL184. Panel D. The anti-allodynic effects of JZL184 do not occur in CB₂ (-/-) mice. ** $p < 0.001$ versus (++)/VEH, ### $p < 0.001$ versus CB₂ (++)/JZL184. Values represent the mean ($\pm$ SEM) mechanical paw withdrawal threshold. N = 6/group.

Fig. 3.

The anti-edematous effects of JZL184 require CB₂ receptors, but CB₁ receptors are expendable. Panel A. Rimonabant (SR1) did not block the anti-edematous effects of JZL184. *** $p < 0.001$ versus VEH/VEH. Panel B. SR144528 (SR2) blocked the anti-edematous effects of JZL184. *** $p < 0.001$ versus VEH/VEH; ## $p < 0.05$ versus VEH/JZL184. Panel C. The anti-edematous effects of JZL184 occur in CB₁ (-/-) mice. ** $p < 0.01$, *** $p < 0.001$ versus (+/+)/VEH. Panel D. The anti-edematous effects of JZL184 do not occur in CB₂ (-/-) mice (Panel D). *** $p < 0.001$ versus (+/+)/VEH; ### $p < 0.001$ versus (+/+)/JZL184. Values represent the mean ($\pm$ SEM) difference in paw thickness. $N = 5-7$ /group.

Fig. 4. JZL184-induced anti-allodynia occurs independently of its anti-edematous effect. Panel A. Neither rimonabant (SR1) nor SR144528 (SR2) blocked the anti-edematous effect of JZL184 when injected 4 h after carrageenan. Panel B. Both rimonabant and SR144528 blocked the anti-allodynic effect of JZL184 when injected 4 h after carrageenan. Values represent the mean ($\pm$ SEM) mechanical paw withdrawal threshold and difference in paw thickness. ** $p < 0.01$, *** $p < 0.001$ versus VEH/VEH; # $p < 0.05$ versus JZL184/VEH. $N = 6-9$ /group.

Fig. 5.

The anti-edematous (Panel A) and anti-allodynic (Panel B) effects of low doses of JZL184 do not undergo tolerance following repeated administration. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ versus VEH/VEH; ### $p < 0.001$ versus acute 40 mg/kg JZL184. Values represent the mean ($\pm$ SEM) mechanical paw withdrawal threshold and difference in paw thickness. $N = 6-8$ /group.

Fig. 6. JZL184 reverses edema (Panel A) and allodynia (Panel B) when administered 3 h after carrageenan. Drug was administered immediately after the 3 h allodynia assessment. Values represent the mean ($\pm$ SEM) mechanical paw withdrawal threshold and difference in paw thickness. ** $p < 0.01$ versus JZL184 at 3 h. ** $p < 0.01$, versus VEH; $N = 6$ /group.

Table 1

The paw withdrawal threshold for the control paw was not altered by any of the treatments as measured with von Frey filaments. Values represent the mean ($\pm$ SEM) mechanical paw withdrawal threshold.

Experiment	Mean (g)	$\pm$ SEM
JZL184 acute dose	3.27	0.142
JZL 184 repeated dosing	3.11	0.119
PF-3845 acute dose	3.20	0.276
Diclofenac	3.14	0.40
Rimonabant	3.35	0.165
SR144528	2.90	0.248
CB ₁ (–/–) mice	3.37	0.297
CB ₂ (–/–) mice	2.96	0.217
Rimonabant or SR144528 after carrageenan	3.44	0.189
JZL184 after carrageenan	3.50	0.435
JZL184 repeated administration	3.21	0.164