Neuropharmacology 81 (2014) 224–230

Contents lists available at ScienceDirect

Neuropharmacology

journal homepage: www.elsevier.com/locate/neuropharm

Actions of the dual FAAH/MAGL inhibitor JZL195 in a murine inflammatory pain model

Wayne B. Anderson*,[1], Michael J. Gould [1], Romeo D. Torres, Vanessa A. Mitchell, Christopher W. Vaughan

Pain Management Research Institute, Kolling Institute of Medical Research, Northern Clinical School, University of Sydney at Royal North Shore Hospital, St Leonards, NSW 2065, Australia

ARTICLE INFO

Article history:
Received 5 August 2013
Received in revised form
5 December 2013
Accepted 19 December 2013
Available online 30 December 2013

Keywords:
Cannabinoid receptors
Inflammation
Pain
Fatty acid amide hydrolase
Monoacylglycerol lipase

ABSTRACT

The analgesic efficacy of cannabinoids in chronic pain models is limited by side-effects. It has been proposed that this might be overcome by using agents which indirectly activate the endocannabinoid system. We examined the analgesic and side-effect profile of the dual FAAH/MAGL inhibitor JZL195 in an inflammatory pain model. The effect of systemic injections of a range of doses of JZL195 and the pan-cannabinoid receptor agonist WIN55212 were performed 1 day following intraplantar injection of CFA in C57BL/6 mice. JZL195 and WIN55212 both reduced mechanical allodynia and thermal hyperalgesia, and produced catalepsy and sedation in a dose dependent manner. Unlike WIN55212, JZL195 reduced allodynia at doses below those at which side-effects were observed. The effects of JZL195 and WIN55212 were abolished by co-application with the CB1 antagonist AM251. The CB2 antagonist also reduced the JZL195 anti-allodynia, and reversed the WIN55212 anti-allodynia. The reduction in allodynia produced by JZL195 was greater than that produced individually by the FAAH and MAGL inhibitors, URB597 and JZL184. These findings suggest that JZL195 reduces inflammation induced allodynia at doses below those which produce side-effects, and displays greater efficacy that FAAH or MAGL inhibitors. Thus, dual FAAH/MAGL inhibition has the potential to alleviate inflammatory pain with reduced cannabinoid-like side-effects.

1. Introduction

The psychoactive component of *Cannabis sativa*, Δ9-tetrahydrocannabinol (THC), and synthetic cannabinoid receptor agonists have well established efficacy in preclinical models of chronic inflammatory pain. A number of animal studies have shown that systemic injection of a range of non-selective cannabinoid CB1/2 agonists reduce inflammation induced allodynia and hyperalgesia (Clayton et al., 2002; De Vry et al., 2004; Elmes et al., 2005; Jayamanne et al., 2006; Kehl et al., 2003; Smith et al., 1998). Cannabinoid agonists, however, also produce a range of side-effects such as motor-incoordination, catalepsy and immobility, and cognitive impairment. While some of these studies have shown that cannabinoid agonists reduce inflammation induced allodynia at doses below those at which they produce side-effects, the therapeutic window between analgesia and side-effects produced by systemically delivered non-selective cannabinoid agonists has not been determined (De Vry et al., 2004; Jayamanne et al., 2006; Kehl et al., 2003).

In order to improve cannabinoid therapies, a number of agents which modulate the endogenous cannabinoid (endocannabinoid) system have been examined (Petrosino and Di Marzo, 2010; Roques et al., 2012). Endocannabinoids play an important role in pain via their actions at cannabinoid CB1 and CB2 receptors, plus other receptors (Bradshaw and Walker, 2005; Hill et al., 2009; Pacher et al., 2006). The two main endocannabinoids, N-arachidonyl ethanolamide (anandamide) and 2-arachidonyl glycerol (2-AG), are synthesized and released on demand, and are then metabolized by fatty acid amide hydrolase (FAAH) and monoacylglyceride lipase (MAGL), respectively (Di Marzo et al., 2005; Piomelli, 2003). A number of agents have now been described which selectively target FAAH and MAGL.

There is substantial evidence that FAAH and MAGL inhibitors reduce inflammatory pain when administered systemically in a therapeutic regimen, i.e. after inflammation is established. In these studies, FAAH inhibitors such as URB597, URB937, PF3485 and

* Corresponding author. Tel.: +61 2 9926 4950; fax: +61 2 9926 4045.
E-mail address: wayne.anderson@sydney.edu.au (W.B. Anderson).
URL: http://www.pmri.med.usyd.edu.au
[1] These authors contributed equally to the study.

0028-3908/$ – see front matter © 2013 Published by Elsevier Ltd.
http://dx.doi.org/10.1016/j.neuropharm.2013.12.018

PF04457845 reduce the allodynia and hyperalgesia induced in a range of models of inflammation (Ahn et al., 2009, 2011; Booker et al., 2012; Clapper et al., 2010; Jayamanne et al., 2006; Kinsey et al., 2011a; Naidu et al., 2010). A smaller number of studies have demonstrated that the MAGL inhibitors URB602 and JZL184 also reduce inflammation induced allodynia and hyperalgesia (Comelli et al., 2007; Ghosh et al., 2013). Like cannabinoid agonists, both FAAH and MAGL inhibitors produce cannabinoid side-effects at high doses, however, the therapeutic window between pain relief and side-effects of these agents has not been directly assessed in an inflammatory pain state (Ahn et al., 2011; Comelli et al., 2007; Jayamanne et al., 2006).

Recently, a dual FAAH and MAGL inhibitor, JZL195 has been described which inhibits the degradation of both anandamide and 2-AG with high potency (Long et al., 2009b). Systemic administration of JZL195 produces analgesia in acute thermal pain assays, but also produces side-effects at high doses (Long et al., 2009b; Wise et al., 2012). Given that both FAAH and MAGL inhibitors have a role in reducing inflammatory pain, we therefore examined the effect of systemic delivery of JZL195 in a mouse model of hind paw inflammation, and compared it to URB597 and JZL184, plus the non-selective cannabinoid receptor agonist WIN55212. In these animals, we obtained their dose-response effects on allodynia, plus a range of cannabinoid-like side-effects in order to compare their relative therapeutic indices.

2. Methods

Experiments were carried out on adult male C57BL/6 mice following the guidelines of the 'NH&MRC Code of Practice for the Care and Use of Animals in Research in Australia' and with the approval of the Royal North Shore Hospital (RNSH) Animal Care and Ethics Committee (ACEC). Mice initially weighed between 20 and 25 g, and were housed in groups of four in individually ventilated cages (23 ± 1 °C, humidity 70%) with environmental enrichment and free access to food and water, in a 12:12 h light–dark cycle.

2.1. Behavioural and pain testing

All testing was carried out in the day cycle, commencing at 9am. Inflammation was measured as paw volume displacement using a plethysmometer (Ugo Basile, Comerio, Italy). To assess mechanical allodynia, the mechanical paw withdrawal threshold (PWT) to mechanical stimulation of the left hind paw was assessed using von Frey hairs (North Coast Medical, San Jose, USA). The mice were allowed to acclimatise for 20–30 min prior to testing in elevated perspex cages (15 × 10 × 10 cm) with a wire mesh floor. A series of von Frey hairs (0.2–8.5 g) were pressed perpendicularly (for 2 s) onto the plantar surface of the hind paw 4 times, and the threshold was calculated using a threshold tracking algorithm (Chaplan et al., 1994). Thermal hyperalgesia was measured using a Hargreaves plantar tester (Ugo Basile). The mice were placed in a perspex enclosure (9 × 9 × 14 cm) and given 20–30 min to acclimatise. A focused infrared beam was directed to the plantar surface of the left hind paw through a glass plate. Thermal paw withdrawal latency (PWL) was measured as the time at which the mouse sharply withdrew its paw, with a cut-off duration of 20 s to prevent tissue injury.

A number of side effect measures were also included during testing. Motor impairment was measured using a rotarod device (Ugo Basile) which gradually accelerated (0–30 rpm, cut-off time 300 s). The time at which the mouse fell off the rotarod, or just held onto the cylinder for 2 consecutive rotations was noted as the rotarod latency. Catalepsy was assessed with the bar test by placing the animal's forepaws on a bar 4.5 cm off the ground. The time taken to remove both forepaws from this position was measured (cut-off time of 120 s). Spontaneous locomotor activity was assessed using the open field test. Each animal was placed in an open topped, opaque, perspex enclosure (40 × 40 × 40 cm) and video recorded for 2 min. For analysis, the area was divided into a 4 × 4 grid and the number of forepaw crossings counted. Unlike other tests, the open field was only tested once; at 2 h post-drug injection. All of the above tests were carried out in low level white light, except for the open field test which was carried out under low level red light (both <3 lux).

2.2. Drug administration

Stock solutions of all agents were prepared in a vehicle solution which comprised 2% randomly-methylated beta-cyclodextrin, 15% dimethylsulfoxide (DMSO) and 5% tween80 in saline. Subcutaneous injections were made in a volume of 0.1, or 0.15 ml per 10 g body weight; the results for vehicle injections of both volumes were not significantly different and were subsequently pooled. Solutions of all agents were made up immediately prior to administration. Drugs were tested in a randomised order and the experimenter was blinded to the drugs being tested.

2.3. Drugs

AM251 (1-(2,4-dichlorophenyl)-5-(4-iodophenyl)-4-methyl-N-(1-piperidyl) pyrazole-3-carboxamide), AM630 (1-[2-(morpholin-4-yl)ethyl]-2-methyl-3-(4-methoxybenzoyl)-6-iodoindole), JZL184 (4-nitrophenyl-4-(dibenzo[d][1,3]dioxol-5-yl(hydroxy)methyl)piperidine-1-carboxylate), JZL195 (4-nitrophenyl 4-(3-phenoxybenzyl)piperazine-1-carboxylate), URB597 ((3′-(aminocarbonyl)[1,1′-biphenyl]-3-yl)-cyclohexylcarbamate) and (+)-WIN55212 mesylate ([(3R)-2,3-dihydro-5-methyl-3-(4-morpholinylmethyl)pyrrolo[1,2,3-de]-1,4-benzoxazin-6-yl]-1-naphthalenyl-methanone, monomethanesulfonate) were obtained from Cayman Chemicals (Ann Arbor, USA). All other reagents were obtained from Sigma (Sydney, Australia).

2.4. Protocol and pain model

All animals were allowed to acclimatize to their holding cages for 4–5 days before any procedures were carried out. Acclimatisation to the testing apparatuses and baseline behavioural data was collected for 2 days. On the next day, 5–50 μl of Complete Freund's Adjuvant (CFA, Sigma, Sydney, Australia) was injected subcutaneously into the plantar surface of the left hind paw under isoflurane anaesthesia (2.5% in saturated O_2, 1 ml min^{-1}) using a 30-gauge needle. 24 h later, behavioural testing was carried out twice over a 30–45 min period, the animal then received a drug injection, and testing was repeated at set intervals for up to 6 h. Animals were euthanized at the end of the testing period.

2.5. Analysis

For the initial time course experiments, comparisons of drug/vehicle treatment effects over time were made using two-way repeated measures ANOVAs, with time and treatment as a within- and between-subjects factors, respectively (Prism, GraphPad Software, La Jolla, USA). When two-way ANOVAs were significant, post-hoc comparisons between treatment groups at individual time points were made using the Bonferroni adjustment for multiple comparisons.

In subsequent experiments, the post-drug measures for mechanical PWT, thermal PWL, bar test and rotarod were taken as the average of measurements at 1 and 2 h post-injection, and values were calculated as a percentage of the maximum possible effect (MPE) = (post-drug−pre-drug)/(cut-off−pre-drug). The cut-off values were 8.5 g, 20 s, 120 s and 300 s for mechanical PWL, thermal PWL, bar test and rotarod, respectively. For the open field crossings, raw values at 2 h post-drug injection were used. Dose response curves were constructed by fitting data to a sigmoidal curve with variable slope (Prism). Statistical comparisons of drug effects alone were made using one-way ANOVAs and when significant, post-hoc comparisons were made using the Newman–Keuls adjustment for multiple comparisons. Statistical comparisons of drug effects in the presence and absence of antagonists were made using two-way ANOVAs and when significant, post-hoc comparisons were made using the Bonferroni adjustment for multiple comparisons. All data is shown as mean ± s.e.mean.

3. Results

We first established the minimal dose of CFA needed to produce inflammation induced mechanical allodynia in C57BL/6 mice. At 24 h after intraplantar injection, 5, 10, 25 and 50 μl CFA produced increases in paw volume of 0.01 ± 0.01, 0.04 ± 0.01, 0.17 ± 0.03 and 0.27 ± 0.04 ml ($n = 4$ each). The mechanical PWT was 5.9 ± 0.6 g prior to CFA injection, and was 1.9 ± 0.6, 1.6 ± 0.5, 0.2 ± 0.1 and 0.2 ± 0.1 g at 24 h after CFA injection. We therefore examined the effect of cannabinoids in animals 24 h after intraplantar injection of 25 μl of CFA.

3.1. Time course of action of JZL195 and WIN55212

We first determined the time course of action of the dual FAAH/MAGL inhibitor JZL195 and the pan-cannabinoid receptor agonist WIN55212 on the inflammation induced allodynia. This was done at doses which were just above their ED_{50} values to avoid ceiling effects which might mask their time course of action. The effect of subcutaneous injection of JZL195 (20 mg kg^{-1}, $n = 6$), WIN55212 (1 mg kg^{-1}, $n = 4$) and vehicle ($n = 4$) on mechanical PWT differed over time ($F_{2,13} = 10.5$, $p = 0.002$). Both JZL195 and WIN55212 produced an increase in mechanical PWT which plateaued within 1–2 h (Fig. 1). JZL195 produced an increase in mechanical PWT