

RESEARCH PAPER

The inhibition of monoacylglycerol lipase by URB602 showed an anti-inflammatory and anti-nociceptive effect in a murine model of acute inflammation

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Background and purpose: 2-arachidonoylglycerol (2-AG) is an endocannabinoid whose hydrolysis is predominantly catalysed by the enzyme monoacylglycerol lipase (MAGL). The development of MAGL inhibitors could offer an opportunity to investigate the anti-inflammatory and anti-nociceptive role of 2-AG, which have not yet been elucidated. On these bases, URB602, a MAGL inhibitor, was tested in a murine model of inflammation/inflammatory pain.

Experimental approach: Acute inflammation was induced by intraplantar injection of λ -carrageenan into mice. The highest dose to be employed has been selected performing the tetrad assays for cannabimimetic activity in mice. URB602 anti-inflammatory and anti-nociceptive efficacy (assessed by plethysmometer and plantar test, respectively) was evaluated both in a preventive regimen (drug administered 30 min before carrageenan) and in a therapeutic regimen (URB602 administered 30 min after carrageenan). To elucidate the cannabinoid receptor involvement, rimonabant and SR144528, CB₁ and CB₂ selective antagonists, respectively, were given 15 min before URB602.

Key results: Systemic administration of URB602 elicited a dose-dependent anti-oedemigen and anti-nociceptive effect that was reversed exclusively by the CB₂ receptor antagonist. The efficacy of URB602 persisted also when the compound was administered in a therapeutic regimen, suggesting the ability of URB602 to improve established disease.

Conclusions and implications: The present report highlighted the ability of the selective MAGL inhibitor, URB602, to prevent and treat an acute inflammatory disease without producing adverse psychoactive effects. The data presented herein also contributed to clarify the physiological role of 2-AG in respect to inflammatory reactions, suggesting its protective role in the body.

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Abbreviations: AEA, arachidonylethanolamide (anandamide); 2-AG, 2-arachidonoylglycerol; CB₁, cannabinoid receptor type 1; CB₂, cannabinoid receptor type 2; DMSO, dimethylsulphoxide; FAAH, fatty acid amide hydrolase; MAGL, monoacylglycerol lipase; PEA, palmitoylethanolamide; rimonabant (SR141716), *N*-piperidino-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methylpyrazole-3-carboxamide; SR144528, *N*-[(1*S*)-endo-1,3,3-trimethyl bicyclo [2.2.1] heptan-2-yl]-5-(4-chloro-3-methylphenyl)-1-(4-methylbenzyl)-pyrazole-3-carboxamide; URB602, [1,1'-biphenyl]-3-yl-carbamic acid, cyclohexyl ester

Introduction

2-Arachidonoylglycerol (2-AG), a monoglyceride of arachidonic acid esterified at the *sn*-2 position, which was isolated from rat brain (Sugiura *et al.*, 1995) and from canine gut

(Mechoulam *et al.*, 1995), is currently recognized as the most abundant and efficacious endocannabinoid. 2-AG is present in rat brain in amounts 170–1000 greater than the other endogenous cannabinoid, anandamide (AEA) (Sugiura *et al.*, 1995; Stella *et al.*, 1997). 2-AG acts as a fully efficacious agonist at both cannabinoid receptor type 1 (CB₁) and type 2 (CB₂) (Stella *et al.*, 1997; Sugiura *et al.*, 1999, 2000; Gonsiorek *et al.*, 2000; Savinainen *et al.*, 2001). In contrast, AEA, is less potent and acts only as a partial agonist at the cannabinoid

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receptors (Sugiura *et al.*, 1995, 2000; Savinainen *et al.*, 2001). 2-AG, as anandamide, produced 'on demand', acts near the site of its synthesis and is promptly metabolized to ensure rapid signal inactivation (Devane *et al.*, 1992; Mechoulam *et al.*, 1995; Sugiura *et al.*, 1995). Although 2-AG might be metabolized by fatty acid amide hydrolase (FAAH) (Di Marzo *et al.*, 1998; Bisogno *et al.*, 2005), a distinct enzyme, monoacylglycerol lipase (MAGL) plays the predominant role in catalysing 2-AG hydrolysis (Dinh *et al.*, 2004). This enzyme, which has been cloned and characterized in rat (Dinh *et al.*, 2002) and human brain (Ho *et al.*, 2002), hydrolyses preferentially 2-acylglycerols such as 2-AG and 2-oleoylglycerol (2-OG), but is unable to metabolize AEA and palmitoylethanolamide (PEA) (Dinh *et al.*, 2002). The role of MAGL as a 2-AG hydrolysing enzyme in the cannabinoid system has only recently started to be elucidated and few MAGL inhibitors have been developed. Among these, URB602 ([1,1'-biphenyl]-3-yl-carbamic acid, cyclohexyl ester) can specifically and noncompetitively inhibit MAGL activity (Makara *et al.*, 2005). In fact, URB602 increases 2-AG levels without altering levels of AEA both *in vitro* and *in vivo* (Hohmann *et al.*, 2005), confirming the selectivity of this agent in inhibiting MAGL relative to FAAH activity. Moreover, URB602 did not affect the activities of lipid-metabolizing enzymes such as diacylglycerol lipase and cyclooxygenase-2 and did not significantly influence binding of [³H]WIN55212-2 to CB₁ or CB₂ receptors (IC₅₀ ≥ 5 μM) or [³⁵S]GTP-γS to rat cerebellar membranes (EC₅₀ > 50 μM) (Hohmann *et al.*, 2005). On these bases, URB602 could offer the opportunity to investigate the functions of 2-AG by blocking its deactivation and thus amplifying its intrinsic actions. To date, this approach led to the demonstration that URB602 magnifies endocannabinoid-dependent stress-induced analgesia (Hohmann *et al.*, 2005; Suplita *et al.*, 2006) and induces anti-nociception in formalin-evoked pain behaviour (Guindon *et al.*, 2007). Notably, the enhanced stress-induced analgesia was CB₁-mediated (Hohmann *et al.*, 2005; Suplita *et al.*, 2006), whereas the anti-nociceptive effect in formalin test knew CB₁ and CB₂ mediation (Guindon *et al.*, 2007).

There are controversial findings concerning the 2-AG role in inflammatory processes. In fact, Oka *et al.* (2005) found that 2-AG levels in mouse ear were markedly augmented following the application of 12-*O*-tetradecanoylphorbol-13-acetate. Moreover, topical application of 2-AG to mouse ear evoked swelling, which was abolished by treatment with the selective CB₂ receptor antagonist *N*-[1-(1*S*)-endo-1,3,3-trimethyl bicyclo [2.2.1] heptan-2-yl]-5-(4-chloro-3-methylphenyl)-1-(4-methylbenzyl)-pyrazole-3-carboxamide (SR144528). In contrast, several investigators reported that 2-AG and the cannabinoid CB₂ receptors are involved in attenuation of inflammatory reactions and immune responses. Particularly, 2-AG has been shown to inhibit cytokine production, including tumour-necrosis factor- α released from both lipopolysaccharide-treated rat microglial cells (Facchinetti *et al.*, 2003) and murine macrophages (Gallily *et al.*, 2000) and interleukin-2 secretion in activated murine splenocytes (Ouyang *et al.*, 1998). In contrast, more clear is the evidence concerning the analgesic property of 2-AG. Recently, Hohmann *et al.* (2005) demonstrated

that the release of 2-AG in periaqueductal grey matter might mediate the stress-induced analgesia in rats and that URB602 local administration enhanced such anti-nociception, identifying MAGL as a potential therapeutic target for pain control.

Therefore, to clarify better the role of 2-AG in the inflammation, and to evaluate the potential pharmacological properties of MAGL inhibitors, we tested the preventive and therapeutic effect of URB602 in a murine model of inflammation/inflammatory pain such as that evoked by carrageenan. In addition, cannabinoid receptor antagonists were used to elucidate which receptor subtype is involved in the anti-inflammatory effect of 2-AG.

Methods

Animals

All experiments were performed in accordance with Italian State and European regulations governing the care and treatment of laboratory animals (permission no. 101/2004B) and conformed to the guidelines for the study of pain in awake animals established by the International Association for the Study of Pain (Zimmermann, 1983). Male C57BL/6J mice (9 weeks old, Harlan, Italy) were housed under controlled temperature (22 ± 1 °C), humidity (60 ± 10%) and light (12 h/day) and allowed to acclimatize for at least 1 week before being used in the experiments. Standard food and water was available *ad libitum*. All the experiments were conducted in a randomized manner by the same experimenter blind to the pharmacological treatments.

Drugs

URB602 was purchased from the Cayman Chemical Co. (Ann Arbor, MI, USA); rimonabant and SR144528 were kindly supplied by Sanofi-Aventis (Montpellier, France).

Evaluation of psychoactive effects

A series of four consecutive observations were conducted on each mouse, according to the standard procedure employed to evaluate psychoactive cannabinoid-induced effects (Compton *et al.*, 1993). Mice were consecutively tested for body temperature, nociceptive threshold, spontaneous locomotor activity and immobility on a ring, 30, 60 and 90 min after i.p. doses (10 and 20 mg kg⁻¹) of URB602. Body temperature was measured with a rectal thermistor probe (Ellab Instruments, Roedrove, Denmark) inserted to a constant depth of 2 cm. To determine thermal nociception, mice were tested for responsiveness to radiant heat with a plantar test (Ugo Basile, Varese, Italy). Spontaneous locomotor activity was counted for 5 min by an activity cage (Ugo Basile, Varese, Italy), which recorded the number of horizontal animal movements through infrared sensors. Immobility time was measured by the 'ring test' described by Pertwee, (1972). The apparatus consisted of a wire ring, 6 cm in diameter, fixed horizontally to a ring stand and 22 cm high. The experimenters placed the mouse across the ring, so that it was supported only by its front and rear paws. Its forepaws were placed on diametrically opposite points on

the ring. The number of seconds the mouse remained motionless on the ring (except for breathing movements) was recorded. The test was conducted for 5 min and catalepsy was measured as immobility index, calculated as % of immobility = (time immobile in seconds/length of session in seconds)*100.

To verify the involvement of CB₁ receptor in the URB602-induced psychoactive effects, mice were pre-treated (15 min) with the selective antagonist rimonabant (0.5 mg kg⁻¹ i.p.) and submitted to behavioural test session, as described previously.

Induction of inflammation

Animals were anaesthetized with sodium pentobarbital (60 mg kg⁻¹ i.p.). Acute inflammation was induced by intraplantar (i.pl.) injection of 20 µl of λ-carrageenan (2% wv⁻¹ in saline), purchased from Sigma-Aldrich (Milano, Italy), into the right hind paw. Control animals received a corresponding i.p. injection of vehicle.

Drug treatment and experimental design

URB602 at non-psychoactive doses (10, 5 and 1 mg kg⁻¹) or an appropriate volume of its vehicle was administered i.p. 30 min before (preventive regimen) or 30 min after (therapeutic regimen) carrageenan injection. URB602 was first dissolved in 10% dimethylsulphoxide (DMSO), then a drop of Tween 80 was added for every 2–3 mg of compound to maintain its solubility when it is diluted with 90% saline. In the antagonism studies, rimonabant 0.5 mg kg⁻¹ i.p. and SR144528 1 mg kg⁻¹ i.p., selective antagonists for CB₁ and CB₂ receptors respectively, were given 15 min before 10 mg kg⁻¹ URB602 administration. Rimonabant and SR144528 were dissolved in a mixture of Tween 80:DMSO:distilled water (1:2:7). The doses of SR144528 and rimonabant used here were based on previous works in rodents (Rinaldi-Carmona *et al.*, 1998; Carta *et al.*, 1999).

Evaluation of paw oedema

The volume of the injected paw as well as the contralateral paw was measured with a plethysmometer (Ugo Basile, Varese, Italy), 2 and 24 h after the carrageenan injection. Data are expressed as oedema (difference in volume between the right and left paws).

Evaluation of thermal hypersensitivity

Responses to thermal stimuli were measured on the same animals used to determine oedema, 5 and 24 h after carrageenan i.pl. injection. Since at the 2 h time point, some animals had not still recovered from anaesthesia, the test was performed when they had completely recovered, about 5 h after carrageenan. Heat hypersensitivity was tested according to the Hargreaves' procedure (Hargreaves *et al.*, 1988) using the plantar test (Ugo Basile, Varese, Italy). Briefly, animals were placed in a clear plexiglass box and allowed to acclimatize. A constant intensity, radiant heat source was aimed at the midplantar area of the hind paw. The

time, in seconds, from initial heat source activation until paw withdrawal was recorded.

Statistical analysis

All data were expressed as the mean ± s.e.m. and analysed using analysis of variance followed by Tukey's *post-hoc* test for multiple comparison. Differences were considered significant at *P* < 0.05. Linear regression analyses were done using GraphPAD Software (San Diego, CA, USA).

Results

Psychoactive effects of URB602

To select a dose of URB602 devoid of psychoactive effects that could be used to study its anti-inflammatory and anti-nociceptive activity, mice were tested in the tetrad of assays used to evaluate cannabinoid psychoactive effects (Compton *et al.*, 1993). At 30, 60 and 90 min after a single i.p. administration of URB602 (10 and 20 mg kg⁻¹), each mouse's body temperature, nociceptive threshold, locomotor activity and ring immobility were consecutively measured. The behavioural evaluations are given in Figure 1. Mice exerted the typical signs of cannabimimetic effects only at the dose of 20 mg kg⁻¹, even if each effect showed a different time course. Particularly, body temperature decreased very early (at 30 min) of about 1°C (Figure 1a) and returned at basal levels already at 60 min. Nociceptive threshold (Figure 1b) increased in a time-dependent manner, reaching a maximum at 90 min time point, demonstrating a marked analgesia. Both hypomotility and catalepsy were always present at all time points; the first one was significantly reduced of about 80% (Figure 1c) and the second one showed an increase of 600% in immobility time (Figure 1d). On the contrary, the mouse tetrad showed that the dose of 10 mg kg⁻¹ possessed no psychoactive effects, leading us to test its anti-inflammatory and anti-nociceptive properties in the mouse model of carrageenan. To ascertain whether the psychoactive effects induced by 20 mg kg⁻¹ URB602 were mediated by cannabinoid receptor CB₁, an antagonism study has been performed employing rimonabant (0.5 mg kg⁻¹ i.p.). Based on the time course data obtained, the effect of rimonabant was evaluated at the peak time of every URB602-induced psychoactive effects (30 min for body temperature and 90 min for the others). Results, shown in Table 1, revealed that rimonabant blocked the hypothermic, cataleptic and analgesic effects of URB602 whereas it only attenuated hypoactivity. The employed dose of rimonabant did not modify the behavioural responses on its own (data not shown).

Anti-inflammatory and anti-nociceptive preventive dose-related effect of URB602

I.pl. injection of carrageenan resulted in an increase in ipsilateral hindpaw volume, expressed as oedema, that is the difference between the ipsilateral injected paw and the contralateral non-injected paw. Paw oedema was present both at 2 and 24 h after carrageenan (Figures 2a and b), even

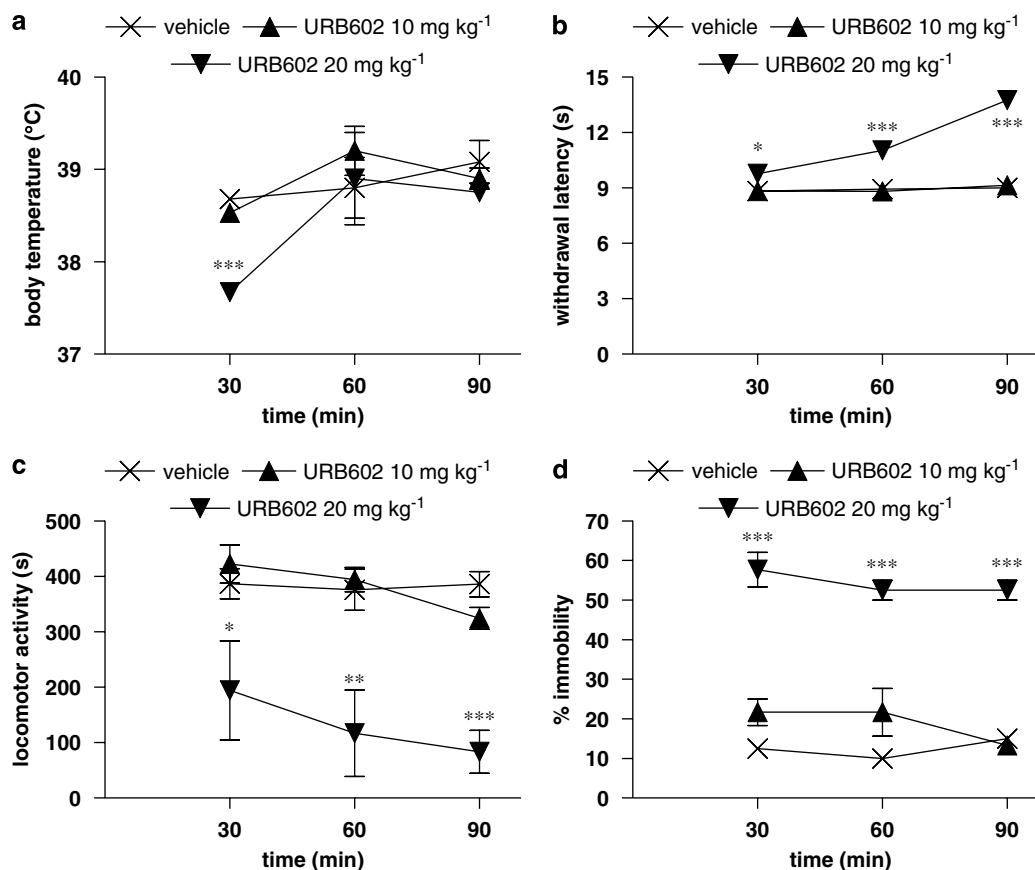


Figure 1 Effect of URB602 (20 and 10 mg kg⁻¹ i.p.) 30, 60 and 90 min after the administration on (a) body temperature, (b) nociceptive threshold, (c) spontaneous locomotor activity, (d) immobility on a ring. Data represent mean \pm s.e.m. of 5–6 mice. *** P < 0.001, ** P < 0.01, * P < 0.05 versus vehicle. URB602, [1,1'-biphenyl]-3-yl-carbamic acid, cyclohexyl ester.

Table 1 Effect of rimonabant administration (0.5 mg kg⁻¹) on URB602 (20 mg kg⁻¹)-induced psychoactive effects

	Vehicle	URB602	Rimonabant/URB602
Body temperature (°C)	38.7 \pm 0.12	37.66 \pm 0.05*	38.9 \pm 0.06°
Nociceptive threshold (s)	8.83 \pm 0.14	13.73 \pm 0.15*	8.30 \pm 0.10°
Spontaneous locomotor activity (s)	361.2 \pm 15.85	83.67 \pm 22.2*	130.0 \pm 18.00*
Immobility on a ring (%)	15.0 \pm 2.04	53.33 \pm 1.67*	12.0 \pm 4.00°

Values shown in the table are the mean \pm s.e.m. * P < 0.001 versus vehicle; ° P < 0.001 versus URB602. Abbreviation: URB602, [1,1'-biphenyl]-3-yl-carbamic acid, cyclohexyl ester.

if at 24 h it was further increased. At 2 h, oedema was abolished by the highest dose (10 mg kg⁻¹) of URB602 administered 30 min before the inflammatory stimulus, it was diminished by the intermediate dose of 5 mg kg⁻¹, while it was not counteracted by the lowest dose (1 mg kg⁻¹). Figure 2a shows this dose-correlated effect ($r^2 = 0.9592$, $P < 0.0001$, $F = 164.6$). At 24 h after carrageenan injection, the animals treated with the highest doses (10 and 5 mg kg⁻¹) were significantly different from both non-inflamed and inflamed mice, suggesting that this effect was still present (Figure 2b). Carrageenan injection also caused a marked thermal hypersensitivity, as shown in Figures 2b and c, that remained unaltered at 24 h. All doses of URB602, except the lowest one (1 mg kg⁻¹), diminished pain hypersensitivity to thermal stimulus, in a dose-correlated manner ($r^2 = 0.9484$, $P < 0.0001$, $F = 128.8$), as shown in Figure 2c. At

the 24 h time point, only the dose of 10 mg kg⁻¹ elicited a partial relief of pain hypersensitivity, even if this effect was diminished versus the 5 h time point. Contralateral hindpaw volume did not change significantly (data not shown) and no differences in paw-withdrawal latencies were found in the contralateral hindpaw (data not shown) at any time point.

Effect of CB₁ and CB₂ receptor antagonists on URB602-induced anti-oedemigen and anti-nociceptive effect

The ability of specific CB₁ and CB₂ antagonists to reverse the effect elicited by URB602 treatment was tested both on oedema and thermal hypersensitivity. Antagonists were administered 15 min before URB602 10 mg kg⁻¹ and oedema evaluations were performed 2 h after drug administration while thermal hypersensitivity was measured 5 h later as

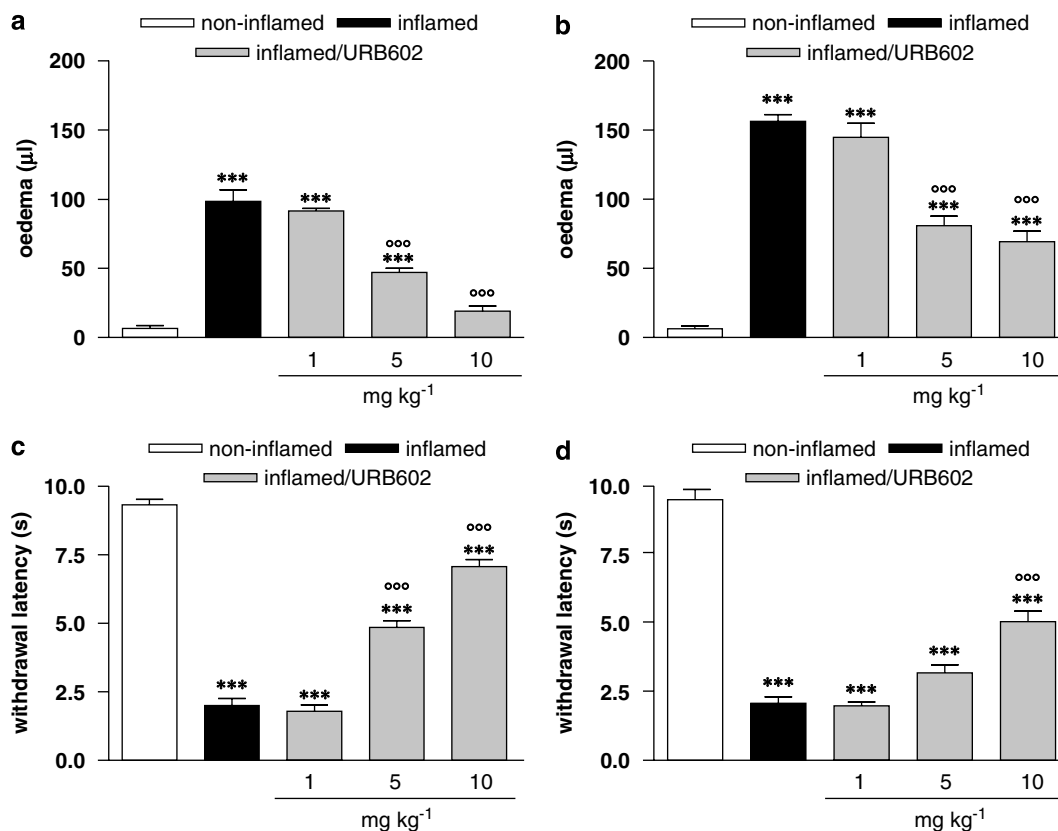


Figure 2 Dose-related effect of URB602 (10, 5 and 1 mg kg⁻¹ i.p.), administered in a preventive regimen, on oedema, evaluated (a) 2 h, and (b) 24 h after carrageenan, and on thermal hypersensitivity, evaluated (c) 5 h and (d) 24 h after carrageenan. Data represent mean ± s.e.m. of 8–10 mice. ****P* < 0.001 versus non-inflamed; °°°*P* < 0.001 versus inflamed. URB602, [1,1'-biphenyl]-3-yl-carbamic acid, cyclohexyl ester.

described previously. Results in Figure 3 show that only SR144528 reversed both URB602-induced anti-oedemigen (Figure 3a) and anti-nociceptive (Figure 3b). Rimonabant and SR144528, given alone, did not affect the oedema paw and the nociceptive response of animals (data not shown).

Anti-inflammatory and anti-nociceptive therapeutic effect of URB602

To test the ability of URB602 to counteract an established inflammatory process, the maximal dose of the drug (10 mg kg⁻¹) was administered in a therapeutic regimen, that is 30 min after carrageenan injection. Figure 4a shows that URB602 partially counteracted oedema (about 60%) evoked by carrageenan injection, 2 h after carrageenan injection. The effect remained unaltered after 24 h. URB602 also showed an anti-nociceptive activity, increasing inflamed paw thermal withdrawal latency of about 170% (Figure 4b), assessed 5 h after carrageenan; 24 h later, thermal hypersensitivity was completely abolished.

Discussion and conclusions

There is evidence supporting the role of the endocannabinoid system in a wide variety of physiological and pathological processes. In fact, endocannabinoid levels appear to

be transiently elevated as an adaptive reaction to re-establish normal homeostasis when this is perturbed. In some chronic states, however, changes in endocannabinoid levels also contribute to the progress or symptoms of diseases. In this latter case, the development of drugs blocking/stimulating the endocannabinoid action could be very useful to restore the physiological endocannabinoid levels thus blocking the progression of the disease. Inhibitors of endocannabinoid hydrolases could offer a rational therapeutic approach in treating certain inflammatory diseases where higher endocannabinoid activity would be beneficial. The development of FAAH inhibitors, together with the generation of mice lacking the FAAH gene, has deepened considerably the understanding of the pathophysiological role of AEA in the central nervous system and in the periphery. By contrast, the functional role of 2-AG is only beginning to be explored. Inhibition of endocannabinoid metabolism is considered a promising therapeutic target on its own with respect to the use of exogenously administered cannabinoid ligands, since it presumably increases the endocannabinoid level mainly at the location where their synthesis has been stimulated, thus minimizing the adverse side effects produced by the direct activation of cannabinoid receptors in the brain. Consistent with the efficacy of this strategy, we have shown previously that the systemic administration of FAAH inhibitor URB597 reduced the oedema response to carrageenan in the mouse via CB₂ receptors (Holt *et al.*, 2005). A very recent report by

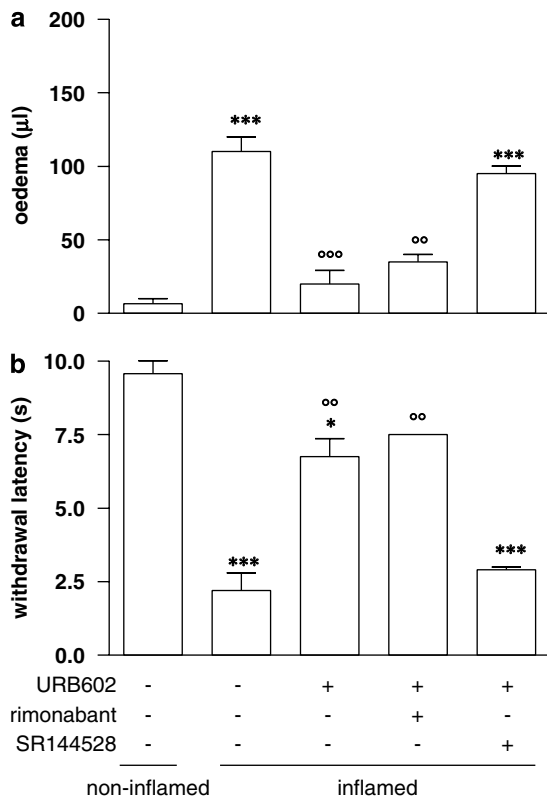


Figure 3 Effect of rimonabant (0.5 mg kg^{-1} i.p.) and SR144528 (1 mg kg^{-1} i.p.) on (a) anti-oedemigen and (b) anti-nociceptive effect evoked by URB602 (10 mg kg^{-1} i.p.). Antagonists were administered 15 min before the drug and behavioural evaluations were assessed 2 h (oedema) and 5 h (thermal hypersensitivity) after carrageenan. Data represent mean \pm s.e.m. of 8–10 mice. *** $P < 0.001$, * $P < 0.05$ versus non-inflamed, ooo $P < 0.001$, oo $P < 0.01$ versus inflamed. URB602, [1,1'-biphenyl]-3-yl-carbamic acid, cyclohexyl ester.

Guindon *et al.* (2007) demonstrated that exogenous 2-AG induced peripheral anti-nociceptive effects in the formalin test and that the MAGL inhibitor URB602, administered locally to the paw, enhanced the anti-nociceptive effect of the exogenously applied 2-AG. On these bases, the work reported here was designed to investigate the efficacy of the MAGL inhibitor URB602 in a model of inflammation/inflammatory pain such as that evoked by the i.p. injection of carrageenan in mice, after systemic administration and, importantly, under both prophylactic and therapeutic regimen.

Since 2-AG acts as potent endogenous ligand for both cannabinoid receptor subtypes, the systemic administration of URB602 at too high doses might enhance the 2-AG level in brain areas, thereby producing adverse psychoactive effects that could limit the therapeutic employment of such a compound. To select a dose of URB602 devoid of psychoactivity which could be used to study its anti-inflammatory and anti-nociceptive efficacy, we first tested mice in the tetrad of assays employed to evaluate cannabinoid central effects (Compton *et al.*, 1993). At 30, 60 and 90 min after i.p. doses of URB602 (10 and 20 mg kg^{-1}) each mouse's body temperature, locomotor activity, nociceptive

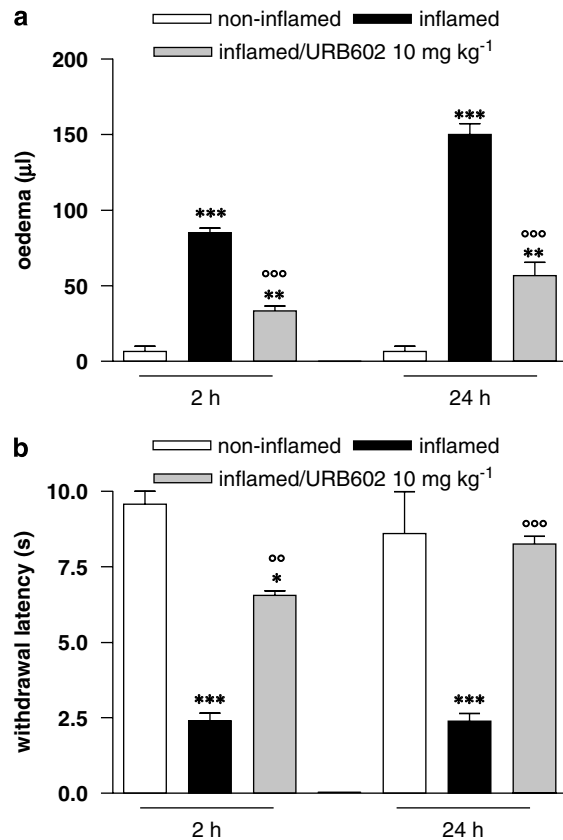


Figure 4 Effect of URB602 (10 mg kg^{-1} i.p.), administered in a therapeutic regimen, on (a) oedema and (b) thermal hypersensitivity, evaluated 2 h (oedema) or 5 h (thermal hypersensitivity) and 24 h after carrageenan. Data represent mean \pm s.e.m. of 8–10 mice. *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$ versus non-inflamed; ooo $P < 0.001$, oo $P < 0.01$ versus inflamed. URB602, [1,1'-biphenyl]-3-yl-carbamic acid, cyclohexyl ester.

threshold and ring immobility were consecutively measured. The data clearly indicated that only mice given 10 mg kg^{-1} URB602 did not show any cannabimimetic activity. Consequently, this dose has been selected as the highest to be administered to mice in the following experimental sessions. However, the finding that 20 mg kg^{-1} URB602 evoked a marked cannabimimetic activity adds an important insight about the efficacy of this MAGL inhibitor, demonstrating that it is able to enhance the endocannabinoid tone also *in vivo*. In addition this result suggests that, although URB602 showed a relative low potency in inhibiting MAGL activity *in vitro* (Hohmann *et al.*, 2005), its systemic administration at high dose led to a significant blockade of 2-AG hydrolysis *in vivo*. Besides the high dose employed and the route of administration (i.p.) causing reasonable bio-availability, the species and strain employed in our experiments could account for the systemic effect observed. To ascertain whether CB₁ receptor mediated the psychoactive effects following URB602 administration, the selective CB₁ receptor antagonist rimonabant was pre-administered to mice. Rimonabant (0.5 mg kg^{-1} i.p.) completely blocked the hypothermic, cataleptic and analgesic effects of URB602 whereas it only attenuated hypoactivity, highlighting the

contribution of cannabinoid CB₁ receptor in the cannabimimetic activity of URB602. The limited antagonism of URB602-induced hypoactivity by rimonabant has been reported previously (Jarbe *et al.*, 2002; McMahon *et al.*, 2005; McMahon and Koek, 2007) suggesting that locomotor activity seems to be differently sensitive to cannabinoid CB₁ receptor activity. The data strongly suggest that 2-AG, whose level increased following URB602 administration, bound to CB₁ receptors inducing cannabimimetic effects; however, in the light of the high dose employed (20 mg kg⁻¹) and of the affinity of URB602 for CB₁ and CB₂ receptors (IC₅₀ ≥ 5 μM) (Hohmann *et al.*, 2005), we cannot exclude a direct action of URB602 upon CB₁ receptors.

We have shown here for the first time that the systemic administration of MAGL inhibitor URB602 prevented the development of carrageenan-induced oedema. A clear dose-response relationship was seen in the present study for the anti-oedema effect of URB602, where a dose of 10 mg kg⁻¹ decreased the oedema development to a level undistinguishable from the non-inflamed animals. The anti-oedema effect was still present at 24 h, suggesting a lasting efficacy of this inhibitor. Of course, the finding that this MAGL inhibitor produces effects in the carrageenan model does not conclusively prove that the key of the efficacy is its inhibition upon MAGL. However, the demonstration reported by Hohmann *et al.* (2005) that *in vivo* microinjection of URB602 into the periaqueductal grey of rats increased 2-AG concentration without affecting AEA levels, strongly suggests that the URB602 anti-inflammatory property could be ascribed to a selective enhancement of 2-AG. The ability of URB602 to prevent the inflammatory process also highlights the significance of 2-AG as an endogenous protective substance against inflammation, pointing to the possibility that 2-AG shares with AEA and PEA the capability to modulate inflammation. With respect to 2-AG physiological role in inflammation, controversial findings have been reported so far. Some studies suggested that CB₂ receptors and 2-AG are involved in the stimulation of different types of inflammatory and immune responses (Smith *et al.*, 2001; Oka *et al.*, 2005, 2006), whereas other investigators reported that 2-AG is involved in the attenuation of both inflammatory and immune reactions (Ouyang *et al.*, 1998; Gallily *et al.*, 2000; Facchinetti *et al.*, 2003), even if these findings have been obtained *in vitro*. The data presented herein strongly support the *in vitro* results, since the URB602-induced inhibition of 2-AG degradation *in vivo* offers the opportunity to investigate the functions of 2-AG by amplifying its intrinsic actions that show anti-inflammatory effects. We demonstrated here that URB602 has dose-dependent action in reducing not only oedema but also thermal hypersensitivity associated with inflammation, extending the recently described ability of this compound to counteract formalin-induced pain behaviour (Guindon *et al.*, 2007) to acute inflammatory pain. Similar to the anti-inflammatory effect, the anti-nociceptive property of URB602 persisted also at 24 h after the administration. In addition, the same treatment did not affect the nociceptive thresholds of the paw contralateral to the carrageenan injection, indicating that the doses employed are not directly analgesic and confirming the results obtained by

us in the tetrad assay with URB602 10 mg kg⁻¹. This finding prompted us to suggest that the enhancement of 2-AG in tissue (that is, spinal cord, skin) in which there is an ongoing production of the endocannabinoid, accounts for the effect observed validating the hypothesis of the beneficial use of the so-called 'indirect agonists'. To ascertain the relative contribution of CB₁ and CB₂ receptors in the anti-oedema and anti-nociceptive effect of URB602, we employed selective antagonists for these receptors. Both the URB602-induced effects in inflamed mice can be prevented by SR144528 but not by rimonabant (at the same dose able to reverse the effect of URB602 on the tetrad assay) clearly indicating that exclusively CB₂ receptor mediated the anti-inflammatory and anti-nociceptive properties of URB602. With respect to inflammation, there is good evidence in the literature that the activation of CB₂ receptors expressed by mast cells and macrophages is involved in downregulation of the inflammatory response. In the light of the potent agonist activity of 2-AG at the CB₂ receptor, its involvement in URB602-induced anti-inflammation was strongly predictable. Conversely, the fact that also the relief of thermal hypersensitivity evoked by the MAGL inhibitor was mediated solely by CB₂ receptors was unexpected but welcomed. We can speculate that the anti-nociceptive effect of URB602 might be mediated by a direct action of 2-AG on CB₂ receptors located in activated microglia within the spinal cord or in the mouse paw tissue. The activation of CB₂ receptors could contribute to the relief of pain by decreasing the release of sensitizing substances from mast and immune cells. In addition, the lack of CB₁ involvement in the URB602-induced anti-nociception confirms that URB602 can be systemically administered without producing central effects. In addition, it has been recently identified a novel MAGL, sensitive to URB602 inhibition, that is mainly expressed in microglia cells (Muccioli *et al.*, 2007). Interestingly, this suggests that the cloned MAGL which is thought to be responsible for the majority of the 2-AG hydrolysis in the brain, does not play a major role in microglia. Consequently, we cannot exclude that the anti-nociceptive property of URB602 in unhealthy animals could be ascribed to a selective inhibition of this novel MAGL.

Finally, our findings demonstrated that the anti-inflammatory and anti-nociceptive efficacy of URB602 persists also when the compound was administered in a therapeutic regimen, suggesting that the ability of URB602 to improve the established disease may have potential therapeutic application.

In conclusion, the present report highlighted the ability of the selective MAGL inhibitor, URB602, to prevent and treat an acute inflammatory disease without producing adverse psychoactive effects. The data presented herein also contributed to clarify the physiological role of 2-AG with respect to inflammatory reactions, suggesting its protective role in the body. More work is necessary to demonstrate that MAGL may be considered a novel molecular target to be exploited for the treatment of inflammation and pain and the development of new more potent and selective inhibitors as well as the generation of mice lacking the MAGL gene could help us to understand better the potential of this pharmacological tool.

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Conflict of interest

The authors state no conflict of interest.

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