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4-Methylbenzenecarbothioamide, a hydrogen sulfide donor, inhibits tumor necrosis factor-α and CXCL1 production and exhibits activity in models of pain and inflammation

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ABSTRACT

The gasotransmitter hydrogen sulfide (H_2S) is known to regulate many pathophysiological processes. Preclinical assays have demonstrated that H_2S donors exhibit anti-inflammatory and antinociceptive activities, characterized by reduction of inflammatory mediators production, leukocytes recruitment, edema and mechanical allodynia. In the present study, the effects induced by 4-methylbenzenecarbothioamide (4-MBC) in models of pain and inflammation in mice, the mechanisms mediating such effects and the H_2S-releasing property of this compound were evaluated. 4-MBC spontaneously released H_2S in vitro in the absence of organic thiols. Intraperitoneal (i.p.) administration of 4-MBC (100 or 150 mg/kg) reduced the second phase of the nociceptive response induced by formaldehyde and induced a long lasting inhibitory effect on carrageenan mechanical allodynia. 4-MBC antiallodynic effect was not affected by previous administration of naltrexone or glibenclamide. 4-MBC (50, 100 or 150 mg/kg, i.p.) induced a long lasting inhibitory effect on paw edema induced by carrageenan. The highest dose (150 mg/kg, i.p.) of 4-MBC inhibited tumor necrosis factor-α and CXCL1 production and myeloperoxidase activity induced by carrageenan. Mechanical allodynia and paw edema induced by carrageenan were not inhibited by the 4-MBC oxo analogue (*p*-toluamide). In summary, 4-MBC, an H_2S releasing thiobenzamide, exhibits antinociceptive and anti-inflammatory activities. These activities may be due to reduced cytokine and chemokine production and neutrophil recruitment. The H_2S releasing property is likely essential for 4-MBC activity. Our results indicate that 4-MBC may represent a useful pharmacological tool to investigate the biological roles of H_2S.

1. Introduction

Hydrogen sulfide (H_2S), along with nitric oxide and carbon monoxide, form the group of gasotransmitters, small signaling molecules that play several biological roles (Wang, 2002, 2003; Szabó, 2007). H_2S is synthesized in mammalian tissues from cysteine by cystathionine gamma lyase, cystathionine beta-synthase or 3-mercaptopyruvate sulfurtransferase (Huang and Moore, 2015).

H_2S was first believed to be a proinflammatory mediator as propargylglycine, an inhibitor of cystathionine gamma lyase, inhibits paw edema induced by carrageenan (Bhatia et al., 2005) and lung inflammation induced by lipopolysaccharide (Li et al., 2005). Other

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studies, however, demonstrated that H_2S exhibits anti-inflammatory activity. H_2S reduces paw (Zanardo et al., 2006), joint (Ekundi-Valentim et al., 2010) and brain (Zhao et al., 2017) edema induced by different stimuli. This anti-inflammatory activity may be due to reduced production of cytokines (Whiteman et al., 2010; Li et al., 2013), cellular recruitment (Ekundi-Valentim et al., 2010; Perna et al., 2013) and adhesion molecules expression (Talaei, 2016). H_2S also plays opposing roles in models of pain. Endogenous H_2S induces pro-nociceptive effects, whereas antinociceptive effects are mainly induced by exogenous H_2S (Cunha et al., 2008; Donatti et al., 2014). H_2S antinociceptive activity was demonstrated by reduction of sensitization induced by carrageenan (Ekundi-Valentim et al., 2010), paclitaxel (Di Cesari Manelli et al., 2017), nerve injury (Kida et al., 2015), bone cancer (Zhuang et al., 2018) and opioid withdrawal syndrome (Yang et al., 2014).

Direct exposure of experimental animals to high concentrations of H_2S can lead to pulmonary edema or death (Zhao et al., 2014; Szabó, 2017). Thus, development of synthetic H_2S donors that mimic the gradual H_2S release of enzymatic synthesis is essential for investigating the biological actions of this gasotransmitter. Substrates of H_2S-generating enzymes (cysteine and its analogues) and agents that release H_2S spontaneously or after bioactivation (GYY4137 and 1,2-dithiole-3-thione derivatives, among others) have been the compounds most commonly employed in these investigations (Papapetropoulos et al., 2015; Hartle and Pluth, 2016).

Although thiobenzamides are not the typical small molecule H_2S donors, 4-hydroxybenzenecarbothioamide (4-HBC) was used to examine the role of H_2S in a model of gastric ulcer and showed to increase the extent of lesions healing (Wallace et al., 2007). Subsequently, 4-HBC was used as an H_2S-releasing moiety for the synthesis of ATB-346 (Wallace et al., 2010), an H_2S-releasing derivative of naproxen currently being evaluated in phase II clinical trials in osteoarthritis patients (Wallace et al., 2018). As other thiobenzamides release H_2S (Martelli et al., 2013), these compounds may represent valuable tools to investigate the biological actions of this gasotransmitter.

To the best of our knowledge, the effects of thiobenzamides in models of pain and inflammation have not been investigated. In the present study, we evaluated the effects of 4-methylbenzenecarbothioamide (4-MBC) in models of pain and inflammation, the mechanisms mediating such effects and the ability of 4-MBC to release H_2S. We aimed to provide further information on the role of H_2S in pain and inflammation and to evaluate whether 4-MBC may represent a useful tool to investigate the biological roles of H_2S.

2. Materials and methods

2.1. Synthesis of 4-MBC

All melting points (m.p.) were determined on a Microquímica MQAPF 301 apparatus. The infrared (IR) spectra were recorded using a PerkinElmer Spectrum One infrared spectrometer and absorptions are reported as wave numbers (cm^{-1}). The NMR spectra were recorded on a Bruker AVANCE DRX200 instrument, using tetramethylsilane as the internal standard. Chemical shifts are given in δ (ppm) scale and J values are given in Hz. All reagents of analytical grade were obtained from commercial suppliers and used without further purification. The synthetic route for the preparation of 4-MBC is depicted in Fig. 1B.

To a solution of *p*-tolunitrile (2 g; 17 mmol) in a mixture of ethanol (50 ml) and dioxane (50 ml), a solution of NaOH (5 g; 125 mmol) in water (50 ml) was added under stirring in ice-bath. Then, 30% hydrogen peroxide solution (42 ml) was added dropwise to the mixture. The reaction mixture was stirred at 60 °C for 2 h. The organic solvents were removed under reduced pressure and the resulting aqueous phase was extracted with 3 × 50 ml AcOEt. The organic layers were dried over anhydrous sodium sulfate, filtered and the solvent was evaporated under reduced pressure. The crude product was recrystallized from ethanol to yield 2.0 g (87%) of a white solid. M.p. 153.9–155.8 °C;

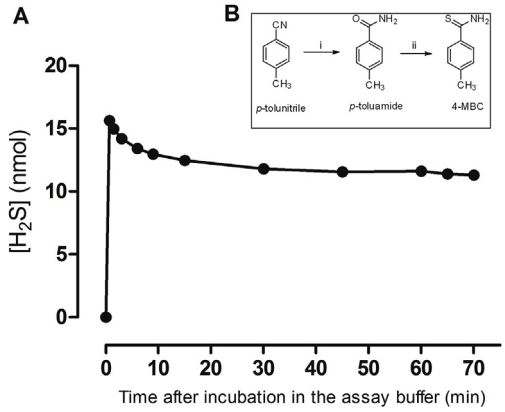

Fig. 1. (A) Ability of 4-MBC to release H_2S as a function of time. Formation of H_2S from 4-MBC was monitored up to 70 min after the preparation of the solution in PBS buffer (pH 6.8) containing 40% PEG 400. The in vitro system contained 10 μmoles of 4-MBC. (B) Chemical structures of *p*-tolunitrile and *p*-toluamide and synthetic route for the preparation of 4-MBC. Reagents and conditions: i = NaOH solution, H_2O_2 30%, dioxane, ethanol (87% yield); ii = Lawesson's reagent, THF, rt (67% yield).

literature m.p. 155–156 °C (Hauser and Hoffenberg, 1955); IR (cm^{-1}): 3337, 3157, 1665, 1610, 1568, 1410, 1395; ^{1}H NMR (200 MHz, acetone-d6), δ/ppm: 7.85 (2H, d, J = 8.0 Hz); 7.26 (2H, d, J = 8.0 Hz), 2.37 (3H, s); ^{13}C NMR (50 MHz, acetone-d6), δ/ppm: 169.0, 142.3, 132.5, 129.6, 128.4, 21.3.

In a round-bottom flask containing the amide (*p*-toluamide; 0.140 g; 1.0 mmol), Lawesson's reagent (0.490 g; 1.20 mmol) and anhydrous tetrahydrofuran (THF; 10 ml) were added. The reaction mixture was stirred at room temperature for 4.5 h. The completion of the reaction was observed by thin-layer chromatography (eluent: EtOAc/hexane 9:1). The solvent was removed under reduced pressure and resulting residue was diluted with sodium bicarbonate 5% solution (25 ml) and extracted with 3 × 25 ml EtOAc. The combined organic layers were dried over anhydrous sodium sulfate, filtered and the solvent was evaporated under reduced pressure. The resulting solid was purified by column chromatography, providing the product as a pale solid (67% yield). M.p. 83.3–84.5 °C; literature m.p. 87.5–88.5 °C (Boudet, 1949); IR (cm^{-1}): 3375, 3275, 3155, 1621, 1604, 1412; ^{1}H NMR (200 MHz, acetone-d6), δ/ppm: 7.92 (2H, d, J = 8.0 Hz); 7.22 (2H, d, J = 8.0 Hz), 2.36 (3H, s); ^{13}C NMR (50 MHz, acetone-d6), δ/ppm: 202.28, 142.53, 137.71, 129.33, 128.17, 21.22.

2.2. In vitro release of H_2S from 4-MBC

Release of H_2S from 4-MBC was examined using a TBR4100 Free Radical Analyzer (WPI, USA) equipped with the amperometric sensor ISO–H_2S-2 (detection limit lower than 5 nM). Standard curve of Na_2S was plotted through current changes (pA) to quantify H_2S, according to the manufacturer instructions, as it follows: H_2S = [Na_2S]/3.04173977. The generation of H_2S from the test compound was monitored 42 s, 1.5, 3, 6, 9, 15, 30, 45, 60, 65 and 70 min after addition of 100 μl of 100 mM 4-MBC (prepared in PEG 400) to PBS (pH 6.8; saline) to obtain PEG 400 at a final concentration of 40% v/v, the same concentration used in the in vivo experiments. Thus, this experiment was performed with 10 μmoles of 4-MBC.

2.3. Animals

Male Swiss mice (25–30 g) were used. The animals were fed standard laboratory chow and tap water and were maintained in a room with a 12 h light-dark cycle for at least three days before the experiment to allow acclimatization. A room temperature of 27 °C, corresponding to the thermoneutral zone for mice, was used. All experiments were

approved by the Ethics Committee on Animal Experimentation of the Federal University of Minas Gerais (Protocol 22/2016) and carried out according to the ethical guidelines for investigation of experimental pain in conscious animals (Zimmermann, 1983).

2.4. Drugs

Dexamethasone 21-phosphate disodium salt, naltrexone, glibenclamide, λ-carrageenan, formaldehyde solution, carboxymethylcellulose (CMC) sodium salt (Sigma-Aldrich, USA), phenobarbital (Sanofi Aventis, Brazil) and polyethylene glycol 400 (PEG400; Synth, Brazil) were used. Suspensions of 4-MBC or *p*-toluamide were prepared in sterile saline (pH 6.8) containing 40% (v/v) PEG 400 and administered intraperitoneally (i.p.) in a volume of 10 ml/kg. Glibenclamide suspension was prepared in CMC (0.5% w/v in sterile saline) and phenobarbital solution was prepared in sterile saline. Glibenclamide and phenobarbital were administered per os (p.o.) in a volume of 10 ml/kg. Dexamethasone and naltrexone solutions were prepared in sterile saline and administered i.p. in a volume of 4 ml/kg. Formaldehyde solution (0.92%) and carrageenan suspension (2%) were prepared in sterile saline and administered via the intraplantar (i.pl.) route. All solutions and suspensions were prepared immediately before each experiment.

2.5. Evaluation of motor coordination

The motor coordination of the animals was evaluated in a rota-rod apparatus. The animals were trained on the apparatus for three days before the experiment. On the experimental day, the animals were placed on the rotating rod (14 rpm) and the time they spent on it was measured. The cut-off time was 120 s. After determination of the baseline values, 4-MBC (50, 100 or 150 mg/kg, i.p.), phenobarbital (50 mg/kg, p.o.) or vehicle (PEG 400 40%, i.p.) were administered. The animals were tested again in the apparatus 0.5, 1.5, 3 and 6 h later. Results were expressed as time (s) spent on the rotating rod.

2.6. Nociceptive response induced by formaldehyde

On the experimental day, the animals were individually placed under glass funnels (18 cm diameter and 14 cm height) about 10 min before injection of formaldehyde to allow acclimatization. 4-MBC (50, 100 or 150 mg/kg) or vehicle (PEG 400, 40%) were administered via the i.p. route 30 min before the intraplantar (i.pl.) injection of formaldehyde (0.92%, 20 µl) into the right hind paw. Immediately after the injection, each mouse was placed again under the glass funnel and the amount of time that the animal licked the injected paw was measured between 0 and 5 min (first phase) and 15–30 min (second phase) (Tjølsen et al., 1992). Results were expressed as time (s) spent licking the injected paw between 0 and 5 min (first phase) and 15–30 min (second phase).

2.7. Mechanical allodynia induced by carrageenan

Mechanical allodynia was measured by using an electronic von Frey apparatus (Model EFF 301, Insight, Brazil).The mice were kept individually in acrylic cages whose floor was a metal grid. The animals were habituated to the experimental apparatus daily, approximately 60 min a day, for two days before the experiments. A hand-held force transducer, fitted with a polypropylene tip (0.5 mm^2), was gradually pressed onto the plantar surface of the right hind paw. The test consisted of evoking a reflex of hind paw flexion. The paw withdrawal threshold was determined by averaging five measurements. On the experimental day, baseline paw withdrawal threshold of each animal was determined. After that, the animals were divided into the experimental groups in such a way that the mean paw withdrawal thresholds of the different groups were similar. On the experimental day, carrageenan (600 µg, 20 µl) was injected via the i.pl. route 30 min after i.p. administration of 4-MBC (50, 100 or 150 mg/kg), vehicle (PEG 400, 40%) or dexamethasone (5 mg/kg). The paw withdrawal threshold of each animal was again measured at 2, 4 and 6 h after carrageenan injection. To evaluate putative mechanisms mediating the activity of 4-MBC in this model, naltrexone (5 or 10 mg/kg, i.p.) or glibenclamide (20 or 40 mg/kg, p.o.) were administered 30 min before 4-MBC (150 mg/kg, i.p.). To evaluate the role of H_2S in the activity of 4-MBC, the effect induced by this compound was compared to that induced by *p*-toluamide, an S-lacking analogue. The results were expressed as the absolute paw withdrawal threshold (grams).

Table 1
Effects of 4-MBC (50, 100 or 150 mg/kg, i.p. - 30 min) or phenobarbital (50 mg/kg, p.o., - 30 min) on the time spent on the rotating rod.

Treatment	Time spent on rotating rod (s)				
	Baseline	0.5	1.5	3	6
Vehicle	120 ± 0	120 ± 0	118 ± 2	118 ± 2	118 ± 2
4-MBC 50 mg/kg	120 ± 0	120 ± 0	119 ± 1	120 ± 0	120 ± 0
4-MBC 100 mg/kg	120 ± 0	120 ± 0	120 ± 0	120 ± 0	120 ± 0
4-MBC 150 mg/kg	120 ± 0	119 ± 1	118 ± 2	120 ± 0	120 ± 0
Phenobarbital 50 mg/kg	120 ± 0	10 ± 5[a]	12 ± 7[a]	20 ± 11[a]	90 ± 18

Motor activity was evaluated 0.5, 1.5, 3 and 6 h after administration of 4-MBC, phenobarbital or vehicle (saline). Statistical comparisons were carried out using Kruskal-Wallis test, followed by Dunn's multiple comparisons test. [a] $P < 0.001$ *vs* vehicle group. n = 7.

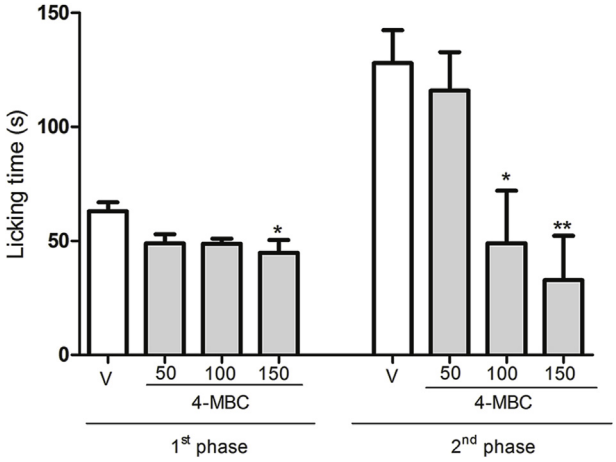

Fig. 2. Effect of 4-MBC (50, 100 or 150 mg/kg, i.p., - 30 min) on the nociceptive response induced by i.pl. injection of formaldehyde (0.92%, 20 µl). Statistical comparison was carried out using one-way ANOVA, followed by Newman-Keuls multiple comparisons test. *P < 0.05 and **P < 0.01 vs vehicle (V) group. n = 7.

2.8. Paw edema induced by carrageenan

Paw edema was measured with a plethysmometer (Model 7140, Ugo Basile, Italy).The basal volume of the right hind paw was measured before administration of any drug. Next, the animals were divided into the experimental groups in such a way that the mean paw volumes of the different groups were similar. On the experimental day, carrageenan (600 µg, 20 µl) was injected via the i.pl. route 30 min after i.p. administration of 4-MBC (50, 100 or 150 mg/kg), vehicle (PEG 400, 40%) or dexamethasone (5 mg/kg).The paw volume of each animal was again measured at 2, 4 and 6 h after injection of the inflammatory stimulus. To evaluate the role of H_2S in the activity of 4-MBC, the effect induced by this compound was compared to that induced by *p*-toluamide, an S-lacking analogue. The results were expressed as the paw

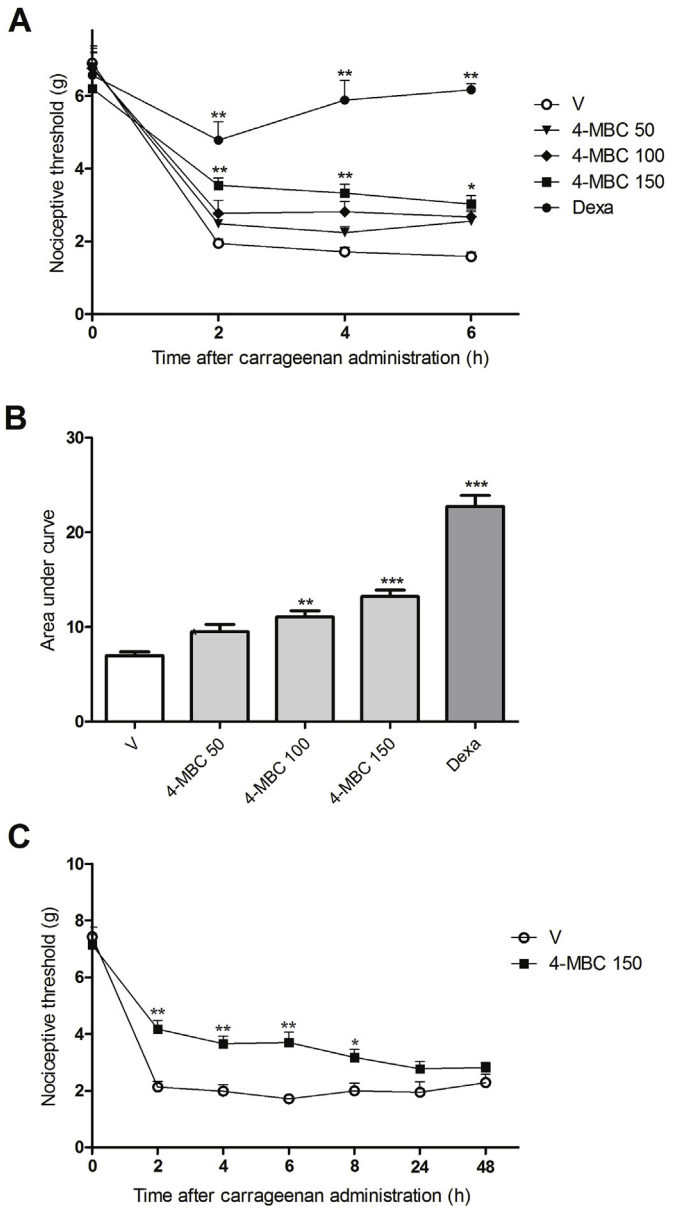

Fig. 3. Effects of 4-MBC (50, 100 or 150 mg/kg, i.p., - 30 min; A and B), 4-MBC (150 mg/kg, i.p., - 30 min; C) or dexamethasone (Dexa; 5 mg/kg, i.p., - 30 min) on the mechanical allodynia induced by i.pl. injection of carrageenan (600 μg). A and C represents the temporal course and B represents the AUC. (A and C) Statistical comparison was carried out using Kruskal-Wallis test, followed by Dunn's multiple comparisons test. (B) Statistical comparison was carried out using one-way ANOVA, followed by Newman-Keuls multiple comparisons test. *P < 0.05, **P < 0.01 and ***P < 0.001 *vs* vehicle (V) group. n = 7.

volume change (μl) in relation to the basal values.

2.9. Tumor necrosis factor-α (TNF-α) and chemokine (C-X-C motif) ligand1 (CXCL-1) concentrations in the paw tissue

Carrageenan (600 μg, 20 μl) was injected via the i.pl. route 30 min after i.p. administration of 4-MBC (150 mg/kg) or vehicle (PEG 400, 40%). TNF-α and CXCL-1 concentrations were measured in the ipsilateral footpad tissue using ELISA assays, following the instructions supplied by manufacturer (DuoSet kits, R&D Systems, Minneapolis, USA).The animals were euthanized by cervical dislocation 4 h later and the paw tissue was removed. The tissues were weighed and homogenized in phosphate buffered saline containing Tween-20 (0.05%), phenylmethylsulphonyl fluoride (0.1 mM), benzamethonium chloride (0.1 mM), EDTA (10 mM), aprotinin A (2 μg/ml) and bovine serum albumin (0.5%), followed by centrifugation (20,124 g) for 15 min at 4 °C. The supernatant samples were stored at −70 °C until analysis of TNF-α and CXCL-1 concentrations. All samples were assayed in duplicate and the results were expressed as pg/100 mg of tissue.

2.10. Evaluation of myeloperoxidase (MPO) activity

The pellet samples collected after the removal of the supernatants (Section 2.9) were frozen and thawed three times in liquid nitrogen. Upon thawing, the samples were centrifuged (20,124 g) for 15 min at 4 °C and 25 μl of the supernatant were used for the MPO assay. The enzymatic reaction was assessed by adding 25 μl of 1.6 mM tetramethylbenzidine prepared in dimethyl sulfoxide. The mixture was incubated for 5 min at 37 °C. Then, 100 μl of 0.02% hydrogen peroxide were added, followed by incubation for 5 min at 37 °C. After 10 min, the reaction was stopped by adding 100 μl of 1 M sulphuric acid. The MPO activity was calculated according to the change in optical density and absorbance was measured at 450 nm.

2.11. Statistical analysis

All data were presented as the mean ± standard error of the mean. Both temporal change and area under the curve (AUC) were shown. AUC was calculated using the trapezoidal rule with the aid of the GraphPrism 5.0 for Windows software. Data were first tested for normality using the Shapiro-Wilk test. When data were found to be normally distributed, differences were evaluated by using one-way ANOVA followed by Newman-Keuls multiple comparisons test. The nonparametric Kruskal-Wallis test, followed by Dunn's multiple comparisons test, was used when data were found to be not normally distributed. The statistical test used is outlined in each figure legend. A $P < 0.05$ was considered significant. Statistical analysis was conducted using GraphPrism 5.0 for Windows software.

3. Results

3.1. In vitro release of H_2S from 4-MBC

Fig. 1A demonstrates that 4-MBC (10 μmoles) spontaneously releases H_2S at pH 6.8 in the presence of 40% PEG 400 along the whole experimental period (70 min). Indeed, 15.6 nmoles of H_2S were released from 4-MBC in the first 0.7 min (42 s) after the preparation of the aqueous solution. From 30 to 70 min after the preparation of the 4-MBC solution, the amount of H_2S in the buffered solution remained constant (11.5 nmoles in average).

3.2. Effect of 4-MBC on the motor coordination

To rule out muscle relaxation or impairment of motor coordination as possible confounding effects when evaluating the activity of 4-MBC in the experimental models of pain, we investigated the effect of this compound on the performance of mice in the rotating rod. The time mice spent on the rotating rod was not altered 0.5, 1.5, 3 and 6 h after administration of 4-MBC (50, 100 or 150 mg/kg, i.p.). Phenobarbital (50 mg/kg, p.o.) markedly reduced the time mice spent on the rotating rod (Table 1).

3.3. Effect of 4-MBC on the nociceptive response induced by formaldehyde

I.pl. injection of formaldehyde (0.92%, 20 μl) induced a biphasic nociceptive response. 4-MBC (100 or 150 mg/kg, i.p.), administered 30 min before formaldehyde injection, markedly inhibited (61.7 and 74.4%, respectively) the second phase of the nociceptive response. However, the first phase of the nociceptive response was partially

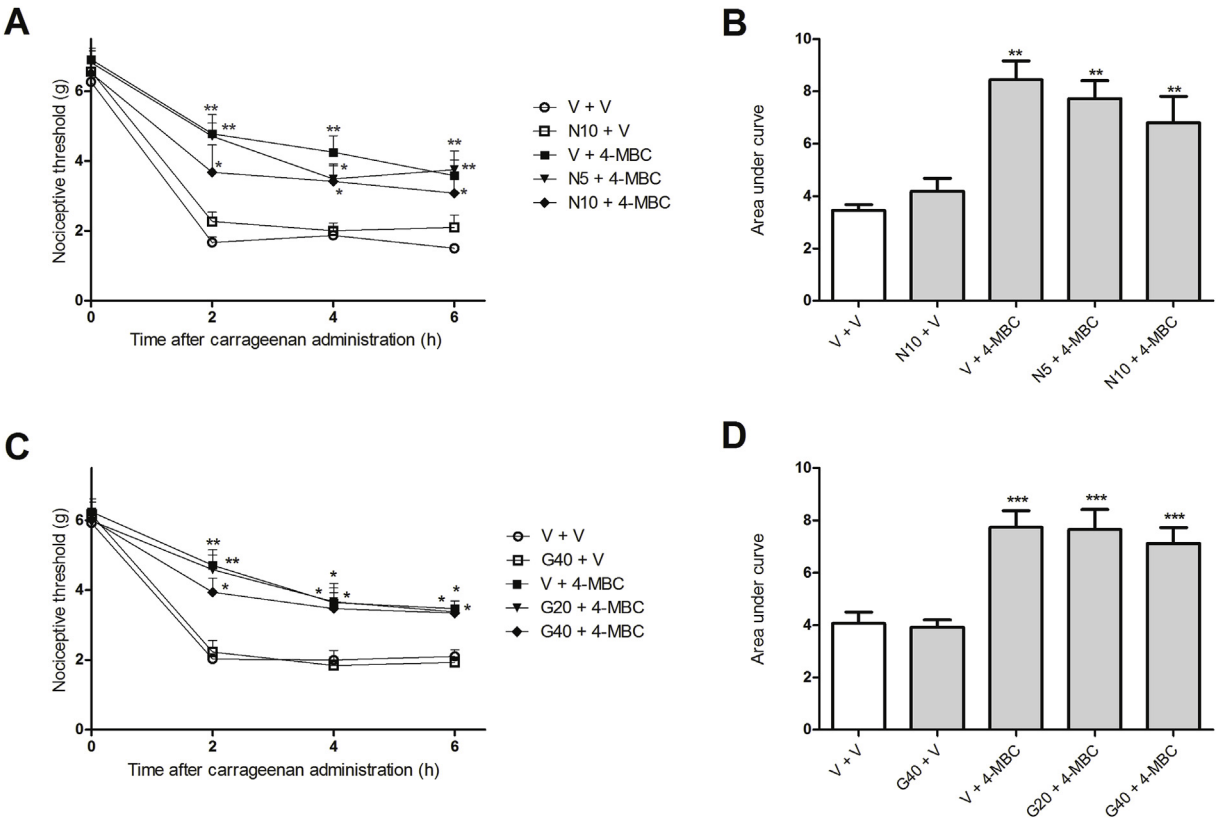

Fig. 4. Effect of 4-MBC (150 mg/kg, i.p., - 30 min) on the mechanical allodynia induced by i.pl. injection of carrageenan (600 μg) in animals previously (30 min) treated with naltrexone (N, 5 or 10 mg/kg, i.p.; A and B) or glibenclamide (G, 20 or 40 mg/kg, p.o.; C and D). A and C represents the temporal course and B and D represents the AUC. (A, B and C) Statistical comparison was carried out using Kruskal-Wallis test, followed by Dunn's multiple comparisons test. (D) Statistical comparison was carried out using one-way ANOVA, followed by Newman-Keuls multiple comparisons test.*P < 0.05, **P < 0.01 and ***P < 0.001 *vs* vehicle + vehicle (V + V) group. n = 7.

inhibited (28.8%) only by the highest dose (150 mg/kg, i.p.) of the compound (Fig. 2). The lowest dose of 4-MBC (50 mg/kg, i.p.) was devoid of effect.

3.4. Effect of 4-MBC on the mechanical allodynia induced by carrageenan

I.pl. injection of carrageenan (600 μg, 20 μl) induced a marked and long lasting mechanical allodynia. Previous (30 min) i.p. administration of the highest dose of 4-MBC (150 mg/kg) or dexamethasone (5 mg/kg) inhibited the mechanical allodynia at 2, 4 and 6 h after injection of carrageenan (Fig. 3A). The analysis of the AUC indicated that 4-MBC and dexamethasone inhibited the nociceptive response by 47.4 and 69.4%, respectively (Fig. 3B). In an additional experiment, it was demonstrated that the antiallodynic activity of 4-MBC (150 mg/kg, i.p.) was observed until 8 h, but not at 24 h, after administration of inflammatory stimulus (Fig. 3C).

3.5. Effects of naltrexone and glibenclamide on the antiallodynic activity of 4-MBC

To investigate putative mechanisms mediating the antiallodynic activity of 4-MBC, an opioidergic antagonist (naltrexone) and an ATP-dependent potassium (K_{ATP}) channels blocker (glibenclamide) were used. Mechanical allodynia induced by carrageenan was evaluated as previously described. Once again, the mechanical allodynia induced by carrageenan was inhibited by previous administration of 4-MBC (150 mg/kg, i.p.). Naltrexone (5 or 10 mg/kg; Fig. 4A and B) and glibenclamide (20 or 40 mg/kg; Fig. 4C and D) did not affect 4-MBC antiallodynic activity. Naltrexone (10 mg/kg, i.p.) and glibenclamide (40 mg/kg, p.o.), per se, did not affect the mechanical allodynia induced by carrageenan (Fig. 4).

3.6. Effect of 4-MBC on the paw edema induced by carrageenan

I.pl. injection of carrageenan (600 μg, 20 μl) induced a marked and long lasting paw edema. The paw edema was inhibited by 4-MBC (50, 100 or 150 mg/kg, i.p.) and dexamethasone (5 mg/kg, i.p.), administered 30 min before carrageenan (Fig. 5A). The analysis of the AUC indicated that 4-MBC (50, 100 or 150 mg/kg) and dexamethasone inhibited the paw edema by 34.7, 45.2, 57.6 and 92.6%, respectively (Fig. 5B). In an additional experiment, it was demonstrated that the antiedematogenic activity of 4-MBC (150 mg/kg, i.p.) was observed until 24 h, but not at 48 h after administration of the inflammatory stimulus (Fig. 5C).

3.7. Effect of 4-MBC on the production of TNF-α and CXCL1 and MPO activity

Four hours after i.pl. injection of carrageenan (600 μg, 20 μl), TNF-α and CXCL-1 concentrations in the paw tissue were increased. Increased MPO activity was also observed at the same time in the paw tissue. Previous (30 min) treatment with 4-MBC (150 mg/kg, i.p.) reduced TNF-α (51.7%) and CXCL-1 (62.1%) concentrations and MPO activity (87.4%) (Fig. 6).

3.8. Effects of 4-MBC and p-toluamide on the mechanical allodynia and paw edema induced by carrageenan

The activity of *p*-toluamide, the 4-MBC oxo analogue, was evaluated in the models of mechanical allodynia and paw edema induced by

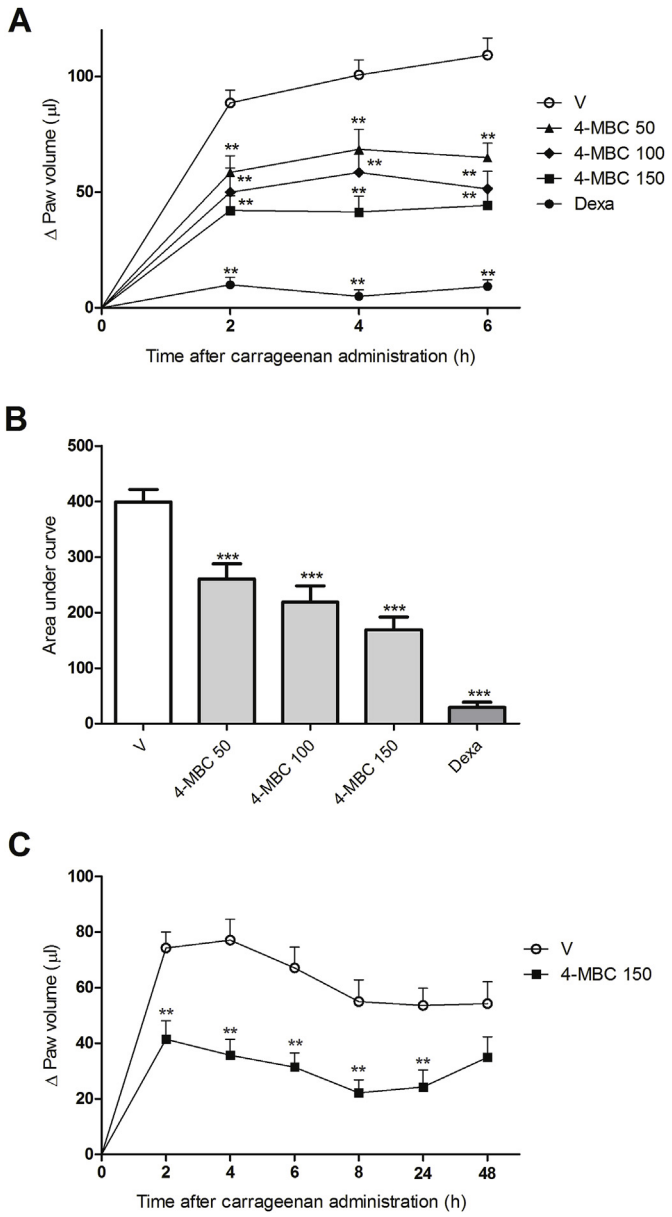

Fig. 5. Effects of 4-MBC (50, 100 or 150 mg/kg, i.p., - 30 min; A and B), 4-MBC (150 mg/kg, i.p., - 30 min; C) or dexamethasone (Dexa; 5 mg/kg, i.p., - 30 min) on the paw edema induced by i.pl. injection of carrageenan (600 μg). A and C represents the temporal course and B represents the AUC. (A and C) Statistical comparison was carried out using Kruskal-Wallis test, followed by Dunn's multiple comparisons test. (B) Statistical comparison was carried out using one-way ANOVA, followed by Newman-Keuls multiple comparisons test. $^{**}P < 0.01$ and $^{***}P < 0.001$ *vs* vehicle (V) group. n = 7.

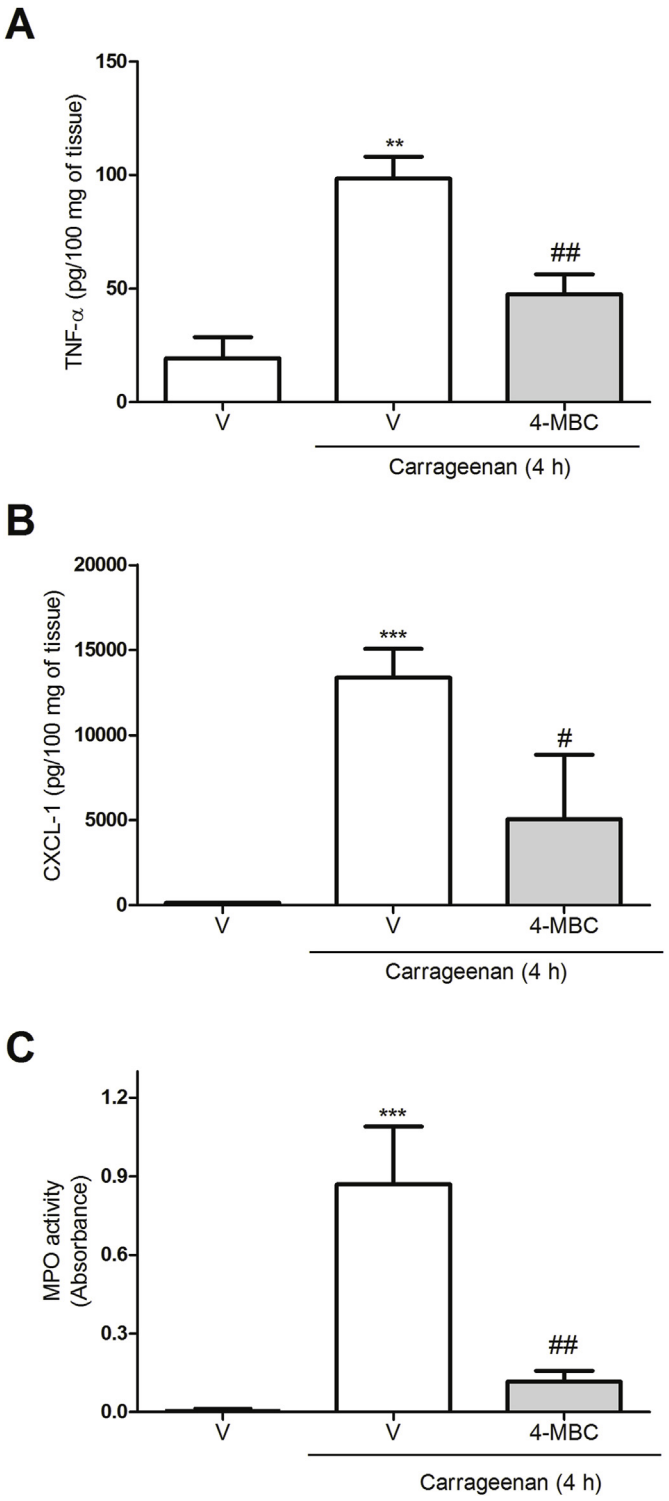

Fig. 6. Effect of 4-MBC (150 mg/kg, i.p., - 30 min) on the concentrations of TNF-α (A) and CXCL1 (B) and MPO activity (C) in the paw 4 h after i.pl. injection of carrageenan (600 μg). Statistical comparison was carried out using Kruskal-Wallis test, followed by Dunn's multiple comparisons test. $^{**}P < 0.01$ and $^{***}P < 0.001$ *vs* vehicle (V) group. $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ *vs* vehicle (V) + carrageenan group. n = 6.

carrageenan (600 μg, 20 μl, i.pl.). The dose of *p*-toluamide (134 mg/kg, i.p.) was equimolar to that of 4-MBC (150 mg/kg, i.p.). 4-MBC, but not *p*-toluamide, inhibited the mechanical allodynia (52.8%) and paw edema (40.4%) induced by carrageenan (Fig. 7).

4. Discussion

In the present study, we provide unequivocal evidence that 4-MBC, a thiobenzamide compound, releases H_2S and exhibits activities in models of pain and inflammation. This thiobenzamide, in contrast to the sulfide salts, Na_2S and NaHS (Li et al., 2008; Whiteman and Winyard, 2011), induced a slow and long lasting H_2S release, thus mimicking the biological effects of endogenously produced H_2S. In addition, the H_2S-releasing property of 4-MBC did not require the presence of organic thiols. This result contrasts with those described by Martelli et al. (2013) for the thiobenzamide 4-HBC, which slowly releases H_2S only in the presence of L-cysteine, an organosulphur compound. These contrasting results may be explained by the fact that 4-

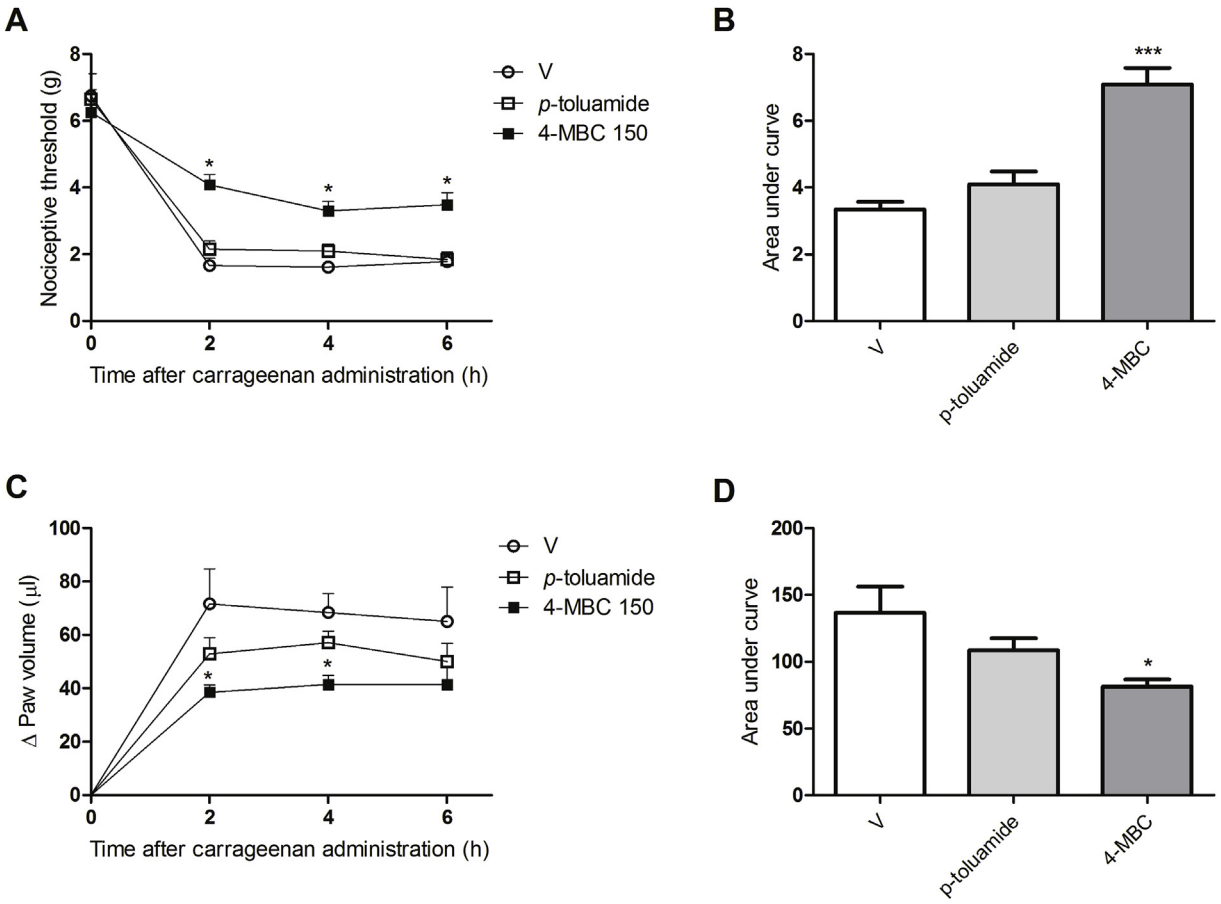

Fig. 7. Effects of 4-MBC (150 mg/kg, i.p., - 30 min) or *p*-toluamide (134 mg/kg, i.p., - 30 min) on the mechanical allodynia (A and B) or paw edema (C and D) induced by i.pl. injection of carrageenan (600 μg/paw). A and C represent the temporal course and B and D represent the AUC. Statistical comparison was carried out using Kruskal-Wallis test, followed by Dunn's multiple comparisons test. *P < 0.05 and ***P < 0.001 *vs* vehicle (V) group. n = 6.

HBC bears a *para*-hydroxyl group at the phenyl ring that, in turn, decreases the electrophilicity of the thiocarbonyl group. This delocalization of electrons reduces the electrophilicity of 4-HBC, then reducing its ability to undergo attack by water. Caliendo et al. (2010), investigating the hydrolysis of thiobenzamides and thioacetamides in an acidic medium, suggested that the mechanisms would be similar, but the thiobenzamides hydrolysis would occur more easily as the presence of the aromatic ring makes the intermediates more stable. Lougiakis et al. (2016) also suggested the same mechanisms after investigating the hydrolysis of these compounds at a pH 7.4, closer to what is expected to occur in vivo.

After demonstrating that 4-MBC releases H_2S, we investigated its effects in models of pain (nociceptive response induced by formaldehyde and mechanical allodynia induced by carrageenan) and inflammation (paw edema induced by carrageenan). The nociceptive response induced by formaldehyde is an experimental model of pain that exhibits both a nociceptive and an inflammatory profile. The first phase of this response has been attributed to direct activation of nociceptors, a chemonociceptive effect, and the second phase is associated with production of inflammatory mediators and, consequently, activation and sensitization of nociceptors (Tjølsen et al., 1992). Analgesic drugs that act predominantly in the central nervous system, such as opioids, usually inhibit both phases, whereas drugs with predominantly anti-inflammatory activity (steroidal and non-steroidal anti-inflammatory drugs) usually inhibit the second phase of the response. As 4-MBC barely affected the first phase and markedly inhibited the second phase of the nociceptive behavior, it is suggested that this compound inhibits mainly the inflammatory component of the response induced by formaldehyde. Similar results have been observed for Na_2S, a fast H_2S-releasing compound that also inhibited only the second phase of the nociceptive response induced by formaldehyde (Donatti et al., 2014).

The anti-inflammatory profile of 4-MBC profile is further supported by the demonstration of the effects of this thiobenzamide on the mechanical allodynia and paw edema induced by carrageenan. Injection of carrageenan triggers neutrophils recruitment and production of several inflammatory mediators that induce a marked sensitization of nociceptors and both thermal and mechanical allodynia (Posadas et al., 2004; Gregory et al., 2013). The myriad of inflammatory mediators induced by carrageenan also induces vasodilation and increases vascular permeability, thus resulting in inflammatory edema (Salvemini et al., 1996). Previous treatment of mice with 4-MBC induced a long lasting effect on the mechanical allodynia and paw edema induced by carrageenan.The antiallodynic effect induced by other H_2S donors, such as Lawesson's reagent (Ekundi-Valentim et al., 2010) and NaHS (Cunha et al., 2008), as well as the Na_2S and NaHS inhibitory effect on the paw edema induced by carrageenan (Zanardo et al., 2006), have also been demonstrated.

Next, we investigated mechanisms that could contribute to the 4-MBC antiallodynic activity in the inflammatory pain model induced by carrageenan. Many studies have shown that activation of opioidergic receptors (Gray et al., 1998; Hernandez-Leon et al., 2016) and K_{ATP} channels (León-Reyes et al., 2009; Hajhashemi and Amin, 2011) contributes to the activity of a wide variety of drugs in different experimental models of pain. However, a non-selective opioid receptor antagonist (naltrexone) and a K_{ATP} channels blocker (glibenclamide) failed to affect 4-MBC antiallodynic activity, indicating that these pathways are not relevant to the activity of this thiobenzamide in the

experimental model inflammatory pain induced by carrageenan.

The antiallodynic and antiedematogenic activities of 4-MBC may also be associated with effects on neutrophils recruitment and inflammatory mediators production. 4-MBC markedly reduced the MPO activity in the inflamed paw, an indirect evidence of an inhibitory effect on neutrophils recruitment. Demonstration of the effect of other H_2S donors on carrageenan-induced MPO activity is lacking. However, by using alternative methods to evaluate chemotaxis, it has been shown that other H_2S donors reduce neutrophils recruitment in different models (Lin et al., 2016; Kayar-Yasar et al., 2017) and that this event may be exacerbated when H_2S synthesis is inhibited (Zanardo et al., 2006).

In addition to inhibiting MPO activity, 4-MBC reduced TNF-α and CXCL1 production in the inflamed paw. These effects may contribute to 4-MBC antiedematogenic and antiallodynic activities. Neutrophils infiltration as well as TNF-α and CXCL1 production are known to play relevant roles in the mechanisms leading to inflammatory edema and mechanical allodynia (Brack et al., 2004; Rocha et al., 2006; Klinke et al., 2011). 4-MBC inhibitory effect on carrageenan-induced TNF-α production is similar to that observed by several authors who also evaluated other H_2S donors. It has been shown that TNF-α concentrations may be reduced by administration of NaHS (Hu et al., 2007; Fan et al., 2013; Rios et al., 2015), GYY4137 (Li et al., 2009, 2013; Whiteman et al., 2010) and H_2S-releasing hybrid compounds (Fiorucci et al., 2007; Dief et al., 2015) in different experimental models. Although there is no previous direct demonstration that H_2S release may be related to reduced production of CXCL1, reduced H_2S production may raise the concentration of other chemokines such as CCL2 and CX3CL1 (Gao et al., 2015). Furthermore, pre-incubation with NaHS inhibits interferon-γ- or lipopolysaccharide-induced CX3CR1 and CX3CL1 expression (Zhang et al., 2012).

In contrast to 4-MBC, *p*-toluamide, the S-lacking analogue, did not induce antiallodynic or antiedematogenic effects in the models used in the present study. Comparison of the effects of an H_2S donor (4-MBC) and its oxo analogue (*p*-toluamide) reinforces the association between the antiallodynic and antiedematogenic effects of 4-MBC and H_2S release. Similar approach was used by Di Cesari Manelli et al. (2017), who demonstrated that allyl isothiocyanate, but not its S-lacking analogue, allyl isocyanate, reduces the cold allodynia induced by oxaliplatin in mice.

In summary, we demonstrated that 4-MBC, an H_2S releasing thiobenzamide, exhibits antinociceptive and anti-inflammatory activities. These activities may be due to reduced neutrophils recruitment and cytokine and chemokine production. The H_2S releasing property is likely essential for 4-MBC activity. Finally, our results indicate that 4-MBC may represent a useful pharmacological tool to investigate the biological roles of H_2S.

Conflicts of interest

The authors declare that they have no conflicts of interest to disclose.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejphar.2019.172404.

References

Bhatia, M., Sidhapuriwala, J., Moochhala, S.M., Moore, P.K., 2005. Hydrogen sulphide is a mediator of carrageenan-induced hindpaw oedema in the rat. Br. J. Pharmacol. 145, 141–144. https://doi.org/10.1038/sj.bjp.0706186.

Boudet, I.R., 1949. The N-benzyl thioamides. Bull. Soc. Chim. Fr. 172–177.

Brack, A., Rittner, H.L., Machelska, H., Leder, K., Mousa, S.A., Schäfer, M., Stein, C., 2004. Control of inflammatory pain by chemokine-mediated recruitment of opioid-containing polymorphonuclear cells. Pain 112, 229–238. https://doi.org/10.1016/j.pain.2004.08.029.

Caliendo, G., Cirino, G., Santagada, V., Wallace, J.L., 2010. Synthesis and biological effects of hydrogen sulfide (H_2S): development of H_2S-releasing drugs as pharmaceuticals. J. Med. Chem. 53, 6275–6286. https://doi.org/10.1021/jm901638j.

Cunha, T.M., Dal-Secco, D., Verri, W.A., Guerrero, A.T., Souza, G.R., Vieira, S.M., Lotufo, C.M., Neto, A.F., Ferreira, S.H., Cunha, F.Q., 2008. Dual role of hydrogen sulfide in mechanical inflammatory hypernociception. Eur. J. Pharmacol. 590, 127–135. https://doi.org/10.1016/j.ejphar.2008.05.048.

Di Cesari Manelli, L., Lucarini, E., Micheli, L., Mosca, I., Ambrosino, P., Soldovieri, M.V., Martelli, A., Testai, L., Taglialatela, M., Calderone, V., Ghelardini, C., 2017. Effects of natural and synthetic isothiocyanate-based H_2S-releasers against chemotherapy-induced neuropathic pain: role of Kv7 potassium channels. Neuropharmacology 121, 49–59. https://doi.org/10.1016/j.neuropharm.2017.04.029.

Dief, A.E., Mostafa, D.K., Sharara, G.M., Zeitoun, T.H., 2015. Hydrogen sulfide releasing naproxen offers better anti-inflammatory and chondroprotective effect relative to naproxen in a rat model of zymosan induced arthritis. Eur. Rev. Med. Pharmacol. Sci. 19, 1537–1546. https://www.europeanreview.org/.

Donatti, A.F., Araujo, R.M., Soriano, R.N., Azevedo, L.U., Leite-Panissi, C.A., Branco, L.G., 2014. Role of hydrogen sulfide in the formalin-induced orofacial pain in rats. Eur. J. Pharmacol. 738, 49–56. https://doi.org/10.1016/j.ejphar.2014.05.023.

Ekundi-Valentim, E., Santos, K.T., Camargo, E.A., Denadai-Souza, A., Teixeira, S.A., Zanoni, C.I., Grant, A.D., Wallace, J.L., Muscará, M.N., Costa, S.K., 2010. Differing effects of exogenous and endogenous hydrogen sulphide in carrageenan-induced knee joint synovitis in the rat. Br. J. Pharmacol. 159, 1463–1474. https://doi.org/10.1111/j.1476-5381.2010.00640.x.

Fan, H., Guo, Y., Liang, X., Yuan, Y., Qi, X., Wang, M., Ma, J., Zhou, H., 2013. Hydrogen sulfide protects against amyloid beta-peptide induced neuronal injury via attenuating inflammatory responses in a rat model. J. Biomed. Res. 27, 296–304. https://doi.org/10.7555/JBR.27.20120100.

Fiorucci, S., Orlandi, S., Mencarelli, A., Caliendo, G., Santagada, V., Distrutti, E., Santucci, L., Cirino, G., Wallace, J.L., 2007. Enhanced activity of a hydrogen sulphide-releasing derivative of mesalamine (ATB-429) in a mouse model of colitis. Br. J. Pharmacol. 150, 996–1002. https://doi.org/10.1038/sj.bjp.0707193.

Gao, L., Xu, Z., Yin, Z., Chen, K., Wang, C., Zhang, H., 2015. Association of hydrogen sulfide with alterations of monocyte chemokine receptors, CCR2 and CX3CR1 in patients with coronary artery disease. Inflamm. Res. 64, 627–635. https://doi.org/10.1007/s00011-015-0844-7.

Gray, A.M., Spencer, P.S., Sewell, R.D., 1998. The involvement of the opioidergic system in the antinociceptive mechanism of action of antidepressant compounds. Br. J. Pharmacol. 124, 669–674. https://doi.org/10.1038/sj.bjp.0701882.

Gregory, N., Harris, A.L., Robinson, C.R., Dougherty, P.M., Fuchs, P.N., Sluka, K.A., 2013. An overview of animal models of pain: disease models and outcome measures. J. Pain 14, 1255–1269. https://doi.org/10.1016/j.jpain.2013.06.008.

Hajhashemi, V., Amin, B., 2011. Effect of glibenclamide on antinociceptive effects of antidepressants of different classes. Clinics 66, 321–325. https://doi.org/10.1590/S1807-59322011000200023.

Hartle, M.D., Pluth, M.D., 2016. A practical guide to working with H_2S at the interface of chemistry and biology. Chem. Soc. Rev. 45, 6108–6117. https://doi.org/10.1039/C6CS00212A.

Hauser, C.R., Hoffenberg, D.S., 1955. Conversion of nitriles to amides and acids by means of boron fluoride. J. Org. Chem. 20, 1448–1453. https://doi.org/10.1021/jo01127a025.

Hernandez-Leon, A., Fernández-Guasti, A., González-Trujano, M.E., 2016. Rutin antinociception involves opioidergic mechanism and descending modulation of ventrolateral periaqueductal grey matter in rats. Eur. J. Pain 20, 274–283. https://doi.org/10.1002/ejp.720.

Hu, L., Wong, P.T., Moore, P.K., Bian, J.S., 2007. Hydrogen sulfide attenuates lipopolysaccharide-induced inflammation by inhibition of p38 mitogen-activated protein kinase in microglia. J. Neurochem. 100, 1121–1128. https://doi.org/10.1111/j.1471-4159.2006.04283.x.

Huang, C.W., Moore, P.K., 2015. H_2S synthesizing enzymes: biochemistry and molecular aspects. Handb. Exp. Pharmacol. 230, 3–25. https://doi.org/10.1007/978-3-319-18144-8_1.

Kayar-Yasar, Y., Karaman, Y., Bozkurt, T.E., Onder, S.C., Sahin-Erdemli, I., 2017. Effects of intranasal treatment with slow (GYY4137) and rapid (NaHS) donors of hydrogen sulfide in lipopolysaccharide-induced airway inflammation in mice. Pulm. Pharmacol. Therapeut. 45, 170–180. https://doi.org/10.1016/j.pupt.2017.06.006.

Kida, K., Marutani, E., Nguyen, R.K., Ichinose, F., 2015. Inhaled hydrogen sulfide prevents neuropathic pain after peripheral nerve injury in mice. Nitric Oxide 46, 87–92. https://doi.org/10.1016/j.niox.2014.11.014.

Klinke, A., Nussbaum, C., Kubala, L., Friedrichs, K., Rudolph, T.K., Rudolph, V., Paust, H.J., Schröder, C., Benten, D., Lau, D., Szocs, K., Furtmüller, P.G., Heeringa, P., Sydow, K., Duchstein, H.J., Ehmke, H., Schumacher, U., Meinertz, T., Sperandio, M., Baldus, S., 2011. Myeloperoxidase attracts neutrophils by physical forces. Blood 117, 1350–1358. https://doi.org/10.1182/blood-2010-05-284513.

León-Reyes, M.R., Castañeda-Hernández, G., Ortiz, M.I., 2009. Pharmacokinetic of

diclofenac in the presence and absence of glibenclamide in the rat. J. Pharm. Pharm. Sci. 12, 280–287. https://doi.org/10.18433/J3S597.
Li, L., Bhatia, M., Zhu, Y.Z., Zhu, Y.C., Ramnath, R.D., Wang, Z.J., Anuar, F.B., Whiteman, M., Salto-Tellez, M., Moore, P.K., 2005. Hydrogen sulfide is a novel mediator of lipopolysaccharide-induced inflammation in the mouse. FASEB J. 19, 1196–1198. https://doi.org/10.1096/fj.04-3583fje.
Li, L., Whiteman, M., Guan, Y.Y., Neo, K.L., Cheng, Y., Lee, S.W., Zhao, Y., Baskar, R., Tan, C.H., Moore, P.K., 2008. Characterization of a novel, water-soluble hydrogen sulfide-releasing molecule (GYY4137): new insights into the biology of hydrogen sulfide. Circulation 117, 2351–2360. https://doi.org/10.1161/CIRCULATIONAHA.107.753467.
Li, L., Salto-Tellez, M., Tan, C.H., Whiteman, M., Moore, P.K., 2009. GYY4137, a novel hydrogen sulfide-releasing molecule, protects against endotoxic shock in the rat. Free Radic. Biol. Med. 47, 103–113. https://doi.org/10.1016/j.freeradbiomed.2009.04.014.
Li, L., Fox, B., Keeble, J., Salto-Tellez, M., Winyard, P.G., Wood, M.E., Moore, P.K., Whiteman, M., 2013. The complex effects of the slow-releasing hydrogen sulfide donor GYY4137 in a model of acute joint inflammation and in human cartilage cells. J. Cell Mol. Med. 17, 365–376. https://doi.org/10.1111/jcmm.12016.
Lin, S., Visram, F., Liu, W., Haig, A., Jiang, J., Mok, A., Lian, D., Wood, M.E., Torregrossa, R., Whiteman, M., Lobb, I., Sener, A., 2016. GYY4137, a slow-releasing hydrogen sulfide donor, ameliorates renal damage associated with chronic obstructive uropathy. J. Urol. 196, 1778–1787. https://doi.org/10.1016/j.juro.2016.05.029.
Lougiakis, N., Papapetropoulos, A., Gikas, E., Toumpas, S., Efentakis, P., Wedmann, R., Zoga, A., Zhou, Z., Iliodromitis, E.K., Skaltsounis, A.L., Filipovic, M.R., Pouli, N., Marakos, P., Andreadou, I., 2016. Synthesis and pharmacological evaluation of novel adenine-hydrogen sulfide slow release hybrids designed as multitarget cardioprotective agents. J. Med. Chem. 59, 1776–1790. https://doi.org/10.1021/acs.jmedchem.5b01223.
Martelli, A., Testai, L., Citi, V., Marino, A., Pugliesi, I., Barresi, E., Nesi, G., Rapposelli, S., Taliani, S., Da Settimo, F., Breschi, M.C., Calderone, V., 2013. Arylthioamides as H_2S donors: L-cysteine-activated releasing properties and vascular effects in vitro and in vivo. ACS Med. Chem. Lett. 4, 904–908. https://doi.org/10.1021/400239a.
Papapetropoulos, A., Whiteman, M., Cirino, G., 2015. Pharmacological tools for hydrogen sulphide research: a brief, introductory guide for beginners. Br. J. Pharmacol. 172, 1633–1637. https://doi.org/10.1111/bph.12806.
Perna, A.F., Sepe, I., Lanza, D., Capasso, R., Zappavigna, S., Capasso, G., Caraglia, M., Ingrosso, D., 2013. Hydrogen sulfide reduces cell adhesion and relevant inflammatory triggering by preventing ADAM17-dependent TNF-α activation. J. Cell. Biochem. 114, 1536–1548. https://doi.org/10.1002/jcb.24495.
Posadas, I., Bucci, M., Roviezzo, F., Rossi, A., Parente, L., Sautebin, L., Cirino, G., 2004. Carrageenan-induced mouse paw oedema is biphasic, age-weight dependent and displays differential nitric oxide cyclooxygenase-2 expression. Br. J. Pharmacol. 142, 331–338. https://doi.org/10.1038/sj.bjp.0705650.
Rios, E.C.S., Szczesny, B., Soriano, F.G., Olah, G., Szabó, C., 2015. Hydrogen sulfide attenuates cytokine production through the modulation of chromatin remodeling. Int. J. Mol. Med. 35, 1741–1746. https://doi.org/10.3892/ijmm.2015.2176.
Rocha, A.C., Fernandes, E.S., Quintão, N.L., Campos, M.M., Calixto, J.B., 2006. Relevance of tumour necrosis factor-alpha for the inflammatory and nociceptive responses evoked by carrageenan in the mouse paw. Br. J. Pharmacol. 148, 688–695. https://doi.org/10.1038/sj.bjp.0706775.
Salvemini, D., Wang, Z.Q., Wyatt, P.S., Bourdon, D.M., Marino, M.H., Manning, P.T., Currie, M.G., 1996. Nitric oxide: a key mediator in the early and late phase of carrageenan-induced rat paw inflammation. Br. J. Pharmacol. 118, 829–838. https://doi.org/10.1111/j.1476-5381.1996.tb15475.x.
Szabó, C., 2007. Hydrogen sulphide and its therapeutic potential. Nat. Rev. Drug Discov. 6, 917–935. https://doi.org/10.1038/nrd2425.
Szabó, C., 2017. A timeline of hydrogen sulfide (H_2S) research: from environmental toxin to biological mediator. Biochem. Pharmacol. 149, 5–19. https://doi.org/10.1016/j.bcp.2017.09.010.
Talaei, F., 2016. Pathophysiological concepts in multiple sclerosis and the therapeutic effects of hydrogen sulfide. Basic Clin. Neurosci. 7, 121–136. https://dx.doi.org/10.15412/J.BCN.03070206.
Tjølsen, A., Berge, O.G., Hunskaar, S., Rosland, J.H., Hole, K., 1992. The formalin test: an evaluation of the method. Pain 51, 5–17. https://doi.org/10.1016/0304-3959(92)90003-T.
Wallace, J.L., Dicay, M., Mcknight, W., Martin, G.R., 2007. Hydrogen sulfide enhances ulcer healing in rats. FASEB J. 21, 4070–4076. https://doi.org/10.1096/fj.07-8669com.
Wallace, J.L., Caliendo, G., Santagada, V., Cirino, G., 2010. Markedly reduced toxicity of a hydrogen sulphide-releasing derivative of naproxen (ATB-346). Br. J. Pharmacol. 159, 1236–1246. https://doi.org/10.1111/j.1476-5381.2009.00611.x.
Wallace, J.L., Vaughan, D., Dicay, M., Macnaughton, W.K., de Nucci, G., 2018. Hydrogen sulfide-releasing therapeutics: translation to the clinic Antioxid. Redox Signal 28, 1533–1540. https://doi.org/10.1089/ars.2017.7068.
Wang, R., 2002. Two's company, three's a crowd: can H_2S be the third endogenous gaseous transmitter? FASEB J. 16, 1792–1798. https://doi.org/10.1096/fj.02-0211hyp.
Wang, R., 2003. The gasotransmitter role of hydrogen sulfide. Antioxidants Redox Signal. 5, 493–501. https://doi.org/10.1089/152308603768295249.
Whiteman, M., Li, L., Rose, P., Tan, C.H., Parkinson, D.B., Moore, P.K., 2010. The effect of hydrogen sulfide donors on lipopolysaccharide-induced formation of inflammatory mediators in macrophages. Antioxidants Redox Signal. 12, 1147–1154. https://doi.org/10.1089/ars.2009.2899.
Whiteman, M., Winyard, P.G., 2011. Hydrogen sulfide and inflammation: the good, the bad, the ugly and the promising. Expert Rev. Clin. Pharmacol. 4, 13–32. https://doi.org/10.1586/ecp.10.134.
Yang, H., Wu, Z.Y., Bian, J.S., 2014. Hydrogen sulfide inhibits opioid withdrawal-induced pain sensitization in rats by down-regulation of spinal calcitonin gene-related peptide expression in the spine. Int. J. Neuropsychopharmacol. 17, 1387–1395. https://doi.org/10.1017/S1461145714000583.
Zanardo, R.C.O., Brancaleone, V., Distrutti, E., Fiorucci, S., Cirino, G., Wallace, J.L., 2006. Hydrogen sulfide is an endogenous modulator of leukocyte-mediated inflammation. FASEB J. 20, 2118–2120. https://doi.org/10.1096/fj.06-6270fje.
Zhang, H., Guo, C., Wu, D., Zhang, A., Gu, T., Wang, L., Wang, C., 2012. Hydrogen sulfide inhibits the development of atherosclerosis with suppressing CX3CR1 and CX3CL1 expression. PLoS One 7, e41147. https://doi.org/10.1371/journal.pone.0041147.
Zhao, H., Pan, P., Yang, Y., Ge, H., Chen, W., Qu, J., Shi, J., Cui, G., Liu, X., Feng, H., Chen, Y., 2017. Endogenous hydrogen sulphide attenuates NLRP3 inflammasome-mediated neuroinflammation by suppressing the P2X7 receptor after intracerebral haemorrhage in rats. J. Neuroinflammation 14, 163. https://doi.org/10.1186/s12974-017-0940-4.
Zhao, Y., Biggs, T.D., Xian, M., 2014. Hydrogen sulfide (H_2S) releasing agents: chemistry and biological applications. Chem. Commun. 50, 11788–11805. https://doi.org/10.1039/C4CC00968A.
Zhuang, L., Li, K., Wang, G., Shou, T., Gao, C., Mao, Y., Bao, M., Zhao, M., 2018. Preconditioning with hydrogen sulfide prevents bone cancer pain in rats through a proliferator-activated receptor gamma/p38/Jun N-terminal kinase pathway. Exp. Biol. Med. 243, 57–65. https://doi.org/10.1177/1535370217740859.
Zimmermann, M., 1983. Ethical guidelines for investigations of experimental pain in conscious animals. Pain 16, 109–110. https://doi.org/10.1016/0304-3959(83)90201-4.