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Activities of 2-phthalimidethanol and 2-phthalimidethyl nitrate, phthalimide analogs devoid of the glutarimide moiety, in experimental models of inflammatory pain and edema

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ABSTRACT

The reintroduction of thalidomide in the pharmacotherapy greatly stimulated the interest in the synthesis and pharmacological evaluation of phthalimide analogs with new and improved activities and also greater safety. In the present study, we evaluated the activities of two phthalimide analogs devoid of the glutarimide ring, namely 2-phthalimidethanol (PTD-OH) and 2-phthalimidethyl nitrate (PTD-NO), in experimental models of inflammatory pain and edema in male C57BL/6J mice. Intraplantar (i.pl.) injection of carrageenan (300 μg) induced mechanical allodynia and this response was inhibited by previous per os (p.o.) administration of PTD-OH and PTD-NO (750 mg/kg) and also by thalidomide (500 or 750 mg/kg). The edema induced by carrageenan was also inhibited by previous p.o. administration of PTD-OH (500 and 750 mg/kg) and PTD-NO (125, 250, 500 or 750 mg/kg), but not by thalidomide. Carrageenan increased tumor necrosis factor (TNF)-α and CXCL1 concentrations and also the number of neutrophils in the paw tissue. Previous p.o. administration of PTD-NO (500 mg/kg) reduced all the parameters, while PTD-OH (500 mg/kg) reduced only the accumulation of neutrophils. Thalidomide, on the other hand, was devoid of effect on these biochemical parameters. Plasma concentrations of nitrite were increased after p.o. administration of the phthalimide analog coupled to a NO donor, PTD-NO (500 mg/kg), but not after administration of PTD-OH or thalidomide. In conclusion, our results show that small molecules, structurally much simpler than thalidomide or many of its analogs under investigation, exhibit similar activities in experimental models of pain and inflammation. Finally, as there is evidence that the glutarimide moiety contributes to the teratogenic effect of many thalidomide analogs, our results indicate that phthalimide analogs devoid of this functional group could represent a new class of analgesic and anti-inflammatory candidates with potential greater safety.

1. Introduction

The best known phthalimide analog is thalidomide (2-phthalimido glutarimide; Fig. 1), originally developed and marketed as a hypnotic, sedative and antiemetic drug in the late 1950s, but withdrawn in 1961 after the association of its use with teratogenic effects (Brynner and Stephens, 2001). Extensive investigations during the next three decades showed the immunomodulatory and anti-inflammatory activities of thalidomide (Asher and Furnish, 2013; Brynner and Stephens, 2001; Matthews and McCoy, 2003) and allowed its reintroduction, initially (1998) in the treatment of patients with erythema nodosum leprosum and later on (2006) in the treatment of patients with multiple myeloma (for a review, see Matthews and McCoy, 2003).

The reintroduction of thalidomide greatly stimulated the interest in the synthesis and pharmacological evaluation of phthalimide analogs with new and improved activities and also greater safety. Usually, phthalimide analogs have been developed aiming anti-inflammatory, analgesic, immunomodulatory, anti-angiogenic and anti-tumor properties. Some of these analogs inhibit the production of the inflammatory cytokine tumor necrosis factor (TNF)-α (Chaulet et al., 2011; de Almeida et al., 2007; Mazzoccoli et al., 2012; Miyachi et al., 1997a, 1997b; Stewart et al., 2007, 2010; Teubert et al., 1998) and also the

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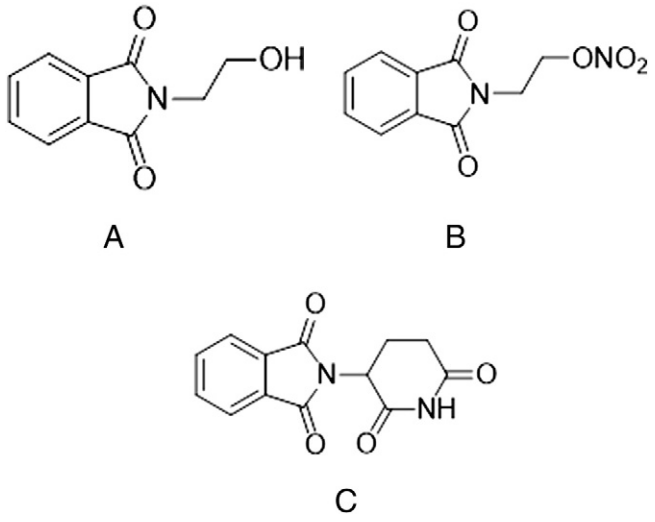

Fig. 1. Chemical structures of (A) 2-phthalimidethanol (PTD-OH), (B) 2-phthalimidethyl nitrate (PTD-NO) and (C) thalidomide (2-phthalimidoglutarimide).

activity of cyclooxygenases (Noguchi et al., 2002; Sano et al., 2004; Suizu et al., 2003) and nitric oxide (NO) synthases (Noguchi et al., 2004; Shimazawa et al., 2004). The antitumoral activity of some phthalimide analogs may be related to their inhibitory effects on angiogenesis (Noguchi et al., 2005; Sano et al., 2006) and also on tubulin polymerization (Iguchi et al., 2008; Li et al., 2006; Yanagawa et al., 2006).

Some investigators, while developing new phthalimide analogs, have also adopted a strategy that has been widely used with steroidal (SAIDs) and non-steroidal (NSAIDs) anti-inflammatory drugs, that is, the coupling of NO donors. This strategy led to the development of new compounds with improved efficacy and greater safety in preclinical assays such as nitroacetylsalicylic acid (Fiorucci et al., 2000; Turnbull et al., 2008), nitroparacetamol (Romero-Sandoval et al., 2007), nitronaproxen (Young et al., 2005) and nitroprednisolone (Tallet et al., 2002). Regarding the phthalimide analogs coupled to NO donors, the investigation is still preliminary but it has been shown that some of them exhibit cytotoxic activity against various cancer cell lines (Wang et al., 2009) and also antinociceptive and anti-inflammatory activities (Dos Santos et al., 2011).

In the present study, we evaluated the activities of two phthalimide analogs devoid of the glutarimide ring, 2-phthalimidethanol (PTD-OH) and 2-phthalimidethyl nitrate (PTD-NO) (Fig. 1), in experimental models of inflammatory pain and edema. These analogs represent much simpler molecules when compared with many of the phthalimide analogs synthesized and evaluated by other investigators (Chaulet et al., 2011; de Almeida et al., 2007; Mazzoccoli et al., 2012; Miyachi et al., 1997a, 1997b; Stewart et al., 2007, 2010; Teubert et al., 1998). In addition, our interest in the phthalimide analogs devoid of the glutarimide moiety derives from the evidence provided by different studies that this functional group may contribute to the teratogenic effect (Huang and McBride, 1997; Lepper et al., 2004).

2. Methods

2.1. Synthesis of phthalimide analogs

2.1.1. Chemicals and reagents

All chemicals were obtained from commercial suppliers and used without further purification. Melting points were determined in a Gehaka PF 1500 apparatus and are uncorrected. ^{1}H nuclear magnetic resonance (NMR) spectra and ^{13}C NMR spectra were recorded on a Bruker Avance DPX/200. Chemical shift values (δ) were given in parts per million (ppm). Infrared (IR) spectra were recorded on a Spectro One Perkin Elmer.

2.1.2. Syntheses of PTD-OH

Phthalimide (1.0 mmol) was added slowly to the ethanolamine (1.25 mmol) at 100 °C and stirred for 4 h. The reaction mixture was stirred at room temperature for 12 h (Scheme 1). The progress of the reaction was monitored by TLC. Boiling water was added to the residue and the obtained white solid was vacuum filtered, with yield 65%; Mp 126.6–128.4 °C. IR (ATR): 3474, 2954, 2944, 2888, 1768, 1694, 1430, 1396, 1378, 1058, 994, 726, 534. ^{1}H NMR (200 MHz, $CDCl_3$): 3.83 (s, 4H), 7.65–7.80 (m, 4H). ^{13}C NMR (50 MHz, $CDCl_3$): 40.6, 60.6, 123.2, 131.8, 134.0, 168.7. Anal. Calcd. for $C_{10}H_9NO_3$. Calcd. (%): C, 62.82; H, 4.74; N, 7.33. Found (%): C, 62.99; H, 4.66; N, 7.13.

2.1.3. Syntheses of PTD-NO

This compound was obtained by mixing fuming nitric acid (1.0 mmol) and the precursor compound (0.10 mmol) at −5 °C and stirring for 2 h (Scheme 1). The reaction mixture was poured into a mixture of water and ice. The pH was adjusted to 6 by adding $CaCO_3$. The obtained white solid was vacuum filtered, with yield 93%; Mp 87.1–89.5 °C. IR (ATR): 3062, 3030, 2978, 2886, 1774, 1716, 1608, 1422, 1402, 1288, 982, 870, 722, 530. ^{1}H NMR (200 MHz, DMSO-d_6): 3.95 (t, 2H), 4.71 (t, 2H), 7.86 (br s,4H). ^{13}C NMR (50 MHz, DMSO-d_6): 34.8, 70.8, 123.1, 131.4, 134.4, 167.5. Anal. Calcd. for $C_{10}H_8N_2O_5$. Calcd. (%): C, 50.85; H, 3.41; N, 11.86. Found (%): C, 51.09; H, 3.32; N, 11.77.

2.2. Animals

Eight-week-old male C57BL/6J mice were used. The animals had free access to food and water and were maintained in a room with a 12 h light-dark cycle for at least 3 days before the experiment to allow acclimatization. The experiments were carried out at a room temperature of 27 °C. This temperature was used because the thermoneutral zone for mice and rats ranges between 26 and 34 °C, a temperature range that markedly differs from that of standard laboratory environments which could be stressful and affect many aspects of physiology and behavior of the rodents (Gaskill et al., 2009; Gordon, 1990). All experiments were conducted according to the ethical guidelines for investigation of experimental pain in conscious animals (Zimmermann, 1983) and by the Ethics Committee on Animal Experimentation of the Federal University of Minas Gerais (CETEA — UFMG. Protocol 48/2011).

2.3. Drugs

Suspensions of PTD-OH, PTD-NO, thalidomide (FUNED, Brazil) and phenobarbital (Aventis Pharma, Brazil) were prepared in 0.5% carboxymethylcellulose (CMC) sodium salt (Sigma, USA) suspension in saline immediately before the experiments. Suspensions were administered per os (p.o.) in a volume of 12 ml/kg. λ-Carrageenan (Sigma, USA) suspension was prepared in saline.

2.4. Evaluation of mechanical allodynia

Tactile thresholds were measured by using an electronic von Frey apparatus (electronic pressure-meter, Insight, Brazil), according to method described by Cunha et al. (2004), with minor modifications. The mice were kept individually in acrylic cages (10 × 10 cm with 18 cm-high walls) whose floor was a metal grid. The hand-held force transducer, fitted with a polypropylene tip (0.5 mm^2) filament, was directed upwards from below the grid and gradually pressed on the plantar surface of the right hind paw. The test consisted of evoking a hind paw flexion reflex. The endpoint was characterized by the removal of the paw usually followed by flinching movements. After the paw withdrawal, the intensity of the pressure was automatically recorded. The value for the response was obtained by averaging five measurements. Mice were habituated to the experimental apparatus daily, approximately 60 min a day, for 4 days before the experiments. On the experimental day, baseline paw withdrawal threshold of each animal