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Pharmacological mechanism underlying the antinociceptive activity of vanillic acid

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ABSTRACT

Vanillic acid is found at high concentrations in many plants used in traditional medicine. It has been associated with a variety of pharmacologic activities such as carcinogenesis inhibition, apoptosis and inflammation; however, it has become most popular for its pleasant creamy odor. Since there are few reports concerning the antinociceptive activity of this phenolic compound, the aim of this work was to study this activity in in vivo animal models. Vanillic acid was administered by the intraperitoneal route producing a dose-dependent inhibition of the acetic acid-induced writhing response (ED_{50}: 9.3 mg/kg). The antinociceptive activity was inhibited by the pretreatment with ondansetron and yohimbine, indicating that the serotoninergic and adrenergic systems could participate in the mechanism underlying the analgesic activity of vanillic acid. This compound was also demonstrated to interact with ASICs (Acid-sensing Ion Channels) as well as with TPRV1, TRPA1, and TRPM8 receptors in vivo. Furthermore, vanillic acid did not interfere with the locomotor function or motor coordination. The plasmatic phenolic content, analyzed by HPLC, showed that its $t_{1/2}$ and AUC were 0.123 h and 1.38 μg·h/mL; respectively. In conclusion, vanillic acid might represent a potential therapeutic option for the treatment of pain.

1. Introduction

Pain is a transitory unpleasant sensation that follows a noxious or injurious stimulus, acting as a warning system. Particularly, the acute nociceptive pain is the consequence of the activation of primary afferent nociceptive fibers produced by mechanical, chemical or thermal stimuli. Both acute and chronic pains remain a significant health problem and although a considerable number of analgesic drugs are available, the development of novel substances that can effectively treat painful states remains as an important challenge. In this context, many plant-derived substances are attractive sources for developing new analgesic agents.

The role of secondary plant products such as phenolic acids, in the prevention of many human diseases has been extensively described. Particularly, vanillic acid (4-hydroxy-3 methoxybenzoic acid), a phenolic derivative found in several plants and fruits, has shown to possess an interesting pharmacological profile. Experimental studies have provided evidence of effectiveness on cardiovascular (StanelyMainzenPrince et al., 2011), gastrointestinal (Kim et al., 2010) and liver diseases (Itoh et al., 2009). The beneficial activity on acute inflammatory processes has also been described (Leal et al., 2011). Furthermore, vanillic acid has been demonstrated to inhibit the synthesis or release of tumor necrosis factor (TNF)-α, interleukin (IL)-6, cyclooxygenase-2 (COX-2) and nitric oxide (NO), which are mediators that are increased during inflammatory processes (Kim et al., 2011).

We have previously shown that *Lithraea molleoides* (Vell). Engl. (Anacardiaceae) was able to reduce the nociceptive effect induced by acetic acid and formalin tests and this effect was partly related to the presence of its main compound, vanillic acid (Morucci et al., 2012). This study suggested a predominant effect of vanillic acid in models with inflammatory components. However, research studies have not clearly demonstrated the underlying mechanisms involved in the antinociceptive effects of the phenolic compound. Therefore, the primary aim of this study was to investigate the mechanism of vanillic acid inducing antinociception. The pharmacokinetic profile of this compound was also evaluated.

2. Material and methods

2.1. Drugs

Amiloride, indomethacin, ketanserin, pindolol, diazepam, yohimbine, ondansetron, Evans blue, thiobarbituric acid (TBA), phosphotungstic acid, butylhydroxytoluene (BHT), capsaicin, cinnamaldehyde, menthol, ruthenium red, morphine, camphor and vanillic acid were purchased from Sigma Chemical Co., St. Louis, MO, USA. Ultrapure quality water (Milli-Q) was employed to prepare the mobile phase. Acetonitrile (HPLC) and butanol were purchased from J. T. Baker. Acetic acid and Sodium dodecyl sulfate (SDS) were purchased from Merck

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(Darmstadt, Germany). All reagents were of analytical grade. The purity of vanillic acid was checked by HPLC analysis and was 97% on the basis of peak area integration.

2.2. Animals

Female Swiss mice weighing 21–26 g were used. The experiments were carried out taking into account international guiding and local regulations concerning the care and use of laboratory animals for biomedical research. The experiments were approved by the local Ethics Committee (Exp-FyB: 0738658/2011). The animals had free access to a standard commercial diet and water ad libitum and were kept in a room maintained at 22 ± 1 °C with a 12-h light/dark cycle.

2.3. Antinociceptive activity

2.3.1. Acetic acid-induced abdominal writhing

The test was performed as described by Collier et al. (1968). Nociception was induced by intraperitoneal injection of 1.0% acetic acid, (0.1 mL/10 g body weight). Mice were treated with vanillic acid 30 min by the intraperitoneal route (i.p.) (1–100 mg/kg) or intracisternal route (i.c.) (10 and 50 μg/10 μL) before acetic acid injection. A group of mice was treated with indomethacin (10 mg/kg i.p.) as a reference drug. Control animals received a similar volume of saline solution (10 mL/kg, i.p.). The intracisternal route was performed during short anesthesia. Ten or 50 μg/10 μL of solution per mouse was injected slowly into the cisterna magna. Distribution of the injected substances was always checked by giving 0.4% methylene blue aqueous solution in the same manner as the drugs and the vehicle controls. Brains were dissected to verify the location and spread of each injection (Ueda et al., 1979). The animals were observed in experimental cages. Hand-operated counters and stopwatches were employed to score the number of abdominal writhes (full extension of both hind paws). The writhes were cumulatively counted over a period of 20 min immediately after the acetic acid injection. Doses of vanillic acid were selected based on pilot experiments. A significant reduction in the number of abdominal contractions between control and pre-treated animals was considered indicative of antinociceptive activity.

2.3.1.1. Pharmacological evaluation of the mechanism of action. To assess the possible participation of different systems on the antinociceptive effect of the phenolic compound, mice were pre-treated with yohimbine (1 mg/kg i.p.), an α_2 adrenoceptor antagonist; ondansetron (0.2 mg/kg i.p.), a 5-HT_3 receptor antagonist; ketanserin (0.3 mg/kg i.p.), a 5-HT_2 receptor antagonist; and pindolol (1 mg/kg i.p.), a β-adrenoceptor blocker/5-$HT_{1A/1B}$ antagonist, 30 min before the administration of vanillic acid (10 mg/kg i.p.). Doses and drug administration schedules were selected based on previous reports and on pilot experiments carried out in our laboratory (Salam, 2006; de Mattos et al., 2007; Spindola et al., 2011; Vidyalakshmi et al., 2012). The nociceptive response was evaluated in the acetic acid-induced abdominal writhing test.

2.3.2. Nociception induced by capsaicin, cinnamaldehyde, menthol and acidified saline

To test whether TRPV1, TRPA1, TRPM8 and ASIC (Acid-sensing Ion Channels) receptors are potential specific targets for the antinociceptive actions of vanillic acid, a single intraplantar (i.pl.) injection of either capsaicin (1.6 μg/paw), cinnamaldehyde (10 nmol/paw), menthol (1.2 μmol/paw), acidified saline (2% acetic acid in 0.9% saline) or the corresponding vehicle was delivered into the ventral surface of the right hind paw. Each animal was then placed, immediately and alone, into a glass cylinder of 20 cm of diameter positioned on a platform in front of a mirror to enable full view of hind paws. The time spent licking, biting or lifting the injected paw in seconds(s) was used as an index of nociceptive behavior intensity. This activity was recorded for 5 min (for capsaicin or cinnamaldehyde), 20 min (for menthol) or 15 min (for acid saline). Thirty minutes prior to i.pl. injection of the nociceptive agent, mice were treated with vanillic acid (1–100 mg/kg i.p.), morphine (5 mg/kg) ruthenium red (nonselective TRP antagonist, RR, 3 mg/kg i.p.), camphor (TRPA1 antagonist, 7.6 mg/kg i.p.) or amiloride (nonselective ASCI inhibitor, AML, 100 mg/kg i.p.). Control animals received a similar volume of saline solution (10 mL/kg) (Santos and Calixto, 1997; Rios et al., 2013).

2.3.3. Antinociceptive action of vanillic acid after local administration

To determine whether vanillic acid acted locally, 20 μl into the plantar surface of their right hind paw of either vehicles or doses of phenolic acid (10, 50 and 150 μg/paw) was administered 20 min before capsaicin injection into the ipsilateral paw. Also, vanillic acid was administered to the left (contralateral) paw 20 min before injection of capsaicin into the right paw.

2.4. Vascular permeability

The test was performed as described by Yu et al., 2012. Animals were pre-treated with vanillic acid (10–100 mg/kg i.p.) or vehicle; 30 min after the last administration, each mouse received an intravenous injection of 0.1 mL/10 g (0.5% W/V Evans blue solution in saline) and then injected with acetic acid 0.8% i.p. Twenty minutes after the administration of acetic acid, animals were sacrificed and the peritoneal cavity was washed with 6 mL of cold saline (divided into several washings), with a gentle manual massage, the exudates were collected and their volume was added up to 10 mL of saline, followed by centrifugation for 15 min at 3000 rpm. The optical density of the supernatant was measured at 590 nm in a spectrophotometer. The dye extravasation was quantified from the standard curve and the percentage of inhibition was calculated.

2.5. Measurement of TBARs

Thiobarbituric acid reactive substances (TBARs) are low molecular weight compounds formed via decomposition of certain primary and secondary lipid peroxidation products that at low pH and at high temperature participate in a nucleophilic addition reaction with thiobarbituric acid generating a red fluorescent complex (Fraga et al., 1987). At the end of the writhing test, mice were anesthetized with pentobarbital and blood samples were obtained. Samples were centrifuged at 3000 rpm for 10 min at 4 °C. Plasma was collected and stored at −80 °C until analysis. One hundred microliters of the plasma was mixed with 4% BHT, 3% SDS, 10.0% phosphotungstic acid and 2 mL of a 0.7% TBA solution, boiled for 45 min and cooled at room temperature. The chromogen was then extracted with 2.0 mL of butanol by vigorous shaking for 1 min. After centrifuging, (10 min, 1500 *g*, 25 °C), MDA (malondialdehyde) was measured spectrofluorimetrically (excitation at 515 nm, emission at 555 nm).

2.6. Behavioral assessment

2.6.1. Rota-rod

To evaluate the possible occurrence of non-specific effects such as muscle-relaxation or sedation, the effect of vanillic acid on motor coordination was assessed in mice subjected to the rota-rod test (Dunham and Miya, 1957). Animals that were able to stay on the bar of the apparatus (2.5 cm diameter bar, 25 cm above the floor, turning at 14 rpm) for two consecutive periods of 120 s were selected to receive i.p. vanillic acid (10, 30, 100 mg/kg), diazepam (2 mg/kg i.p.) or saline solution (10 mL/kg). Thirty minutes after the treatment, animals were placed on the apparatus for up 120 s, and the time each animal remained on the bar during each trial was recorded.