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Toxicological, toxicokinetic and gastroprotective evaluation of the benzaldehyde semicarbazone

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ABSTRACT

Benzaldehyde semicarbazone (BS) has presented positive results in several pharmacological models, including anticonvulsivant and anti-inflammatory models. The present study evaluated the preclinical toxicity (acute and subchronic), as well as the toxicokinetic and gastroprotective effects of BS against ethanol lesions. Oral doses of 300 and 2000 mg/kg were used in the preclinical acute toxicity study; 100, 200, and 300 mg/kg were used in both the subchronic toxicity evaluation and the gastric study; and 300 mg/kg was used in the toxicokinetic study. No impact from the dose of 300 mg/kg could be identified; while, one animal died at 2000 mg/kg in the acute toxicity test. In the subchronic toxicity test, changes in the biochemical parameters of the liver, as well as in the histopatological evaluation, demonstrated that BS is a hepatotoxic drug. BS proved to be effective for moderate and severe gastric lesions. In the toxicokinetics study, BS presented a low concentration and rapid plasma disappearance. Several results also indicate that BS is likely to be mostly eliminated from the liver and may well undergo a first-pass effect after oral absorption. It was impossible to estimate the noobserved-adverse-effect-levels (NOAEL) and lowest-observed-adverse-effect-levels (LOAEL) due to the presence of hepatotoxicity in all tested doses.

1. Introduction

According to the International Union of Pure and Applied Chemistry (IUPAC), semicarbazones are formed by the condensation of aldehydes or ketones with semicarbazide. Semicarbazones are low-cost drugs that are easily administered *per os* and commonly present a higher anticonvulsive activity than do phenitoine, carbamazepine and sodium valproate in animals (Dimmock and Baker, 1995; Beraldo et al., 2002; Teixeira et al., 2003). Benzaldehyde semicarbazone (BS), a member of the semicarbazone group, is shown in Fig. 1. BS was tested in pain models including: edema induced by carrageenan, mechanical allodynia induced by carrageenan, thermal hyperalgesia induced by carrageenan, nociceptive response induced by formaldehyde, quantification of angiogenesis by hemoglobin measurement, nociceptive response of mice in the hot-plate model, which demonstrated a satisfactory antinociceptive activity illustrating that this molecule could also be used as a treatment of neuralgias, such as trigeminal neuralgia (Rocha et al., 2006).

All new molecules, after being approved in pharmacological tests, must undergo preclinical safety testing. However, anti-inflammatory drugs must also go through gastrointestinal assessments.

Currently, peptic ulcer disease is due mostly to the widespread use of low-dose aspirin and nonsteroidal anti-inflammatory drugs. Gastric irritations affect 15–30% of all patients undergoing continuous use of anti-inflammatory drugs (Cellotti and Laufer, 2001). In addition, gastric irritation most commonly affects patients that use the following anticonvulsivants: carbamazepin, valproic acid, ethosuximide, gabapentin, and felbamate (Lowenstein, 1998).

Preclinical studies serve several purposes such as providing the kinetics data which will define the initial parameters of a drug's characteristics in order to estimate the proper dose for humans (Peck et al., 1996). Kaiser et al. (2010) reported on toxicokinetics using BS; however, that involved lower doses than the highest dose from the subchronic study (300 mg/kg).

Therefore, the aim of the present study was to evaluate the preclinical toxicity (acute, subchronic, and toxicokinetics of the highest dose) and the gastric clinical consequences of BS.

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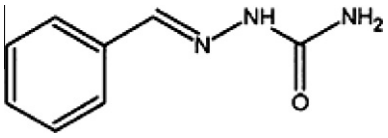

Fig. 1. Structure of benzaldehyde semicarbazone (BS).

2. Materials and methods

2.1. Drugs

Semicarbazide hydrochloride (SEM-HCl, CAS No. 563-41-7) was purchased from Sigma–Aldrich Co. (USA). BS was obtained as described in the literature (Dimmock et al., 1993), and characterized by its melting point (216–217 °C), by its infrared and ^{1}H (hydrogen atomic weight 1) and ^{13}C (carbon atomic weight 13) nuclear magnetic resonance (NMR) spectra.

2.2. Preparation of doses

The suspentions were prepared just before the administration of the doses. The administration was achieved through gavage in constant volumes. Since BS is not soluble in water, it was dispersed in 1% dimethylsulfoxide (DMSO) (Synth, Brazil) and 0.13% Tween 80 (Synth, Brazil) diluted in purified water (Milli Q®) (Cavaletti et al., 2000; Authier et al., 2002). Test and control groups received the same vehicle so that the differences between the effects observed in both groups could be attributed only to the use of the drug.

2.3. Animals

The animals (Wistar rats) were provided by Animal House of Faculdade de Farmácia da Universidade Federal Minas Gerais (UFMG), Belo Horizonte, Minas Gerais, Brazil. Rats were maintained under controlled conditions of temperature (22 ± 3 °C) and humidity (50–60%), with a 12-h light–dark cycle (07:00am – 7:00pm), free access to water and standard rat chow. The experimental protocols, 025/08 (preclinical toxicity), 208/09 (gastric lesions) and 12474 (toxicokinetics), have been approved by the Ethics Committee on Animal Experimentation.

2.4. Acute toxicity studies in rats

Acute toxicity tests were based on the OECD guideline 420 (Fixed Dose Procedure) (OECD, 2001). The compounds were administered in a single dose (300 mg/kg or 2000 mg/kg) through gavage.

After the administration of the doses, the rats were submitted to close clinical observation during the first 30 min and periodically during the first day. The animals were then observed twice daily for toxic effects, time of their onset, duration and time necessary for recovery during the following 13 days, with animal food and water supplied *ad libitum*. After that time, the animals were sacrificed in a carbon dioxide (CO_2) chamber and submitted to macroscopic and microscopic examination (Hilaly et al., 2004).

2.5. Subchronic toxicity studies in rats

Subchronic toxicity tests were based on the OECD guideline 408 (OECD, 1998). Male (180–232 g) and female (178–215 g) Wistar rats, aged 8–9 weeks, were used in this model. The animals were randomly divided into six groups: control group (with 10 males and 10 females), three test groups (with 10 males and 10 females in each group) and two satellite groups (with 6 males and 6 females in each group). A constant volume of the test product was administered daily (7:00a.m. to 9:00a.m) by gavage, for 90 days, with one dose for each group. During the administration period, the animals were observed daily. The animals were weighed weekly, so that the dose (mg/kg body weight) could be adjusted if necessary. The consumption of food and water was evaluated weekly.

In a previous subacute study (Rodrigues et al., 2005), BS presented a 13.6% loss in body weight, which could be was observed in the female group treated with the highest dose of BS (500 mg/kg). Therefore, the doses 100, 200 and 300 mg/kg were chosen for the subchronic study. Three doses (100, 200 and 300 mg/kg) and a control solution (1% DMSO and 0,13% Tween 80) were administered, according to the OECD protocol 408, paragraph 16, which only allows for testing of up to three doses when signs of toxicity have not observed in prior studies using 1000 mg/kg dose. The satellite control group received the control solution, while the satellite highest-dose group received the dose of 300 mg/kg.

After treatment, animals were anesthetized by intraperitoneal injection of ketamine (70 mg/kg) and xylazine (15 mg/kg), and approximately 5 mL of blood was removed by direct puncture of the abdominal vena cava (Melo et al., 2008). The blood samples were divided into two aliquots: one was transferred to an empty tube and the other to a tube containing EDTA for biochemical and hematological assays. The animals were subjected to fasting for 12 h prior to this procedure (OECD, 1998). The animals were euthanized by an overdose of anesthesia for removal of organs and future macroscopic and histopathological analyses.

2.5.1. Laboratory analyses

Laboratory analyses were performed for subchronic toxicity (biochemical, hematological, microscopic and macroscopic analyses of the organs). At the end of the sub-chronic toxicity test, the animals were weighed, subjected to fasting for 12 h, anesthetized to collect blood, and euthanized for necropsy. Hematological analyzes were performed on the whole blood collected in tubes with EDTA, using the Abacus Junior Vet apparatus (Veterinary School – UFMG – Brazil).

Biochemical analyses were performed in serum obtained after centrifugation (2500 rpm/15 min) of whole blood samples without anticoagulants. Standardized Synermed® diagnostic kits and the Cobas Mira® equipment were employed for spectrophotometrical determination of the following biochemical parameters: uric acid (AUR), albumin (ALB), alanine aminotransferase (ALT), aspartate aminotransferase (AST), cholesterol (COLT) , creatinine (CREA), alkaline phosphatase (ALP), glucose (GLU), total proteins (PROT), urea (URE), Gamma GT (GGT), creatine kinase (CK), triglycerides (TRI), total bilirubin (BilT), direct bilirubin (BilD), amylase (AML), calcium (CA), chloride (Cl) and phosphorus (P).

Hearts, lungs, kidneys, adrenal glands, spleens, livers, stomachs, pancreas, small intestines, uterus, ovarys, testes, epididymides, brains and thymus were removed for macroscopic examination. Fragments of these organs were fixed in 10% formalin for histopathological examination (Palmeiro et al., 2003; Kanjanapothi et al., 2004).

2.6. Toxicokinetics

Ten male Wistar rats (250–350 g) were used to determine the BS pharmacokinetic profile. A BS oral dose of 300 mg/kg was administered by gavage to animals deprived of food 12 h before experimentation and up to 4 h after dosing. Water was allowed *ad libitum*.

BS plasma concentrations were determined by previously validated HPLC-UV method (Kaiser et al., 2008). Analyses were performed on an HPLC Waters® system using a C_{18} column and isocratic elution with mobile phase composed of water–methanol–acetonitrile (65:30:5 v/v/v , adjusted to pH 9.0) at a flow rate of 1.1 mL/min. The BS and the internal standard were monitored at 282 nm. The method was linear in the concentration range of 0.1–16.0 μg/mL and the accuracy was within 15%. Intra and inter-day relative standard deviations were equal or less than 10.19% and 2.10%, respectively.

On test day, the animals were confined in an acrylic device. The blood samples (200–250 μL) were withdrawn via lateral tail vein puncture into heparinized tubes after BS oral administration at predetermined times (0.25, 0.5, 1, 2, 4, 6, 8, 10 and 12 h). The plasma was separated through centrifugation and stored at −20 °C until analysis, when, 100 μL aliquot of the biological samples were spiked with 10 μL of internal standard solution and deproteinized by adding 300 μL of methanol. The samples were vortex mixed for 20 s and centrifuged at 6800*g*, 4 °C for 10 min. 25 μL of supernatant was injected into the HPLC system and the BS quantification were acquired by a calibration curve.

2.7. Evaluation of gastric lesions in rats

Thirty-six male Wistar rats (220–250 g), randomly divided into six groups containing six animals each ($n = 6$) (La Casa et al., 2000), were used: Group 1: negative control solution (5% Tween 80); Group 2: positive control solution (500 mg/kg of sucralfate); and Group 3: 100 mg/kg of a BS suspension; Group 4: 200 mg/kg of BS suspension; Group 5: 300 mg/kg of BS suspension; Group 6: 68 mg/kg of semicarbazide hydrochloride. The volume (1 mL) administered by gavage was equal for all animals. During the 24 h preceding the beginning of the experiment, the animals were kept in cages with netwired floor without sawdust, without food *ad libitum* access to filtered water. Six hours after the beginning of the fasting process, a condensed milk (2:1) preparation was furnished to avoid the accumulation of food in the stomach (Jorge, 2004).

Absolute ethanol (1 mL) was given 60 min after the administration of the control solution, product suspensions, and sucralfate (Alvarez et al., 1999; Seito et al., 2002; Tan et al., 2002). The animals were euthanasiated 60 min after the application of ethanol and their stomachs were removed. A small incision was made near the pylorus to extract the gastric juice to measure the pH level and gastric volume. Next, the stomachs were opened along the greater curvature and immersed in saline solution for preservation. Shortly thereafter, they were opened and placed on a platform to examine the lesions (Jorge, 2004).

The lesions were classified according to severity levels: 1 (redness and/or petechiae), 2 (moderate erosion with bleeding), 3 (hemorrage with extensive and severe lesions), according to that established by Szelenyi and Thiemer (1978). The ulcer index was calculated by adding the largest diameters of the lesions (Makovek et al., 1999; Perera et al., 2001). The readings were all performed by the same analyst, who was unaware of the treatment received by the animals. After the lesions had been evaluated, the stomachs were fixed and preserved in a 10% phosphate-buffered formalin, embedded in paraffin and cut into 0.5 μm sections.