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Pre-clinical pharmacokinetics evaluation of an anticonvulsant candidate benzaldehyde semicarbazone free and included in β-cyclodextrin

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ABSTRACT

The study aimed to investigate the pharmacokinetics and tissue distribution of the benzaldehyde semicarbazone (BS) a potential antiepileptic drug, administered as a free drug or complexed β-cyclodextrin (BS/β-CD). Free BS and BS/β-CD were administered to male Wistar rats as a 10 mg/kg intravenous bolus dose. For the oral route, 50 mg/kg and 100 mg/kg doses of the free drug and 50 mg/kg of the complex were administrated and plasma concentrations were determined by a validated HPLC-UV method. Individual profiles were evaluated by non-compartmental and compartmental analysis using Excel® and Scientist®, respectively. Free BS plasma protein binding was 34 ± 5%. A one-compartmental model adequately described all the plasma profiles for both formulations. After intravenous (10 mg/kg) and oral (50 mg/kg) administration, the V_d (1.6 ± 0.5 and 2.2 ± 0.8 L/kg, respectively) and the Cl_{tot} (1.4 ± 0.5 and 1.8 ± 0.5 L/h kg, respectively) determinated for the BS/β-CD complex were higher than those obtained for the free drug, but the $t_{1/2}$ (0.8 ± 0.1 h) was similar ($p < 0.05$). The oral bioavailability of the BS/β-CD complex (~37%) was approximately 2-fold of the free BS (~20%). The higher drug brain penetration (2.8) after BS/β-CD dosing and the longer mean residence time in this organ, regardless of the administration route, reveals that the complex may be a potential drug carrier for the central nervous system delivery of BS.

1. Introduction

Epilepsy is a neurological brain disorder characterized by periodic and unpredictable occurrence of seizures which can lead to lack of consciousness, exposing the patients to possible physical injuries and social restrictions (WHO, 2001a). The treatment of epilepsy is based on the symptoms. Therefore, there is no prophylaxis or cure. Although the current drugs provide adequate seizure control in many patients, it is roughly estimated that up to 25% of the patients do not show any clinical improvement during current drug therapy (WHO, 2001b; Mccorry et al., 2004). Moreover, many antiepileptic drugs present serious side effects and a long period of medication may be required (Mccorry et al., 2004). Thus, the search for a new anticonvulsant prototype continues to be an area of intense investigation in medicinal chemistry.

In the course of investigations aiming the development of structurally novel anticonvulsants, a number of aryl semicarbazones were found to display significant activity in different experimental models of epilepsy with low or absent neuro and hepatotoxicity (Dimmock et al., 1993, 1995, 2000; Yogeeswari et al., 2005). Aryl semicarbazones are not structurally similar to classical anticonvulsants, and their mechanism of action is not completely elucidated, although it is probably associated to the broad-spectrum blocker of mammalian voltage-gated sodium channels (Ilyin et al., 2005).

The benzaldehyde semicarbazone (BS) (Fig. 1) is an aryl semicarbazone that present a low cost of synthesis, can be orally administrated and exhibit a great antiepileptic activity in the maximal electroshock screen model (MES) in rats, with doses ranging from 50 to 100 mg/kg p.o. (Dimmock et al., 1993, 2000). In addition to the anticonvulsant activity, it was been reported that BS has an analgesic effect, demonstrated by different experimental models in doses ranging from 10 to 50 mg/kg i.p. (Rocha et al., 2006), which is a property that has also been reported for several anticonvulsant drugs, through the blockade of the sodium channel mechanism, as described to carbamazepine (Bianchi et al., 1995). The acute and subacute toxicological studies in rats did not reveal histopathological and hematological side effects at higher doses, though some insignificant and reversible biochemical changes were observed (unpublished results).

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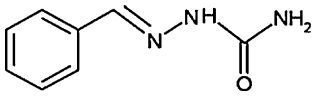

Fig. 1. Chemical structure of benzaldehyde semicarbazone (BS).

The disadvantages of this drug are its very low water solubility and short duration of action. To circumvent these biopharmaceutical problems BS complexation with β-cyclodextrin (β-CD) was investigated (Teixeira et al., 2003). In the MES model of epilepsy, free BS at 100 mg/kg p.o. dose blocked the hind limb extension in about 100% of the animals, 30 min after administration (Dimmock et al., 1993). The minimum dose necessary to produce the same anticonvulsant activity decreased from 100 mg/kg p.o. to 25 mg/kg p.o. (75% of reduction), when the compound was included in β-CD. Similar results in the literature were obtained using hydroxypropyl-β-cyclodextrin (HP-β-CD) at 35 mg/kg p.o dose (65% of reduction). In addition, whereas free BS exhibits no activity 4 h after administration, the BS/HP-β-CD complex protected 60% of the animals against seizures, indicating a possible change in pharmacokinetic parameters (Beraldo et al., 2002).

In this context, the purpose of the present study is to describe the plasma pharmacokinetics and tissue distribution of the BS free and complexed with β-CD (BS/β-CD) after administration of various doses by different routes to rodents.

2. Materials and methods

2.1. Chemical and reagents

BS was synthesized at the Chemistry Department of the Federal University of Minas Gerais (Brazil) as described previously (Dimmock et al., 1993). The BS complex with β-CD (1:1 ratio) was prepared according to the literature (Teixeira et al., 2003). Sodium diclofenac was purchased from Delaware (Porto Alegre, Brazil). LC grade methanol and acetonitrile were purchased from Tedia® (Fairfield, USA). Ammonium hydroxide 30% was purchased from Merck® (Darmstat, Germany). Distillated water was obtained by Milli-Q System (Millipore®). All other products were of analytical or LC grade.

2.2. Animals

Male Wistar rats (250–350 g), purchased from the State Foundation for the Research and Production in Health (FEPPS, Porto Alegre, Brazil), were used to determine the pharmacokinetic profile of BS. The rats were housed under standard conditions: room temperature (22 ± 2 °C) and the humidity (65%) were controlled and a 12-h light:12-h dark cycle was provided. Water was freely available. The animals that received the oral doses were deprived of food 12 h before experimentation and 4 h after dosing. Water was allowed *ad libitum*. The protocols for animal experiments were approved by the Ethics in Research Committee of the Universidade Federal do Rio Grande do Sul (Protocol 2007794, Porto Alegre, Brazil).

2.3. Pharmacokinetic study design

The groups used to investigate free BS and BS/β-CD complex pharmacokinetics, together with the doses preparation are described in Table 1. The i.v. doses were injected into the lateral tail vein and the oral doses, by gavage. The doses were chosen based on the pharmacodynamic experiments conducted previously (Dimmock et al., 1993; Teixeira et al., 2003).

At predetermined times (30 min before dosing and at 0.08, 0.17, 0.25, 0.5, 1, 1.5, 2, 3 and 4 h) after BS or BS/β-CD intravenous administration, blood samples (200–250 μl) were withdrawn via puncture in the lateral tail vein, opposite to the tail vein used for drug i.v. administration, and put into heparinized tubes. The same procedure was carried out after oral administration of BS, with sampling harvesting at 0.25, 0.5, 1, 2, 4, 6, 8 and 10 h. After BS/β-CD dosing, blood samples were collected at 0.25, 0.30, 0.75, 1, 2, 3, 4 and 6 h. The plasma was separated through centrifugation (6800 × *g*, 4 °C for 10 min) and stored at −20 °C until analysis by a validated HPLC-UV method.

2.4. Pharmacokinetic analysis

BS pharmacokinetic parameters after free BS and the BS/β-CD dosing were determined from individual plasma profiles by non-compartmental approach using EXCEL® v.7.0 software (Microsoft, USA). The peak BS plasma concentration (C_{max}) and the time of maximum concentration (t_{max}) were obtained by visual inspection of the data from the plasma concentration–time after oral dosing. Pharmacokinetic parameters such a elimination rate constant (k_e), area under the curve ($AUC_{0-\infty}$), clearance (Cl_{tot}), half-time ($t_{1/2}$), volume of distribution (V_d), mean residence time (MRT) and bioavailability (F_{abs}) were determinated using classical equations (Shargel et al., 2005).

The compartmental analyses were performed using SCIENTIST® v.2.0.1 software (MicroMath®, USA). One- and two-compartment models with or without weighting schemes were evaluated. The best model to fit the data was chosen based on the random distribution of residuals, the correlation coefficient and the model selection criterion (MSC) given by the software.

The individual plasma profiles of BS and BS/β-CD obtained after intravenous and extravascular administrations were best described by the one-compartmental open model with first-order elimination (Eq. (1)) and one-compartment open model with first-order elimination and first-order absorption (Eq. (2)), respectively:

$$C = C_0 e^{-k_e t} \quad (1)$$

where C is the total plasma concentration over time, C_0 is the concentration at time zero and k_e is the elimination rate constant.

$$C = \frac{DF_{abs}k_a}{V_d(k_a - k_e)}(e^{-k_e t} - e^{-k_a t}) \quad (2)$$

Table 1
Animal experimental groups used to investigate free BS and BS/β-CD complex pharmacokinetics.

Groups	Dose[a] and route	Dose preparation	Total volume administrated (ml/kg)
G1	10 mg/kg i.v.	Free BS dissolved in physiological solution (3.0 mg/ml) with 10% of Tween 80® and 25% of DMSO	1.0
G2	10 mg/kg i.v.	BS/β-CD complex suspended in physiological solution (1.5 mg/ml) with 10% of DMSO	2.0
G3	50 mg/kg p.o.	Free BS suspended in physiological solution (10.0 mg/ml) with 10% of Tween 80® and 25% of DMSO	1.5
G4	100 mg/kg p.o.	Free BS suspended in physiological solution (10.0 mg/ml) with 10% of Tween 80® and 25% of DMSO	3.0
G5	50 mg/kg p.o.	BS/β-CD complex suspended in distillated water (5.0 mg/ml)	3.0

[a] All doses are based free BS; i.v. = intravenous; p.o. = oral; n = 8/group.