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Pharmacokinetic study of a carbamazepine nanoemulsion in beagle dogs

Gislaine Kuminek[a], Jadel M. Kratz[a], Rodrigo Ribeiro[a], Regina G. Kelmann[a], Bibiana V. de Araújo[b], Helder F. Teixeira[b], Cláudia M.O. Simões[a], Letícia S. Koester[a,*]

[a] Programa de Pós-Graduação em Farmácia, Centro de Ciências da Saúde, Departamento de Ciências Farmacêuticas, Universidade Federal de Santa Catarina, 88.040-900 Florianópolis, SC, Brazil
[b] Programa de Pós-Graduação em Ciências Farmacêuticas, Faculdade de Farmácia, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil

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ABSTRACT

This work describes the pharmacokinetics of a novel carbamazepine nanoemulsion. The plasma concentration profiles were determined in beagle dogs after i.v. *bolus* administration of a 5 mg/kg carbamazepine nanoemulsion and compared to the corresponding carbamazepine/hydroxypropyl-β-cyclodextrin complex solution. Both formulations showed similar pharmacokinetic profiles and could represent valuable formulations in case of emergencies, when a rapid action in the central nervous system is desirable.

Carbamazepine (CBZ) is a worldwide used anticonvulsant whose poor solubility in water impairs the development of an intravenous formulation. Two approaches have been extensively studied in order to achieve this objective: (1) CBZ complexation with hydroxypropyl-β-cyclodextrin (HPβCD) (Brewster et al., 1991, 1997; Löscher et al., 1995), and more recently (2) CBZ incorporation into nanoemulsions (Becirevic-Lacan et al., 2002; Akkar and Müller, 2003; Madhusudhan et al., 2007).

In addition, our group has been investigating the development of CBZ nanoemulsions by spontaneous emulsification method (Kelmann et al., 2007a, 2008). In the present study, a novel CBZ nanoemulsion formulation was developed; the *in vivo* pharmacokinetics was assessed in beagle dogs and compared to that of a CBZ/HPβCD complex. Although the pharmacokinetics of CBZ/HPβCD complexes was extensively investigated in the 1990s, particularly in dogs, there is no study reporting the pharmacokinetic parameters and overall concentration–time profiles for CBZ nanoemulsions in dogs.

The CBZ nanoemulsion (CBZ/Nano) investigated in this study was prepared by spontaneous emulsification procedure (Kelmann et al., 2007a,b, 2008): 80 mg of drug (Henrifarma, Brazil) and 2.8 g of 1:1 castor oil:MCT (w/w) (Lipoid, Germany) were mixed and added to a 200 ml of acetone:ethanol (1:1, v/v) containing 2.0 g of soy lecithin (Lipoid, Germany). The oily phase was slowly added under magnetic stirring into a 400 ml of aqueous phase containing poloxamer 188 (600 mg; BASF, Germany) and glycerol (900 mg; Nuclear, Brazil), forming the nanoemulsion. Solvents and most water were removed under reduced pressure resulting in a 40 ml formulation containing theoretically 2 mg/ml of CBZ and pH was adjusted to 7.0 with NaOH. Nanoemulsions batches ($n = 3$) were characterized with respect to drug content by HPLC ($95.28 \pm 2.06\%$, Kelmann et al., 2007b); mean particle size (125.6 ± 11.6 nm) and polydispersity index (0.294 ± 0.06) by a Nanosizer (ND4, Coultronics). After 3 months of storage under 25 °C, the formulations did not present any sign of physical instability.

CBZ/cyclodextrin complex (CBZ/HPβCD) was prepared by dissolving 1.4016 g of HPβCD in 40 ml ultrapure water. CBZ (80 mg) was added and the dispersion was stirred until a clear solution was formed, and pH was adjusted to 7.0 with NaOH.

Six female beagle dogs (13.55 ± 1.23 kg) were fasted overnight and provided water *ad libitum*. According to a randomized, crossover, design (protocol 23080.029451-13, approved by the Animal Ethics Committee, Federal University of Santa Catarina), each dog received recently prepared batches of either CBZ/Nano or CBZ/HPβCD (5 mg/kg) with a *washout* period of 1 week. Dog's legs were shaven and cannulated in each of the two cephalic veins: one for formulation administration and one for blood sampling. Blood samples (2 ml) were withdrawn prior to administration and after 10, 20, 30, 40, 50, 60, 75, 90, 105, 120 and 135 min into heparinized tubes, centrifuged (3000 rpm, 15 min) and plasma was stored at −80 °C until analysis.

* Corresponding author. Tel.: +55 4837215066; fax: +55 4837219542.
E-mail address: leticiakoester@gmx.net (L.S. Koester).

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Table 1
Pharmacokinetic parameters of CBZ (5 mg/kg) after i.v. *bolus* administration of CBZ/HPβCD or CBZ/Nano in beagle dogs ($n = 6$).

Parameters	CBZ/HPβCD	CBZ/Nano
$AUC_{0-\infty}$ (μg min/ml)	153.43 ± 17.04	177.41 ± 20.59
Cp_0 (μg/ml)	3.87 ± 0.36	4.57 ± 0.91
$t_{(1/2)}$ (min)	40.15 ± 10.17	41.81 ± 19.38
MRT (min)	39.91 ± 3.48	37.81 ± 3.30
ke (min^{-1})	0.0183 ± 0.0052	0.0196 ± 0.0082
Vd_{ss} (ml/kg)	1304.21 ± 57.68*	1072.27 ± 106.56*
Cl_{TOT} (ml min/kg)	32.92 ± 3.64	28.47 ± 3.03

* Significantly different, Student's *t*-test ($\alpha = 0.01$).

CBZ was assayed in plasma using a validated method (Koester et al., 2004), with no changes relating to the sample processing (extraction of plasma by precipitation with acetonitrile containing internal standard, centrifugation, evaporation under vacuum and reconstitution in mobile phase) and the chromatographic conditions (column: Shim-pack CLC-ODS (M) RP18 5 μm, 250 mm × 4 mm; mobile phase: phosphate buffer (0.05 M):acetonitrile:methanol, 56:28:16 (pH 4.0); flow rate: 1.0 ml/min; detection wavelength: 286 nm; injection volume: 50 μl), but the used equipment was now a Shimadzu LC-10A coupled to a LC-10AT pump, a SPD-10AV detector, a SCL-10Avp controller and a Rheodyne 7725 injection valve. The method was revalidated and proved reproducible with respect to specificity (considering both plasma profile and components of the formulation), linearity over the concentration range of 0.125–8.0 μg/ml ($r^2 > 0.999$), intermediate precision (R.S.D. < 9.6%) and accuracy (CBZ recoveries ranged from 100.8 to 126.9% for the three concentrations analyzed: 0.150, 1.5 and 6.4 μg/ml). CBZ eluted at approximately 12 min, and the internal standard at 20 min.

CBZ pharmacokinetic parameters were calculated using a non-compartmental analysis with standard equations (Gibaldi and Perrier, 1982), for each animal and the data presented as arithmetic mean ± S.D. in Table 1. The treatments were compared for statistical significance by using the Student's *t*-test ($\alpha = 0.01$).

Similar mean plasma concentration–time profiles were observed for CBZ/HPβCD and CBZ/Nano in Fig. 1A and B, respectively. There were no significant differences between the area under the concentration–time curve ($AUC_{0-\infty}$), drug concentration at zero time (Cp_0), half-life ($t_{1/2}$), elimination rate constant (*ke*), mean residence time (MRT) and clearance (Cl_{TOT}) (Table 1). The average $t_{(1/2)}$ was in agreement with values reported by Löscher et al. (1995) and Brewster et al. (1997) (36–38 min) for the i.v. administration of CBZ/HPβCD complex in dogs. On the other hand, a significantly lower volume of distribution (Vd_{SS}) was observed for the emulsion compared to the corresponding solution, which was also observed by other authors (Hwang et al., 2004; Zhang et al., 2008; Lu et al., 2009).

Hwang et al. (2004) compared a microemulsion and a sodium salt solution of all-*trans*-retinoic acid (ATRA) administered i.v. to 5 male Sprague–Dawley rats. Although the plasma concentration profiles and most pharmacokinetic parameters of the ATRA microemulsion were not significantly different from those of the ATRA sodium solution, its initial plasma concentration was slightly higher and its Vd_{SS} was significantly lower, as in the present study. It is worth emphasizing that such microemulsions were composed by phospholipids and soybean oil, and presented a mean particle size around 230 nm, being equivalent to nanoemulsions developed in this study.

Zhang et al. (2008) observed similar pharmacokinetic profiles for vinorelbine in rats, except for Vd_{SS}, which was 1.5 times higher for an aqueous solution when compared to a lipid microsphere formulation (~180 nm), which in this work is a synonym for a drug-loaded lipid emulsion, that is, a nanoemulsion. The authors

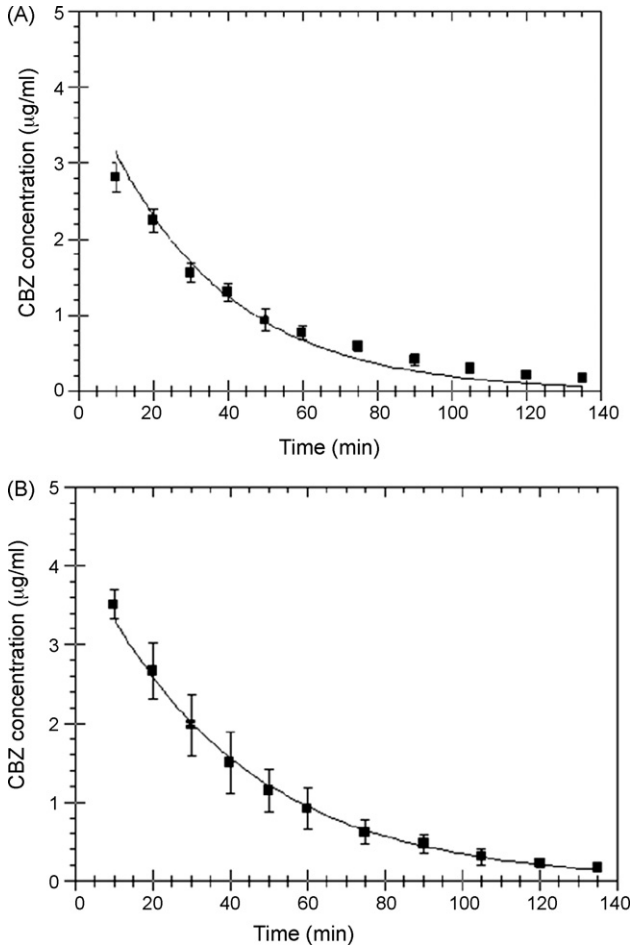

Fig. 1. Plasma profiles of CBZ (5 mg/kg) after i.v. *bolus* administration of: (A) CBZ/HPβCD and (B) CBZ/Nano in beagle dogs ($n = 6$) (mean ± S.D.).

postulate two theories for such variation: (1) the incorporation of the drug to the lipid core of the emulsion may reduce its penetration into the tissues, producing higher plasma concentration (and consequently a lower Vd_{SS}) and (2) vinorelbine-loaded lipid microspheres may distribute preferably in plasma, while free vinorelbine may distribute mostly in platelets, which are removed during sampling processing (centrifugation).

Similarly, Lu et al. (2009) compared the pharmacokinetics of clarithromycin–phospholipid complex emulsion (~140 nm) and a clarithromycin solution in 6 male Wistar rats, and found no statistically difference between most parameters except Vd_{SS}, which was 1.7 times higher for the solution as well.

Analyzing the obtained results and comparing them with those cited above, it was not possible to postulate whether the smaller distribution of CBZ from the nanoemulsion compared to the HPβCD complex (addressed by the Vd_{SS}) may have any clinical relevance, such as a faster effect in the central nervous system. Notwithstanding, the fact that a CBZ-loaded nanoemulsion did not exhibit a delayed release compared to the respective cyclodextrin complex may be considered advantageous if a fast brain access is concerned, and considering that this is a central compartment.

Madhusudhan et al. (2007) observed that nanoemulsions improved the availability of CBZ in mice tissues, but after 2 h, the CBZ levels in all tissues studied were negligible with the control solution and the lecithin nanoemulsion whereas they were considerable with the 1-O-alkylglycerol/soya-lecithin nanoemulsions. The rapid clearance of serum CBZ following the administration of the lecithin nanoemulsion was attributed to a higher extravascu-

lar distribution which did not lead to higher tissue levels since lecithin does not interact with capillary endothelial cell surfaces as the 1-O-alkylglycerol stabilized oil droplets do.

In conclusion, nanoemulsions have been investigated as intravenous carriers for CBZ and in the present study, the similarity observed between the overall plasma profiles from such colloidal system and the solution formed at the expenses of HPβCD was found advantageous if a fast brain access is concerned, as in seizure disorders, and considering that this is a central compartment.

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