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Dose-dependent pharmacokinetics and brain penetration of rufinamide following intravenous and oral administration to rats

Zsolt Gáll [a,b], Szende Vancea [c,*], Tibor Szilágyi [a,d], Orsolya Gáll [d], Melinda Kolcsár [b]

[a] University of Medicine and Pharmacy of Tirgu Mures, Faculty of Medicine, Department of Physiology, Doctoral School, Romania
[b] University of Medicine and Pharmacy of Tirgu Mures, Faculty of Pharmacy, Department of Pharmacology and Clinical Pharmacy, Romania
[c] University of Medicine and Pharmacy of Tirgu Mures, Faculty of Pharmacy, Department of Physical Chemistry, Romania
[d] University of Medicine and Pharmacy of Tirgu Mures, Faculty of Medicine, Department of Physiology, Romania

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ABSTRACT

Rufinamide is a third-generation antiepileptic drug, approved recently as an orphan drug for the treatment of Lennox–Gastaut syndrome. Although extensive research was conducted, its pharmacokinetics in rats was not described. This work addresses that area by describing in a rapid pharmacokinetic study the main pharmacokinetic properties of rufinamide at three different doses of 1 mg/kg body weight (bw), 5 mg/kg bw, and 20 mg/kg bw. Furthermore, total brain concentrations of the drug were determined in order to characterize its brain-to-plasma partition coefficient. Adult Wistar male rats, weighing 200–450 g, were administered rufinamide by intravenous and oral routes. Rufinamide concentrations from plasma samples and brain tissue homogenate were determined using a liquid chromatography–mass spectrometric method and pharmacokinetic parameters were calculated. The mean half-life was between 7 and 13 h, depending on route of administration – intravenously administered drug was eliminated faster than orally administered drug. Mean (S.E.M.) total plasma clearance was 84.01 ± 3.80 ml/h/kg for intravenous administration, while the apparent plasma clearance for oral administration was 95.52 ± 39.45 ml/h/kg. The mean (S.E.M.) maximum plasma concentration reached after oral administration of 1 mg/kg bw and 5 mg/kg bw was 0.89 ± 0.09 μg/ml and 3.188 ± 0.71 μg/ml, respectively. The median (range) time to reach maximum plasma concentration (t_{max}) was 4 (2–8) h. Mean (S.E.M.) brain-to-plasma concentration ratio of rufinamide was 0.514 ± 0.036, consistent with the brain-to-plasma ratio calculated from the area under curves (AUC_{0-t}) of 0.441 ± 0.047. No influence of dose, route of administration, or post-dosing time was observed on brain-to-plasma ratio.

1. Introduction

Rufinamide, a new structurally unique antiepileptic drug, was extensively studied in the 1990s for efficacy in animal models (Schmutz et al., 1993) and in healthy volunteers (Cardot et al., 1998; Cheung et al., 1995; Waldmeier et al., 1996). It entered clinical trial phase in the 2000s and several reports confirmed the efficacy by reducing the number of seizures in patients with epilepsy (Pålhagen et al., 2001). Finally in 2007, the EMA granted marketing authorization and later in 2008 it was approved for the treatment of Lennox-Gastaut syndrome by the FDA. Preclinical data regarding the anticonvulsant activity and the toxicity in mice and rats were published (McLean et al., 2005; White et al., 2008), but the pharmacokinetic (PK) parameters of the drug were not described in these species.

Recent evidence suggests a unique mechanism of action for rufinamide and structurally similar compounds (Gilchrist et al., 2014). A new direction for antiepileptic drug development was identified and rufinamide, as the basic compound of the triazole class, will serve as reference for the preclinical studies of the new derivatives.

In this study the main PK parameters – area under curves (AUCs), elimination half-life ($t_{1/2}$), maximum plasma concentration (C_{max}), and time to reach maximum plasma concentration (t_{max}) – of rufinamide after intravenous and oral administration at three different doses were determined in rats.

In order to evaluate the dose dependency of the anticonvulsant activity, the kinetics of brain penetration should be elucidated. An *in vitro* study suggests no relevant interference between rufina-

* Corresponding author at: University of Medicine and Pharmacy of Tirgu Mures, Faculty of Pharmacy, Gh. Marinescu street, 38, 540139 Tg. Mures, Romania. Tel.: +40 265 215551/182; fax: +40 265 210407.
E-mail address: vancea.szende@umftgm.ro (S. Vancea).

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mide and P-glycoprotein (Chan et al., 2014) but, several other transport mechanisms may be involved. The total brain concentrations of the drug were determined in order to calculate the brain-to-plasma partition coefficient (*in vivo* K_p) in rats. The influence of dose, time, and route of administration on *in vivo* K_p was also evaluated. A potential application of the total brain concentrations is to estimate unbound brain concentrations by using the free fraction of the compound in the brain (Fridén et al., 2007; Kalvass et al., 2007).

Allometric scaling is a successfully utilized tool to predict PK parameters between different species, even the selected first-in-human dose is based on data obtained from small animals (Mahmood et al., 2006). Recently, the concept of refractory epilepsy turned up in veterinary medicine. There is a clear need for new therapeutic options in the treatment of canine and feline epilepsy (Muñana, 2013). PK data is available for dogs (Wright et al., 2012), but not for cats. This work establishes an allometric relationship by using the available data in humans (Perucca et al., 2008) and in dogs combined with the obtained results in rats. This equation can be used to predict clearances in felines and other animal species.

2. Materials and methods

2.1. Drugs

Rufinamide was supplied by MSN Laboratories Ltd. (Hyderabad, India). Several solubilizing agents (e.g. propylene glycol, polyethylene glycols and Tween 80) were tested for use as intravenous and oral solutions of rufinamide, but the drug was not sufficiently soluble at the desired concentrations. Therefore, rufinamide solutions for intravenous administrations were prepared in 100% dimethyl sulfoxide (DMSO) (Sigma–Aldrich, Saint-Louis, MO, USA) at concentrations of 5 and 20 mg/ml. The total amount of DMSO administered was 1 ml/kg bw, the intravenous LD50 being 7.4 ml/kg bw (Kelava et al., 2011). For oral administration 1 mg/ml suspensions were prepared from 20 mg/ml solution in DMSO (5.5% w/w), 2-hydroxyethyl cellulose (Sigma–Aldrich, Saint-Louis, MO, USA) 0.5% (w/w) as suspending agent, and deionized (DI) water (94.0% w/w) produced by a Millipore Direct Q5 Ultrapure Water System (Millipore SA, Molsheim, France). Ketamin hydrochloride (Kepro BV, Deventer, Netherland) and xylazine (Alfasan, Woerden, Netherland) were used for anesthesia. Methanol and formic acid used for analysis were HPLC grade products (Merck KgaA, Darmstadt, Germany).

2.2. Animals

The study was approved by the Ethics Committee for Scientific Research of University of Medicine and Pharmacy of Tîrgu Mure?. Experimental animals were male adult Wistar rats, weighing 200–450 g. All animals were housed in accordance with Directive 2010/63/EU and received standard chow and tap water *ad libitum* prior to experiments. They were removed 4 h prior to drug administration and replaced 8 h after drug administrations. All efforts were made to minimize pain or discomfort of the animals. Anesthesia was achieved after intraperitoneal injection of ketamine hydrochloride (90 mg/kg) and xylazine (10 mg/kg).

2.3. Experiment design and sample collection

2.3.1. Pharmacokinetic study

Rufinamide was administered orally to animals ($n = 3$) at a dose of 1 mg/kg bw to examine the concentration–time curve and the main PK parameters. The conventional full PK study design as described by Li et al. (2013) was used. After drug administration, samples of approximately 200 μl of blood were collected by serial sampling at 0.5, 1, 2, 4, 6 and 24 h from the tail vein. Blood samples were collected in K3 EDTA coated tubes and centrifuged at 3000*g* for 10 min within 2 h of collection. The collected plasma samples were stored at −20 °C until analysis. Individual plasma samples were analyzed and corresponding PK curves were reported for each animal.

2.3.2. Evaluation of brain-to-plasma concentration ratio

Based on the pharmacokinetic study results, a sparse sampling approach, as described previously by Rani and Padh (2001), was implemented. At each time point, two animals were used. One blood sample and brain tissue sample from each animal was obtained. This type of design can be described as a 2–2–2–2–2–2. Animals were administered a single dose of rufinamide by either intravenous or oral routes. To verify the dose-dependency of brain penetration, rats were randomly assigned to dose groups. Rufinamide doses of 5 ($n = 12$) and 20 ($n = 12$) mg/kg bw were administered via tail vein over a one minute period at a volume of 1 ml/kg bw. The influence of the route of administration was investigated by administering orally rufinamide suspension in a dose of 5 mg/kg bw by gavage at a volume of 5 ml/kg bw ($n = 14$). Terminal sampling of blood and brain tissue, respectively, were performed at 0.5, 1, 2, 4, 6 (p.o. only), 8 and 24 h. Animals were anesthetized as described above and thoracotomy was performed. Blood was collected by cardiac puncture in K3 EDTA tubes and centrifuged at 3000*g* for 10 min within 2 h of collection. Harvested plasma samples were stored at −20 °C until analysis. Blood was withdrawn slowly, preventing the heart from collapsing. After blood collection, the animals were perfused transcardially with normal saline until total blood volume was removed (approximately 30 ml). Using this technique eliminates contributions from brain vasculature ensuring the measured concentrations reflect the amount of compound in the brain tissue. Each brain was removed within 7 min following thorax opening. After weighing, tissue samples were homogenized with 20 ml phosphate buffer (PBS), ultrasonicated for 10 min, and stored at −70 °C until analysis. The final concentrations of the homogenates were approximately 0.1 g brain tissue/ml.

2.4. Analysis of drug concentrations

Rufinamide was assayed by a previously developed and published liquid chromatography–mass spectrometric (LC–MS/MS) method (Gáll et al., 2013). For plasma samples, a dilution factor of 10 was applied. Aliquots of 10 μl rat plasma were diluted with blank plasma to 100 μl and mixed with 300 μl internal standard (IS) working solution (lacosamide in methanol) to induce plasma protein precipitation. The mixture was vortex-mixed for 10 s, then centrifuged for 10 min at 9167*g*. One hundred micro-liters of supernatant was transferred to an autosampler vial, 100 μl formic acid 0.1% in water was added, vortex-mixed for 10 s, and 5 μl were injected into the LC–MS/MS system. Homogenized brain tissue samples in a volume of 300 μl were mixed with 900 μl IS working solution to precipitate tissue proteins. The remaining steps were the same as plasma sample preparation.

2.5. Pharmacokinetic analysis

Plasma concentrations obtained from the conventional full PK study were analyzed with the commercially available software Kinetica 5.0 (Thermo Fisher Scientific, MA, USA) using the built-in *Extravascular* method of calculation. In this method the areas under concentration–time curves (AUC) were calculated by mixed-log linear model. The observed PK parameters were the maximum concentration of the drug in plasma (C_{max}), the time to