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Identification of the primary determining factor(s) governing the oral absorption of edaravone in rats

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ABSTRACT

This study was performed to determine the primary factor(s) governing the oral absorption of edaravone, a novel anti-oxidant for the treatment of amyotrophic lateral sclerosis, in rats. While the aqueous solubility of edaravone widely varied depending on the vehicle used, the oral bioavailability of the drug was not low when it was adequately solubilized, as evidenced by the fact that the oral exposure was high (in terms of the absolute bioavailability of 50–90%) at all dose ranges (i.e., 0.5–27 mg/kg) under solubilized conditions in rats. The sum of the in vitro clearance values for edaravone, 12.7 mL/(min × kg), obtained from metabolic stability studies with tissue-homogenates from the rat liver, kidney, intestine, and with the rat plasma, was found to be virtually identical to the systemic clearance of the drug in rats. It was noted that the liver represented over 83.9% of the total elimination with a hepatic extraction ratio of approximately 0.137, indicative of the minor role of hepatic first pass metabolism in the systemic absorption of edaravone after its oral administration. In studies with Ussing chamber with rat intestinal segments and Madin-Darby canine kidney (MDCKII) cells, edaravone was found to be highly permeable (i.e., P_{app} over 10×10^{-6} cm/s), and appeared to be a substrate for rat P-glycoprotein (P-gp; estimated K_m of 421 μM). In contrast, however, the drug did not appear to be a substrate for human P-gp in transport studies with MDCKII-hMDR1 cells. Collectively, these observations suggest that the primary determining factor for the intestinal absorption of edaravone is its solubilization in vehicle/intestinal fluids, rather than permeability, pre-systemic first-pass metabolism, or efflux transport. Considering the fact that the newly approved indication of the drug would require prolonged administration, probably via oral administration, the findings reported herein provide relevant information regarding its use.

1. Introduction

Edaravone (3-methyl-1-phenyl-2-pyrazolin-5-one, Radicava/Radicut®), a free radical scavenger, was originally developed for the treatment of acute strokes (Kikuchi et al., 2013; Lapchak, 2010; Watanabe et al., 2008; Yoshida et al., 2006). Since the treatment of a brain infarction would necessitate an acute exposure of the drug, the only reasonable route of administration would be intravenous (iv) administration via infusion at a dose of 30 mg twice a day for 14 days (i.e., approximately 1 mg/kg/day for humans) (Lapchak, 2010; Sinha et al., 2009). The intravenous infusion of edaravone was also recently approved for the treatment of amyotrophic lateral sclerosis (i.e., Lou Gehrig's disease), a chronic and neurodegenerative disease, by the United States Food and Drug Administration (FDA) (Abe et al., 2017; Hardiman and van den Berg, 2017; Yoshino and Kimura, 2006). For the case of this new application, however, an oral administration, rather than parenteral administration, would be preferable, since amyotrophic lateral sclerosis is a chronic disease. Unfortunately, however, an oral

Abbreviations: A to BA, pical to Basolateral; ANOVA, Analysis of variance; AUC, Area under the curve; B to A, Basolateral to Apical; BA, Bioavailability; CL, Clearance; CMC, Carboxymethyl cellulose; DMSO, Dimethyl sulfoxide; ER, Extraction ratio; FDA, Food and Drug Administration; iv, Intravenous; LC-MS/MS, Liquid chromatography-mass spectrometry; MDCKII, Madin-Darby canine kidney; MDR1, Multidrug resistance protein 1; NaOV, Sodium vanadate; P_{app}, Apparent permeability; P-gp, Permeability glycoprotein; PO, Per oral; SD, Sprague-Dawley; $t_{1/2}$, half-life; V_{ss}, Volume of distribution at steady state

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formulation of edaravone is not currently commercially available, although such a form of edaravone (TW001) is currently under development/study in the form of a phase 1 clinical study [TW001, (Mohamed et al., 2017)]. The pharmacokinetic characteristics for the experimental formulation have not yet been published in the literature.

Pre-formulation/formulation studies, however, have been carried out for edaravone (Parikh et al., 2016, 2017; Rong et al., 2014), including the determination of its oral bioavailability in rats. The oral bioavailability of edaravone was reported to be quite low (i.e., 5.23%) in rats (Rong et al., 2014), suggesting that this pharmacokinetic insufficiency could be a major limiting factor for the development of an oral formulation for this drug. However, Rong et al. (2014) reported on the bioavailability by comparing the systemic exposure in rats that were receiving markedly different dosage levels via different administration routes (i.e., an oral dose at 27 mg/kg vs an iv dose at 9 mg/kg). Furthermore, the dosage used in the study was quite high in comparison to the recommended clinical dosage. As a reference, the approved dose for human use in clinical practice would be close to 1 mg/kg (60 mg per day), and, even if the pharmacokinetic data based on a rat brain ischemia model were compared to humans (Doi et al., 2004; Shibata et al., 1998; Takamatsu et al., 1997), the dosage would not exceed 3 mg/kg. Considering the fact that the underlying mechanism by which edaravone is absorbed across the intestine is not fully understood, the difference in the dosage between the two administration routes may have affected the estimation of the oral bioavailability of the drug. In addition, the results of single-pass intestinal perfusion studies with rats indicate that the permeability of edaravone is enhanced by a co-treatment with verapamil, a standard inhibitor of the P-glycoprotein (P-gp), suggesting P-gp mediated efflux is involved in the transport of edaravone across the rat intestine (Rong et al., 2014). Obviously, the involvement of P-gp mediated efflux in this process would be expected to limit the bioavailability of the drug and would have an effect on its estimation. In addition to the potential involvement of an efflux process, the limited solubility of the drug in the dosing solution and/or the intestinal fluid may also contribute to the low oral bioavailability in rats, since the drug has limited solubility in aqueous media [1.85 mg/mL, (Parikh et al., 2017)]. Based on the above findings, it is clear that the determining factor(s) that govern the low oral bioavailability of edaravone are not completely understood.

The results of our routine pharmacokinetics studies suggest that the oral bioavailability of edaravone vastly exceeds the estimates reported in the literature (i.e., approximately 5%; Rong et al., 2014) in rats receiving a clinical dosage of the drug in the range of 0.5–5 mg/kg. Considering the differences in the experimental designs, the kinetic discrepancies may be manifested by multiple factors. The objective of the study, therefore, was to identify the primary factor(s) that govern the oral bioavailability of this antioxidant in rats regarding four major aspects of oral absorption, i.e., solubility, metabolism, permeability, and P-gp mediated efflux.

2. Materials and methods

2.1. Chemicals and reagents

Verapamil, metoprolol, and digoxin were purchased from Sigma-Aldrich (St Louis, USA). Edaravone (3-methyl-1-phenyl-5-pyrazolone) was obtained from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Radiolabeled [^{3}H]-digoxin (specific activity: 29.8 Ci/mmol; Perkin Elmer Life or Analytical Sciences (MA, USA) was also used in this study. All other chemicals were of the highest grade available and were used without further purification.

2.2. Animal

Male Sprague-Dawley (SD) rats [7–8 weeks old, Orient Bio Inc. (Gyeonggi-do, Korea)] were housed under a 12 h light/dark cycle for equilibration, and fasted overnight before the animal experiments (i.e., all in vivo studies and the collection of blood/tissue samples). The protocols for all animal studies were reviewed and approved by the Institutional Animal Care and Use Committees of Seoul National University, Seoul, Republic of Korea, according to the guidance (Number 85-23), Principles of Laboratory Animal Care (revised in 1985), from the National Institutes of Health Publication.

2.3. Dose-ranging study

Dose dependent pharmacokinetic studies were performed according to previously described methods (Jeong et al., 2017; Lee et al., 2015a; Ryu et al., 2017). Briefly, male SD rats, weighing 235–250 g, were anesthetized by an intramuscular administration of 40 mg/kg tiletamine-HCl/zolazepam-HCl (Zoletil 50) and 10 mg/kg xylazine-HCl (Rompun). After confirming the induction of anesthesia, the femoral artery (for collecting blood samples) and vein (for administering and supplementing body fluids) were catheterized with polyethylene tubing (PE 50; Clay Adams, Parsippany, NJ) filled with heparinized saline (20 U/mL; for arterial cannulae) and normal saline (for venous cannulae), respectively. Edaravone was dissolved in saline containing 2% DMSO and the solution administered via intravenous/oral routes at doses of 0.5, 1, 2, and 5 mg/kg (the volume of the dosing solution was fixed at 2 mL/kg for both routes). Blood samples (150 μL) were collected in heparinized tubes via the right femoral artery at 2, 5, 15, 30, 60, 90, 120, 180, and 240 min after being administered to rats. Immediately after blood collection, a volume of saline identical to the volume of the blood sample was administered to the animal to compensate for the fluid loss. Plasma samples, obtained by the centrifugation of blood samples at 16,100*g* for 5 min at 4 °C, were collected and the concentration of the drug in the plasma was determined using an LC-MS/MS assay for edaravone (see Section 2.8).

2.4. Solubility study

In this study, the solubility of edaravone in various aqueous vehicles was determined using a previously described method (Parikh et al., 2016; Yin et al., 2009b). The test vehicles used included double distilled water, 0.5% sodium carboxymethyl cellulose (CMC-Na) [for an in vivo study in the literature (Parikh et al., 2016; Rong et al., 2014)], 2% DMSO in saline (the primary vehicle used in this study), simulated gastric fluid, and simulated intestinal fluid. For solubility measurements, an excess amount of edaravone was added into 1 mL of test vehicle, followed by mixing in a shaking incubator at 25 °C for 24 h. The solution was then centrifuged at 16,100*g* for 5 min at room temperature. The supernatant was filtered to remove excess edaravone, and the concentration of the drug in the filtrate was determined by an LC-MS/MS assay (see Section 2.8).

2.5. Stability of edaravone in tissue homogenates and plasma of the rat

In this study, the metabolic stability of edaravone was determined in homogenates of the liver, kidney, and intestine from the rats using procedures described in the literature (Ao et al., 2015; Lee et al., 2016). Briefly, after recovering from the anesthesia, the rats were immediately sacrificed by cervical dislocation for the collection of the liver, kidney, and intestine. The wet weight of tissue was determined, and the tissue sample was then homogenized in a 2-fold volume of Dulbecco's phosphate-buffered saline using a homogenizer (Ultra Turrax model, T25, IKA Works, Inc., Cincinnati, OH). An aliquot (499 μL) of pre-warmed (37 °C) tissue homogenate was mixed with 1 μL of a solution containing 5 mM edaravone for the initiation of the metabolic reaction. The mixture was incubated at 37 °C and aliquots (50 μL) of the homogenate were collected at various times (i.e., at 0, 5, 10, 15, and 20 min). The concentration of the drug in the sample was immediately determined using an LC-MS/MS assay (see Section 2.8) and the percent of

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