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Gastric retention pellets of edaravone with enhanced oral bioavailability: absorption mechanism, development, and in vitro/in vivo evaluation

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Abstract

Absorption mechanism of edaravone (EDR) was studied to inform the preparation of gastric retention pellets with the aim to enhance its oral bioavailability. Three different models, namely, Caco-2 cells model, in situ single-pass intestinal perfusion model, and everted gut sac model in rats, were employed to characterize the gastrointestinal absorption kinetics of EDR. And it was found that passive transfer plays a vital role for the transport of EDR, and acidic condition is preferable for EDR absorption. Further, it is likely that EDR acts as a substrate for P-glycoprotein and multidrug-resistance protein. And hence, an orally available gastric retention pellets were developed accordingly. Pharmacokinetic experiments performed with rats and beagles showed that the absolute bioavailability of EDR solution and enteric-coated pellets following oral administration were 33.85% ± 2.45% and 7.64% ± 1.03%, indicating that stomach absorption is better than intestinal adsorption for EDR. However, the gastric retention pellets resulted in 68.96% absolute bioavailability and about 200% relative bioavailability in comparison to EDR solution, which was 9 times that of enteric-coated pellets. The present work demonstrates that gastric retention pellets has excellent potential as oral administration route for EDR.

Keywords: Edaravone; High density gastric retention pellets; pH dependent; Absorption mechanism; Oral bioavailability.

1. Introduction

It is widely accepted that excessive amount of free radical is associated with cerebral ischemia and reperfusion, inducing oxidative damage and causing activation of certain signaling pathways (Sano et al., 2010). These changes could cause cell damage, necrosis, and apoptosis, and consequently result in ischemic brain injury (Suzuki, 2009). Edaravone (EDR), a free radical scavenger, has been used in the treatment for acute ischemic stroke (Watanabe et al., 2008), with its efficacy demonstrated in both animal experiments and clinical studies (Amemiya et al., 2005; Otomo et al., 2003; Zhang et al., 2005).

Despite the great potential it has shown, EDR is currently administered through injection due to its poor oral bioavailability. However, comparing with injection, oral administration could provide a much more convenient route for EDR, as has been shown with a wide range of medicines. It is therefore necessary to develop an oral formulation of EDR so that patients could benefit from the advantages of oral administration, such as convenient medication, avoiding injection pain and improving patient compliance (Bala et al., 2016).

There have been limited studies concerning oral administration route for EDR. Sato and colleagues demonstrated the possibility of an oral mucosal and rectal administration of EDR with pharmacokinetics and bioavailability study (Sato et al., 2010). It has been suggested that the combination of metabolic inhibitors and EDR can be used as a new transdermal therapeutic agent for skin diseases (Sato et al., 2009). Rong and colleagues designed HP-SBE-βCD as a carrier and regulator of the efflux pump of P-glycoprotein (P-gp) to prepare EDR/HP-SBE-βCD inclusion complex, which improves the absolute bioavailability of EDR by 53.8% in rats (Rong et al., 2014). This is because the formed

complex could improve the solubility and inhibit the expression of intestinal P-glycoprotein. However, the effect of physiological environment (particularly the pH) on the stability and solubility of EDR, which a significant factor determining the efficiency and effectiveness of the oral formulation, has not been examined systematically.

Possessing a phenol like structure, EDR has a pKa value of 7 and tends to ionise in basic environment (Higashi et al., 2006; Yoshida et al., 2006), which enhances the solubility of EDR (Zhang et al., 2013). However, it seems that EDR may degrade rapidly due to alkaline hydrolysis, as is the case for other drugs with phenolic structures (Friedman and Jürgens, 2000). Therefore, acidic conditions may be beneficial to EDR. In a recent study concerning the influence of pH (between pH 2-10) on the stability of EDR, it was found that EDR is relatively stable with less than 8% degradation in acidic conditions (pH 2-6) within 21 days, while ≤20% in basic conditions (pH 8-10), and < 10% in neutral conditions (pH 7) (Robinson et al., 2015). Taking into account of factors that determine both solubility and stability of EDR, a novel oral delivery system consisted of labrasol and aqueous buffer system (pH 4) was prepared to improve the oral bioavailability of EDR by 5.7 folds (Parikh et al., 2016). In another study, lipid-based nanosystem (LNS) loaded with EDR has been reported to enhance the oral bioavailability (Parikh et al., 2017). LNS is composed of oil, surfactants and co-surfactants which selected by their potential to improve physicochemical parameters of EDR, including solubility, stability and metabolism. In vivo performance of L-LNS (liquid LNS) and S-LNS (solid LNS) showed excellent potential for further development of liquid and solid dosage form by enhancing 10.79-fold and 9.29-fold oral bioavailability of EDR, respectively.

ATP-binding cassette (ABC) superfamily of proteins serve as drug efflux transporters and have a great influence on the bioavailability of most drugs. Besides, the side effects and toxicity risk of the drug are also related to the role of ABC transporters. P-glycoprotein (P-gp) and multidrug resistance-associated protein (MRP) are both parts of the ABC proteins. Gastrointestinal P-gp and MRP are major routes of elimination for orally administered drugs. Inhibiting the expression of gastrointestinal P-gp and MRP could be an important means for enhancing the oral bioavailability of EDR (Schinkel and Jonker, 2012; Wacher et al., 2001).

In addition to solubility and stability, absorption of a drug in the body is a main factor determining oral absolute bioavailability and also influences the distribution, metabolism, and excretion of the drug (Hidalgo, 2001). Previous studies have been focusing on the enhancement of oral bioavailability by inhibiting the expression of P-gp or increasing the solubility of EDR (Rong et al., 2014), without considering the absorption mechanism of EDR after oral administration.

In the present work, a gastric retention drug system, different from the general gastric floating agent, was prepared as high-density pellets to stay in the stomach for a prolonged period by the characteristics of animals and bedridden. The absorption mechanism of EDR was investigated systematically by examining the effects of the drug concentration, the pH of the surrounding medium, the p-glycoprotein inhibitors, and multidrug-resistance protein inhibitors. Three different models, including Caco-2 cells model, in situ single-pass intestinal perfusion model, and everted gut sac model, were employed to characterize the gastrointestinal absorption kinetics of EDR, with details included in the Supplementary

Material. In vivo X-ray imaging was deployed to verify the retention characteristics of the developed pellets. The bioavailability and pharmacokinetic parameters of the formulation were compared with other dosage forms to further evaluate the gastrointestinal absorption characteristics of EDR, which underpins the pathway for its oral administration.

2. Materials and methods

2.1. Materials

EDR (purity >99%) was purchased from Jinan Xuan Hong Pharmaceutical Technology Co., Ltd (Jinan, China). Bicun (a commercial injection of EDR) was obtained from Simcere Pharmaceutical (Nanjing, China). HPLC-grade acetonitrile and methanol were purchased from Oceanpak Alexative Chemical Co., Ltd (Sweden). Transwell plates were purchased from Corning Costar (Cambridge, MA, USA). Dulbecco's modified Eagle's medium (DMEM) was purchased from Gibco (USA). Sodium pentobarbital, formic acid, diethyl ether, and heparin sodium were supplied from Aladdin Reagent Database Inc. (Shanghai, China). Iron powder and lactose were purchased from Tianjin Damao Chemical Reagent Factory (Tianjin, China). Microcrystalline cellulose (MCC, PH 301) was purchased from Asahi Kasei Co., Ltd (Japan). Sodium hydrogen sulfite was purchased from Tianjin Fuchen Chemical Reagent Co., Ltd (Tianjin, China). Hydroxy Propyl Methyl Cellulose (HPMC, 90SH-100000) was purchased from Shin-Etsu Chemical Co., Ltd (Japan). CsA and Probenecid were purchased from Shanghai Mindray Chemical Technology Co., Ltd (Shanghai, China). Urethane was purchased from Sinopharm Chemical ReagentCo., Ltd (Shanghai, China). All other reagents were purchased from standard sources and were

analytical grade.

2.2. Chemical analysis

2.2.1. HPLC assay

HPLC analysis was carried out using an LC-10A (Shimadzu Ltd, Japan). The separation was performed on a Thermo ODS-2 HYPERSIL C18 column (250 mm × 4.6 mm i.d., 5 μm, Thermo Scientific, Pittsburgh, PA, USA) at 25 °C. The mobile phase consisted of methanol-water (containing 0.1% formic acid) at a volume ratio of 45:55. The flow rate was 1 ml/min, and the UV absorption wavelength was 235 nm. The injection volume was 20 μL.

2.2.2. HPLC-MS/MS assay

Samples were analyzed by HPLC-MS/MS using a TSQ Quantum Access Max HPLC-MS/MS (Thermo Fisher Scientific). The mass spectra were acquired using a triple quadrupole instrument equipped with an electrospray ionization (ESI) source operated in positive ionization mode. The separation was carried out on an ultra-performance liquid chromatography (UPLC) system (Thermo Fisher Scientific) using a Hypersil GOLD column (particle size, 1.9 μm; pore size, 175 Å; column dimensions: 100 × 2.1 mm) at 25°C. The mobile phase consisted of methanol-water (containing 0.1% formic acid) at a volume ratio of 90:10. The flow rate was set at 0.3 ml/min, the injection volume was 2 μL, and the analysis time was 2 min per sample. The following parameters were optimized for the analysis of EDR. The electrospray voltage was set at 3.5 kV, and the capillary temperature was maintained at 350°C. Nitrogen was used as the sheath gas (30 Arb) and

auxiliary gas (5 Arb) for nebulization and desolvation. Argon was used as the collision gas (1.0 mTorr) for collision-induced dissociation. Acquisition was performed in a selected reaction monitoring mode (SRM) using m/z 175→133 (EDR) and m/z 152→110 (acetaminophen) for IS. The calibration curves were linear over the concentration range of 0.05 to 20 μg/ml. The intraday and interday accuracy and precision of the assay were less than 10 %.

2.3. Preparation of gastric retention pellets and enteric-coated pellets

The gastric retention pellet were prepared by extrusion-spheronization of a mixture containing EDR 10 wt%, Iron Powder 70 wt%, MCC PH 301 10 wt%, Lactose 9 wt%, $NaHSO_3$ 1 wt% (w/w), and an additional small amount of water (~ 2 g for 100 g mixture). After the pharmaceutic adjuvants and EDR were blended homogeneously by means of grinding whilst water was added gradually. The formed mixtures was passed through a cylinder extruder (1 mm diameter holes, rotation speed = 30 rpm), subsequently spheronized at 480 rpm for 6 min, and dried at 40 °C for 8 h. Pellets were sieved to obtain that with a size range of 700-1000 um.

The enteric-coated pellet were prepared with EDR 10 wt%, MCC PH 301 65 wt%, Lactose 24 wt%, $NaHSO_3$ 1wt%, and additional amount of water (~10 g for 100 g mixture) by extrusion-spheronization. The pellet cores were prepared in the same manner as gastric retention pellets, and then coated with EudragitRS30D: EudragitRL30D blends at the weight ratio of 5: 1 by bed coating technology. 80% ethanol and 20% water mixture was used as moistening agent sprayed before scatter the adjuvant mixtures. The pellet cores

were preheated in coating pan for 10 min prior to coating. Coating temperature maintained 40±3 °C by the heating mantle. The pellet core is coated with enteric material until a weight gain of 25% (w / w) is reached. At last, the pellets after coating were transfered to an oven at 40 °C and dried for 12 hours to achieve a satisfactory state.

The density of enteric-coated pellet and gastric retention pellet prepared in the present study is 1.2 g/cm^3 and 2.4 g/cm^3 respectively.

2.4. In vitro release

The USP dissolution apparatus I (Basket type) were employed for release tests, which at 100 rpm, 37 ± 0.5 °C. 500 mg gastric retention pellets (equivalent to edaravone 50 mg) were added into 900 ml artificial gastric juice (pH 1.2), rotated at 100 rpm and 37 °C for 8h. The same amount of the enteric-coated pellets was examined using 900 ml of artificial gastric juice (pH 1.2) for 2h, and then the media was replaced by phosphate buffer (pH 6.8) maintained over a 6 h period. Sink conditions were maintained throughout the test. 3 ml sample was withdrawn at predetermined time intervals. The drug content of samples were analyzed by HPLC after filtration through a 0.45 μm millipore filter. Meanwhile, 3 ml fresh media was added back. The data represents mean ± standard deviation from three independent experiments.

2.5. In vivo X-ray imaging

As the gastric retention pellets containing iron powder, and the X-ray cannot penetrate the iron powder, the position of the pellet in the rats can be positioned under the X-ray. In

vivo X-ray images were acquired with an IVISR Lumina XRMS Series III (Perkin Elmer) that integrates the best in class in vivo bioluminescence and fluorescence imaging with 2D X-ray capability. Three male Sprague-Dawley rats were administrated of ten gastric retention pellets by gavage respectively. And the rats were under isoflurane anesthesia prior of and within imaging. After that, the rats quickly regained consciousness and moved freely. In vivo X-ray imaging were performed at time of 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 h to study the gastric retention time of the prepared gastric retention pellets.

2.6. Pharmacokinetics study

2.6.1. In vivo absorption in sprague- dawley rats

Male Sprague-Dawley rats (180–220 g, 8 weeks) were supplied by the Animal Experimental Center at Guangzhou University of Chinese Medicine. The rats were kept in a room at a temperature of 22–25 °C, with 50%–60% relative humidity, a 12-h light-dark cycle. Water and food were available libitum. All animal treatments were performed in accordance with the Regulations of the Administration of Affairs Concerning Experimental Animals and were approved by the Animal Research Ethics Committee at Guangzhou University of Chinese Medicine.

24 healthy rats were randomly divided into four groups (n = 6 rats per group), food was returned 2 h after dosing. The rats were access to water throughout the experiments. The first group received the commercial EDR formulation (Bicun®) at a single dose of 16 mg/kg by intravenous injection (i.v.) into the caudal vein. The infusion time was about 20 s. The second group received EDR, which was dissolved in normal saline (1.5 mg/mL) at a single dose of 16 mg/kg by liquid gavage (equivalent to solution volume of 2.13 mL/200 g). The third and fourth groups were given oral enteric-coated pellets and gastric retention

pellets (prepared in our laboratory) respectively, at the same dose as the other groups by intragastric administration (i.g.). Blood samples (500 μL) were collected in heparinized tubes from ophthalmic venous plexus with a heparinized glass tube at 0.033, 0.083, 0.167, 0.333, 0.5, 0.667, 1.0, 2.0, 4.0, 8.0, and 12 h after administration. Each sample was immediately centrifuged at 4,000 rpm for 10 min. The plasma obtained after centrifugation was immediately stored at -80°C until analysis.

2.6.2. In vivo absorption in beagles

Six male beagle dogs (10.0-12.0 kg) were purchased from Guangzhou General Pharmaceutical Research Institute Co., Ltd (Guangzhou, China). The dogs were randomly divided into two groups (n = 3 dogs per group). Food, but not water, was withheld for 12 h before drug administration. And food was returned 2 h after dosing. Water was available throughout the experiments. The first group received a single dose of EDR (10 mg/kg), which was dissolved in normal saline (1.5 mg/mL) by liquid gavage (equivalent to solution volume of 66.67 mL/10 kg). The second group received a single dose of EDR oral gastric retention pellets (10 mg/kg, prepared in our laboratory) by the same way as the first group. Blood samples (2 mL) were collected in heparinized tubes from forearm vein with a heparinized glass tube at 0.083, 0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 6.0, 8.0, and 12 h after administration. The blood samples treatment were performed as the method of rats.

2.6.3. Sample treatment

The concentrations of EDR in plasma were determined by HPLC/electrospray ionization mass spectrometry (HPLC/ESI-MS). One hundred microliters of each plasma sample was spiked with 100 μL of acetaminophen solution (2 μg/mL) as an internal standard, and then 100 μL of methanol and 200 μL of acetonitrile were added. The mixture was vortex for 2

min and shaken on an orbital shaker for 10 min, followed by centrifugation at 12,000 rpm for 10 min.

The supernatant (100 μL) was transferred to another clean tube then evaporated to dryness with a gentle stream of nitrogen (about 5 L/min) at 40 °C. The residue was reconstituted in 100 μL of the mobile phase, then vortexed and centrifuged at 12,000 rpm for 10 min. Then, 2 μL of the supernatant was injected into the HPLC / ESI-MS system for analysis. A quality control sample was prepared by evaporating a series of stock solutions to dryness with a gentle stream of nitrogen (about 5 L/min) at 40 °C and processed as described above.

2.6.4. Pharmacokinetic data analysis

A pharmacokinetic software, DAS 2.0, was used to establish the appropriate compartment model. The time to peak of plasma concentrations (t_{max}) and peak plasma levels (C_{max}) were both obtained directly from the concentration-time data. K_a was obtained from the linear regression of the terminal log-linearity of the concentration-time curve, while the calculated half-life ($t_{1/2}$) was $0.693/K_a$, and the area under the concentration-time curve ($AUC_{0-\infty}$) was calculated by the statistical moment method. The absolute bioavailability (F_{abs}) of the formulations for oral administration is defined as the ratio of their AUC to that of intravenous injection, while the relative bioavailability (F_{rel}) is calculated as the ratio of their AUC (A) after oral administration to that of the EDR solution (B). F_{rel} (%) is obtained by the formula $[(AUC_A \times Dose_B) / (Dose_A \times AUC_B)]$.

2.7. Statistical analysis

The results were expressed as the mean ± standard deviation (SD). For comparison

between each test group and the control, statistical analysis was carried out using one-way analysis of variance (ANOVA) with Dunnett's test in SPSS version 20 (SPSS Inc., USA) or by a student's t-test. The differences in *P* values less than 0.05 were considered significant.

3. Results

3.1. In vitro release

In order to evaluate the release of the drug in stomach and intestine after administration of the pellets, an in vitro release test was performed. The mean percent release-time curves of both gastric retention pellets and enteric-coated pellets are presented in Fig. 1. It was found that the release amount of EDR from gastric retention pellets was over 90% within the first 2.5 hours. This suggests that most of EDR contained within the pellets can be released before gastric emptying. However, as for the enteric-coated pellets, there was only limited amount of EDR released (less than 10%) in the first 2 hours, which shows the pellets have a significant resistance to acid when being exposed to artificial gastric juice (pH 1.2) for at least 2 hours. When being exposed to phosphate buffer (pH 6.8) at a later stage, rapid release of EDR from the enteric-coated pellets was observed, which suggests that more than 90% of EDR is released in intestine other than stomach.

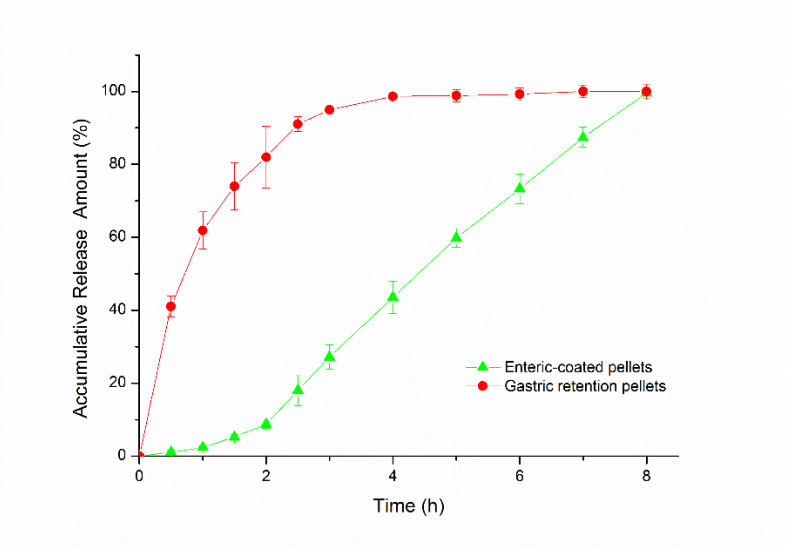

Fig. 1. In-vitro release profile of EDR for gastric retention pellets and enteric-coated pellets. Each point indicates the mean ± SD (n = 3).

3.2. In vivo X-ray imaging

X-ray imaging was carried out to visually observe the retention of the pellets in the stomach after oral administration. The acquired X-ray images are presented in Fig. 2. By comparing the position of gastric retention pellets at different time points in the images, it was found that the pellets were distributed in the whole stomach after the oral administration of the pellets. Then, the pellets settled down to the lower part of the stomach and no significant change in position during 1-3 hours. At the 4th hour, the pellets began to be slowly discharged out of stomach. Almost all the pellets were observed entering the intestine from stomach in 7 hours or so. Follow that, the pellets were further moved to the lower part of the intestinal tract. There was no notable reduction of the pellets throughout the imaging period.

To prepare the gastric retention agents, iron powder of high density was included in the pellets, so that the pellets could be deposited at the bottom of the stomach for a period of time, instead of being removed quickly as the conventional float approach. The developed design appears to be promising as a suitable platform as gastric retention agents for those bedridden patients because it offers a prolonged period for the release of EDR. The images

strongly support the hypothesis that the developed formulation could ensure the pellets to retain in the stomach long enough allowing most of the drug (94.96±0.58%) to be released and absorbed in the stomach.

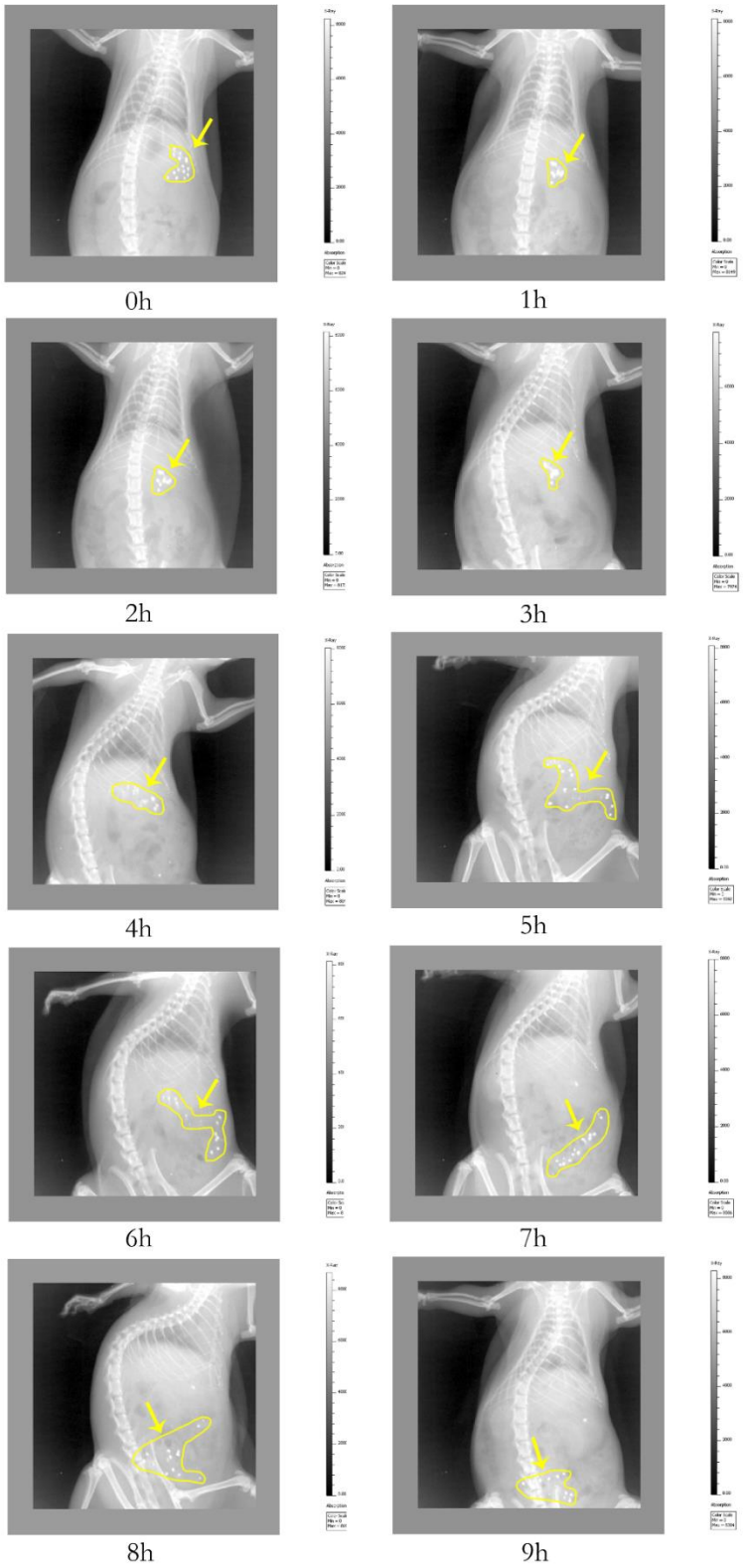

Fig. 2. In vivo X-ray images of rats before and after intra-gastric administration of gastric retention pellets at time of 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 h.

3.3. Pharmacokinetics study

3.3.1. In vivo absorption in Sprague-Dawley rats

Pharmacokinetic experiments were performed in rats to quantitatively evaluate the pharmacokinetic behavior and bioavailability of EDR with different dosage forms. The mean plasma concentration is presented as a function of time in Fig.3. Of the four administration routes, intravenous administration results in a high concentration within the half hour, but declined significantly by one hour; both EDR solution and gastric retention pellets produced a concentration spike at 0.24 and 0.70 hour respectively, whilst there is no notable spike induced by the enteric-coated pellets.

The in vivo pharmacokinetic characteristics of EDR by oral administration is consistent with a two-compartment model with first order absorption. The pharmacokinetic parameters of EDR in rats are listed in Table 1, with the oral absolute bioavailability of EDR solution and enteric-coated pellets is 33.85% ± 2.45% and 7.64% ± 1.03% respectively. The measured oral absolute bioavailability of EDR gastric retention pellets is 68.96% ± 5.66%, which is significantly greater than that of EDR solution and enteric-coated pellets. The oral pharmacokinetic study shows that about 200% relative bioavailability with EDR gastric retention pellets compared to EDR solution. The results confirm that EDR can be absorbed after oral administration, with rapid distribution and elimination. The oral bioavailability of enteric-coated pellets is much lower than EDR solution, indicating stomach absorption is better than intestinal absorption for EDR, which confirms that the effectiveness of EDR gastric retention pellet is appropriate.

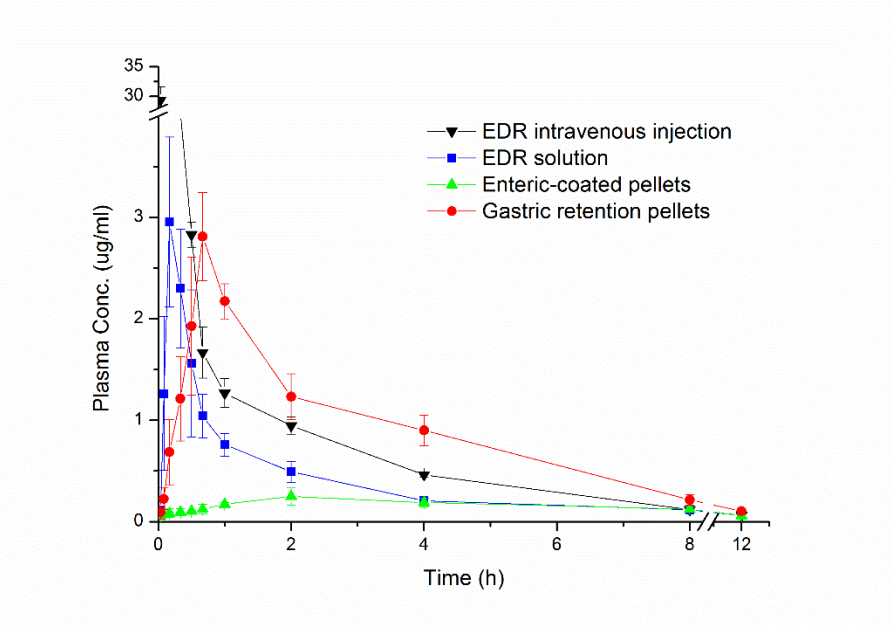

Fig. 3. The mean plasma concentration-time curves of EDR after intravenous and oral administration in rats. Each point indicates the mean ± SD (n = 6).

Table 1

Pharmacokinetic parameters of EDR after intravenous and oral administration in rats. Values are means ± SD (n = 6).

Parameters	i.v. 16 mg/kg	p.o. Solution 16 mg/kg	p.o. Enteric-coated pellets 16 mg/kg	p.o. Gastric retention pellets 16 mg/kg
$t_{1/2}$ (h)	2.94 ± 0.30	3.48 ± 0.79	3.40 ± 0.32	3.54 ± 0.37
CL (L/h/kg)	8.35 ± 0.57	24.77 ± 1.95	9.68 ± 0.19	11.79 ± 1.02
$MRT_{0-\infty}$ (h)	1.57 ± 0.12	3.54 ± 0.52	6.77 ± 0.44	4.02 ± 0.52
$AUC_{0-\infty}$ (mg/L*h)	11.98 ± 1.10	4.06 ± 0.31	0.92 ± 0.28	8.26 ± 0.70
C_{max} (mg/L)	-	3.30 ± 0.52	0.26 ± 0.05	2.96 ± 0.33

T_{max} (h)	-	0.24 ± 0.15	2.35 ± 0.65	0.70 ± 0.16
F_{abs} (%)	100	33.85 ± 2.45	7.64 ± 1.03	68.96 ± 5.66
F_{rel} (%)	-	100	22.58	203.70

"-": no value

3.3.2. In vivo absorption in beagles

To verify the pharmacokinetic results acquired from Sprague-Dawley rats, studies in beagle dogs were also carried out. The mean plasma concentration-time curves of EDR in pharmacokinetic study performing in dogs are presented in Fig.4, with corresponding pharmacokinetic parameters listed in Table 2. Of the two administration routes, EDR solution produced a concentration spike at 0.38 hour, but declined significantly by 1.5 hours, while the gastric retention pellets produced a concentration spike at 0.88 hour and declined rapidly by 4 hours. The relative bioavailability of EDR gastric retention pellets is 211.02% ± 9.29% in comparison with EDR solution. The result was in agreement with that obtained from the experiments carried out on rats, which confirms that stomach is the best site for EDR absorption, and the gastric retention pellets are a promising dosage form for EDR oral administration.

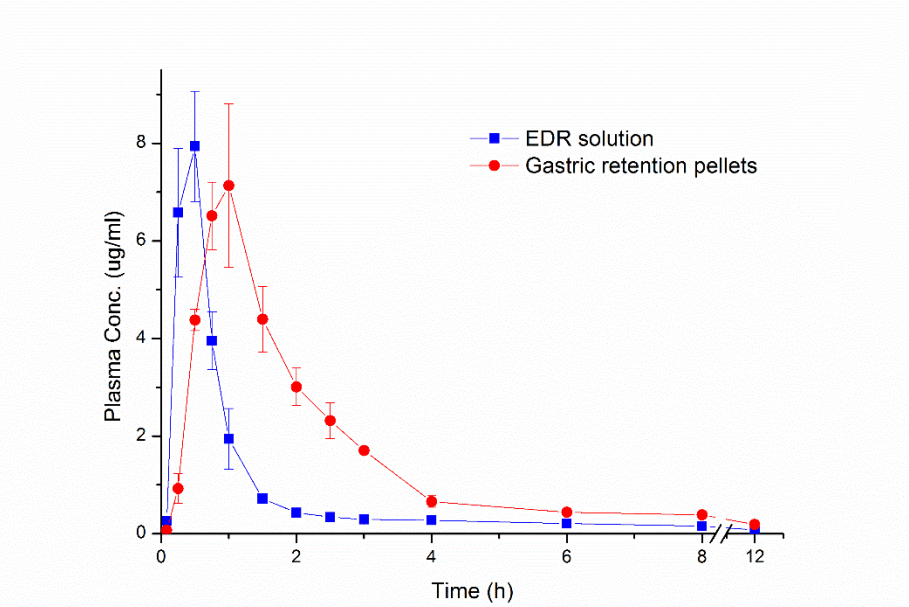

Fig. 4. The mean plasma concentration-time curves after oral administration of EDR solution and gastric retention pellets in dogs respectively. Each point indicates the mean ± SD (n = 6).

Table 2

Pharmacokinetic parameters of EDR after oral administration in Beagles. Values are means ± SD (n = 3).

Parameters	p.o. Solution 10 mg/kg	p.o. Gastric retention pellets 10 mg/kg
$t_{1/2}$ (h)	4.13 ± 0.36	4.59 ± 0.22
CL (L/h/kg)	12.55 ± 0.23	6.09 ± 0.27
MRT $_{0-\infty}$ (h)	2.84 ± 0.40	3.90 ± 0.37
AUC $_{0-\infty}$ (mg/L*h)	7.78 ± 0.12	16.42± 0.72
C_{max} (mg/L)	8.12 ± 0.87	7.66 ± 0.93

T_{max} (h)	0.37 ± 0.18	0.88 ± 0.18
F_{rel} (%)	-	211.02± 9.29

"-": no value

4. Discussion

4.1. Absorption mechanism

To validate the possibility of oral administration of EDR and to optimize the efficiency and effectiveness of the formulation developed, it is necessary to understand the absorption mechanism of EDR. P-gp and MRPs both acts as drug flux transporters by extruding drugs out of cells (Dean and Allikmets, 2001; Ruetz and Gros, 1994; van Helvoort et al., 1996), and their distribution along the length of the intestine is uneven. The expression of P-gp and MRPs in the stomach was found higher than that in gut (Sodani et al., 2012; Keppler, 2011; Ono et al., 2007; Gillet et al., 2007; Fojo et al., 1987; Bera et al., 2002; Bera et al., 2001; Brady et al., 2002; Nakayama et al., 2000; Wagner et al., 2001). It is considered that the non-uniform distribution of P-gp and MRPs has a significant effect on the uptake of their substrates, which was confirmed in clinical studies (Fricker et al., 1996). In suit single-pass intestinal perfusion study has been shown about 1.4- fold increase in K_a and P_{app} by co-perfusion with Cyclosporine A (P-gp inhibitor) or probenecid (MRPs inhibitor), which indicated that EDR was a substrate of P-glycoprotein and multidrug-resistance protein. Make EDR be absorbed in the stomach rather than the intestine, can reduce the efflux of EDR and improve its oral bioavailability.

Oral bioavailability of drugs is affected by many factors, such as drug solubility, dissolution

rate, intestinal permeability and so on. The pH distribution theory shows that only non-ionizable forms of ionizable drug molecules can diffuse across the cell membrane. Although it has been shown from later studies that ionized forms of drug molecules can also be assigned to phosphatidylcholine bilayers (Avdeef et al., 1998; Iseki et al., 1992; Ottiger and Wunderli-Allenspach, 1997), much less than non-ionized forms. In earlier studies, it was found that non-ionized forms of weak acids transported through rat intestinal tissue with a rate more than 10,000 times faster than the ionized form (Tai and Jackson, 1981). However, Palm et al. found that the transport rates of non-ionized drug were 150 to 30 times higher than those ionized forms of the lipophilic drug alfentanil and the hydrophilic drug cimetidine, respectively (Palm et al., 1999). In the present work, Caco-2 cell monolayer model was used to study the effect of pH on EDR transport across the gastrointestinal epithelium. The cell monolayer permeability coefficients (P_{app}) of EDR were determined in the pH range 5.5 to 8.0. The result shows that the cell monolayer permeability coefficients (P_{app}) in the AP-BL direction is significantly increased when the pH was reduced to 5.5, which is attributed to the reduced degree of EDR ionization at pH 5.5. Therefore, the acidic environment is favorable for the permeability of EDR.

4.2. High density gastric retention pellets improve the oral bioavailability

Being able to deliver drug to any specific region of the gastrointestinal tract offers many advantages, and it is critical for the drugs that exhibit an absorption window in the gastrointestinal tract or drugs with poor stability. It has been reported that pellets of high-density are able to resist gastric peristaltic movements due to their retention in the antrum rugae or folds, increasing the gastrointestinal tract time (Garg, 2008). The threshold for

density required to result in an increased gastric residence time range is between 2.4 and 2.8 g/cm^3 (Clarke, 1995). The density of enteric pellets and gastroretentive pellets prepared in the present study is 1.2 g/cm^3 and 2.4 g/cm^3 respectively, which is much greater than that of gastric juice (1.004 g/cm^3) (Bardonnet et al., 2006). Therefore, the gastric retention pellets can extend the retention time to more than 4h, while the enteric pellets could retain in stomach for 2-3 hours because it has a similar density to gastric juice (Hoffman et al., 2004; Singh and Kim, 2000).The increased expression rate of intestinal P-glycoprotein (P-gp) and multidrug resistance protein (MDR) could inhibit the absorption of EDR. Furthermore, Parikh and colleagues systematically examined the stability of edaravone in buffer system with pH between 2 and 10, and reported that EDR is relatively stable in acidic conditions (pH 2-6) with <8% degradation within 21 days, in comparison to <10% degradation in neutral conditions (pH 7), and ≤20% degradation in basic conditions (pH 8-10). It is highly likely that EDR is a tautomer that is more likely to be free in the form of dissociation at increased pH environment, with improved solubility but reduced stability (Parikh et al., 2016) . While EDR is absorbed via passive absorption mechanism in the gastrointestinal tract, the dissociation form can hardly be absorbed through the intestinal epithelial cells. With the increased pH in the intestine, the absorption rate is reduced whilst the degradation rate is increased, which results in low oral bioavailability.

Results of pharmacokinetic studies carried out on rats and beagle dogs confirm that the absorption and elimination of EDR are rapid. In detail, EDR solution produced a concentration spike at 0.24 hour and declined significantly by 1 hour in rats, while spiked at 0.38 hour and declined rapidly by 1.50 hours in dogs. The results show that the

elimination half-life of EDR by intravenous infusion, liquid gavage, and enteric-coated pellets are similar. The rapid dispersion to different regions of the body may have affected the mean plasma concentration-time curves of EDR, as evidenced by the reduction of several folds in plasma drug concentrations within a few minutes.

The dose used in rats for both liquid gavage and enteric-coated pellets was 16 mg/kg, with C_{max} values of 3.30 ± 0.52 and 0.26 ± 0.05 mg/L, respectively. These data could provide a reference for future studies of the most appropriate administration dosage.

Moreover, the absolute bioavailability of gastric retention pellets, peroral solution and enteric coated pellets in rats were 68.96%, 33.85% and 7.64%, respectively. The absolute bioavailability of edaravone gastric retention pellets was about 9 times of enteric coated pellets in rats. Compared with the peroral solution, the relative bioavailability of the gastric retention pellets was 203.70 in rats, which is very close to 211.02% in the beagle dogs. While absolute bioavailability is low, that of liquid gavage is 4 times greater than that of enteric-coated pellets, demonstrating that liquid gavage may be more effective than enteric-coated pellets. The results showed that edaravone had good absorption and stability in the stomach, but it is hardly absorbed and easily degraded in the intestinal tract. Interestingly, it is observed a significant difference between the clearance value of peroral solution and gastric retention pellets. This is due to the peroral solution has a very obvious absorption fluctuation curve, and it can also clearly show that there is a significant difference absorption between in stomach and intestine. The above results confirmed the absorption in the stomach is significantly better than that in small intestine, which suggests that the gastric retention pellet is a promising formulation for EDR with high oral bioavailability.

5. Conclusion

In this study, we examined the oral bioavailability and pharmacokinetics of EDR, a potent free radical scavenger used to treat ischemia. Three different models, namely the Caco-2 cells model, in situ single-pass intestinal perfusion model, and everted gut sac model were used to investigate the gastrointestinal absorption characteristics of EDR and its transmembrane delivery. Results showed that EDR was absorbed after oral administration and exhibited rapid distribution and elimination. Moreover, the absorption of EDR was enhanced under acidic condition, suggesting that stomach is the optimal site for EDR absorption, which occurs via passive diffusion. It is possible that P-glycoprotein (P-gp) and multidrug resistance protein (MDR)-mediated efflux mechanisms are involved in the transportation of EDR. These data provide important insights into the development of an orally available EDR formulation. Subsequently, gastric retention pellets of EDR was prepared based on the findings listed above. The newly developed formulation shows a much improved performance as oppose to conventional EDR solution, with 68.96% absolute bioavailability and about 200% relative bioavailability, according to the pharmacokinetic measurements. This confirms that gastric retention pellet is a promising platform for oral administration of EDR instead of the conventional injection route.

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References

Amemiya S., Kamiya T., Nito C., Inaba T., Kato K., Ueda M., Shimazaki K., Katayama Y., 2005. Anti-apoptotic and neuroprotective effects of edaravone following transient focal ischemia in rats. Eur. J. Pharmacol. 516, 125-130.

Avdeef A., Box K.J., Comer J., Hibbert C., Tam K.Y., 1998. pH-metric logP 10. Determination of liposomal membrane-water partition coefficients of ionizable drugs. Pharm. Res.-Dordr. 15, 209-215.

Bala V., Rao S., Bateman E., Keefe D., Wang S., Prestidge C.A., 2016. Enabling oral SN38-based chemotherapy with a combined lipophilic prodrug and self-microemulsifying drug delivery system. Mol. Pharmaceut. 13, 3518-3525.

Bardonnet P.L., Faivre V., Pugh W.J., Piffaretti J.C., Falson F., 2006. Gastroretentive dosage forms: overview and special case of Helicobacter pylori. J. Control. Release. 111, 1-18.

Bera T.K., Iavarone C., Kumar V., Lee S., Lee B., Pastan I., 2002. MRP9, an unusual truncated member of the ABC transporter superfamily, is highly expressed in breast cancer. Proc Natl Acad Sci U S A. 99, 6997-7002.

Bera T.K., Lee S., Salvatore G., Lee B., Pastan I., 2001. MRP8, a new member of ABC transporter superfamily, identified by EST database mining and gene prediction program, is highly expressed in breast cancer. Mol. Med. 7, 509-516.

Brady J.M., Cherrington N.J., Hartley D.P., Buist S.C., Li N., Klaassen C.D., 2002. Tissue distribution and chemical induction of multiple drug resistance genes in rats. Drug Metab. Dispos. 30, 838-844.

Clarke G.M.N.J., 1995. Comparative gastrointestinal transit of pellet systems of varying density. Int. J. Pharm. 114, 1-11.

Dean M., Allikmets R., 2001. Complete characterization of the human ABC gene family. J. Bioenerg. Biomembr. 33, 475-479.

Fojo A.T., Ueda K., Slamon D.J., Poplack D.G., Gottesman M.M., Pastan I., 1987. Expression of a multidrug-resistance gene in human tumors and tissues. Proc Natl Acad Sci U S A. 84, 265-269.

Fricker G., Drewe J., Huwyler J., Gutmann H., Beglinger C., 1996. Relevance of P-glycoprotein for the enteral absorption of cyclosporin A: in vitro-in vivo correlation. Br J Pharmacol. 118, 1841-1847.

Friedman M., Jürgens H.S., 2000. Effect of pH on the stability of plant phenolic compounds. J. Agr. Food Chem. 48, 2101-2110.

Garg R.G.D.G., 2008. Progress in controlled gastroretentive delivery systems. Trop. J. Pharm. Res. 7, 1055-1066.

Gillet J., Efferth T., Remacle J., 2007. Chemotherapy-induced resistance by ATP-binding cassette transporter genes. Bba.-Rev. Cancer. 1775, 237-262.

Hidalgo I.J., 2001. Assessing the absorption of new pharmaceuticals. Curr. Top. Med. Chem. 1, 385-401.

Higashi Y., Jitsuiki D., Chayama K., Yoshizumi M., 2006. Edaravone (3-methyl-1-phenyl-2-pyrazolin-5-one), a novel free radical scavenger, for treatment of cardiovascular diseases. Recent Pat Cardiovasc Drug Discov. 1, 85-93.

Hoffman A., Stepensky D., Lavy E., Eyal S., Klausner E., Friedman M., 2004. Pharmacokinetic and pharmacodynamic aspects of gastroretentive dosage forms. Int J Pharm. 277, 141-153.

Iseki K., Hirano T., Fukushi Y., Kitamura Y., Miyazaki S., Takada M., Sugawara M., Saitoh H., Miyazaki K., 1992. The pH dependent uptake of enoxacin by rat intestinal brush-border membrane

vesicles. J. Pharm. Pharmacol. 44, 722-726.

Keppler D., 2011. Multidrug resistance proteins (MRPs, ABCCs): importance for pathophysiology and drug therapy. Handb Exp Pharmacol., 299-323.

Nakayama A., Saitoh H., Oda M., Takada M., Aungst B.J., 2000. Region-dependent disappearance of vinblastine in rat small intestine and characterization of its P-glycoprotein-mediated efflux system. Eur. J. Pharm. Sci. 11, 317-324.

Ono N., Van der Heijden I., Scheffer G.L., Van de Wetering K., Van Deemter E., De Haas M., Boerke A., Gadella B.M., De Rooij D.G., Neefjes J.J., Groothuis T.A.M., Oomen L., Brocks L., Ishikawa T., Borst P., 2007. Multidrug resistance-associated protein 9 (ABCC12) is present in mouse and boar sperm. Biochem. J. 406, 31-40.

Otomo E., Tohgi H., Kogure K., Hirai S., Takakura K., Terashi A., Gotoh F., Maruyama S., Tazaki Y., Shinohara Y., Ito E., Sawada T., Yamaguchi T., Kikuchi H., Kobayashi S., Fujishima M., Nakashima M., 2003. Effect of a novel free radical scavenger, edaravone (MCI-186), on acute brain infarction - Randomized, placebo-controlled, double-blind study at multicenters. Cerebrovasc. Dis. 15, 222-229.

Ottiger C., Wunderli-Allenspach H., 1997. Partition behaviour of acids and bases in a phosphatidylcholine liposome-buffer equilibrium dialysis system. Eur. J. Pharm. Sci. 5, 223-231.

Palm K., Luthman K., Ros J., Grasjo J., Artursson P., 1999. Effect of molecular charge on intestinal epithelial drug transport: pH-dependent transport of cationic drugs. J. Pharmacol. Exp. Ther. 291, 435-443.

Parikh A., Kathawala K., Tan C.C., Garg S., Zhou X., 2016. Development of a novel oral delivery system of edaravone for enhancing bioavailability. Int. J. Pharmaceut. 515, 490-500.

Parikh A., Kathawala K., Tan C.C., Garg S., Zhou X.F., 2017. Lipid-based nanosystem of edaravone:

development, optimization, characterization and in vitro/in vivo evaluation. Drug Deliv. 24, 962-978.

Robinson K., Mock C., Liang D., 2015. Pre-formulation studies of resveratrol. Drug Dev. Ind. Pharm. 41, 1464-1469.

Rong W., Lu Y., Tao Q., Guo M., Lu Y., Ren Y., Yu S., 2014. Hydroxypropyl-Sulfobutyl-beta-Cyclodextrin improves the oral bioavailability of edaravone by modulating drug efflux pump of enterocytes. J. Pharm. Sci.-US. 103, 730-742.

Ruetz S., Gros P., 1994. Phosphatidylcholine translocase - a physiological-role for the MDR2 gene. Cell. 77, 1071-1081.

Sano H., Kamijo T., Ino T., Okamoto M., 2010. Edaravone, a free radical scavenger, in the treatment of idiopathic sudden sensorineural hearing loss with profound hearing loss. Auris Nasus Larynx. 37, 42-46.

Sato T., Mizuno K., Ishii F., 2009. A novel administration route for edaravone: I. Effects of metabolic inhibitors on skin permeability of edaravone. Int. J. Pharmaceut. 372, 33-38.

Sato T., Mizuno K., Ishii F., 2010. A novel administration route of edaravone - II: Mucosal absorption of edaravone from edaravone/hydroxypropyl-beta-cyclodextrin complex solution including l-cysteine and sodium hydrogen sulfite. Pharmacology. 85, 88-94.

Schinkel A.H., Jonker J.W., 2012. Mammalian drug efflux transporters of the ATP binding cassette (ABC) family: an overview. Adv. Drug Deliver. Rev. 64, 138-153.

Singh B.N., Kim K.H., 2000. Floating drug delivery systems: an approach to oral controlled drug delivery via gastric retention. J. Control. Release. 63, 235-259.

Sodani K., Patel A., Kathawala R.J., Chen Z.S., 2012. Multidrug resistance associated proteins in multidrug resistance. Chin J Cancer. 31, 58-72.

Suzuki K., 2009. Anti-oxidants for therapeutic use: Why are only a few drugs in clinical use? Adv. Drug

Deliver. Rev. 61, 287-289.

Tai C.Y., Jackson M.J., 1981. Weak-acid transport in the small intestine: discrimination in the lamina propria. J Membr Biol. 59, 35-43.

van Helvoort A., Smith A.J., Sprong H., Fritzsche I., Schinkel A.H., Borst P., van Meer G., 1996. MDR1 P-glycoprotein is a lipid translocase of broad specificity, while MDR3 P-glycoprotein specifically translocates phosphatidylcholine. Cell. 87, 507-517.

Wacher V.J., Salphati L., Benet L.Z., 2001. Active secretion and enterocytic drug metabolism barriers to drug absorption. Adv. Drug Deliver. Rev. 46, 89-102.

Wagner D., Spahn-Langguth H., Hanafy A., Koggel A., Langguth P., 2001. Intestinal drug efflux: formulation and food effects. Adv Drug Deliv Rev. 50 Suppl 1, S13-S31.

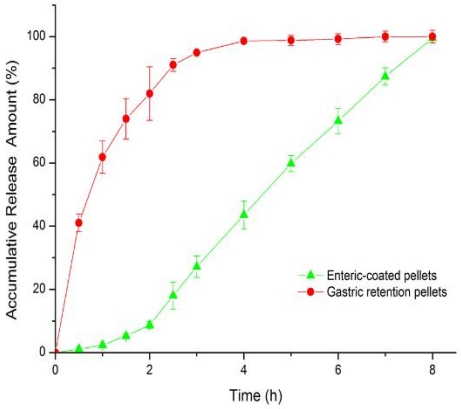
Watanabe T., Tahara M., Todo S., 2008. The novel antioxidant edaravone: from bench to bedside. Cardiovasc. Ther. 26, 101-114.

Yoshida H., Yanai H., Namiki Y., Fukatsu-Sasaki K., Furutani N., Tada N., 2006. Neuroprotective effects of edaravone: a novel free radical scavenger in cerebrovascular injury. CNS Drug Reviews. 12, 9-20.

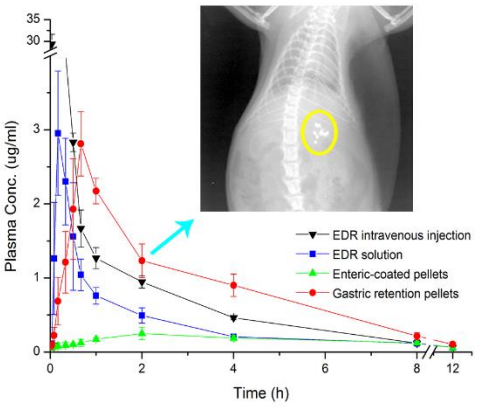
Zhang N., Komine-Kobayashi M., Tanaka R., Liu M., Mizuno Y., Urabe T., 2005. Edaravone reduces early accumulation of oxidative products and sequential inflammatory responses after transient focal ischemia in mice brain. Stroke. 36, 2220-2225.

Zhang W., Parniak M.A., Mitsuya H., Sarafianos S.G., Graebing P.W., Rohan L.C., 2013. Preformulation studies of EFdA, a novel nucleoside reverse transcriptase inhibitor for HIV prevention. Drug Dev. Ind. Pharm. 40, 1101-1111.

Graphical abstract



In vitro release



In vivo bioavailability