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Research Paper

Pharmacokinetic interaction between puerarin and edaravone, and effect of borneol on the brain distribution kinetics of puerarin in rats

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Abstract

Objectives The aim was to investigate the pharmacokinetic interaction between puerarin and edaravone, and the effect of borneol on the brain distribution kinetics of puerarin in rats.
Methods A reversed-phase high performance liquid chromatography method was developed and validated for the simultaneous determination of puerarin and edaravone in rat plasma. The detection method was successfully applied to compare the pharmacokinetic interaction and brain distribution kinetics of puerarin and edaravone using in-situ microdialysis sampling in rats after intravenous administration and co-administration with a single dose.
Key findings The method gave good linearity and no endogenous material interfered with the two target compounds and internal standard peaks. The limit of detection of puerarin and edaravone was 0.03 and 0.05 μg/ml, respectively. The average recovery of the two compounds from rat plasma was >94%. The precision of the test was determined to be within 10%. The combination of puerarin and edaravone reduced drug elimination rates, gave a wider distribution, and the disposition of both drugs in rats was optimized. The distribution of puerarin in brain tissues was significantly increased and its elimination was noticeably slower with borneol pretreatment.
Conclusions The results provide important information for the improved combined use of puerarin and edaravone with borneol pretreatment in clinical practice.
Keywords brain distribution kinetics; borneol; edaravone; microdialysis; pharmacokinetics interaction; puerarin

Introduction

Puerarin (7,4-dihydroxyisoflavone-8-glucopyranoside, daidzein 8-*C*-glucoside) is one of the main bioactive components isolated from the roots of *Pueraria lobata* (Willd) Ohwi and *Pueraria thomsonni* Benth. Both roots are well-known traditional Chinese medicinal herbs and have been used for various medicinal purposes. Puerarin belongs to the isoflavone compounds, which are present in some foods derived from plant origins and comprise one of the distributed groups of secondary plant metabolites.[1] Puerarin has been reported to exhibit notable pharmacological effects in cardiac and cerebral vascular diseases such as antihypertensive and anti-arteriosclerosis effects, and dilation of coronary arteries and cerebral blood vessels. Intake of puerarin has been reported to decrease myocardial oxygen consumption and improve microcirculation in patients with cardiovascular and cerebral vascular diseases. Puerarin also protects cells against oxidative stress.[2–4] The antioxidant activity is achieved through multiple mechanisms such as reducing H_2O_2-induced elevation of caspase-3 activation, scavenging free radicals, increasing superoxide dismutase activity and decreasing malonaldehyde.[5] Puerarin has been shown to reduce serum cholesterol levels.[6] Puerarin may protect the myocardium against ischaemia reperfusion injury via opening of the calcium-activated potassium channel and activating protein kinase C.[7] Puerarin inhibits endothelial progenitor cell senescence *in vitro*. Such an effect is potentially important in cell therapies targeting cardiocerebrovascular disease.[8]

Edaravone (3-methyl-1-phenyl-2-pyrazolin-5-one) is a novel free radical scavenger and brain protectant. Edaravone inhibits both the non-enzymatic lipid peroxidation and

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lipoxygenase pathways by arachidonate cascade reactions, and has potent antioxidant activity.[9] Thus, edaravone has several protective effects against ischaemia reperfusion-induced damage, including vascular endothelial cell injury,[10] delayed neuronal death,[11] brain oedema,[12,13] infarct,[14,15] and concomitant neurological deficits. Following a multicentre, placebo-controlled and double-blind clinic trial, edaravone has been commercialized for the treatment of acute ischaemic stroke.[16–21]

For some years, puerarin and edaravone have been widely used for treating ischaemic encephalopathy in Oriental countries. In clinical practice, puerarin is usually prescribed in combination with edaravone to obtain synergistic effects and diminish adverse reactions.[22,23] However, due to its low lipid solubility, orally administered puerarin undergoes poor absorption, resulting in low bioavailability in different species (3.95–5.45%).[24–27] Therefore, although the combined administration of puerarin and edaravone is usually via the intravenous route in clinic practice, it is still difficult for puerarin to penetrate the blood–brain barrier and produce its therapeutic effect.[25,28] In this study, the pharmacokinetic interaction between puerarin and edaravone via intravenous administration was investigated in rats. Borneol was combined with puerarin in order to promote puerarin passage across the blood–brain barrier. The effect of borneol on the brain distribution kinetics of puerarin was also studied. Before these investigations were performed, a simple gradient high performance liquid chromatography (HPLC) method was developed for the simultaneous determination of puerarin and edaravone in biological samples. The detection method was successfully applied to compare the pharmacokinetic interaction and brain distribution kinetics of puerarin and edaravone using in-situ microdialysis sampling in rats after intravenous administration and co-administration with a single dose. The results provide information on the combined use of puerarin, edaravone and borneol in the clinical setting.

Materials and Methods

Chemicals and reagents

Puerarin, edaravone and borneol were purchased from the National Institute for Control of Pharmaceuticals and Biological Products (Beijing, China). The internal standard (*p*-hydroxybenzoic acid) was purchased from Beijing Reagent Chemical Company (Beijing, China). Puerarin injection was purchased from Shandong Ruiyang Pharmaceutical Co. Ltd (Ruiyang, Shandong, China). Edaravone injection was purchased from Nanjing Xiansheng Pharmaceutical Co. Ltd (Nanjing, Jiangsu, China). The artificial cerebrospinal fluid (aCSF) buffer (124 mM NaCl, 5 mM KCl, 1.25 mM NaH_2PO_4, 2 mM $MgSO_4$, 2 mM $CaCl_2$, 26 mM $NaHCO_3$, 10 mM D-glucose) was prepared weekly, filtered, degassed to obtain a pH of 7.1–7.4, and used as the perfusate. HPLC grade methanol was used (Fisher Scientific Products, Fair Lawn, NJ, USA). Water was triply distilled. The other chemicals, reagents and solvents used were of analytical grade.

HPLC assay

All chromatography was performed using an Agilent HPLC system (Series 1100; Agilent Technology, Palo Alto, CA, USA), comprising a G1310A quaternary pump, G1322A vacuum degasser, G1316A column thermostat, and G1314A VWD and G1329A auto sample injector. The chromatography data were recorded and processed using HP Chemstation software (Agilent Technology). The analytical column was an Agilent Zorbax SB-C_{18} (150 mm ∞ 4.6 mm, i.d. 5 μm) column. All chromatography was performed at 32°C.

The mobile phase was a mixture of methanol and water containing 0.5% (v/v) glacial acetic acid, employing a gradient elution (from 10 : 90 to 70 : 30, v/v) at a flow rate of 0.7 ml/min. The solvent was filtered through a 0.45-μm filter and degassed. Both puerarin and edaravone were spectroscopically measured at 252 nm. The sample injection volume was 20 μl.

The brain microdialysis systems comprised a CMA/102 microinjection pump (CMA, Stockholm, Sweden) and microdialysis probes with dialysing membranes. A dialysing membrane with a length of 2 mm and an outer diameter of 0.5 mm (CMA/12, CMA, Stockholm, Sweden) was used for brain sampling for freely moving animals.

Preparation of samples

Standard stock solutions containing puerarin (1 mg/ml) and edaravone (1 mg/ml) were prepared in methanol. Working standard solutions were prepared by serially diluting the stock solutions using methanol. The internal standard stock solution (*p*-hydroxybenzoic acid; 50 μg/ml) was prepared in methanol. Calibration samples in blood were prepared by mixing solutions of the standard mixture and internal standard with blank rat plasma at a volume ratio of 100 : 10 : 100 μl to form a concentration series of 100, 50, 10, 1, 0.1 and 0.05 μg/ml for puerarin, 100, 50, 10, 5, 1, 0.5 and 0.1 μg/ml for edaravone and 5 μg/ml for *p*-hydroxybenzoic acid. Quality control samples (50, 10, 0.1 and 0.05 μg/ml for puerarin and 50, 10, 0.2 and 0.1 μg/ml for edaravone) were also prepared in a similar manner. All solutions were stored at 4°C before use.

Blood samples, collected in heparinized tubes, were immediately centrifuged at 2500*g* for 10 min. The resulting supernatant plasma was separated and stored at –40°C until further analysis. The proteins were precipitated with perchloric acid (0.58 M) and the remaining solution used for sample preparation. A plasma sample (0.1 ml) was spiked with 100 μl each of the working solutions containing puerarin and edaravone and 10 μl of the internal standard stock solution and vortex-mixed for 5 min. Subsequently, 0.1 ml of perchloric acid solution (0.58 M) was added to precipitate the proteins, vortex-mixed for 2 min and centrifuged at 3500*g* for 15 min. The supernatant was transferred to a clean vial and a 20 μl aliquot of this solution was injected directly into the HPLC system for analysis.

Assay performance

The method was validated for its specificity, linearity, limit of detection (LOD), limit of quantification (LOQ), accuracy, precision, recovery and stability. To evaluate the specificity, five portions of blank rat plasma were analysed by comparison with the plasma-spiked analytes to exclude endogenous material interference. Quantification was based on the internal standard method of plotting the peak area ratios of the

analyte/internal standard against the concentration of the samples with a weighting factor of 1. The calibration curves were repeated five times. The LOD was considered as the final concentration that produced a signal-to-noise ratio of 3. The LOQ was considered as the final concentration that produced a signal-to-noise ratio of 10. The precision and accuracy of the method were assessed by performing replicate analyses of the quality control samples against the calibration standards. The interday and intraday precision and accuracy were determined using five determinations of low, middle and high concentrations and expressed as the relative standard deviation (RSD%). The recovery was determined by calculating the ratio of the amount of the compounds from processed precipitated protein samples with known amounts of puerarin and edaravone at three concentrations.

Animal studies

Pharmacokinetic interaction of puerarin and edaravone

Male Sprague–Dawley rats, 0.3 ± 0.02 kg, were obtained from the Laboratory Animals Center of Capital Medical University (Beijing, China). The animals were pathogen-free and allowed to acclimate in environmentally controlled quarters (22 ± 1°C) for at least 7 days before being used for experiments. The rats had unlimited access to food except for fasting 12 h before the experiment; water was available *ad libitum*. Pooled drug-free plasma was obtained from the healthy rats. After aliquoting, plasma controls were stored at –40°C and then thawed at roomed temperature for use in the calibration curves and quality control samples. The experiments were carried out in accordance with current guidelines for the care of laboratory animals and ethical guidelines for investigations of experimental pain in conscious animals. In addition, the protocols were approved by the Animal Care and Use Committee of Capital Medical University.

Six rats were randomly divided into three groups (groups A, B and C) according to the crossover design. Rats in the three groups were used for comparison with each other and administered alternatively after each wash-out period. Drugs were administered via the sublingual vein. In the first experiment, group A were given puerarin (62.5 mg/kg, i.v.), group B were given edaravone (3.75 mg/kg, i.v.), and group C were intravenously co-administered with puerarin (62.5 mg/kg) and edaravone (3.75 mg/kg). After 2 weeks of drug elimination, group A were given edaravone, group B were co-administered with puerarin and edaravone, and group C were given puerarin in the second experiment. After the second wash-out period, group A were co-administered with puerarin and edaravone, group B were given puerarin, and group C were given edaravone in the third experiment. The dose of each drug was the same in the three experiments.

The blood samples were collected from the orbital vein of the rats before and after administration at 1, 2, 5, 10, 30, 60, 90, 120 and 240 min after receiving a single intravenous dose of puerarin and/or edaravone. Plasma (100 μl) was separated by centrifugation at 2500*g* for 10 min and the plasma stored at –40°C until analysis. A rat plasma sample (0.1 ml) was spiked with 100 μl methanol and 10 μl of internal stock solution and vortex-mixed for 5 min. Then, 0.1 ml of perchloric acid solution (0.58 M) was added to precipitate the proteins, mixed for 2 min and centrifuged at 3500*g* for 15 min. The supernatant was transferred to a clean vial and a 20-μl aliquot of this solution was injected directly into the HPLC system for analysis.

The reversed-phase HPLC procedure was successfully applied to investigate the plasma concentration–time profiles of puerarin, edaravone and the combination of puerarin and edaravone in rats. The pharmacokinetic model and the parameters were calculated by the practical pharmacokinetic program version 87 (3P87), edited by the Committee of Mathematics and Pharmacology, Chinese Society of Pharmacology. The compartment model was established by the methods of the survival square sum and the fitted degree (r^2).

Microdialysis sampling and brain distribution kinetics of puerarin

Another 36 rats were randomly assigned into six groups. The rats were allowed to recover for 12 h from the cannula implantation before the microdialysis injection. Groups A and D were given puerarin (62.5 mg/kg, i.v.), groups B and E were given edaravone (3.75 mg/kg, i.v.), and groups C and F were administered with puerarin (62.5 mg/kg) and edaravone (3.75 mg/kg, i.v.), respectively. Borneol was dissolved in 1% sodium carboxymethylcellulose water solution for an oral dose (300 mg/kg) before administration. Borneol was administrated orally to rats in groups D, E and F, and puerarin and edaravone were administered intravenously 30 min later. The rats were initially anesthetized with chloral hydrate (10%, 0.4 ml/100 g, i.p.), and remained anesthetized throughout the experimental period. Rats were mounted on a stereotaxic frame (Anhui, China) for brain microdialysis and body temperature was maintained at 37°C with a heating light. An incision was made in the scalp, the skull was exposed and a small hole was drilled. A CMA/12 guide cannula was implanted into the cortex and secured permanently in position with dental cement. The microdialysis probe was implanted via the guide cannula into the frontal cortex (coordinates: AP, –1.2 mm; LAT, –2 mm; VERT, 4 mm). The positions of the probes were verified by standard histological procedures at the end of the experiments.

The microdialysis probe was perfused with aCSF via a tube connected to a CMA/100 pump, at a flow rate of 1.0 μl/min. Outflows from brain microdialysis probes were connected to a microfraction collector (CMA/140) and collected every 20 min. After 20 μl of drug-free samples were collected, the drugs were administered intravenously, and the collection times of the microdialysate were 20, 40, 60, 100, 140 and 180 min, and 5, 7, 15, 24 and 50 h. The rats were awake and active during the microdialysis sampling period. A total of 20 μl of each sample microdialysate was collected for 50 h and stored at –20°C until analysis. Brain blank samples from microdialysis were spiked with each drug and internal standard stock solution. The procedure was the same as that described above.

Microdialysis probe in-vivo relative recovery was calibrated by measurements of in-vitro recovery and in-vitro and in-vivo loss. In-vitro relative recovery was determined by

placing the microdialysis membrane in a 50-μg/ml puerarin or 5-μg/ml edaravone solution in aCSF, and perfusing with aCSF at 1 μl/min. Samples of the dialysate were collected at 20-min intervals and puerarin and edaravone concentrations were analysed by HPLC to give the relative recovery *in vitro*. The in-vitro relative loss was determined by perfusing the analyte solution in aCSF into a microdialysis probe, the dialysis membrane of which was placed in a vial containing aCSF at 37°C. Relative loss *in vitro* was calculated using the different puerarin and edaravone concentrations in the perfusate and microdialysate. In a similar manner to the determination of the in-vitro loss, the in-vivo loss was calculated by perfusing the analyte solution in aSCF into a microdialysis probe, the dialysis membrane of which was inserted into rat brain. The probe relative recovery (RR) and relative loss (RL) were defined by the following equations, in which C_d is the concentration in the microdialysate, C_s is the concentration of the sample, and C_p is the concentration in the perfusate. The probe in-vivo relative recovery was calculated by equation 2.

$$\mathrm{RR} = (C_d/C_s)\times 100\%;\ \mathrm{RL} = (C_p - C_d)/C_p \times 100\% \quad (1)$$

$$\mathrm{RR_{in\ vivo}}/\mathrm{RL_{in\ vivo}} = \mathrm{RR_{in\ vitro}}/\mathrm{RL_{in\ vitro}} \quad (2)$$

Statistical analysis

Statistical analysis was performed using SPSS software (SPSS Inc., Chicago, IL, USA) on an IBM microcomputer. An unpaired *t*-test was used for pharmacokinetic data of plasma drug concentration against time. The parametric analysis of variance was performed for the distribution kinetics data in brain tissue, Individual differences between the treatments were identified using Dunnett's post-hoc test. Data are expressed as the mean ± SD and the statistical significance was determined at the $P < 0.05$ and $P < 0.01$ level.

Results

Quantitative basis and assay validation

The reversed-phase HPLC method described was selective and specific. The analysis of the plasma samples showed that no endogenous material or drug metabolite peaks interfered with the analytes and internal standard at the retention times. The retention times were 10.25, 11.54 and 13.28 min for internal standard, puerarin and edaravone, respectively.

The linear regression of puerarin and edaravone in rat plasma displayed a good linear relationship between the ratios of the peak areas of the analytes to the internal standard over the range of concentrations studied. For a standard curve, the ratios of the chromatographic peak areas (analytes/internal standard) were plotted against the concentration of puerarin and edaravone. The standard calibration for puerarin and edaravone was linear over the ranges 0.03–100 and 0.05–100 μg/ml, respectively. The mean values of the regression equation of the analytes in rat plasma were: $y = 3.436x + 0.113$ ($r = 0.9999$, puerarin), $y = 2.363x + 0.0112$ ($r = 0.9999$, edaravone).

Sensitivity was evaluated by determining the LOD and LOQ of the analytes. To determine the LOD, pooled plasma samples were spiked to contain 0.03 μg/ml puerarin and 0.05 μg/ml edaravone and were analysed on 5 different days. To determine the LOQ, pooled plasma samples were spiked to contain 0.05 μg/ml puerarin and 0.1 μg/ml edaravone and were analysed on 5 different days.

The precision and accuracy of the method were determined in rat plasma by performing replicate analyses of spiked samples against calibration standards. The procedure was repeated on the same day and on five different days on the same spiked standard series. The precision (RSD%) was less than 10%. According to FDA guidance for bioanalytical method validation, the data indicated that the precision and accuracy were acceptable.

The recoveries were determined for five replicates of rat plasma spiked with low (0.05 μg/ml), medium (10 μg/ml) and high (50 μg/ml) concentrations of the two analytes. The mean recoveries (%) of puerarin were 102.3 ± 0.84, 101.4 ± 4.72 and 97.49 ± 1.40, and the mean recoveries (%) of edaravone were 104.3 ± 1.27, 94.28 ± 3.32 and 102.0 ± 6.09. The RSD% of puerarin was 0.82–4.65% and the RSD% of edaravone was 1.22–5.97%. The data indicated that the recoveries of puerarin and edaravone over the concentration range were acceptable.

For microdialysis, the relative in-vitro recovery, in-vitro loss and in-vivo loss of puerarin was 27.50, 22.24 and 12.60%, respectively, the relative in-vitro recovery, in-vitro loss and in-vivo loss of edaravone was 16.46, 24.88 and 31.30%, respectively. The in-vivo recovery of puerarin and edaravone was calculated according to Equation 1 to be 15.58% and 20.70%, respectively. The concentrations of puerarin and edaravone in rat CSF were calculated using the in-vivo recovery by multiplying the concentration determined in the microdialysate.

Comparison of pharmacokinetic interaction of puerarin and edaravone

The reversed-phase HPLC method showed satisfactory results for the simultaneous determination of puerarin and edaravone in rat plasma and was successfully used for the investigation of the pharmacokinetic interaction of puerarin and edaravone following intravenous administration and co-administration in rats. The plasma concentration–time profiles for puerarin alone and puerarin co-administered with edaravone are shown in Figure 1. The plasma concentration–time profiles for edaravone alone and edaravone co-administered with puerarin are shown in Figure 2. Pharmacokinetic investigations were performed on rats and the plasma concentration–time data of puerarin and edaravone were best fitted to a two-compartment intravenous open model. The main pharmacokinetic parameters of puerarin alone and puerarin co-administered with edaravone are presented in Table 1. The main pharmacokinetic parameters of edaravone alone and edaravone co-administered with puerarin are presented in Table 2. The pharmacokinetic interaction comparison of puerarin and edaravone from a single dose and a single combination dose in rats using the *t*-test is also presented (Tables 1 and 2).

From the comparison of the main pharmacokinetic parameters between puerarin alone and puerarin co-administered

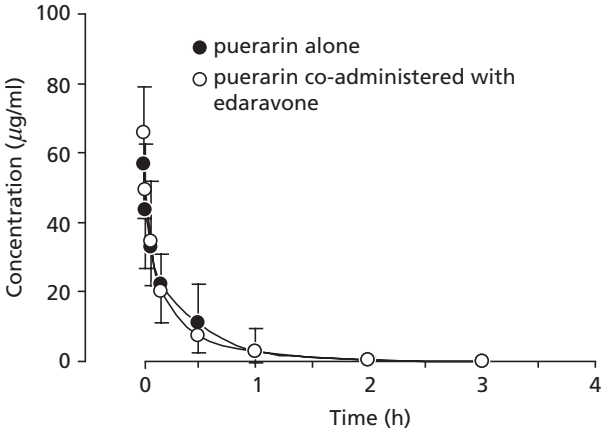

Figure 1 Plasma concentration–time profiles of puerarin. Puerarin (62.5 mg/kg) was administered intravenously to rats in the presence and absence of edaravone (3.75 mg/kg). Values are the mean ± SD, $n = 6$

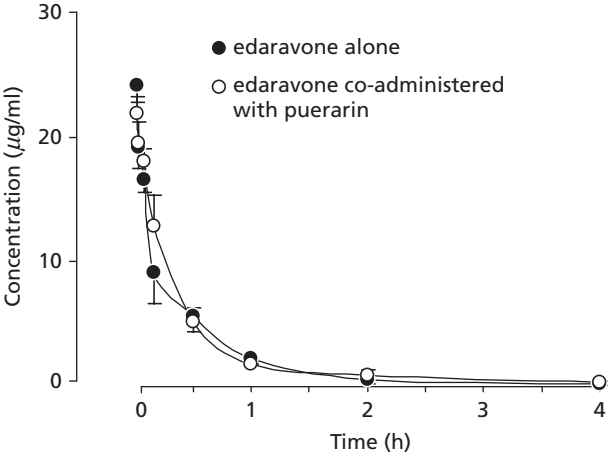

Figure 2 Plasma concentration–time profiles of edaravone. Edaravone (3.75 mg/kg) was administered intravenously to rats in the presence and absence of puerarin (62.5 mg/kg). Values are the mean ± SD, $n = 6$

with edaravone, the values of the half-life of the distribution ($t1/2_\alpha$), the half-life of the elimination ($t1/2_\beta$) and the central volume of distribution (V_c) of puerarin co-administered with edaravone were greater than that of puerarin alone, and there were statistically significant differences in these parameters ($P < 0.05$ or $P < 0.01$). The results showed that the distribution and elimination of puerarin co-administered with edaravone were slower than those for puerarin alone and the distribution in tissues was wider. There was no statistically significant difference in the area under the plasma drug concentration–time curve (AUC) between the two administrations. From the comparison of the main pharmacokinetic parameters between edaravone alone and co-administration with puerarin, the values of $t1/2_\alpha$ and $t1/2_\beta$ of edaravone co-administered with puerarin were notably greater than those of edaravone alone, and there were statistically significant differences in these parameters ($P < 0.01$). The results showed that the distribution and elimination of edaravone co-administered with puerarin were also markedly slower than those for edaravone alone. There was no statistically significant difference in V_c and AUC between the two administrations. From the results above, the combination injection of puerarin and edaravone could significantly increase the values of the $t1/2_\beta$ of each drug and the V_c of puerarin, so the combination of puerarin and edaravone noticeably reduced the rate of elimination and gave a wider distribution, and the disposition of both drugs was optimized.

Table 1 Pharmacokinetic parameters of puerarin in plasma

Parameter	Puerarin	Puerarin + edaravone
V_c (l/kg)	0.80 ± 0.09	1.43 ± 0.16**
$t1/2_\alpha$ (h)	0.05 ± 0.02	0.22 ± 0.03**
$t1/2_\beta$ (h)	0.32 ± 0.03	0.77 ± 0.19*
K_{21} (1/h)	6.47 ± 2.88	1.09 ± 0.45*
K_{10} (1/h)	5.80 ± 0.81	2.87 ± 0.19**
K_{12} (1/h)	7.39 ± 6.02	0.18 ± 0.14
AUC (μg/ml h)	13.80 ± 1.71	15.38 ± 1.59
Cl_s (l/(kg h))	4.59 ± 0.58	4.10 ± 0.42

V_c, apparent volume of distribution at the central compartment; $t1/2_\alpha$, half-life of the distribution; $t1/2_\beta$, half-life of the elimination; K_{21}, rate constant for movement of drug from compartment 2 to compartment 1; K_{10}, elimination rate constant of drug from compartment 1; K_{12}, rate constant for movement of drug from compartment 1 to compartment 2; AUC, area under the plasma drug concentration–time curve; Cl_s, clearance. Puerarin (62.5 mg/kg, i.v.) was administered to rats in the presence and absence of edaravone (3.75 mg/kg, i.v.). Values are mean ± SD, $n = 6$. $^{*}P < 0.05$, $^{**}P < 0.01$, significantly different compared with the puerarin group.

Table 2 Pharmacokinetic parameters of edaravone in plasma

Parameter	Edaravone	Edaravone + puerarin
V_c (l/kg)	0.14 ± 0.02	0.16 ± 0.01
$t1/2_\alpha$ (h)	0.08 ± 0.04	0.17 ± 0.04**
$t1/2_\beta$ (h)	0.61 ± 0.12	1.58 ± 0.51**
K_{21} (1/h)	4.59 ± 2.71	0.94 ± 0.44*
K_{10} (1/h)	3.04 ± 0.29	2.12 ± 0.55**
K_{12} (1/h)	4.67 ± 2.50	1.53 ± 0.77*
AUC (μg/ml h)	8.76 ± 0.89	10.59 ± 1.80
Cl_s (l/(kg h))	0.43 ± 0.05	0.36 ± 0.06

V_c, apparent volume of distribution at the central compartment; $t1/2_\alpha$, half-life of the distribution; $t1/2_\beta$, half-life of the elimination; K_{21}, rate constant for movement of drug from compartment 2 to compartment 1; K_{10}, elimination rate constant of drug from compartment 1; K_{12}, rate constant for movement of drug from compartment 1 to compartment 2; AUC, area under the plasma drug concentration–time curve; Cl_s, clearance. Edaravone (3.75 mg/kg, i.v.) was administered to rats in the presence and absence of puerarin (62.5 mg/kg, i.v.). Values are the mean ± SD, $n = 6$. $^{*}P < 0.05$, $^{**}P < 0.01$, significantly different compared with the edaravone group.

Effect of borneol on the brain distribution kinetics of puerarin

In this study borneol was combined with puerarin and the effect of borneol on the brain distribution kinetics of puerarin was studied in rats. Using in-situ microdialysis sampling,

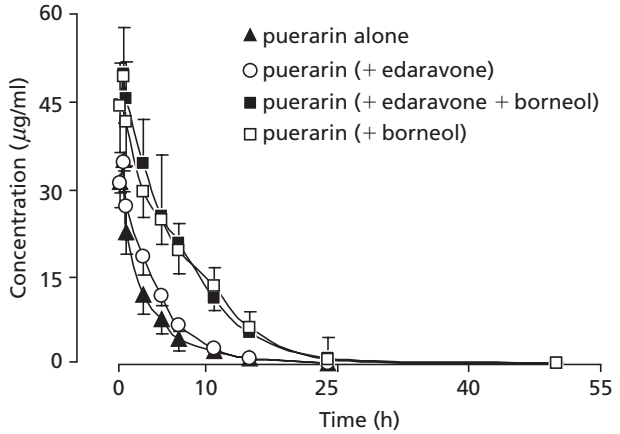

Figure 3 Brain tissue concentration–time profiles of puerarin. Puerarin (62.5 mg/kg) was administered intravenously to rats in the presence and absence of edaravone (3.75 mg/kg), with or without borneol (300 mg/kg) pretreatment. Values are the mean ± SD, $n = 6$

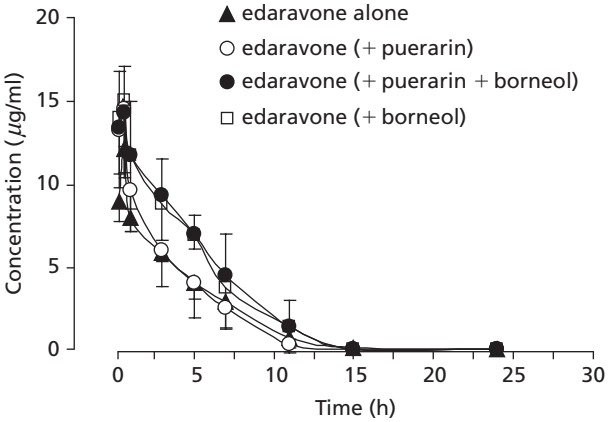

Figure 4 Brain tissue concentration–time profiles of edaravone. Edaravone (3.75 mg/kg) was administered intravenously to rats in the presence and absence of puerarin (62.5 mg/kg), with or without borneol (300 mg/kg) pretreatment. Values are the mean ± SD, $n = 6$

the brain distribution kinetics of puerarin and edaravone following intravenous administration and co-administration in rats were also investigated.

The brain tissue concentration–time profiles of puerarin alone, puerarin co-administered with edaravone, puerarin co-administered with edaravone and pretreated with borneol, and puerarin with borneol pretreatment are showed in Figure 3. The brain distribution kinetic parameters of puerarin are presented in Table 3. The brain tissue concentration–time profiles of edaravone alone, edaravone co-administered with puerarin, edaravone co-administered with puerarin and pretreated with borneol, and edaravone with borneol pretreatment are shown in Figure 4. The brain distribution kinetic parameters of edaravone are presented in Table 4. The comparison of the distribution kinetic interaction of these drugs from a single dose, a single combination dose and a pretreated dose with borneol in rats using the one-way analysis of variance and Dunnett's post-hoc test are also presented (Table 3).

Discussion

Puerarin is usually prescribed in combination with edaravone, but owing to its low lipid solubility, orally administered puerarin undergoes poor absorption, resulting in low bioavailability.[22–27] Although the concomitant administration of puerarin and edaravone is usually via the intravenous route in clinic practice, it is still difficult for puerarin to penetrate the blood–brain barrier.[25,28] The primary objective of this study was to investigate the pharmacokinetic interaction between puerarin and edaravone and the effect of borneol on the brain distribution kinetics of puerarin and on the concomitant use puerarin and edaravone.

The endothelial cells lining brain capillaries are joined to each other by tight junctions to produce an unbroken cell membrane lining that is the main element of the blood–brain barrier. This prevents passive entry into the brain tissue of lipophobic or ionised molecules. Puerarin is a polyhydroxy, hydrophilic drug and the absorption rate and distribution level of puerarin in the brain is relatively low via the oral route, resulting in poor therapeutic efficacy on cerebrovascular disease.[24–28] Generally, the membrane of cells and tissues constitutes a hydrophobic lipid barrier and drug permeation can mainly occur by direct diffusion through the lipid; diffusion through membrane lipids depends on the lipid/water partition coefficient and structure of the drug.[29–30] Borneol is a monoterpene compound and highly lipid-soluble; borneol easily penetrates the blood–brain barrier and also promotes

Table 3 Brain distribution kinetic parameters of puerarin

Parameter	Puerarin	Puerarin + edaravone	Puerarin + edaravone + borneol	Puerarin + borneol
V_c (l/kg)	1.60 ± 0.23	1.66 ± 0.11	3.80 ± 3.08	1.18 ± 0.44
$t_{1/2\alpha}$ (h)	0.97 ± 0.32	2.26 ± 1.23	$4.61 \pm 0.35^{**\dagger}$	$4.80 \pm 0.26^{**\dagger\dagger}$
$t_{1/2\beta}$ (h)	3.67 ± 0.41	3.18 ± 0.36	$7.28 \pm 2.51^{**\dagger\dagger}$	$5.97 \pm 1.73^{*\dagger}$
AUC (μg/ml h)	117.6 ± 11.92	149.9 ± 13.89	$354.4 \pm 49.81^{**\dagger\dagger}$	$341.8 \pm 57.92^{**\dagger\dagger}$
Cl_s (l/(kg h))	0.54 ± 0.05	0.42 ± 0.04	0.55 ± 0.43	$0.19 \pm 0.04^{**\dagger\dagger}$

V_c, apparent volume of distribution at the central compartment; $t_{1/2\alpha}$, half-life of the distribution; $t_{1/2\beta}$, half-life of the elimination; AUC, area under the plasma drug concentration–time curve; Cl_s, clearance. Puerarin was administered intravenously at a dose of 62.5 mg/kg to rats. Values are the mean ± SD, $n = 6$. $^{*}P < 0.05$, $^{**}P < 0.01$, significantly different compared with the puerarin group; $^{\dagger}P < 0.05$, $^{\dagger\dagger}P < 0.01$, significantly different compared with the puerarin + edaravone group.

Table 4 Brain distribution kinetic parameters of edaravone

Parameter	Edaravone	Edaravone + puerarin	Edaravone + puerarin + borneol	Edaravone + borneol
V_c (l/kg)	0.34 ± 0.19	0.25 ± 0.19	0.23 ± 0.36	0.23 ± 0.27
$t_{1/2\alpha}$ (h)	3.14 ± 2.06	2.50 ± 0.79	3.05 ± 0.95	2.93 ± 0.86
$t_{1/2\beta}$ (h)	3.20 ± 1.93	2.67 ± 1.11	3.19 ± 1.58	3.03 ± 1.01
AUC (μg/ml h)	50.83 ± 28.77	53.94 ± 33.26	71.14 ± 31.07	70.29 ± 56.78
Cl_s (l/(kg h))	0.07 ± 0.03	0.07 ± 0.06	0.05 ± 0.05	0.05 ± 0.04

V_c, apparent volume of distribution at the central compartment; $t½_\alpha$, half-life of the distribution; $t½_\beta$, half-life of the elimination; AUC, area under the plasma drug concentration–time curve; Cl_s, clearance. Edaravone was administered intravenously at dose of 3.75 mg/kg to rats. Values are the mean ± SD, $n = 6$.

many other drugs into brain tissue.[31–33] Borneol loosens the intercellular tight junctions of the tissue of the blood–brain barrier and accelerates the transportation of some substances, especially hydrophilic substances, resulting in enhanced absorption of some drugs.[34–36]

From the comparison of the main brain distribution kinetic parameters among the puerarin alone group, the puerarin plus edaravone group, the puerarin plus edaravone with borneol pretreatment group, and the puerarin with borneol pretreatment group, the values of $t½_\alpha$, $t½_\beta$ and AUC of the puerarin with borneol pretreatment group and the puerarin plus edaravone with borneol pretreatment group were obviously greater than that of the puerarin alone group or the puerarin plus edaravone group, and there were statistically significant differences among these parameters ($P < 0.05$ or $P < 0.01$). The results indicated that the rates of distribution and elimination of the puerarin with borneol pretreatment group and the puerarin plus edaravone with borneol pretreatment group were obviously slower than those of the puerarin alone group and the puerarin plus edaravone group. There was also a statistically significant difference in the AUC between the with borneol pretreated group and the group without borneol pretreatment ($P < 0.05$ or $P < 0.01$). These results suggest that the distribution of puerarin in brain tissue was significantly increased with borneol pretreatment; concurrent use of borneol effectively enhanced the effect of puerarin and prolonged drug action, thus reducing the frequency of administration. The data also showed that co-administration of edaravone did not significantly alter the brain distribution kinetic parameters of puerarin compared with the administration of puerarin alone.

From the comparison of the main brain distribution parameters among the edaravone alone, the edaravone plus puerarin, the edaravone plus puerarin with borneol pretreatment, and the edaravone with borneol pretreatment groups, there were no statistically significant differences in the main brain distribution kinetic parameters among the four groups. The data indicated that co-administration of puerarin and pretreatment with borneol did not markedly alter the brain distribution kinetic parameters of edaravone compared with the administration of edaravone alone.

From the results above, the combination of puerarin and edaravone noticeably reduced the rate of elimination and gave a wider distribution, and the disposition of both drugs was optimized. In brain tissue, the distribution of puerarin was significantly increased and the elimination was slower with borneol pretreatment.

Conclusions

A reversed-phase HPLC method was successfully applied to characterize the pharmacokinetic interaction of puerarin and edaravone in rats. In plasma, the combined administration of puerarin and edaravone significantly altered the half-lives of each drug. In the brain however, significant changes were observed only with puerarin. The combination of puerarin and edaravone reduced drug elimination rates, gave a wider distribution and the disposition of both drugs was optimized. The distribution of puerarin in brain tissue was significantly increased and the elimination of puerarin was slower with borneol pretreatment. The combined application of borneol, edaravone and puerarin increased the concentration of puerarin in the brain. The results provide an important basis for the combination of these drugs in clinical practice, especially in the treatment of ischaemic encephalopathy. The clinical importance of these findings requires further investigation in clinical trials.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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