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In-vivo and in-vitro studies on the effect of Huang-Lian-Jie-Du-Tang on nimodipine transport across rat blood–brain barrier

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Abstract

Huang-Lian-Jie-Du-Tang (HLJDT), an aqueous extract of *Rhizoma Coptidis, Radix Scutellariae, Cortex Phellodendri* and *Fructus Gardeniae* (3:2:2:3) is an important multi-herb remedy in traditional Chinese medicine (TCM). The aim of this study was to evaluate the effect of HLJDT on nimodipine transport across rat blood–brain barrier (BBB). It was found that in-vivo the brain concentrations of nimodipine significantly increased when rats were pretreated with HLJDT. In-vitro, the serum of HLJDT-treated rats increased the accumulation of nimodipine in primary cultured rat brain microvessel endothelial cells (rBMECs) and decreased the expression of P-glycoprotein (P-gp) on rBMECs. Our previous study showed that the peak concentration of baicalin and berberine in rats after administration of HLJDT was 5 μg mL^{-1} and 10 ng mL^{-1}, respectively. This study showed that 5 μg mL^{-1} baicalin significantly increased the accumulation of nimodipine in rBMECs, while 10 ng mL^{-1} berberine had no effect on the accumulation of nimodipine in rBMECs. Both the in-vivo and in-vitro experimental findings indicated that HLJDT pretreatment may alter the transport of nimodipine across rat BBB.

Introduction

Nimodipine is a dihydropyridine calcium-channel blocker and it is used currently to prevent and treat the ischaemic damage caused by cerebral arterial spasm in subarachnoid haemorrhage (Ljunggren et al 1987). Nimodipine has also been used in other cerebrovascular disorders, such as ischaemic stroke (Mohr et al 1994) and multi-infarct dementia (Pantoni et al 2000).

Huang-Lian-Jie-Du-Tang (HLJDT) is an important multi-herb remedy in traditional Chinese medicine (TCM) and was first described by Wang Tao (in the Chinese Tang Dynasty) in his treatise entitled Wai Tai Mi Yao. The traditional remedy is an aqueous extract of *Rhizoma Coptidis* (*Coptis chinensis* Franch, Ranunculaceae), *Radix Scutellariae* (*Scutellaria baicalensis* Georgi, Labiatae), *Cortex Phellodendri* (*Phellodendron amurense* Rupr, Rutaceae) and *Fructus Gardeniae* (*Gardenia jasminoides* Ellis, Rubiaceae) in the ratio 3:2:2:3 and all the herbs are officially listed in the Chinese Pharmacopoeia. There have been many reports on the pharmacological activity of HLJDT with respect to gastrointestinal disorders (Takase et al 1991a, b; Yamasaki et al 1998), inflammatory disease (Mizukawa et al 1993; Wang & Mineshita 1996), acute liver injury (Ohta et al 1997, 1998), vasorelaxation (Higasa et al 1992) and other cardiovascular diseases (Fujiwara & Iwasaki 1993). Recently, the efficacy of this decoction on central nervous system disorders has been attracting attention. HLJDT has been used for the treatment of various clinical symptoms associated with hypertension and cerebral apoplexy. It has a protective effect against impairment of learning and memory function induced by transient cerebral ischaemia in mice (Xu et al 2000). Besides, it protects against ischaemic neuronal death and reduces the exposure of hippocampal neurons to oxidative stress (Kondo et al 2000). It has also been reported to have a protective effect on ischaemia–reperfusion brain injury (Hwang et al 2002).

As both nimodipine and HLJDT have beneficial effects on brain ischaemic damage, the opportunity to combine them would seem attractive. A major concern is that a herb–drug

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interaction may occur. A significant increase in the maximum plasma concentration (C_{max}) and area-under-the-curve (AUC) of nimodipine when co-administered with grapefruit juice to healthy subjects was reported and a more pronounced haemodynamic response of nimodipine was observed in the grapefruit-treated group (Fuhr et al 1998). The metabolism of nimodipine is mediated primarily by cytochrome P-450 (CYP3A4) and numerous herb–drug interactions mediated by CYP3A4 have been reported (Dhananjay & Ashim 2006; Raman et al 2006). However, little information focuses on whether the transport of nimodipine across rat blood–brain barrier (BBB) would be affected by herbs. As brain permeability is a key factor in the therapeutics of central nervous system disorders, the transport of nimodipine across rat BBB is of great value for the reason that the brain is the target organ in many diseases that nimodipine treats.

In this study, we evaluated the effect of HLJDT on the transport of nimodipine across rat BBB. We also conducted in-vitro studies using primary cultured rat brain microvessel endothelial cells (rBMECs), to investigate whether HLJDT affected the transport of nimodipine across rat BBB.

Materials and Methods

Reagents

Huanglian (*Rhizoma Coptidis*), Huangqin (*Radix Scutellariae*), Huangbai (*Cortex Phellodendri*) and Zhizi (*Fructus Gardeniae*) were purchased from Kai-Xin Herbal Shop (Nanjing, China) and identified by Doctor Li-Na Chen (Department of Pharmacognosy, China Pharmaceutical University, Nanjing, China). Nimodipine and felodipine were provided by Shandong Xinhua Pharmaceutical Factory (Shandong, China). Baicalin and berberine standards were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Rhodamine-123 (Rho123) was purchased from Sigma-Aldrich (St Louis, MO). All other chemicals were of analytical grade and commercially available.

Animals

The animal studies were approved by the Animal Ethics Committee of China Pharmaceutical University. Male Sprague–Dawley rats, 220–250 g, were supplied by Sino-British Sippr/BK Lab Animal Ltd (Shanghai, China). The rats were maintained in an air-conditioned animal quarter at a temperature of 22 ± 2°C and a relative humidity of 50 ± 10%. Water and food (laboratory rodent chow, Nanjing, China) were freely accessible. The rats were acclimatized to the facilities for five days, and then fasted with free access to water for 12 h before each experiment. The neonates (7–10 days old) of Sprague–Dawley rats were supplied by the Center of Experimental Animals, China Pharmaceutical University.

Preparation of HLJDT extract powder

Huanglian 30 g, Huangqin 20 g, Huangbai 20 g and Zhizi 30 g were extracted twice by refluxing with boiling water (1:10 and then 1:5, w/v) for 1 h, and the solution obtained was concentrated to give an extract (21.9 g). The dried powder was stored at 4°C before use.

Content of baicalin, wogonoside, berberine and palmatine in HLJDT extract powder

Assay of the main ingredients in HLJDT dried powder was carried out according to previous reports (Lu et al 2005, 2006). As baicalin and wogonoside are the two main effective ingredients in *Radix Scutellariae*, while berberine and palmatine are the phytochemical markers for the quality control of *Rhizoma Coptidis*, we determined the contents of them in HLJDT extract powder using HPLC methods. For baicalin and wogonoside, HPLC conditions were as follows: the column was a Shimadzu Shim-pack VP-ODS column (150 mm × 4.6 mm, 5 μm); the mobile phase consisted of A (0.05% phosphoric acid and 5 mM monosodium phosphate) and B (acetonitrile) (63:37, v/v); the detection wavelength was set at 276 nm. For berberine and palmatine, HPLC separation was achieved using a Diamonsil ODS (250 mm × 4.6 mm, 5 μm) column maintained at 40°C. The mobile phase was composed of A (25 mM potassium dihydrogen phosphate and 5 mM heptyl sodium sulfate, pH adjusted to 3.0 with phosphoric acid) and B (acetonitrile) (68:32, v/v). The analytical wavelength was 345 nm.

In-vivo pharmacokinetic experiments

Sixty rats were randomly divided into two groups of thirty. One group was given intravenous nimodipine 2 mg kg^{-1} and the other group was given intravenous nimodipine 2 mg kg^{-1} 8 h after the administration of HLJDT (4.38 g kg^{-1}). The dose of HLJDT and the 8-h time interval were chosen according to previous research in our lab (Lu et al 2005, 2006). Five rats in each group were sacrificed by femoral artery bleeding at 5, 10, 20, 40, 60 and 90 min after injection of nimodipine under ether anaesthesia. Heparinized blood was collected and centrifuged to obtain plasma. The brain was immediately removed, sliced, blotted and weighed, and then was homogenized in 1 mL distilled water. The plasma and brain tissue samples were stored at −20°C until assays.

Isolation and culture of rBMECs

Primary rBMECs isolation and culture were operated according to a method previously used in our laboratory (Zhang et al 2003). The primary cultured cells were identified as capillary endothelial cells by an immunostaining method using Factor-VIII related antigen (Audus et al 1986). The rBMECs were cultured in Dulbecco's modified Eagle medium (high glucose) (DMEM) and Nutrient mixture F-12 Ham's (F-12) supplemented with 20% cosimic calf serum under the conditions of 37°C with 95% air and 5% CO_2. Uptake experiments were performed in 24-well plates.

Cellular uptake of nimodipine and rhodamine-123 by primary cultured rBMECs

When cells reached confluence in 12–14 days, uptake experiments were performed. Cultured rBMECs were pre-incubated

at 37°C in 1 mL buffer (0.137 M NaCl, 5.37 mM KCl, 1.26 mM $CaCl_2$, 0.81 mM $MgSO_4 \cdot 7H_2O$, 0.37 mM $Na_2HPO_4 \cdot H_2O$, 0.44 mM KH_2PO_4, 4.17 mM $NaHCO_3$ and 2.92 mM D-glucose) for 30 min. After the pre-incubation, the solution was removed, and the buffer (1 mL) containing nimodipine (10 mg L^{-1}) (or rhodamine-123, Rho123, 0.1 mg L^{-1}) in the absence and presence of test agents was added to each incubation well. The uptake was terminated at 90 min for nimodipine (Zhang et al 2003) and 120 min for Rho123 (Zhang et al 2007) by washing cells three times with 1 mL of ice-cold buffer. Then the blank buffer (0.3 mL) was added to each incubated well, frozen and melted repeatedly four times to break cells. Protein content in cultured cells was measured by the method of Bradford (1976) using bovine serum albumin as the standard. Net uptake, expressed as the nimodipine concentration ratio (ng (μg protein)$^{-1}$), was obtained by dividing the apparent uptake amount of nimodipine (ng mL^{-1}) by protein content ((μg protein) mL^{-1}).

Analysis for concentration of nimodipine and Rho123

The plasma and brain homogenate were analysed for nimodipine concentration by HPLC (Liu et al 2003). Internal standard (felodipine, 10 μg mL^{-1}, 10 μL), 2 M NaOH (200 μL), and 5 mL of ether–cyclohexane (1:1) were added to the plasma and brain homogenate sample (1 mL) and mixed vigorously. After centrifugation (3500 rev min^{-1}, 5 min), the supernatant was transferred to another tube and evaporated to dryness under N_2. The residue was reconstituted in 120 μL of mobile phase and 20 μL was injected onto a HPLC system (LC-10AT, Shimadzu, Japan), which was equipped with a VP-ODS column (4.6 × 150 mm, 5 μm; Shimadzu) and a UV-detector (SPD-10Avp; Shimadzu, Japan). The mobile phase consisted of MeOH–H_2O (70:30, v/v), the flow rate was set at 1.0 mL min^{-1} and the analytical wavelength was 238 nm. Linear regression was calibrated based on the peak-area ratio of nimodipine to the internal standard. The LLOQ (lowest limit of quantification) was 5 ng mL^{-1} and a good linearity was obtained over the range 5–1000 ng mL^{-1}. The extraction recovery of nimodipine was 85–90%.

The concentration of nimodipine in cell suspension was measured as for plasma. The concentrations of Rho123 in cell suspension was determined by HPLC (Ando et al 2001). Twenty microlitres of cell suspension were injected into a Shimadzu LC-10A_{VP} system consisting of an LC-10A liquid pump, a CTO-10AS_{VP} column oven and a fluorescence detector (RF-10A_{XL}) set at an excitation wavelength of 485 nm and emission wavelength of 565 nm. Conditions were as follows: column, a Shim-pack ODS (4.6 μm, 150 mm × 4.6 mm i.d.) (Shimadzu, Japan); mobile phase, 1‰ HAc (pH 4.0)–acetonitrile (3:2, v/v); column temperature, 40°C; flow rate, 1 mL min^{-1}. The LLOQ of Rho123 was 0.5 ng mL^{-1}. The linear range of Rho123 was 0.5–50 ng mL^{-1}.

Effect of serum from HLJDT-treated rats on NMD accumulation in rBMECs

It is well known that the effective ingredients of TCM in-vivo may not always be the same ingredients as in raw medical material. For this reason, the serum obtained from HLJDT-treated rats was used instead of HLJDT decoction in-vitro in order to simulate the true in-vivo state as much as possible. The serum obtained from vehicle-treated rats was designed as control. Rats were sacrificed by femoral artery bleeding under ether anaesthesia 8 h after oral administration of vehicle (control) or HLJDT (4.38 g kg^{-1}) to obtain blank or drug-containing serum. The serum (1 mL) was pretreated with acetone (3 mL) to remove protein, evaporated to dryness under N_2, reconstituted in 1 mL buffer solution, then nimodipine was added to obtain a concentration of 10 mg L^{-1}. The uptake experiment procedure was the same as described in the section on cellular uptake of nimodipine by primary cultured rBMECs, above.

Effects of serum from HLJTD-treated rats on rBMEC cytoactivity

To investigate whether serum from rats affected cell cytoactivity, cells were incubated for 24 h with 20% serum from HLJTD-treated or vehicle-treated rats. Cell activity was measured using 3-(4, 5-dimethyl-2thiazoyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) assay. Cells incubated with 20% cosimic calf serum were set as the control group. No differences ($P > 0.05$) were found relative to the control group (data not shown). Thus, we believed that the serum from rats did not damage the cells.

Effect of serum from HLJDT-treated rats on function and expression of P-gp in rBMECs when co-cultured with the serum for 24 h

A P-glycoprotein (P-gp) substrate, Rho123, was used as positive control to evaluate the P-gp function, and western blot was adopted to measure the P-gp protein expression on rBMECs. The rBMECs were cultured with 20% serum from HLJDT-treated rats for 24 h. Some cells were used to measure uptake of Rho123 and the remainder were used to measure P-gp protein expression using western blot.

Western blot analysis of P-gp expression in rBMECs

The cultured cells (density 1×10^6 mL^{-1}) were homogenized in ice-cold cell lysis buffer containing 10 mM Tris-HCl (pH 7.5), 1 mM EGTA, 1 mM $MgCl_2$, 1 mM mercaptoethanol, 1% glycerol and protease inhibitor cocktail (1 mM dithiothreitol, 2 mM phenylmethylsulfonylfluoride; Sigma Chemical Co. Ltd, St Louis, MO). The homogenate was centrifuged at 15 000 rev min^{-1} g for 10 min at 4°C. The soluble fractions were obtained as membrane fractions for western blot. The protein concentration in the solution was measured by the Bio-Rad Protein Assay (Bio-Rad Labs, Richmond, CA). A portion of tissue sample was diluted with a volume of 4 × sodium dodecyl sulfate (SDS) sample buffer containing 0.1 M Tris-HCl (pH 6.8), 4% SDS, 200 mM DTT, mercaptoethanol, 20% glycerol and 0.2% bromophenol blue. Proteins (25 μg/lane) were separated by electrophoresis on 8% SDS-polyacrylamide gel. After electrophoresis, the proteins were electrophoretically

transferred to a nitrocellulose membrane. The membrane was blocked in phosphate-buffered saline (PBS) containing 0.1% Tween-20 and 5% dried skimmed milk for 60 min at room temperature and washed three times for 15 min in PBS containing 0.1% Tween-20. Then the membrane was incubated with the primary monoclonal antibody C219 (Calbiochem-Novabiochem, Seattle, WA), diluted 200 fold in PBS containing 0.1% Tween-20, overnight at 37°C. After washing the membrane with PBS containing 0.1% Tween-20, it was incubated in the appropriate HRP-conjugated goat anti-mouse secondary antibody at room temperature for another 1 h and washed again three times in PBS containing 0.1% Tween-20. The transferred proteins were incubated with ECL substrate solution (Cell Signaling, USA) for 5 min according to the manufacturer's instructions and visualized with autoradiography X-film. The relative expressions were quantified densitometrically by using the quantity one software (Bio-Rad Labs, Richmond, CA) and calculated according to the reference bands of β-actin (Boshide Biotech Co.,Wuhan, China).

Data analysis

Data are expressed as mean ± standard deviation (s.d.). For in-vivo studies, area under the concentration–time curve (AUC^{90}) was calculated by the linear trapezoidal method. Half-life (t½) and k_e were calculated using one-compartmental analysis. The maximum concentration (C_{max}) was obtained from the observed data. Statistical analysis was performed by using the repeated measure analysis of variance or Student's *t*-test. $P < 0.05$ was considered statistically significant.

Results and Discussion

Content of baicalin, wogonoside, berberine and palmatine in HLJDT extract powder

The content of baicalin and wogonoside was determined to be 4.42 g and 1.08 g in 100 g HLJDT decoction extract, respectively. The content of berberine and palmatine was 5.6 g and 1.36 g per 100 g extract, respectively.

Effect of HLJDT on distribution of nimodipine in rat brain

The concentration vs time profiles of nimodipine in plasma and brain following 2 mg kg^{-1} intravenous nimodipine were measured (Figure 1). Concentrations of nimodipine in the plasma of HLJDT-treated rats were significantly higher than those in control rats at 10, 20 and 60 min following intravenous nimodipine. Similar to plasma, the concentrations of nimodipine in the brain of HLJDT-treated rats were higher than those in control rats. The corresponding pharmacokinetic parameters were estimated (Table 1). The AUC^{90} values of nimodipine in the plasma and brain of HLJDT-treated rats were higher than those in control rats and the ratio of brain AUC^{90}/plasma AUC^{90} in HLJDT-treated rats was higher than that in control rats. This indicated that HLJDT may increase the transport of nimodipine across the BBB.

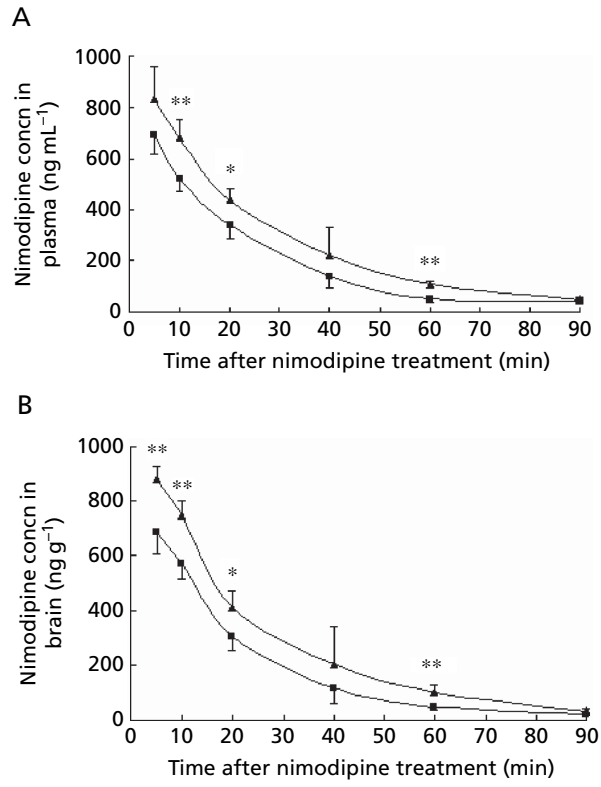

Figure 1 Effect of HLJDT on nimodipine concentration in rat plasma (A) or brain (B). Each point represents the mean ± s.d. of five rats. HLJDT (4.38 g kg^{-1}) or the vehicle was orally administered 8 h before intravenous injection of nimodipine (2 mg kg^{-1}). ▲, HLJDT-treated group; ■, control. *$P < 0.05$, **$P < 0.01$ vs control.

Table 1 Effect of HLJDT (4.38 g kg^{-1}) on pharmacokinetic parameters of nimodipine in rats

	Control group	HLJDT-treated group
Plasma		
C_{max} (ng mL^{-1})	696.44	828.93
AUC^{90} (ng min mL^{-1})	17232	23678
t½ (min)	15.91	19.19
Brain		
C_{max} (ng g^{-1})	686.44	876.03
AUC^{90} (ng min g^{-1})	16155	23260
t½ (min)	14.04	16.93
AUC^{90} (brain)/AUC^{90} (plasma) (mL g^{-1})	0.93	0.98

Effect of serum from HLJDT-treated rats on nimodipine accumulation in rBMECs

The steady-state uptake of nimodipine was significantly higher in the serum of HLJDT-treated rats than that of control rats (Figure 2). This indicated that there were some compounds in the serum of HLJDT-treated rats that could

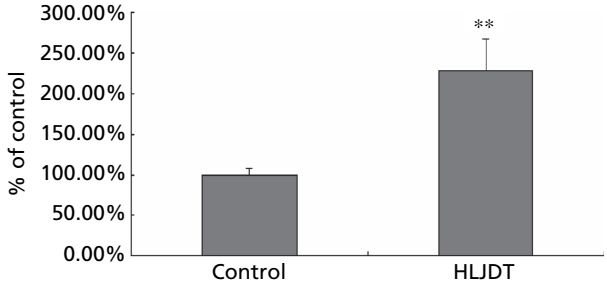

Figure 2 Effect of serum obtained from HLJDT-treated rats on the steady-state uptake of nimodipine by primary cultured rBMECs. Control, serum from control rats; HLJDT, serum from HLJDT-treated rats. The amount of nimodipine taken up in the control group was 5.81 ng (μg protein)$^{-1}$ (n = 5). $^{**}P < 0.01$ vs control.

increase the accumulation of nimodipine in primary cultured rBMECs.

Baicalin is used as a phytochemical marker for the quality control of *Radix Scutellariae*, a key ingredient herb in HLJDT, in the Chinese Pharmacopoeia. Berberine, which belongs to the quaternary protoberberine-type alkaloids family, is the phytochemical marker for the quality control of *Rhizoma Coptidis*, another ingredient in HLJDT, in the Chinese Pharmacopoeia. In our previous research, we evaluated the effect of baicalin and berberine on nimodipine uptake by primary cultured rBMECs in-vitro (Zhang et al 2007). The results of that research showed that baicalin (2–5 μg mL^{-1}) increased the uptake of nimodipine and baicalin (10–20 μg mL^{-1}) decreased the uptake, while berberine (10 ng mL^{-1}) had no effect.

Considering that the peak concentration of baicalin and berberine after oral administration of HLJDT (4.38 g kg^{-1}) in rats was about 5 μg mL^{-1} (Lu et al 2005) and 6 ng mL^{-1} (Lu et al 2006), respectively, we may speculate that baicalin increased the distribution of nimodipine to brain tissue in-vivo, while berberine had no effect on the distribution of nimodipine to the brain. So the increased transport of nimodipine across the BBB observed in-vivo may be, at least partly, caused by baicalin.

Effect of serum from HLJDT-treated rats on function and expression of P-gp in rBMECs when co-cultured with the serum for 24 h

The amount of nimodipine uptake by rBMECs incubated for 24 h in serum from HLJDT-treated rats was significantly higher than that by the rBMECs incubated in serum from vehicle-treated rats (Table 2).

The transport of nimodipine across rBMECs was reported to be restricted by P-gp (Zhang et al 2003). To clarify whether the effect of serum from HLJDT-treated rats on nimodipine uptake was via P-gp, a P-gp substrate (Rho123) was used as positive control to evaluate the P-gp function, and western blot was adopted to measure the P-gp protein expression on rBMECs. It was found that the amount of Rho123 uptake by rBMECs incubated for 24 h in serum from HLJDT-treated rats was significantly higher than that by the rBMECs incubated in serum from vehicle-treated rats (Table 2). Western blotting showed that P-gp protein expression in rBMECs incubated for 24 h in serum from HLJDT-treated rats was down-regulated compared with that in rBMECs incubated in serum from vehicle-treated rats (Figure 3). These two consistent results indicated that the effect of serum from HLJDT-treated rats on nimodipine transport may partly be due to the regulation of P-gp function and expression on rBMECs.

Table 2 Effect of serum obtained from HLJDT-treated rats (incubated with rBMECs for 24 h) on the steady-state amount of nimodipine and Rho123 taken up by primary cultured rBMECs (n = 5)

	NMD (ng (μg protein)$^{-1}$)		Rho123 (ng (μg protein)$^{-1}$)	
	Control group	HLJDT-treated group	Control group	HLJDT-treated group
Mean	5.87	9.80*	0.21	0.26**
s.d.	2.01	1.66	0.01	0.01

$^{*}P < 0.05$ vs control; $^{**}P < 0.01$ vs control.

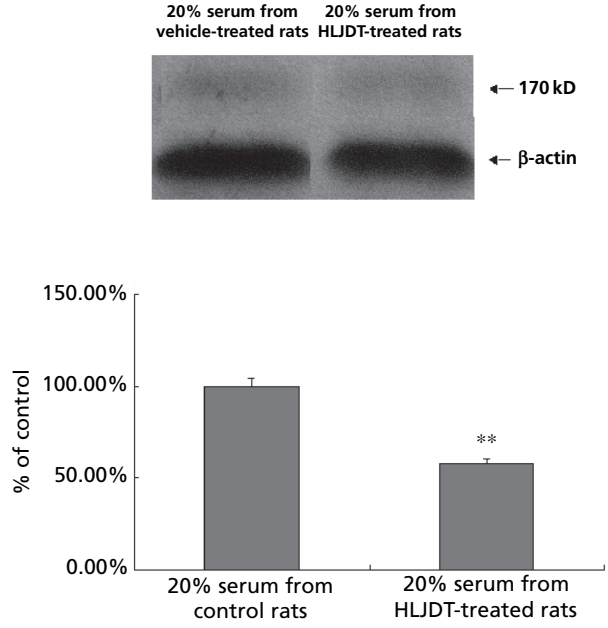

Figure 3 Effect of serum obtained from HLJDT-treated rats (incubated with rBMECs for 24 h) on P-gp protein expression on primary cultured rBMECs (n = 3). $^{**}P < 0.01$ vs control.

Conclusions

The findings suggested that pre-treatment with HLJDT may alter nimodipine distribution in rat brain. Baicalin might be one of the compounds in HLJDT that increased the transport of nimodipine across the BBB. Co-culture of rBMECs with serum from HLJDT-treated rats for 24 h down-regulated the expression of P-gp. Nowadays herb–drug interactions are of

great interest. Most studies focus on metabolism-mediated interactions. Few reports on herb–drug interactions occurring in BBB were found. This study found that HLJDT affected nimodipine transport across rat BBB both in-vivo and in-vitro. However, whether the same interaction will occur in the clinical setting still needs further research.

References

Ando, H., Nishio, Y., Ito, K., Nakao, A., Wang, L., Zhao, Y. L. (2001) Effect of endotoxin on P-glycoprotein mediated biliary and renal excretion of rhodamine-123 in rats. *Antimicrob. Agents. Chemother*. **45**: 3462–3467

Audus, K. L., Borchardt, R. T. (1986) Characterization of an in vitro blood-brain barrier model system for studying drug transport and metabolism. *Pharm. Res*. **3**: 81–87

Bradford, M. M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem*. **72**: 248–254

Dhananjay P., Ashim K. (2006) MDR- and CYP3A4-mediated drug–herbal interactions. *Life Sci*. **78**: 2131–2145

Fuhr, U., Maierbruggemann, A., Blume, H., Muca, W., Unger, S., Kuhcmann, J. (1998) Grapefruit juice increases oral nimodipine bioavailability. *Int. J. Clin. Pharmacol. Ther*. **36**: 126–132

Fujiwara, M., Iwasaki, K. (1993) Toki-shakuyaku-San and Oren-Gedoku-To improve the disruption of spatial cognition induced by cerebral ischemia and central cholinergic dysfunction in rats. *Phytother. Res*. **7**: S60–S62

Higasa, K., Hatake, K., Higasa, M., Hishida, S. (1992) Vasorelaxant effects of Oren-gedoku-to and its four constituent herbs. *J. Med. Pharm. Soc*. **9**: 169–174

Hwang, Y., Shin, C., Huh, Y., Ryu, J. (2002) Hwangryun-Hae-Dok-tang (Huanglian-Jie-Du-Tang) extract and its constituents reduce ischemia-reperfusion brain injury and neutrophil infiltration in rats. *Life Sci*. **71**: 2105–2117

Kondo, Y., Kondo, F., Asanuma, M., Tanaka, K., Ogawa, N. (2000) Protective effect of oren-gedoku-to against induction of neuronal death by transient cerebral ischemia in the C57BL/6 mouse. *Neurochem. Res*. **25**: 205–209

Liu, X., Zhang, L., Xie, L. (2003) Effect of P-glycoprotein inhibitors erythromycin and cyclosporin A on brain pharmacokinetics of nimodipine in rats. *Eur. J. Drug Metab. Pharmacokinet*. **28**: 309–313

Ljunggren, B., Brandt, L., Saveland, H., Romner, B., Ryman, T., Anderson, K. E. (1987) Aneurysmal subarachnoid hemorrhage: prevention of delayed ischemic dysfunction with intravenous nimodipine. *Neurosurg. Rev*. **10**: 255–263

Lu, T., Song, J., Xie, L., Wang, G., Liu, X. (2005) Simultaneous determination of baicalin and wogonoside by HPLC in rat plasma administrated with Huangqin decoction and its pharmacokinetic study. *Zhong Cao Yao*. **36**: 870–873

Lu, T., Liang, Y., Song, J., Xie, L., Wang, G., Liu, X. (2006) Simultaneous determination of berberine and palmatine in rat plasma by HPLC-ESI-MS after oral administration of traditional Chinese medicinal preparation Huang-Lian-Jie-Du decoction and the pharmacokinetic application of the method. *J. Pharm. Biomed. Anal*. **40**: 1218–1224

Mizukawa, H., Yoshida, K., Honmura, A., Uchiyama, Y., Kaku, H., Nakajima, S., Haruki, E. (1993) The effect of orengedokuto on experimentally-inflamed rats. *Am. J. Chin. Med*. **21**: 71–78

Mohr, J. P., Orgogozo, J. M., Harrison, M. J. G., Hennerici, M., Wahlgren, N. G., Gelmers, H. I. (1994) Meta-analysis of oral nimodipine trials in acute ischemic stroke. *Cerebrovasc. Dis*. **4**: 177–210

Ohta, Y., Sasaki, E., Nishida, K., Hayashi, T., Nagata M., Ishiguro, I. (1997) Preventive effect of Oren-gedoku-to (Huang-lian-jie-du-tang) extract on progression of carbon tetrachloride-induced acute liver injury in rats. *Am. J. Chin. Med*. **25**: 57–68

Ohta, Y., Sasaki, E., Nishida, K., Kongo, M., Hayashi, T., Nagata, M., Ishiguro, I. (1998) Inhibitory effect of Oren-gedoku-to (Huanglian-jie-du-tang) extract on hepatic triglyceride accumulation with the progression of carbon tetrachloride-induced acute liver injury in rats. *J. Ethnopharmacol*. **61**:75–80

Pantoni, L., Bianchi, C., Beneke, M., Inzitari, D., Wallin, A., Erkinjuntti, T. (2000) The Scandinavian multi-infarct dementia trial: a double-blind, placebo-controlled trial on nimodipine in multi-infarct dementia. *J. Neurol. Sci*. **175**: 116–123

Raman, V., Bernard, K. Stephen, S. (2006) In vitro and in vivo assessment of herb drug interactions. *Life Sci*. **78**: 2105–2115

Takase, H., Inoue, O., Saito, Y., Yumioka, E., Suzuki, A. (1991a) Roles of sulfhydryl compounds in the gastric mucosal protection of the herb drugs composing oren-gedoku-to (a traditional herbal medicine). *Japan. J. Pharmacol*. **56**: 433–439

Takase, H., Tatsumi, Y., Miura, O., Yumioka, E., Suzuki, A. (1991b) The mechanism of the inhibitory effects of oren-gedoku-to (OGT) on gastric acid secretion in rats. *Fol. Pharmacol. Japon*. **97**: 97–103

Wang, L. M., Mineshita, S. (1996) Preventive effects of unsei-in and oren-gedoku-to, Chinese traditional medicines, against rat paw oedema and abdominal constriction in mice. *J. Pharm. Pharmacol*. **48**: 327–331

Xu, J., Murakami, Y., Matsumoto, K., Tohda, M., Watanable, H., Zhang, S., Yu, Q., Shen, J. (2000) Protective effect of orengedoku-to (Huang-Lian-Jie-Du-Tang) against impairment of learning and memory induced by transient cerebral ischemia in mice. *J. Ethnopharmacol*. **73**: 405–413

Yamasaki, K., Kajimura, K., Nakano, M., Yokoyama, H., Yoneda, K., Umezawa, C., (1998) Effects of preparations of Chinese medicinal prescriptions on digestive enzymes in vitro and in vivo. *Biol. Pharm. Bull*. **21**: 133–139

Zhang, L., Liu, X., Xie, L., Wang, G. (2003) P-glycoprotein restricted transport of nimodipine across blood-brain barrier. *Acta Pharmacol. Sin*. **24**: 903–906

Zhang, D., Liu, H., Xie, Liu, X (2007) Effect of baicalin and berberine on transport of nimodipine on primary cultured, rat brain microvascular endothelial cells. *Acta Pharmacol. Sin*. **28**: 573–578