Effect of Huang-Lian-Jie-Du-Decoction on pharmacokinetics of verapamil in rats

Shan-Shan Xie[a], Nan Hu[a], Xin-Yue Jing[a], Xiao-Dong Liu[a], Lin Xie[a], Guang-Ji Wang[a] and Chang-Hui Liu[a,b]

[a]Key Laboratory of Drug Metabolism and Pharmacokinetics, China Pharmaceutical University, Nanjing and [b]Institute of Clinical Pharmacology, Guangzhou University of Chinese Medicine, Guangzhou, China

Abstract

Objectives The aim was to investigate the effect of Huang-Lian-Jie-Du-Decoction (HLJDD) on the pharmacokinetic behaviour of verapamil in rats.

Methods Rats orally received 3.33 g/kg of HLJDD extract for 14 days, and pharmacokinetics of verapamil was investigated after oral and intravenous verapamil. Norverapamil formation for assessing cytochrome P450 3A activity in hepatic and intestinal microsomes of the HLJDD-treated rats was investigated. The inhibitory effect of berberine on the formation of norverapamil in intestinal and hepatic microsomes was also evaluated.

Key findings HLJDD treatment increased the plasma concentration of verapamil and decreased the plasma concentration of norverapamil, resulting in a 24% increase in the AUC_{0-480} of verapamil and a 25% reduction in the AUC_{0-480} of norverapamil after oral administration. However, HLJDD did not alter the pharmacokinetic behaviour of verapamil after intravenous administration. Norverapamil formation showed biphasic kinetics in both intestinal and hepatic microsomes. HLJDD treatment significantly decreased the intrinsic clearance of verapamil in intestinal microsomes, but had no effect on the hepatic metabolism of verapamil. Berberine also inhibited norverapamil formation in both intestinal and hepatic microsomes; the extent of inhibition was larger in intestinal microsomes.

Conclusions HLJDD displayed a route-dependent effect on the pharmacokinetics of verapamil in rats. HLJDD treatment increased the bioavailability of verapamil partly via inhibiting first-pass verapamil metabolism in the intestine.

Keywords first-pass metabolism; herb–drug interaction; Huang-Lian-Jie-Du-Decoction; norverapamil; pharmacokinetics; verapamil

Introduction

Herb–drug interactions have become a major concern in recent years due to a greater awareness in clinical research and the growing popularity of herbs as complementary medicines. To date, a series of severe events regarding herb–drug interactions have been reported. For example, St John's wort may reduce the bioavailability of ciclosporin, amitriptyline, digoxin, indinavir, nevirapine, oral contraceptives, warfarin, phenprocoumon, theophylline or simvastatin, probably via inducing cytochrome P450 (CYP) activity.[1] Grapefruit juice may increase the plasma concentration of CYP3A4 substrates, such as dihydropyridine calcium channel blockers, ciclosporin, midazolam and some HMG-CoA reductase inhibitors, probably via a selective downregulation of CYP3A4 in the small intestine.[2]

Verapamil, a phenylalkylamine calcium channel blocking agent, is widely used in the treatment of various important cardiovascular disorders, including angina pectoris, coronary artery disease, cardiac arrhythmias and hypertension.[3] Verapamil, a typical substrate of both CYP3A and P-glycoprotein (P-gp), undergoes extensive first-pass metabolism and is mainly metabolized by CYP3A in both liver and small intestine to form a primary N-dealkylated derivative, norverapamil, leading to a low absolute bioavailability (10–20%) in humans.[4] Apart from in the liver, CYP3A is highly expressed in the jejunum and duodenum of the small intestine, suggesting that it plays an important role in the

Correspondence: Xiao-Dong Liu, Key Laboratory of Drug Metabolism and Pharmacokinetics, China Pharmaceutical University, Nanjing 210009, China.
E-mail: xdliu@cpu.edu.cn

intestinal metabolism of drugs such as ciclosporin and verapamil after oral administration.[5–7] Rifampin leads to increase in the presystemic metabolism of verapamil without affecting systemic verapamil clearance, predominantly by inducing CYP3A4 activity in the intestine.[8] Similarly, St John's wort administration may decrease the area under the plasma concentration–time curve (AUC) and the maximum concentration (C_{max}) of verapamil in humans by induction of CYP3A4-catalysed first-pass metabolism.[9] Co-administration of atorvastatin, an inhibitor of CYP3A4 and P-gp, may significantly increase verapamil oral bioavailability, accompanied by a significant decrease in norverapamil accumulation.[10]

Huang-Lian-Jie-Du-Decoction (HLJDD), consisting of *Rhizoma coptidis*, *Radix scutellariae*, *Cortex phellodendri* and *Fructus gardeniae*, is one of the most popular prescriptions in traditional Chinese medicine and has been extensively used in clinical practice for the treatment of gastrointestinal disorders, inflammation, hepatic disease, hypertension and cardiovascular disease. Recent studies showed that berberine, one of the major effective components in HLJDD, may increase the blood concentration of ciclosporin[11,12] by inhibiting CYP3A4 or P-gp function. As both verapamil and HLJDD have beneficial effects on cardiovascular disorders, there may be occasion for patients to use HLJDD during their verapamil regimen. Under that condition, the pharmacokinetics of verapamil may be altered by HLJDD, causing potential herb–drug interactions. However, the effect of HLJDD on the pharmacokinetics of verapamil has not yet been reported.

The aim of this study was to investigate whether repeated oral administration of HLJDD affects the pharmacokinetic behaviour of verapamil in rats after oral and intravenous administration. The results from animal experiments were further confirmed by in-vitro study using rat hepatic and intestinal microsomes.

Materials and Methods

Materials

Rhizoma coptidis (voucher number No. 040720), *Radix scutellariae* (No. 041208), *Cortex phellodendri* (No. 020906) and *Fructus gardeniae* (No. 021202) were purchased from Kai-Xin Herbal Shop (Nanjing, China) and identified by Doctor Li-Na Chen (Department of Pharmacognosy, China Pharmaceutical University, Nanjing, China). HLJDD extract was prepared according to our previous report.[13] The content of the four main ingredients in HLJDD extract, baicalin, wogonoside, berberine and palmatine, were reported to be 4.42 g, 1.08 g, 5.6 g and 1.36 g in 100 g HLJDD extract, respectively.[14] The HLJDD extract was suspended in 0.25% of carboxymethyl cellulose solution before use. Verapamil and propranolol were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Norverapamil, protease inhibitor cocktail p8340 (PI), glucose 6-phosphate dehydrogenase, α-nicotinamide adenine dinucleotide phosphate (NADP) and glucose 6-phosphate were purchased from Sigma Chemical Co. (St Louis, USA). All the other reagents were of analytical grade and were commercially available.

Animals

Male Sprague–Dawley rats, 180–220 g, were purchased from SLAC Laboratory Animal Co. Ltd (Shanghai, China). All rats were housed in an environmentally controlled room at 20–22°C with a 12-h light–dark cycle. They were allowed free access to standard rodent chow. The experimental protocol was approved by Animal Ethics Committee of China Pharmaceutical University.

Effect of HLJDD on the pharmacokinetics of verapamil in rats

Rats were randomly divided into three groups. Group I for multiple-dose HLJDD orally received 3.33 g/kg of HLJDD extract for 14 days, once a day; Group II for single-dose HLJDD were orally treated with vehicle for 13 days and 3.33 g/kg of HLJDD extract on day 14; Group III, the age-matched control group, received only vehicle. On day 14, all rats were orally administered 10 mg/kg of verapamil 2 h after the last treatment. Blood samples (0.25 ml) were collected under light ether anaesthesia via the oculi chorioideae vein at 10, 20, 30, 60, 120, 180, 240 and 480 min post dose. After centrifugation at 3000*g* for 10 min, plasma samples were obtained and stored at –20°C until analysis.

Another experiment was designed to investigate the effect of HLJDD on the intravenous pharmacokinetics of verapamil in rats. Control rats and rats treated with HLJDD for 14 days were prepared as described above. On day 14, 2 h after the last administration, 1 mg/kg of verapamil was given to the rats via the tail vein. Blood samples were collected at 5, 10, 20, 30, 60, 120 and 180 min post dose and plasma samples were obtained.

Preparation of rat hepatic and intestinal microsomes

Hepatic and intestinal microsomes were prepared freshly from HLJDD-treated rats and control rats that were obtained according to procedures described above. Rats were sacrificed under light ether anaesthesia 24 h after the last treatment. The liver and intestine were excised quickly. Rat hepatic and intestinal microsomes were prepared according to the methods described previously.[15,16] The microsomal pellets were re-suspended in Tris-HCl buffer (pH 7.4) containing PI and 20% glycerol, then stored at –80°C until use. The protein concentration of the microsomes was measured by the method of Bradford.[17]

Metabolism of verapamil in HLJDD-treated rat hepatic and intestinal microsomes

Verapamil is mainly metabolized into norverapamil via CYP3A-mediated N-dealkylation, and the formation of norverapamil is used as a marker for assessing CYP3A activity. Preliminary experiments showed that the formation of norverapamil was linear against incubation time for up to 15 min for 0.2 mg/ml of rat hepatic microsomes and 1 mg/ml of rat intestinal microsomes.

The incubation mixture (final volume: 200 μl) consisted of an NADPH-generating system containing 500 μM NADP, 10 mM glucose 6-phosphate, 1 U/ml glucose 6-phosphate

dehydrogenase, 5 mM $MgCl_2$ in 100 mM phosphate buffer (pH 7.4), and either 0.2 mg/ml of rat hepatic microsomes or 1 mg/ml of intestinal microsomes. After a 5-min pre-incubation at 37°C, the reaction was initiated by adding 10 μl verapamil. The reaction was terminated by adding 20 μl of 2 M NaOH after incubation at 37°C for 10 min. The final concentrations of verapamil were set to be 2.5, 5, 10, 20, 40, 100, 200 and 300 μM in hepatic incubation mixture or 5, 10, 40, 100, 150, 200, 300, 400, 800 and 1600 μM in intestinal incubation mixture.

Inhibition of verapamil metabolism by berberine in rat hepatic and intestinal microsomes

The incubation mixture (final volume: 200 μl) consisted of the NADPH-generating system, 0.2 mg/ml of rat hepatic microsomes or 1 mg/ml of rat intestinal microsomes and different concentrations of berberine. The final concentrations of berberine were set to be 0, 5, 20, 50, 100 and 300 μM for all incubation mixtures. After a 5-min pre-incubation at 37°C, the reaction was initiated by adding 10 μl of verapamil. The final concentration of verapamil was set to be 10 μM for the hepatic incubation mixture and 40 μM for the intestinal incubation mixture. After incubation at 37°C for 10 min, the reaction was terminated by adding 20 μl of 2 M NaOH.

Drug assays

Verapamil and norverapamil in plasma or incubation mixture were determined by an HPLC method described previously with a minor modification.[18] The HPLC system was equipped with an LC-10A pump (Shimadzu Ltd, Japan), a CTO-10AS$_{VP}$ column oven, a RF-10AXL fluorescence detector (Shimadzu) set at an excitation wavelength of 280 nm and emission wavelength of 310 nm, and a Diamonsil C18 (150 × 4.6 mm i.d., 5 μm; Dikma Technologies, Beijing, China). The mobile phase consisted of 20 mM potassium dihydrogen phosphate buffer and acetonitrile (70 : 30, v/v), and the flow rate was set at 1.0 ml/min.

One hundred microlitres of plasma were placed in the tube, followed by addition of 10 μl of 2 μg/ml propranolol (internal standard), 10 μl of 2 M NaOH and 2 ml ether. After mixing for 5 min, the mixture was centrifuged at 20 000g for 10 min, the organic layer was transferred and evaporated to dryness under a stream of nitrogen gas in a water-bath at 40°C, the residue was reconstituted in 100 μl of mobile phase and 20 μl was injected into the HPLC. The assay for verapamil and norverapamil in plasma was linear over a range of 15.6–1000 ng/ml and 15.6–250 ng/ml, respectively. The limit of quantification for both verapamil and norverapamil was 7.8 ng/ml.

For the incubation mixture preparation, 1.5 ml ether was added to 200 μl microsomal suspension and prepared according to the procedure described above. The assay for norverapamil in the hepatic and intestinal incubation mixture was linear over the range 0.26–4.2 μM and 0.032–2.1 μM, respectively. The limit of quantification for norverapamil in both hepatic and intestinal incubation mixture was 0.016 μM.

Data analysis and statistical analysis

Pharmacokinetic parameters were estimated by non-compartmental analysis using DAS 2.0 software package (purchased from Wannan Medical College). The peak plasma concentration (C_{max}) and the time required to reach Cmax (T_{max}) of drugs were obtained from the observed data. The area under the concentration–time curve (AUC) was calculated using the linear trapezoidal rule. The elimination rate constant (ke) was determined with a linear regression analysis of the ln-linear phase of the plasma drug concentration–time curve; t½ was calculated as t½ = 0.693/ke.

Enzyme kinetic parameters were estimated by non-linear least-squares regression using a programming solver (Microsoft Excel 2003). The Michaelis–Menten equation was used to calculate apparent K_m and V_{max} values for single-enzyme systems. Eadie–Hofstee plots were used to check for biphasic kinetics. If Eadie–Hofstee plots indicated biphasic kinetics, the following equation was used to estimate kinetic parameters for two enzymes systems:

$$v = \frac{V_{max,1}S}{K_{m,1} + S} + \frac{V_{max,2}S}{K_{m,2} + S} \tag{1}$$

Where subscripts 1 and 2 denoted the high- and low-affinity enzymes of the reaction, respectively. S was the concentration of verapamil. Intrinsic clearance ($Cl_{int,i}$) was defined as being $V_{max,i}/K_{m,i}$.

All results were expressed as mean ± standard deviation. Statistical differences between groups were evaluated by one-way of analysis of variance. If analysis was significant, the differences between groups were estimated using Student–Newman–Keuls multiple comparison post-hoc test. $P < 0.05$ indicated a significant difference.

Results

Effect of HLJDD on the pharmacokinetics of verapamil and its active metabolite norverapamil in rats

To evaluate the effect of HLJDD on the pharmacokinetics of verapamil and its active metabolite norverapamil, plasma concentrations of verapamil (Figure 1a) and norverapamil (Figure 1b) in rats were measured after oral administration of verapamil (10 mg/kg). The corresponding pharmacokinetic parameters were estimated and listed in Table 1.

Compared with control rats, HLJDD treatment for 14 days increased the plasma concentration of verapamil, characterized by a 23% increase in AUC_{0-480}, though no significant difference was found in AUC_{0-480} values. The maximum concentration of verapamil was achieved markedly late (61.11 min for HLJDD-treated rats vs 22.86 min for control rats, $P < 0.05$) after an oral dose of verapamil, with lower maximum concentration ($P > 0.05$). HLJDD treatment for 14 days significantly decreased plasma concentrations of norverapamil, resulting in a reduction of 25% and 54% in AUC_{0-480} and C_{max}, respectively. The maximum concentration of norverapamil was also notably delayed from 35.7 min in control rats to 93.3 min in HLJDD-treated rats. To exclude
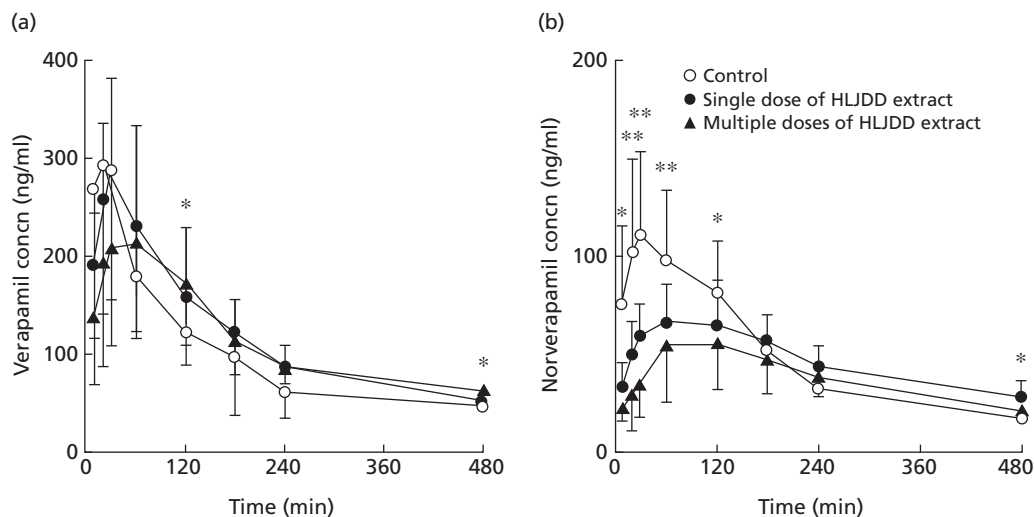
Figure 1 Effect of Huang-Lian-Jie-Du-Decoction (HLJDD) on the plasma concentrations of verapamil (a) and norverapamil (b) after oral administration to rats. Rats were given 10 mg/kg verapamil (control rats) and treatment groups either received a single dose of HLJDD extract or multiple doses of HLJDD extract. HLJDD-treated rats were prepared as described in Materials and Methods. Data points represent mean ± SD, $n = $ 8–9. $^{*}P < 0.05$, $^{**}P < 0.01$ vs control rats

Table 1 Pharmacokinetic parameters of verapamil and its metabolite norverapamil after oral administration of 10 mg/kg verapamil in rats treated with a single dose or multiple doses of HLJDD

	C_{max} (ng/ml)	T_{max} (min)	AUC_{0-480}(ng · min/ml)	AUC(nor)/AUC(ver)	MRT (min)
Verapamil					
Control	315.52 ± 144.57	22.86 ± 5.18	43374 ± 10600	n.a.	285.43 ± 93.43
	[215.34, 415.70]	[19.27, 26.45]	[36029, 50719]		[220.69, 350.17]
Single-dose	288.58 ± 98.87	31.25 ± 12.46	56962 ± 16362	n.a.	270.15 ± 72.15
	[220.07, 357.09]	[22.62, 39.88]	[45623, 68300]		[220.15, 320.15]
Multi-dose	247.80 ± 99.76	61.11 ± 45.67*	53999 ± 11589	n.a.	497.99 ± 238.64*
	[182.62, 312.98]	[31.37, 90.85]	[46427, 61570]		[342.08, 653.90]
Norverapamil					
Control	127.29 ± 43.58	35.71 ± 16.04	24029 ± 6605	0.524 ± 0.107	249.61 ± 44.38
	[97.09, 157.49]	[24.60, 46.82]	[19452, 28605]	[0.45, 0.60]	[218.86, 280.36]
Single-dose	73.62 ± 19.93*	97.50 ± 44.64*	22404 ± 4145	0.403 ± 0.056*	378.81 ± 138.44*
	[59.81, 87.43]	[66.57, 128.43]	[19531, 25276]	[0.36, 0.44]	[282.88, 474.74]
Multi-dose	59.18 ± 26.60**	93.33 ± 43.59*	17957 ± 5557	0.329 ± 0.045$^{**\#}$	455.85 ± 193.42**
	[41.80, 76.56]	[66.85, 121.81]	[14326, 21587]	[0.30, 0.36]	[329.48, 582.22]

AUC, area under concentration–time curve; C_{max}, peak plasma concentration; T_{max}, time required to reach C_{max}; MRT, mean residence time; n.a., not applicable. Data are presented as means ± SD [95% confidence intervals], $n = $ 8–9. $^{*}P < 0.05$, $^{**}P < 0.01$ vs control rats; $^{\#}P < 0.05$ vs rats treated with single dose of HLJDD. Rats treated with multiple-dose HLJDD orally received 3.33 g/kg of HLJDD extract once a day for 14 days. Rats treated with a single dose of HLJDD received vehicle for 13 days and on day 14 received orally 3.33 g/kg of HLJDD extract. Control rats only received vehicle. On day 14, all rats were orally administered with 10 mg/kg of verapamil 2 h after the last treatment.

the possibility that the decrease in norverapamil AUC resulted from verapamil absorption, the ratio of AUC (nor)/ AUC (ver) was also estimated. The results showed that the ratio of AUC (nor)/AUC (ver) was significantly decreased by 37% ($P < 0.01$) after 14 days of HLJDD treatment. Similar results were found in rats treated with a single dose of HLJDD, although the extent of alteration in pharmacokinetic parameters of norverapamil was less than that in rats treated with HLJDD for 14 days. The C_{max} of norverapamil and the ratio of AUC (nor)/AUC (ver) were only reduced by 43% and 23% in rats after single dose of HLJDD, respectively.

To investigate whether the pharmacokinetic alteration of verapamil in rats treated with HLJDD arose from alteration of its systematic clearance, the plasma concentration of verapamil in both HLJDD-treated rats and control rats was measured after intravenous verapamil (Figure 2). In contrast to oral administration of verapamil, 14 days of HLJDD treatment did not affect the plasma concentration of verapamil following intravenous administration of verapamil (1 mg/kg). Estimated AUC_{0-480}, $t\frac{1}{2}$ and Cl values in HLJDD-treated rats were 23216.95 ± 6337 ng min/ml, 20.93 ± 4.91 min and 45.64 ± 10.75 ml/min/kg, respectively.
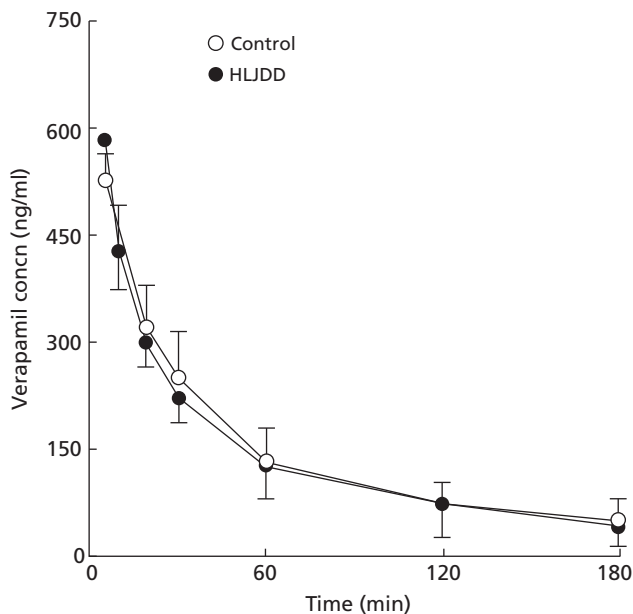
Figure 2 Effect of Huang-Lian-Jie-Du-Decoction (HLJDD) on the plasma concentrations of verapamil after intravenous administration to rats. Rats were administered with 1 mg/kg verapamil (control rats) and the treatment group also received multiple doses of HLJDD. HLJDD-treated rats were prepared as described in Materials and Methods. Data points represent mean ± SD, n = 8–9

These values were similar to the corresponding values in control rats ($AUC_{0–480}$, 24261 ± 6812 ng min/ml; $t\frac{1}{2}$, 23.78 ± 5.29 min; and Cl, 43.32 ± 8.92 ml/min/kg), suggesting that HLJDD treatment did not significantly alter systematic clearance of verapamil.
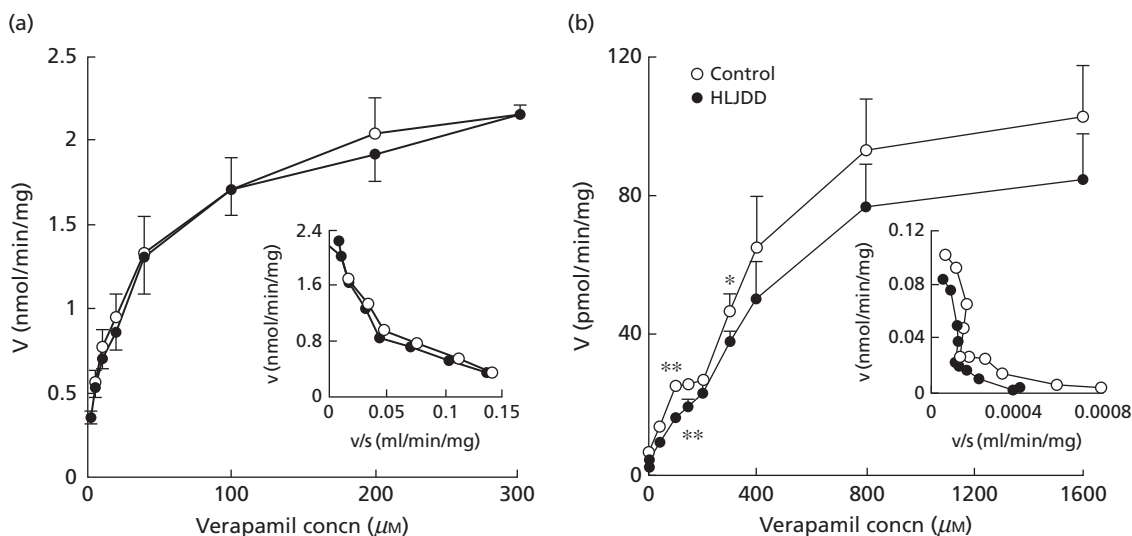
Metabolism of verapamil in hepatic and intestinal microsomes of HLJDD-treated rats

To further confirm in-vivo results, metabolism of verapamil was measured in HLJDD-treated rat hepatic and intestinal microsomes using formation of verapamil metabolite, norverapamil. Formation rates of norverapamil in rat liver microsomes (Figure 3a) and intestinal microsomes (Figure 3b) were obtained and the corresponding kinetic parameters were summarized in Table 2. It is worth noting that the intestinal metabolism of verapamil showed biphasic kinetic characteristics, suggesting the involvement of two different enzymes. The apparent $K_{m,1}$ value (high-affinity component) was approximately 53.77 μM, whereas the $K_{m,2}$ value (low-affinity component) was higher than 1 mM. The apparent maximum velocity ($V_{max,2}$) for low-affinity components was larger than that ($V_{max,1}$) for the high-affinity component, but the intrinsic clearance ($V_{max,2}/K_{m,2}$) of low-affinity components was significantly lower than that ($V_{max,1}/K_{m,1}$) for the high-affinity component (Table 2). Fourteen-day HLJDD treatment significantly decreased the formation of norverapamil in intestinal microsomes and resulted in decrease of intrinsic clearance for the two components. The decrease in intrinsic clearance for the high-affinity component (about 50%) was larger than that for the low-affinity component (about 29%).

The phenomenon of biphasic kinetics for norverapamil formation was also noted in hepatic microsomes. However 14-day HLJDD treatment did not affect formation of norverapamil in hepatic microsomes (Figure 3a), suggesting differential effects on the intestinal and hepatic metabolism of verapamil after 14-day HLJDD treatment.

Inhibition of verapamil metabolism by berberine in rat hepatic and intestinal microsomes

Berberine, a quaternary protoberberine-type alkaloid, is the major ingredient in HLJDD and is often used as a

Figure 3 Formation of norverapamil in hepatic (a) and intestinal (b) microsomes of Huang-Lian-Jie-Du-Decoction (HLJDD)-treated and control rats. Insets represent the corresponding Eadie–Hofstee plots. Incubation mixture consisted of microsomal protein (0.2 mg/ml for liver or 1 mg/ml for intestine). HLJDD-treated rats were prepared as described in Materials and Methods. Data represent the mean ± SD of three different preparations. [*]$P < 0.05$, [**]$P < 0.01$ vs control rats

Table 2 Kinetic parameters for norverapamil formation in hepatic and intestinal microsomes of HLJDD-treated rats

	Rat hepatic microsomes		Rat small intestinal microsomes	
	Control	Treatment	Control	Treatment
$K_{m,1}$ (μM)	5.68 ± 0.98	5.38 ± 0.91	53.77 ± 10.27	101.65 ± 21.65[*]
	[4.27, 6.49]	[4.35, 6.41]	[42.15, 65.39]	[77.15, 126.15]
$V_{max,1}$ (pmol/min/mg)	1142.35 ± 158.42	1076.49 ± 141.11	35.59 ± 2.27	33.19 ± 4.32
	[963.08, 1321.62]	[916.81, 1236.17]	[33.02, 38.16]	[28.30, 38.08]
$V_{max,1}/K_{m,1}$ (μl/min/mg)	203.11 ± 29.53	203.43 ± 25.96	0.68 ± 0.14	0.34 ± 0.11[*]
	[169.69, 236.53]	[174.05, 232.81]	[0.52, 0.84]	[0.22, 0.46]
$K_{m,2}$ (μM)	232.97 ± 15.13	227.14 ± 24.73	1145.8 ± 302.7	1391.5 ± 476.7
	[215.85, 250.09]	[199.16, 255.12]	[803.27, 1488.33]	[852.07, 1930.93]
$V_{max,2}$ (pmol/min/mg)	2053.62 ± 239.69	2094.46 ± 177.63	123.00 ± 14.11	99.81 ± 18.51
	[1782.39, 2324.85]	[1893.46, 2295.46]	[107.03, 138.97]	[78.86, 120.76]
$V_{max,2}/K_{m,2}$ (μl/min/mg)	8.83 ± 0.96	9.21 ± 0.28	0.12 ± 0.047	0.079 ± 0.041
	[7.74, 9.92]	[8.89, 9.53]	[0.07, 0.17]	[0.03, 0.13]

K_m, Michaelis–Menten constant; V_{max}, maximum enzyme velocity; V_{max}/K_m, intrinsic clearance; the subscript 1 and 2 denoted the high- and low-affinity enzymes of the reaction, respectively. Data represent the means ± SD [95% confidence intervals] of three different preparations. [*]P <0.05 vs control rats.

phytochemical marker in the quality control of *Rhizoma coptidis*. In this study, berberine was used as a representative ingredient to investigate whether these protoberberine-type alkaloids inhibited the metabolism of verapamil using rat hepatic and intestinal microsomes.

The inhibitory effect of berberine on norverapamil was expressed as a ratio of norverapamil formation in the presence/absence of berberine. The differential inhibition of berberine on the intestinal and hepatic metabolism of verapamil is illustrated in Figure 4. Berberine inhibited
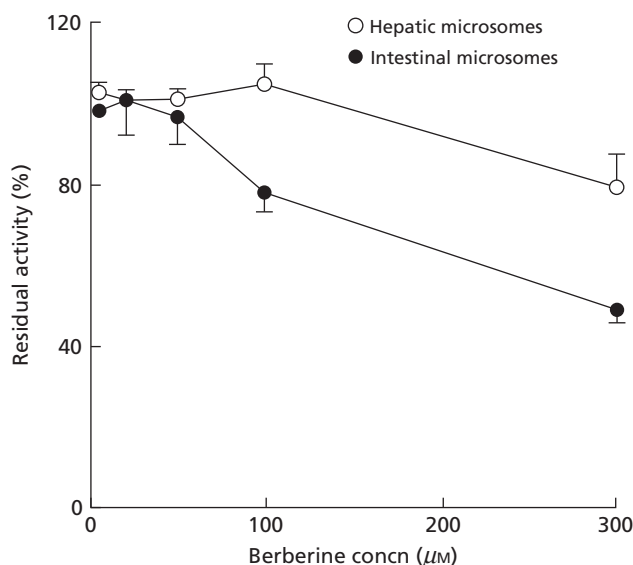
Figure 4 Inhibitory effects of berberine on formation of norverapamil in rat hepatic and intestinal microsomes. Incubation mixture consisted of 0.2 mg/ml hepatic or 1 mg/ml intestinal microsomal protein. The concentrations of verapamil were 10 μM for hepatic microsomes and 40 μM for intestinal microsomes. Control activity of norverapamil by rat hepatic and intestinal microsomes determined in the absence of berberine was 539 and 6.87 pmol/min/mg, respectively. Data represent the mean ± SD of three different preparations

norverapamil formation in intestinal microsomes in a concentration-dependent manner, and about 52% decrease of norverapamil formation was observed in the presence of 300 μM berberine. However, berberine showed a weaker inhibitory effect on the metabolism of verapamil in hepatic microsomes, and only a 21% decrease of norverapamil formation was found in the presence of 300 μM berberine.

Discussion

In this study, the main finding was that HLJDD displayed a route-dependent effect on the pharmacokinetics of verapamil in rats. HLJDD treatment may increase bioavailability of verapamil, but did not alter the pharmacokinetic behaviour of verapamil following intravenous administration. This indicated that metabolism of verapamil in the gut was more susceptible to drugs and the gut wall is a potential site for drug–verapamil interactions. Several studies demonstrated that selective inhibition/induction of gastrointestinal enzymes could significantly alter the pharmacokinetics of drugs after oral administration, whereas systemic clearance remains relatively unaffected. Rifampin, a typical inducer of CYP450, may increase presystemic metabolism of verapamil in the intestine to a larger extent than that in liver.[8] St John's wort administration significantly decreased the bioavailability of verapamil, but did not affect the absorbed fraction of verapamil.[9] St John's wort administration may cause more than 50% decrease in the AUC of midazolam (CYP3A4 substrate) after oral administration, while resulting in a 20% decrease in the AUC after intravenous midazolam.[19] Grapefruit juice treatment had little influence on the systemic clearance of ciclosporin but markedly increased its bioavailability.[20]

This study showed that HLJDD treatment significantly decreased exposure (C_{max} and AUC) to norverapamil after oral administration of verapamil. To investigate whether this decrease in exposure arose from absorption of verapamil, the ratio of AUC (nor)/AUC (ver) was calculated. The results showed that the ratio of AUC (nor)/AUC (ver) in

HLJDD-treated rats was significantly lower than that in control rats, which indicated the decrease in exposure to norverapamil did not result from absorption of verapamil but from decrease of norverapamil formation. It was also found that the maximum concentration of verapamil (T_{max}) was markedly delayed in HLJDD-treated rats. The delay in T_{max} may result from decrease in elimination or speed of absorption. A report showed that berberine, a major component of HLJDD extract, may inhibit intestinal movement in mice and guinea-pig,[21] leading to slowing of verapamil delivery in the intestine.

It is well known that metabolism of verapamil to form norverapamil is mediated by CYP3A. The increased AUC of verapamil and decreased AUC of norverapamil during concomitant HLJDD can be explained by inhibition of their CYP3A-mediated first-pass metabolism in the intestinal wall.

In-vitro study further confirmed that HLJDD treatment resulted in significant decreases in CL_{int} for the intestinal metabolism of verapamil but had no effect on the hepatic metabolism of verapamil. The decrease in intestinal CYP3A activity caused by HLJDD might account for the dramatic decrease in the metabolism of verapamil found in the in-vivo study. It was also found that the metabolism of verapamil in both hepatic and intestinal microsomes showed biphasic kinetic characteristics. As the metabolism of verapamil is well characterized, norverapamil, an N-dealkylated product, is the major metabolite of verapamil mediated by CYP3A4 and CYP3A5 in humans.[22–25] Thus, gut wall represents an important site for drug interactions after oral administration. The effect of HLJDD on drug-metabolizing enzymes may not be limited to the CYP3A subfamily. Inhibition of intestinal P-gp function may be involved in the increase in the oral bioavailability of verapamil. HLJDD has recently been shown to inhibit the transport of nimodipine across the rat blood–brain barrier *in vivo* and *in vitro*.[14]

Berberine can inhibit the metabolism of verapamil in rat intestinal microsomes at high concentrations, while having a weaker inhibitory effect on the metabolism of verapamil in rat hepatic microsomes at the same concentrations. The inhibition of verapamil metabolism by berberine indicated that the effects of HLJDD were, at least in part, caused by its major constituents of protoberberine-type alkaloids.

Conclusions

In conclusion, multiple-dose HLJDD for 14 days decreased the bioavailability of verapamil to the some extent by inhibiting the verapamil N-dealkylation metabolism, probably in the gut rather than in the liver. These results indicate that HLJDD may lead to raising the potential for herb–drug interactions for all CYP3A substrates, which represent at least half of the marketed medications; this is especially relevant for drugs with narrow therapeutic indices.

Declarations

Conflict of interest

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

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