Pre-treatment with Curcumin Enhances Plasma Concentrations of Losartan and Its Metabolite EXP3174 in Rats

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Received July 23, 2011; accepted November 22, 2011; published online November 25, 2011

The study was carried out in the Wistar rats to investigate the effect of curcumin pre-treatment on the pharmacokinetics of the hypertension-treating drug losartan and its metabolite EXP3174 following single oral administration. In the treatment group, rats were gavaged with losartan 10 mg/kg after repeat oral doses of curcumin (100 mg/kg, for 7 d), while rats in the control group were administrated only with the same dose losartan. The results showed that curcumin significantly increased the plasma concentrations of losartan and its metabolite EXP3174. The present study implicated the existence of herb-drug interaction between curcumin and losartan, and further evaluation of the possible interaction during curcumin administration needs to be considered.

Key words losartan; curcumin; interaction

Losartan, a selective non-peptide angiotensin receptor antagonist, is an alternative of antihypertensive agents of angiotensin converting enzyme inhibitors (ACE-I) used in treating hypertension and heart failure. In recent years, the discovery of its antioxidative activity has enhanced the protection of cardiovascular injury and improved the treatment of hypertension.^{1–4} Researches have showed that losartan-antioxidant hybrid may be more useful than losartan alone or controlling hypertension and preventing hypertension-induced cardiovascular damage.⁵

Curcumin (Fig. 1), a polyphenol extracted from the rhizomes of turmeric (*Curcuma longa* L.), is widely used as a food coloring agent and spice in many countries. Interests in this dietary polyphenol have grown in recent years due to its pharmacological effects. Curcumin is known for its anti-carcinogenicity,⁶ ability to trigger anti-inflammatory responses^{7,8} and antioxidative bioactivity.^{9,10} On this basis, Phase I clinical trials of curcumin have been initiated in the United Kingdom¹¹ and Taiwan¹² and also Phase II trials in the United States.¹³ However, the safety concern of curcumin used as a food additive to the drugs is less studied. Research has suggested that it was imperative to conduct evaluation of curcumin-drug interactions to provide a better understanding of the effects of curcumin on pharmacokinetics of drugs.¹⁴ The European Commission has requested related departments to reevaluate the safety of curcumin (E 100) when used as a food coloring substance. Without a doubt, the safety of curcumin use has begun to draw more and more attention.¹⁵

Curcumin is often used in common dietary and may be considered as potential therapeutic agent for cardiovascular disease in recent years,^{16,17} which possible result in curcumin being taken concomitantly with losartan as a combination therapy to treat or prevent hypertension and related cardiovascular damage. As known, in the clinic, the effect of herb

on drug maybe easy to be found by the exaggerative pharmacodynamic effect. But slightly changing of pharmacokinetics of drugs, which can not be obviously observed in pharmacodynamic effects, may also increase the risk of side effects. The present study focused on whether curcumin affects the pharmacokinetics of losartan in order to avoid the potential adverse drug reaction of losartan in clinical therapy.

MATERIALS AND METHODS

Chemicals and Agents Losartan was supplied by Dawnrays Pharmaceutical (Holding) Limited (Suzhou, China), EXP3174 was purchased from International Laboratory Limited (San Bruno, CA, U.S.A.) and irbesartan was obtained from National Institutes for Food and Drug Control (Beijing, China). *Curcuma longa* L. was purchased from Jianlian traditional Chinese medicine store. Acetonitrile, ammonium acetate and formic acid (HPLC grade) were supplied by Tedia Co., Inc. (Fairfield, OH, U.S.A.). Deionized water was obtained from a Millipore Milli-Q Gradient Water Purification System (Molsheim, France). Chemical structures of losartan and EXP3174 are shown in Fig. 2.

Animals Specific-pathogen-free grade healthy Wistar rats (180–220 g, 7 males and 7 females) were obtained from Beijing Medical Laboratory Animal Center (Beijing, P.R. China). Rats were kept in an environmentally controlled breeding room (temperature 25±2°C, humidity 60±5%, 12 h dark/light cycle) for 1 week before the experiment. They were fed a soy-free custom diet (Beijing Medical Laboratory Animal Center, Beijing, P.R. China) and water *ad libitum*.

Fig. 1. Chemical Structure of Curcumin

Fig. 2. Chemical Structures of Losartan and EXP3174

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The animal experiment followed the guidelines of the National Research Council.

Preparation of Curcumin The powder of *Curcuma longa* L. (5 kg) was suspended and extracted with 75% ethanol (5L) for 5 times by ultrasonic extraction, 2h for each time. The filtrates were combined together and offered 500g residue after concentration by a rotary vacuum evaporator. The crude extract was separated into three partition with a silica gel column eluted gradually with petroleum ether, mixture of petroleum ether and chloroform (3:1), and chloroform. The second partition (petroleum ether–chloroform 3:1) was subjected to a silica gel column with a gradient of petroleum ether in acetone. Four fractions were collected (volatile oil and compounds CI–CIII). Compound CI was identified as pure curcumin. The purity of curcumin was measured by HPLC analysis.

Pharmacokinetic Interactions between Curcumin and Losartan All rats were randomly divided into two groups, six rats in the control group and eight in the treatment group, and each group was consisting of either sex. Rats in the treatment group were gavaged with curcumin (100 mg/kg/d, with 0.5% carboxymethyl cellulose as the vehicle) for seven consecutive days. Rats in the control group were gavaged with an equivalent volume of the vehicle for the same days. All animals were allowed free access to food and water, and were fasted overnight before pharmacokinetic experiment with free access to water. On day 7, half an hour after the regular administration, all of the rats were administered with 10 mg/kg losartan dissolved in distilled water. Blood samples (approximate 0.1 mL) were obtained from the orbital vein before dosing and at 0.25, 0.5, 1, 2, 3, 4, 6, 8, 12 h after dosing of losartan. The samples were put into heparinized tubes and plasma was isolated by centrifugation at 12000 rpm for 10 min. All plasma samples were stored at -20°C until analysis.

Liquid Chromatography-Mass Spectrometry. Instruments and Chromatographic Conditions Liquid chromatography-tandem mass spectrometry assay for the quantitation of losartan and its oxidative metabolite EXP3174 was performed on an Agilent 1200 system equipped with an autosampler, a vacuum degasser unit, and a binary pump. The HPLC system was coupled to an Agilent G6410B triple-quadrupole mass spectrometer (Agilent Technologies, U.S.A.), equipped with electrospray ionization (ESI) run by Agilent Mass Hunter Workstation B.01.03. Chromatographic separation was performed using a ProntoSIL C18 column (150 mm $\times$ 3 mm, 3 μm , Bischoff, Germany). The mobile phase was water with 0.1% formic acid and acetonitrile (40:60, v/v). The separation was performed under isocratic conditions with a constant flow rate of 0.4 mL/min, and the injection volume was 20 μL .

Standard Solutions and Calibrators Stock standard solutions of losartan (103.2 $\mu\text{g/mL}$), EXP3174 (57.8 $\mu\text{g/mL}$) were freshly prepared in acetonitrile. A series of working standard solutions were dissolved in acetonitrile and water (1:1, v/v) to produce eight standard solutions ranging 1–2064 ng/mL for losartan, and 0.6–1156 ng/mL for EXP3174, respectively. Matrix based calibrators were prepared by spiking 25 μL of each standard solution to a final volume of 25 μL plasma. Quality control samples were prepared at 2.6, 51.6 and 1858 ng/mL for losartan and 1.45, 28.9 and 1040 ng/mL for EXP3174. The working standard solution of irbesartan (internal standard (IS)) at 200 ng/mL was diluted with acetonitrile.

The inter-batch precision and accuracy were evaluated by processing a set of calibration and quality control samples in six separate batches. All solutions were stored at -20°C until assay.

Sample Preparation and Analysis All frozen plasma samples were thawed at room temperature. For sample preparation, 25 μL of plasma sample was added into a 1.5 mL centrifuge tube, followed by 25 μL of acetonitrile–water solution (v/v, 50:50) and 25 μL of the IS solution (irbesartan, 200 ng/mL). After vortex mixing for 30 s, 80 μL of acetonitrile was added to the mixture to precipitate the plasma protein. After vortex mixing for 1 min, the mixture was centrifuged at 11000 rpm for 5 min, the separated supernatant was analyzed immediately by LC-MS/MS system after preparation.

Pharmacokinetic Parameters The main pharmacokinetic parameters of losartan and EXP3174 were obtained by a non-compartmental model with the help of a computer designed program DAS (version 2.0, Anhui, China). Maximum concentration (C_{max}) and time to reach maximum concentration (T_{max}) were obtained directly from experimental observations. The area under the plasma concentration–time curve from zero to the last sampling time (AUC_{0-t}) was calculated using the linear trapezoidal method.

Statistical Analysis All mean values were expressed as mean $\pm$ S.D. The data for pharmacokinetics in rats were analyzed with DAS 2.0 and the pharmacokinetic parameters were compared using Student *t*-test. Differences were considered to be significant at a level of $p < 0.05$.

RESULTS

Chromatographic and Mass Spectrometric Experimental Conditions The method of simultaneous determination of losartan and EXP3174 in rat plasma was developed and optimized. The compounds were ionized in the positive ion mode. The spray voltage was set at 4000 V. Nitrogen was used as nebulizer gas and nebulizer pressure was set at 35 p.s.i. Desolvation gas (nitrogen) was heated to 300°C and delivered at a flow rate of 10 L/min. For collision-induced dissociation (CID), high purity nitrogen was used as collision gas at a pressure of 0.15 MPa. Quantification was performed using multiple reaction monitoring (MRM) mode at m/z 423.1 $\rightarrow$ 207.1 for LOS, m/z 437.1 $\rightarrow$ 207.1 for EXP3174 and m/z 429.1 $\rightarrow$ 195.1 for irbesartan (IS), respectively (Fig. 3). Retention times for losartan, EXP3174 and irbesartan were at 2.94, 2.98 and 3.85 min, respectively, with a total run time of 4.0 min. The optimized collision energies of 20, 24 and 25 eV were used for LOS, EXP3174 and irbesartan, respectively. The dwell time was set at 200 ms for each analyte.

Method Validation. Selectivity and Matrix Effects No endogenous interference was observed at retention times of losartan, EXP3174 and IS in this method. Analytical signals from blank rat plasma were compared with those obtained from the rat plasma spiked with losartan and EXP3174 at the LLOQ and the IS. The representative chromatograms of the plasma samples are shown in Fig. 3. In addition, chromatograms obtained from rat plasma samples at 2 h after dosing with 10 mg/kg losartan are illustrated in Fig. 3.

Three replicate analyses of samples at 2.6 and 1860 ng/mL in plasma and in acetonitrile were performed. The ratios of the absolute peak areas at the same concentrations were

Fig. 3. Chromatograms of Rat Plasma

(a) Blank, (b) blank spiked with losartan and EXP3174 at LLOQ and IS, (c) sample at 2h after dosing with 10mg losartan.

Table 1. Precision and Accuracy of the Method for Determining Losartan and EXP3174 Concentrations in Rats Plasma

Analyte	Concentration added (ng/mL)	Intra-batch			Inter-batch		
		Concentration measured (mean $\pm$ S.D.; ng/mL)	CV (%)	RE (%)	Concentration measured (mean $\pm$ S.D.; ng/mL)	CV (%)	RE (%)
Losartan	2.58	2.63 $\pm$ 0.09	3.42	1.94	2.54 $\pm$ 0.12	4.72	-1.55
	51.6	52.5 $\pm$ 1.4	2.67	1.74	52.2 $\pm$ 1.39	2.66	1.09
	1860	1780 $\pm$ 22.9	1.29	-4.43	1770 $\pm$ 24.4	1.38	-4.74
	1.45	1.40 $\pm$ 0.12	8.57	-3.45	1.48 $\pm$ 0.11	7.43	2.07
EXP3174	28.9	30.1 $\pm$ 0.67	2.22	4.26	30.0 $\pm$ 0.59	1.97	3.77
	1040	1070 $\pm$ 14.6	1.37	2.67	1050 $\pm$ 22.9	2.18	1.00

CV (%)=S.D. of measured value/mean measured value $\times$ 100. RE (%)=(measured concentration-nominal concentration)/nominal concentration.

within the range 0.95–1.09 and 0.99–1.28 for losartan and EXP3174, respectively. These results showed that matrix effects are negligible and can be ignored.

Calibration and Linearity The calibration curve was linear over the concentration range 1.03–2060 ng/mL for losartan and 0.578–1160 ng/mL for EXP3174. The best linear fit and least-squares residual for the calibration curve were achieved with a $1/x^2$ weighing factor. The mean correlation coefficient (r) of the weighted calibration curve generated during the validation was ≥ 0.9985 for losartan and EXP3174.

Precision and Accuracy The intra- and inter-batch precision and accuracy of the method are illustrated in Table 1. Six sets of quality control samples (low, medium and high concentration) were analyzed with calibration samples in one batch.

Recovery Recoveries of the analytes and IS were high

and consistent. The recoveries of losartan and EXP3174 were 95.7% and 83.3%, respectively.

Stability Stock solutions were established and tested at room temperature for 0 and 24 h, and under refrigeration (-20°C) for 24 d. Stabilities of the analytes and IS in rat plasma were assessed for 2 h, 24 h. The freeze-thaw stabilities were measured after three freeze-thaw cycles (-20°C to room temperature) on consecutive days. All the stabilities were conducted at two QC concentration levels (low and high). No significant change ($p < 0.05$) of the analytes concentration was observed.

Pharmacokinetics of Losartan and EXP3174 The plasma concentration-time profiles of losartan after an oral dose of 10 mg/kg to rats, with or without 7 d curcumin, are shown in Fig. 4. The corresponding pharmacokinetic parameters are

listed in Table 2. Compared with the control group, rats prior exposure to curcumin had significantly increased in AUC_{0-t} and C_{max} of the oral losartan. Administration of 10 mg/kg losartan in the treatment group resulted in a marked increase in the AUC_{0-t} and C_{max} from 2080 ± 837 to $3460 \pm 1350 \mu\text{g/L}\cdot\text{h}$ and 475 ± 63.1 to $1660 \pm 892 \mu\text{g/L}$, respectively. An approximate 1.7-fold increase of the AUC_{0-t} and 3.5-fold increase of the C_{max} in treatment group.

It can be seen in Fig. 5 and Table 2, compared with the control group, the AUC_{0-t} and C_{max} of EXP3174 of treatment group were significantly increased from 766 ± 416 to $1460 \pm 818 \mu\text{g/L}\cdot\text{h}$ and 108 ± 61.1 to $341 \pm 187 \mu\text{g/L}$, respectively. T_{max} of EXP3174 of the control group was nonsignificantly 78% higher than that of group pre-treated with curcumin (3.33 ± 2.34 to

$1.88 \pm 0.64 \text{ h}$).

DISCUSSION

Change of a drug delivery to its site of action may result in pharmacokinetic interaction, which probably caused by minor alteration of any step of absorption, distribution, metabolism, or elimination.¹⁸⁾ Losartan has been shown to take effect in drug interactions mostly owing to its metabolism.¹⁹⁾ Present study was carried out in Wistar rats to investigate effect of pre-treatment with curcumin on the pharmacokinetic of losartan following single oral administration. The reason we chose rats as animal model for the interaction investigation is its similarity to humans on the pharmacodynamic and pharmacokinetic actions of losartan.

The pharmacokinetic of losartan and EXP3174 after a single oral dose were analyzed in rats with or without prior repeat doses of curcumin for seven days. Losartan is converted to an active metabolite, EXP3174 (about 14%),²⁰⁾ which is the main activity metabolite of losartan. EXP3174 has stronger pharmacological effects than the parent compound,²¹⁾ and it mediates the mainly pharmacological influences of losartan. Thus, in present investigation, the pharmacokinetic interaction were studied by measuring the concentration of losartan and EXP3174, respectively.

The dosages of curcumin and losartan used in study were based on similar researches,^{22,23)} respectively. The pharmacokinetic behaviors of losartan and EXP3174 were described using non-compartment model. Our findings revealed that co-administration with curcumin may result in higher concentrations of losartan and EXP3174 in plasma. In the present study, the pharmacokinetic profiles of losartan and EXP3174 were significantly altered in rats treated with curcumin compared with the control. The AUC_{0-t} s of losartan and EXP3174 of the group pre-treatment with curcumin were about 70% and 90%, respectively, greater than those of losartan administration alone. The C_{max} s of losartan and EXP3174 were significantly increased by curcumin to 3.5-fold and to 3.2-fold of their control values, respectively.

This pharmacokinetic modulation of losartan might be attributed to changes in the absorption and metabolism. Losartan is a drug transporter protein (P-gp) substrate,²⁴⁾ and undergoes extensive metabolism involving both oxidation and glucuronidation. The oxidation metabolism of losartan *in vivo* is catalyzed by cytochrome P450 (CYP) 2C and 3A.²⁵⁾ Moreover, losartan glucuronidated mainly by UDP-glucuronosyltransferases (UGTs) 1A1 and 2B7. Thus, the modulation of P-gp or CYPs or UGTs may affect the pharma-

Fig. 4. Mean Plasma Concentration-Time Curves of Losartan in Rats after Oral Administration of Losartan in Single Dose and after 7d Repeated Administration of Curcumin

Fig. 5. Mean Plasma Concentration-Time Curves of EXP3174 in Rats after Oral Administration of Losartan in Single Dose and after 7d Repeated Administration of Curcumin

Table 2. The Main Pharmacokinetic Parameters of Losartan and EXP3174 at Losartan Alone and after Repeated Administration of Curcumin

Analyte	Parameter	Unit	Value (mean $\pm$ S.D.)	
			Control	Treated
Losartan	$AUC_{(0-t)}$	$\mu\text{g/L}\cdot\text{h}$	2080 ± 837	3460 ± 1350
	T_{max}	h	0.38 ± 0.14	0.38 ± 0.13
	C_{max}	$\mu\text{g/L}$	475 ± 63.1	$1660 \pm 892^{**}$
	$AUC_{(0-t)}$	$\mu\text{g/L}\cdot\text{h}$	766 ± 416	$1460 \pm 818^*$
EXP3174	T_{max}	h	3.33 ± 2.34	1.88 ± 0.64
	C_{max}	$\mu\text{g/L}$	108 ± 61.1	$341 \pm 187^{**}$

* Comparing to control group, p value < 0.05 ; ** Comparing to control group, p value < 0.01 .

cokinetic profile of losartan.

Curcumin has been demonstrated to down regulate the P-gp levels in rat,^{16,26)} and the cell-based assay using rhodamine 123 indicated that curcumin significantly inhibited the activity of P-gp with a decrease in P-gp protein and multidrug resistance gene MDR1 mRNA levels. Referring to the studies,^{22,27)} pre-treatment with curcumin could alter the oral bioavailability of docetaxel and norfloxacin, which are substrates of P-gp. Therefore, the improved absorption of losartan caused by inhibition of P-gp is likely to result in higher increased *AUC* and *C_{max}*. Furthermore, the ability of curcumin to suppress 10 members of UGTs including UGT1A1 and 2B7²⁸⁾ might have delayed the glucuronidation process of losartan.

Previous reports have proved that EXP3174 detected in rats is most likely of hepatic origin.^{25,29)} However, curcumin attenuated the CYP3A level in the small intestine but not in the liver.¹⁴⁾ Therefore, it can be hypothesized that the systematic exposure of curcumin is inadequate to inhibit the activity of CYPs in liver, and do little effect on the metabolism of losartan.

The further elimination of EXP3174 also depends on UGTs.³⁰⁾ In the investigation, the plasma concentration of EXP3174 is higher in treatment group than that in control one. Firstly, the increase of losartan result from the above reason may produce more volume of metabolite. Secondly, the inhibition of curcumin on UGTs contribute to the less elimination of EXP3174.

In the present study the *t*_{1/2} of losartan and EXP3174 were not significantly changed, which suggested that the increased plasma concentration observed in the study may be mainly attributable to the improved absorption of losartan. The inhibition of P-gp by curcumin is the main pathway affecting the pharmacokinetics of losartan and EXP3174 in rat pretreated with curcumin, while inhibition of UGTs and CYPs play a minor role.

The increased plasma levels of losartan and EXP3174 indicates that prior administration of curcumin may result in strong pharmacological actions such as blood pressure decrease, and lead to adverse drug reaction. On the one hand, we should be kept in mind that losartan with curcumin may cause herb-drug interaction. On the other hand, from a practical point of view, pre-treatment with curcumin can be developed as enhancer agent of losartan to reduce the drug dose on the base of appropriate dose titration studies.

In addition, a highly sensitive and specific LC/MS/MS method for the quantitation of losartan and EXP3174 has been developed, in the present study. The method has been validated with a routine sensitivity limit of 1.03 ng/mL in 25 μ L rat plasma. Using this method, pharmacokinetics of losartan and EXP3174 following an administration of losartan 10 mg/kg was investigated in rats. The plasma levels of the two drugs have been found at lower level and shorter duration than previously reported.³¹⁾ The developed method could be efficiently used in losartan therapeutic drug monitoring in clinical situation.

In conclusion, the pharmacokinetic profile of losartan affected by curcumin was revealed in rat, which may help to guide the clinical administration of losartan in hypertension patients with further investigation in clinical trials. Furthermore, the developed method could be used in losartan therapeutic drug monitoring in clinical settings.

Acknowledgement The study was supported by The National Natural Science Foundation of China (No. 31101241).

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