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ORIGINAL RESEARCH

Effect of Da-Cheng-Qi decoction on the pharmacokinetics of ranitidine in rats

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ABSTRACT: Da Cheng Qi decoction (DCQD) is composed of Dahuang, Houpu, Zhishi and Mangxiao. It is a formula created under the theory of Chinese medicine to purge the 'evil heat' in the gastrointestitinal tract, which arises from the ileus and acute pancreatitis. The present study was conducted to evaluate the herb–drug interaction between DCQD and ranitidine, which are often co-administered in clinical practice. Ranitidine was administered orally alone or together with DCQD to rats, and plasma ranitidine concentrations were measured by HPLC. Following oral administration, ranitidine plasma levels revealed curves characterized by peaks at 1.8 and 4.2 h corresponding to ranitidine alone and ranitidine with DCQD at mean concentrations of 16.315 and 1.455 μg/mL, respectively. After ranitidine was orally dosed alone or with DCQD, the half-lives were 1.787 and 3.758 h, while the area under the concentration–time curve (0–12 h) was 28.083 and 9.826 μg/L h, respectively, suggesting that DCQD might significantly affect the pharmacokinetics of ranitidine in rats. When physicians or pharmacists administer DCQD and ranitidine, they must make a careful effort to adjust the dosage of the drug and Chinese decoction, or avoid the herb–drug co-administration.

KEYWORDS: ranitidine; Da Cheng Qi decoction; pharmacokinetics; HPLC

INTRODUCTION

During the past three decades, important progress in understanding of the pathophysiology of severe acute pancreatitis has changed the management of choice (Beger and Rau, 2007). This change has led to a marked reduction in morbidity and mortality after mainly conservative treatment and intensive monitoring and therapy (Ribeiro *et al.*, 2002), including the adjuvant use of purgative herbal medicine in China (Xia *et al.*, 2000). Because there are no specific drugs or treatments for the disease (Beger and Rau, 2007), Chinese herbal decoction is accepted throughout the country (Xia *et al.*, 1999; Tang *et al.*, 2005).

Da-Cheng-Qi decoction (DCQD), a well-known and popular traditional Chinese medicine formula in China and East Asia, is traditionally used as a purgative for clearing internal heat in the stomach and intestine and treating constipation (Katakai *et al.*, 2002). It is composed of Dahuang (Radix et Rhizoma Rhei), Houpu (*Magnolia officinalis* Rehd.), Zhishi (Fructus Aurantii Immaturus) and Mangxiao (Natrii Sulfas). In the past, DCQD has been particularly used to treat acute pancreatitis in China according to the purgative theory of Chinese medicine. This prescription is orally administered to relieve the paralytic ileus in acute pancreatitis (Xia *et al.*, 1999).

In China, Chinese herbal medicine was co-administered with antibiotics, proton pump inhibitor, H_2 receptor antagonists and other modern medicines for the treatment of severe acute pancreatitis. Ranitidine was used to protect gastric mucosa and inhibit gastrointestinal and pancreatic secretion in patients with acute pancreatitis. We have reported the pharmacokinetic study of DCQD previously (Tang *et al.*, 2007). The addition of sodium sulfate promotes the absorbance of rhein and affects the pharmacokinetics of rhein after oral administration of DCQD and Xiao Cheng Qi decoction. Many studies focusing on the pharmacokinetic interaction between ranitidine and other medicines have been reported (Kenawi *et al.*, 2005). A few studies have reported that Chinese herbal formula had effect on the pharmacokinetics of drugs such as warfarin and ticlopidine hydrochloride (Makino *et al.*, 2002, 2003). Even though there were no serious adverse interactions between drug and herbal prescription medications in patients (Bush *et al.*, 2007), there were still life-threatening herb–drug interactions in rats (Chiang *et al.*, 2005). In

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Abbreviations used: DCQD, Da-Cheng-Qi decoction.

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this study, *Pueraria lobata* root decoction increased the area under the concentration–time curve, AUC(0–*t*), and prolonged the mean residence time of methotrexate, which resulted in surprisingly high mortalities in rats. In clinical practice, the lack of research on the herb–drug intercation suggests that patients may be at risk (Rogers *et al.*, 2001). Therefore, it is necessary to determine the interaction between Chinese herbal formula and drugs, focusing on their pharmacokinetic and pharmacodynamic intercations when they are co-administered. Then we could adjust dosages or avoid the incorrect coadministration with the Chinese herbal formula. However, there have been no studies on the effect of Chinese herbal formula DCQD on the pharmacokinetics of ranitidine when they are co-administered for the treatment of acute pancreatitis. It is still unknown whether the pharmocokinetics of ranitidine and components from DCQD would be mutually affected when they are administered simultaneously.

Thus, it is important to perform a preclinical study to compare the pharmacokinetics of ranitidine in the plasma when ranitidine is administered orally alone or with DCQD. Furthermore, it would be practical to optimize the oral dosage schedule of ranitidine in clinical practice when used with DCQD. This study aims to explore the effect of DCQD on the pharmacokinetics of ranitidine in rats by HPLC.

EXPERIMENTAL

Materials and reagents. The dried powder extracts of Dahuang, Houpu, Zhishi and Mangxiao were purchased from Chengdu Green Herbal Pharmaceutical Co. Ltd (Chengdu, Sichuan, China). They were spray-dried and stored at 4°C until use, and the voucher specimen of the prescription for this study was an aliquot from the same batch; its code number is 20060625, and it has been deposited in the storeroom of our laboratory. Ranitidine was commercially purchased from Chong Qing Huapont Pharm Co. Ltd.

The reference standard of ranitidine and the internal standard (IS) trimethoprim (Fig. 1) were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Methanol was HPLC-grade (Tedia Company Inc., USA). Acetic acid was obtained from Chongqing Chemistry Co. Ltd (Chongqing, China). Water was triple-distilled and filtered with 0.45 μm membranes.

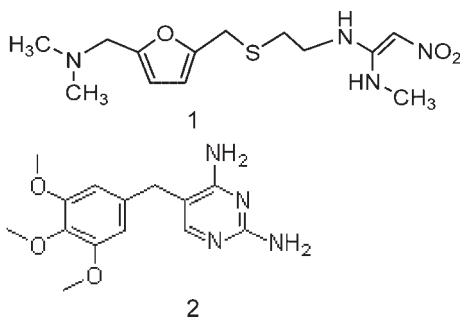

Figure 1. Chemical structures of ranitidine (**1**) and trimethoprim (**2**).

Chromatographic conditions. The HPLC system consisted of a Waters 1525 Binary HPLC pump, a Waters 717^{plus} autosampler and a Waters 2487 Dual λ absorbance detector with Empower chromatographic working station (Waters, Milford, MA, USA). The analytical column, a YMC-Pack RP-C_{18} (150 × 4.6 mm) and the guard column were purchased from GL Sciences Inc. (Japan). The mobile phase was methanol–0.1 mol/L sodium acetate (40:60, v/v) with a flow rate of 0.5 mL/min. The detection wavelenth was 320 nm. To balance the system, a 10 min lag was maintained between each sampling. The chromatographic separation was performed at 23°C. The chromatographic data were recorded and processed with Waters Millennium 2000 software.

Dosage of DCQD and ranitidine. The crude drug ratio of Dahuang, Houpu, Zhishi and Mangxiao in DCQD was 1:1:1:1. According to this ratio, the dosages of spray-dried powder for each herb in the prescription were determined, mixed with distilled water and diluted to 1 g/mL. The dosage of DCQD was 10 g/kg. The dosage of ranitidine for rats was 150 mg/kg, 30 times that for humans. Ranitidine was added to the solution of DCQD and vortex for 10 min before use when they were co-administered. It was dissolved in water with an end concentration of 15 mg/mL when used alone.

Animals, drug administration and plasma collection. Ten male Sprague–Dawley rats (body weight, 280 ± 10 g) that were born, housed, fed and handled according to the university guidelines and the Animal Ethics Committee guidelines in the animal facility of West China Hospital. The rats were maintained in air-conditioned animal quarters under the following conditions: temperature, 22 ± 2°C; relative humidity of 65 ± 10%; free access to water; and feeding with laboratory rodent chow (Chengdu, China). The animals were acclimatized to the facilities for one week and were then fasted with free access to water for 24 h prior to the experiment. They were divided into two groups.

Group A was orally administered with ranitidine alone while group B was dosed with a mixture of ranitidine and DCQD, as described above. Blood samples (0.5 mL) were collected through the tail vein at time points of 0 (prior to administration), 0.25, 0.5, 1, 2, 3, 4, 6, 8 and 12 h after a single dose. The rats had free access to water during the experiment. The blood samples were immediately heparinized and centrifuged at 3000 rpm for 10 min, and the supernatant was separated into 0.2 mL aliquots and stored in 1 mL polypropylene tubes at –80°C prior to analysis.

Extraction procedure. All the standards and the spiked plasma were similarly treated. An aliquot of 0.15 mL plasma and 5 μL of IS (2 μg/mL) was placed in a 1.5 mL polypropylene centrifuge tube and thoroughly homogenized by vortexing, mixed with 100 μL of 2 M sodium hydroxide, and vortex-mixed for 30 s. The analytes were extracted by the addition of 0.7 mL dichloromethane, vortex-mixed for 2 min, and centrifuged at 4000 rpm for 15 min to achieve separation. The

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Table 1. Recoveries of ranitidine in rats plasma (mean ± SD, $n = 5$)

Concentration added (μg/mL)	Concentration measured (μg/mL)	Recovery[a] (%)	Average (%)	RSD[b] (%)	Average (%)
0.4	0.42 ± 0.01	105.0 ± 2.3	99.8	2.3	2.1
2.4	2.31 ± 0.07	96.3 ± 1.5	99.8	3.0	2.1
9.6	9.43 ± 0.11	98.2 ± 3.1	99.8	1.1	2.1

[a] Recovery (%) = (measured concentration/nominal concentration) × 100.
[b] RSD (%) (relative standard deviation) = (SD/mean) × 100.

supernatant was discarded and the subnatant was dried in a 37°C water bath under nitrogen gas. The dried residue was reconstituted in 150 μL of methanol, and 20 μL of this solution was injected into the HPLC system.

Calibration procedure. Stock solutions of ranitidine and IS were prepared by dissolving each of the substances in methanol. The calibration curves were constructed based on analysis of various concentrations (0.1, 0.4, 1.6, 3.2, 4.8, 12 and 20 μg/mL) spiked in drug-free blank plasma (IS 2 μg/mL) by HPLC.

Recovery. Drug-free plasma samples of rats were spiked with three different concentrations of ranitidine (0.4, 2.4 and 9.6 μg/mL). Then fixed amounts of IS were added to plasma for normalization. The plasma samples were processed according to the extraction method above. The recovery was calculated with the internal standard method.

Precision and accuracy. Plasma samples with ranitidine (at concentrations of 0.4, 2.4 and 9.6 μg/mL) were prepared. Intra- and inter-day assays were repetitively carried out on the plasma samples at six different times of the same day and three different days, respectively. Analytical precision was evaluated by calculating the RSD of variances intra- and inter-day, while accuracy was assessed by expressing the mean calculated concentration as a percentage of the nominal concentration, reflecting the difference between the measured concentration and spiked concentration.

Pharmacokinetics analysis. The concentration–time data were computer fitted using the Pharmacokinetics 3p97 program edited by the Mathematics Pharmacological Committee, Chinese Pharmacological Society. The following pharmacokinetic parameters were calculated: peak concentration (C_{max}), time of maximum plasma concentration (T_{max}), half-life ($t_{1/2}$) and AUC (0–12 h). In addition, other parameters were also investigated.

Statistical analysis. Data are expressed as mean ± SD. Comparisons of pharmacokinetic parameters between two groups were performed using Student's *t*-test. A difference of $p < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Extraction and recovery

The method for the determination of ranitidine required only 0.15 mL plasma to be extracted using a simple dichloromethane extraction procedure. The mean recoveries of ranitidine from rat plasma were 99.8% with mean RSD of 2.1% (Table 1).

Chromatographic selectivity

Figure 2 shows the chromatograms obtained following the analysis of drug-free plasma, drug-free plasma spiked with ranitidine and IS and plasma sample collected at 3 h after oral administration of ranitidine alone or ranitidine with DCQD, respectively. Based on the chromatographic behavior of pure reference standards, peaks labeled 1 in Fig. 3 were identified as ranitidine with a retention time of 9.97 min and IS with a retention time of 12.71 min. Under the chromatographic conditions described, complete resolution was achieved and no interfering peaks were observed within the time frame in which ranitidine and IS were detected. The method used in this study is very rapid, with a run-time of 14 min for each analysis. This suggested an efficient sample-preparation and clean-up method.

Calibration curves

The calibration curve for ranitidine was linear ($r^2 = 0.9999$) over the concentration range 0.1–20 μg/mL; a regression equation of $y = 3.1564x + 0.0901$ (where x is the peak area ratio and y the concentration of analyte) was obtained. Its detection limit based on a signal-to-noise ratio of 3 was 25 ng/mL and the quantitation limit was 50 ng/mL with an RSD of 12.38%. These ranges were found to be adequate for the concentrations observed from the analysis of collected plasma samples. It showed considerable linearity and had similar precision and accuracy to those reported in a study by Lee (Schaiquevich *et al.*, 2000).

Precision and accuracy

The reproducibility of the method was defined by examining both intra- and inter-day variance. The intra- and inter-day precision assay also gave satisfactory results (Table 2). The method was found to be highly precise, with an mean RSD <3% and accuracy in the range of 97.9–103.2% for each of the concentrations

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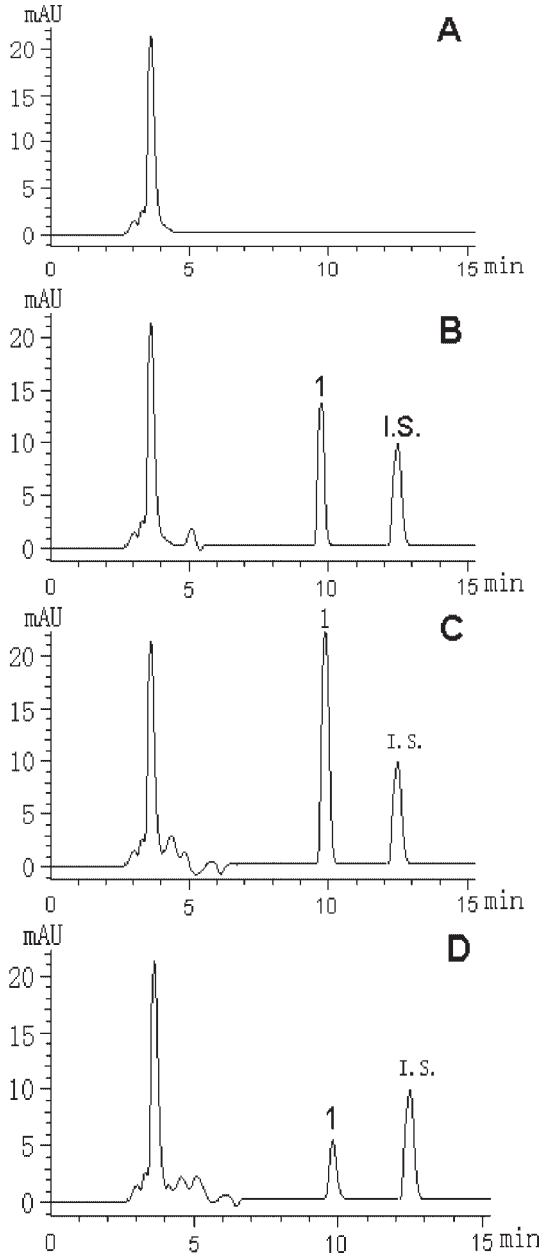

Figure 2. Typical chromatograms of ranitidine in plasma samples: (A) chromatogram of a blank plasma sample; (B) chromatogram of a plasma sample spiked with ranitidine (**1**) and IS; (C, D) chromatogram of the plasma sample collected 3 h after oral administration of ranitidine alone or with DCQD, respectively.

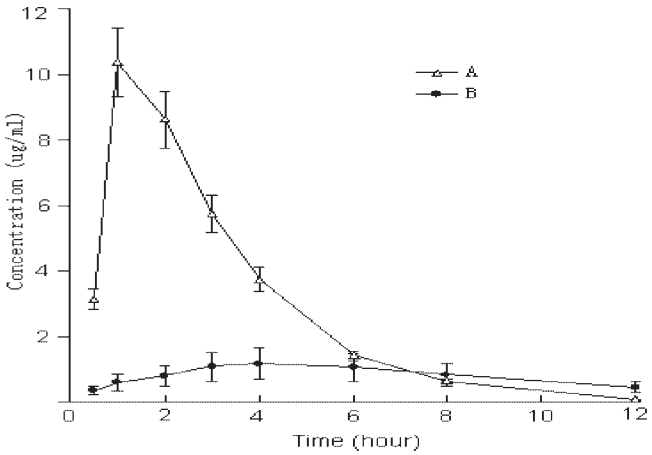

Figure 3. Plasma concentration–time curves of ranitidine after oral dosing with ranitidine at doses of 150 mg/kg body weight. A, Ranitidine alone; B, ranitidine with DCQD. Each point and bar represent the mean ± SD (n = 5).

tested. The results showed that the method has good reproducibility.

Pharmacokinetic comparison

In the study, the established method successfully determined ranitidine in rats after oral administration of ranitidine alone or with DCQD, and the mean estimated pharmacokinetic parameters are listed in Table 3. Following oral administration ranitidine alone, its plasma levels revealed curves characterized by peak at 1.8 h with mean concentration of 16.315 μg/mL. However, when ranitidine was dosed to rats with DCQD simultaneously, the plasma level reached its peak of 1.455 μg/mL at 4.2 h. The half-life of ranitidine in group B was much longer than that in group A.

Although the dosages of ranitidine in the two groups were both 150 mg/kg, the estimated pharmacokinetic parameters between two groups were very different. After the addition of DCQD, the T_{max} of rantidine was delayed, with longer half-life compared with ranitidine alone (Fig. 3). The mean plasma concentration and $AUC_{(0-12\,h)}$ of ranitidine in group A were much larger than those from group B ($p < 0.05$). This contributed to the presence of DCQD, suggesting that DCQD could

Table 2. Intra- and inter-day variability for ranitidine (n = 6)

Variable	Added concentration (μg/mL)	Accuracy[a] (%)	Average (%)	RSD[b] (%)	Average (%)
Intra-day	0.4	101.6	101.5	3.1	2.5
	2.4	99.8	101.5	2.5	2.5
	9.6	103.2	101.5	2.0	2.5
Inter-day	0.4	98.4	99.7	3.1	2.3
	2.4	102.8	99.7	2.6	2.3
	9.6	97.9	99.7	1.3	2.3

[a] Accuracy (%) = (mean of measured concentration/nominal concentration) × 100.
[b] RSD (%) (relative standard deviation) = (SD/mean) × 100.

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Table 3. Pharmacokinetic parameters of ranitidine in rats ($n = 5$)

Parameters	Units	Group A	Group B	p-value
$t_{1/2}$	h	1.787 ± 0.856	3.758 ± 1.635	0.0440
T_{max}	h	1.8 ± 1.304	4.2 ± 1.095	0.0136
C_{max}	mg/L	16.315 ± 9.514	1.455 ± 0.499	0.0082
$AUC_{(0-12)}$	mg/L h	28.083 ± 16.728	9.826 ± 1.911	0.0415
$AUC_{(0-\infty)}$	mg/L h	29.284 ± 5.975	20.835 ± 3.905	0.0294
$MRT_{(0-t)}$	h	3.198 ± 0.54	5.705 ± 0.591	0.0001
$MRT_{(0-\infty)}$	h	3.711 ± 0.733	17.302 ± 10.655	0.0216

Group A: ranitidine alone; Group B: administered with ranitidine and DCQD.

significantly affect the pharmacokinetics of ranitidine in rats.

When ranitidine was orally administered to volunteers alone, its T_{max} was about 3 h, and the half-life increased to 2.78 h (Gschwend *et al.*, 2007), which was substantially greater than the duration observed in this study. However, when ranitidine was co-administered with DCQD to rats at dosages of 150 mg/kg, its plasma concentration reached a C_{max} of 1.455 ± 0.499 mg/L at 4.2 ± 1.095 h, suggesting much lower concentrations and longer T_{max} than from ranitidine alone. This effect may first be attributed to the lower bioavailability of ranitidine after the addition of DCQD, caused by the inter-competitive absorbance between ranitidine and components from DCQD (Flores-Murrieta *et al.*, 2006). As far as we know, herbal extract or formula may affect cytochrome P-450 and then change the process of drug metabolism (Markowitz *et al.*, 2003; Modarai *et al.*, 2007). Components from DCQD affect cytochrome P-450 (Zheng and Zhou, 2007), which may affect the metabolism of ranitidine (Rendic, 1999). Therefore, the varied pharmacokinetic parameters of ranitidine after co-administration may be partly attributed to the effect of DCQD on cytochrome P-450. This is similar to the interaction among Chinese herbal formulas (Huang *et al.*, 2000). Then the dosage of ranitidine co-administered orally with DCQD should be based on the pharmacokinetic parameters in the current study; otherwise co-administration should be avoided.

CONCLUSIONS

This is the first study to compare the pharmacokinetics of ranitidine in rat after oral administration alone or with DCQD. The pharmacokinetic parameters showed that ranitidine alone was absorbed well with higher concentrations and larger $AUC_{(0-12\,h)}$ in plasma than when co-administered with DCQD. The T_{max} and half-life of ranitidine after co-administration with DCQD were both longer than with ranitidine alone in male rats after a single oral dose. The pharmacokinetic parameters may be used to guide the clinical administration of ranitidine with traditional Chinese prescriptions related to DCQD, or herb–drug co-administration can be avoided.

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REFERENCES

Beger HG and Rau BM. Severe acute pancreatitis: clinical course and management. *World Journal Gastroenterology* 2007; **13**: 5043–5051.

Bush TM, Rayburn KS, Holloway SW, Sanchez-Yamamoto DS, Allen BL, Lam T, So BK, Tran de H, Greyber ER, Kantor S and Roth LW. Adverse interactions between herbal and dietary substances and prescription medications: a clinical survey. *Alternative Therapies in Health and Medicine* 2007; **13**: 30–35.

Chiang HM, Fang SH, Wen KC, Hsiu SL, Tsai SY, Hou YC, Chi YC and Chao PD. Life-threatening interaction between the root extract of *Pueraria lobata* and methotrexate in rats. *Toxicology and Applied Pharmacology* 2005; **209**: 263–268.

Flores-Murrieta FJ, Toledo A, del Carmen Carrasco-Portugal M, Reyes-Garcia G, Rodriguez-Silverio J, Medina-Santillan R and Herrera JE. Comparative bioavailability of two oral formulations of ranitidine. *Biopharmaceutics & Drug Disposition* 2006; **27**: 23–27.

Gschwend MH, Guserle R, Erenmemisoglu A, Martin W, Tamur U, Kanzik I and Hincal AA. Pharmacokinetics and bioequivalence study of ranitidine film tablets in healthy male subjects. *Arzneimittelforschung* 2007; **57**: 315–319.

Huang X, Ren P, Wen AD, Wang LL, Zhang L and Gao F. Pharmacokinetics of traditional Chinese syndrome and recipe: a hypothesis and its verification (I). *World Journal of Gastroenterology* 2000; **6**: 384–391.

Katakai M, Akamaru T and Tani T. An analysis of the frequency of formulations and crude drugs described in Shan-Han-Lun. *Yakushigaku Zasshi* 2002; **37**: 28–35.

Kenawi IM, Barsoum BN and Youssef MA. Drug–drug interaction between diclofenac, cetirizine and ranitidine. *Journal of Pharmaceutical and Biomedical Analysis* 2005; **37**: 655–661.

Makino T, Wakushima H, Okamoto T, Okukubo Y, Deguchi Y and Kano Y. Pharmacokinetic interactions between warfarin and kangen-karyu, a Chinese traditional herbal medicin and their synergistic action. *Journal of Ethnopharmacology* 2002; **82**: 35–40.

Makino T, Wakushima H, Okamoto T, Okukubo Y, Deguchi Y and Kano Y. Pharmacokinetic and pharmacological interactions between ticlopidine hydrochloride and Kangen-Karyu Chinese traditional herbal medicine. *Phytotherapy Research* 2003; **17**: 1021–1024.

Markowitz JS, Donovan JL, DeVane CL, Taylor RM, Ruan Y, Wang JS and Chavin KD. Effect of St John's wort on drug metabolism by

induction of cytochrome P450 3A4 enzyme. *JAMA* 2003; **290**: 1500–1504.

Modarai M, Gertsch J, Suter A, Heinrich M and Kortenkamp A. Cytochrome P450 inhibitory action of *Echinacea* preparations differs widely and co-varies with alkylamide content. *Journal of Pharmacy and Pharmacology* 2007; **59**: 567–573.

Rendic S. Drug interactions of H2-receptor antagonists involving cytochrome P450 enzymes: from the laboratory to the clinic. *Croatian Medical Journal* 1999; **40**: 357–367

Ribeiro MD, Paiva JA, Landeiro N and Duarte J. Patients with severe acute pancreatitis should be more often treated in an intensive care department. *Revista Española de Enfermedades Digestivas* 2002; **94**: 523–532.

Rogers EA, Gough JE and Brewer KL. Are emergency department patients at risk for herb-drug interactions? *Academic Emergency Medicine* 2001; **8**: 932–934.

Schaiquevich P, Niselman A and Rubio M. Comparison of two compartmental models for describing ranitidine's plasmatic profiles. *Pharmacology Research* 2002; **45**: 399–405.

Tang WF, Wan MH, Zhu L, Chen GY, Xia Q and Huang X. Immuno-modulatory effect of somatostatin combined with traditional Chinese medicine on severe acute pancreatitis at early stage: a randomized control trial. *Zhong Xi Yi Jie He Xue Bao* 2005; **3**: 103–107.

Tang WF, Yu Q, Wan MH, Qin F, Wang YG and Chen GY. Simultaneous determination and pharmacokinetic studies of aloe emodin and chrysophanol in rats after oral administration of Da-Cheng-Qi decoction by high-performance liquid chromatography. *Biomedical Chromatography* 2007; **21**: 701–707.

Xia Q, Gong X, Jiang J, Chen G and Huang Z. Experimental study of da chengchi tang decoction for relieving lung injury during acute necrotizing pancreatitis. *Hua Xi Yi Ke Da Xue Xue Bao* 1999; **30**: 397–400.

Xia Q, Jiang JM and Gong X. Experimental study of 'TongXia' purgative method in ameliorating lung injury in acute necrotizing pancreatitis. *World Journal of Gastroenterology* 2000; **6**: 115.

Zheng J and Zhou HH. Effects of the flavonoids on cytochrome P-450 CYP1, 2E1, 3A4 and 19. *Yao Xue Xue Bao* 2007; **42**: 8–12.

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