ORIGINAL ARTICLE

Effect of Red Ginseng Extract on the Pharmacokinetics of Aspirin Metabolite in Sprague Dawley Rats

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Key words: red ginseng extract; aspirin; salicylic acid; pharmacokinetics

Objective To investigate whether red ginseng extract can affect the pharmacokinetics of aspirin in Sprague Dawley (SD) rats.

Methods Totally, 12 male SD rats were randomly and uniformly divided into the aspirin group (aspirin 10.42 mg·kg⁻¹) and the combined group (red ginseng extraction 0.5 mg·g⁻¹ + aspirin 10.42 mg·kg⁻¹). After intragastric administration of drugs, blood samples (0.5 ml once) were drawn from orbit at 0.083, 0.25, 0.5, 1, 2, 4, 6, 8, 10 and 12 hours after dosing. Plasma concentration of salicylic acid (metabolite of aspirin) was detected with ultraviolet-visible high performance liquid chromatography (HPLC). The reliability of the procedure was verified with respect to specificity, linearity, accuracy, precision, extraction recovery and matrix effect, and stability. Pharmacokinetics of salicylic acid was evaluated by using non-compartmental model.

Results The method was proved to be validated. Compared with the aspirin group, area under the curve (AUC_{0-t}) and maximum concentration of salicylic acid in rats of the combined group increased obviously ($P < 0.01$), while clearance rate (CL_r/F) decreased clearly ($P < 0.05$).

Conclusion The *in vivo* study showed that red ginseng extract can help the internal absorption of aspirin, and delay the *in vivo* metabolism of aspirin.

CARDIOVASCULAR and cerebrovascular diseases (CCVD) are the leading cause of mortality for middle-aged and elderly people.¹ The best way to reduce the incidence of CCVD is to give appropriate medicines. It has been

proved that aspirin is effective for primary and secondary prevention of CCVD. Long-term use of low dose aspirin (75-150 mg) can distinctly reduce the risk of many vascular diseases.^{2, 3} It has been suggested that people aged 40 to 69 should use aspirin chronically for primary CCVD prevention.^{4, 6} Oral administration of aspirin is rapidly converted into active metabolite salicylic acid after absorption, so it is difficult to detect *in-vivo* aspirin rapidly and accurately. Therefore, pharmacokinetics of aspirin is often studied by measuring the concentration of salicylic acid in blood.^{7, 8}

Red ginseng is produced by steaming and drying from ginseng (the root of *Panaxginseng* C.A. Mey.). It

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has been generally used in the older people for health care as a tonic medicine and nutraceutical food in Asian countries, such as China, Korea and Japan. It has been estimated that about 10% people take red ginseng everyday and more than 50% people eat red ginseng every month in Korea.⁹ As a nutraceutical food, red ginseng has many beneficial properties, including anti-inflammation, anti-hyperlipidemic, anti-thrombotic, anti-platelet, anti-aging and anti-oxidative.¹⁰⁻¹⁴ In practice, many middle-aged and elderly people in Asia often use aspirin and red ginseng together. However, there is no related report on the effects of drug combination. In this study, pharmacokinetics of salicylic acid after co-administering aspirin with red ginseng extract to Sprague Dawley (SD) rats was investigated.

MATERIALS AND METHODS

Drugs and reagents

Standard salicylic acid (Lot No. 100106-201104) was provided by the National Institute for Food and Drug Control, China. Aspirin powder was purchased from Xi'an Yue Lai Medical Technology Co., Ltd (China). Other reagents including methanol and acetonitrile were of HPLC grade.

Red ginseng slices (Baishan, Jilin Province, China) were bought from Tongrentang Pharmaceutical Factory (China). Red ginseng extract was prepared as follows: red ginseng powder (4.8 g) diluted in 48 ml water was extracted by using refluxing extraction method for 30 minutes for twice. The decoction was concentrated up to 32 ml (equivalent to the crude drug of 0.15 g·ml⁻¹).

Instrumentation and chromatographic conditions

Chromatography was carried out on a Shimadzu HPLC LC-20A system (Japan) equipped with a LC-20AT pump, a DGU-20A₅ online degasser, a SPD-20A ultra-violet-visible (UV) dual-wavelength detector, a SIL-20A auto-sampler, and a CTO-10AS VP column oven (at 30°C). Samples were separated on a Welchrom C₁₈ column (4.6 mm×150 mm, 5 µm particle size) with mobile phase consisting acetonitrile, acetic acid, and water (25:2:73, v/v/v) at a flow rate of 1.0 ml·min⁻¹ at room temperature (25°C±2°C). UV detection was performed at a wavelength of 303 nm. HPLC data were analyzed by LC solution software.

Validation of the HPLC-UV method

We investigated the parameters including specificity,

linearity, accuracy, precision, extraction recovery and matrix effect, and stability to validate the reliability of the method.

Preparation of standard solution

Standard stock solution of salicylic acid was prepared by dissolving 10.03 mg of salicylic acid in 10 ml acetonitrile. The working standard solutions of salicylic acid was prepared at the concentrations of 2.508, 5.015, 10.03, 20.06, 30.09, 50.15, 100.30, 200.60, and 300.90 µg·ml⁻¹ respectively by diluting stock solution in acetonitrile.

Specificity

Specificity of the proposed method was verified through chromatographic runs of blank plasma solution, salicylic acid standard solution and plasma sample solution to check the interference from the components of plasma solution and salicylic acid solution.

Linearity

The standard salicylic acid working solution of different concentrations (10 µl) was diluted with blank plasma (100 µl) and swirled for 30 seconds to reach the final concentrations of 250.75, 501.5, 1003, 2006, 3009, 5015, 10 030, 20 060, and 30 090 ng·ml⁻¹ of salicylic acid respectively. The mixture solution was added to acetonitrile (190 µl), then swirled for 30 seconds and centrifugated at 2331×g for 10 minutes. Finally, 10 µl supernatant was used to quantify salicylic acid concentration by using HPLC. The limit of quantification (LOQ) was defined as the minimum level that the salicylic acid can be quantified. The limit of detection (LOD) was defined as the minimum level that the salicylic acid can be detected.

Accuracy

Accuracy of the proposed method was determined by adding known amounts of salicylic acid standard solution to plasma sample solution. The measured value of this solution was compared with the known value. Three concentration levels of salicylic acid in plasma (501.5, 3009, and 20 060 ng·ml⁻¹ respectively) were assessed, and each was analyzed in quintuplicate. Accuracy was reported as relative error (RE).

Precision

Precision of the proposed method was studied by determining intra-day and inter-day repeatability. Intra-day precision was determined by carrying out five

independent assays of the test sample in a day and calculating relative standard deviation (RSD) of five standard replicates.

Inter-day precision was determined by evaluating three different concentrations of salicylic acid (501.5, 3009, and 20 060 ng·ml⁻¹, respectively) for three days and calculating RSD between three replicates for each concentration.

Extraction recovery and matrix effect

Extraction recovery and matrix effect of the proposed method was measured as follows. Firstly, three different salicylic acid blank sample solutions (501.5, 3009, 20 060 ng·ml⁻¹ respectively) were prepared by diluting salicylic acid standard stock solution in acetonitrile; secondly, three different salicylic acid plasma sample solutions (501.5, 3009, 20 060 ng·ml⁻¹ respectively) were obtained by adding salicylic acid standard stock solution to the blank plasma; thirdly, three different salicylic acid precipitated plasma sample solutions (501.5, 3009, 20 060 ng·ml⁻¹ respectively) were prepared by adding salicylic acid standard stock solution to the blank plasma precipitated by acetonitrile.

Extraction recovery was measured by comparing the responses of salicylic acid plasma sample solution and precipitated plasma sample solution. The matrix effect was calculated by comparing the responses of salicylic acid blank sample solution and plasma sample solution. Each level was made in quintuplicate.

Stability

Stability of the plasma sample solution was determined by comparing the responses of three different concentrations (501.5, 3009, 20 060 ng·mL⁻¹, respectively) of salicylic acid plasma sample solution placed at different temperatures (4°C, 25°C) for 12 hours with sample that is not placed. Each level was made in quintuplicate, and RE and RSD were calculated to evaluate stability.

Grouping and drug administration

SD male rats ($n=12$) weighing 250 ± 10 g were provided by Beijing Vital River Laboratory Animal Technology Co., Ltd. [animal license No. SCXX (JING) 2012-0001]. Animals were housed in the Beijing University of Chinese Medicine Laboratory. Rats were acclimatized for 7 days before the experiments. All rats were fasted for 12 hours before experiment. All the animal studies were performed under the Guidelines for the Care and Use of Laboratory Animals and the Experimental Protocols.

They were randomly and uniformly divided into the aspirin group ($n=6$) and combined group ($n=6$). Rat in the aspirin group was intragastrically administered 10.42 mg·kg⁻¹ aspirin (equivalent to 100 mg per 60 kg body weight, conversion factor 6.25). Rat in the combined group was given 0.5 mg·g⁻¹ red ginseng extract (equivalent to 4.8 g per 60 kg body weight, conversion factor 6.25) and 10.42 mg·kg⁻¹ aspirin by gavage.

Collection of blood samples

Blood samples (0.5 ml once) were drawn from orbit at 0.083, 0.25, 0.5, 1, 2, 4, 6, 8, 10, and 12 hours after drug administration and anticoagulated with heparin. All blood samples were centrifugated at 2331×*g* for 10 minutes. Transfer supernatant plasma 100 μl in 0.5 ml centrifuge tube, and add acetonitrile 200 μl. The mixture was swirled for 30 seconds and centrifugated at 2331×*g* for 10 minutes. An aliquot of 10 μl supernatant was analyzed by using HPLC-UV.

Data analysis

Plasma concentration—time curve of salicylic acid of the aspirin group and combined group was constructed. The pharmacokinetic parameters of salicylic acid including $AUC_{(0-t)}$, C_{max} , $MRT_{(0-t)}$, T_{max} , $t_{1/2z}$, V_z/F , and CL_z/F were determined according to non-compartmental model. $AUC_{(0-t)}$ reflects the relative amount of the drug entering the systemic circulation; C_{max} indicates the highest concentration of the drug in the blood; T_{max} (the peak time) reflects the speed at which the drug enters the body; $MRT_{(0-t)}$ (mean residence time) indicates the average retention time of the drug *in vivo*; $t_{1/2z}$ (half-life) refers to the time reducing the concentration of plasma drugs in half; CL_z/F reflects the clear rate of the drug from the body.

Statistical analysis

All data was analyzed by using DAS 3.0 software. Pharmacokinetic parameters data were shown as the mean ± SD. The differences between groups were determined by two independent samples *t*-test. Linear regressive analysis was carried out to analyze the correlation between peak area and concentration of salicylic acid. $P<0.05$ was considered as statistically significant.

RESULTS

Parameters of methodology

In this study, we developed a simple, sensitive and ac-

curate HPLC method for determination of salicylic acid in plasma. In order to observe symmetrical peaks with better mobile phase, we optimized the chromatographic conditions, such as the ratio of acetonitrile, acetic acid and water. **Fig. 1** showed no interference from the components of plasma solution and salicylic acid solution was observed and the peaks of salicylic acid were obtained at retention time of 10.00 minutes.

Linear regressive analysis revealed a linear correlation between peak area and concentration in the range of 250.75–30 090 ng·mL⁻¹ for salicylic acid. The linear regression equation was $Y=6.6766 X+ 899.58$ with correlation coefficient equal to 0.9998, where X is the concentration and Y is the absolute peak area. The LOQ and LOD were respectively 250.8 ng·mL⁻¹ and 75.2 ng·mL⁻¹.

The results for the accuracy and precision investigations are shown in **Table 1**. Values of RE and RSD were well within the acceptable range of $\pm 15\%$. The results indicated the analytical methods had a good accuracy and precision.

The results ($\geq 70\%$) shown in **Table 2** indicated that the pretreatment of sample had a good extraction recovery and acceptable matrix effect.

Table 3 illustrated that values of RE and RSD were well within the acceptable range of $\pm 15\%$, and

Figure 1. The representative chromatogram of blank plasma and salicylic acid solutions. A. blank plasma; B. salicylic acid standard solution; C. salicylic acid plasma solution.

the concentrations of salicylic acid in plasma under different conditions had a good stability.

Effects of red ginseng extract on pharmacokinetics of salicylic acid

Plasma concentration—time curves of the aspirin group and combined group are shown in **Fig. 2**. As demonstrated in **Table 4**, compared with the aspirin group, AUC_(0-t) and C_{max} of the combined group increased ob-

Table 1. Accuracy and precision of the method (%)

Concentration of salicylic acid (ng·mL ⁻¹)	RE	RSD	
		Intra-day	Inter-day
501.5	-2.58	11.82	6.99
3009	0.20	1.63	3.81
20 060	-2.43	1.27	1.28

RE: relative error; RSD: relative standard deviation.

Table 2. Extraction recovery and matrix effect evaluated with salicylic acid sample solution at the three concentration levels in rat plasma[§] (%)

Actual concentration of salicylic acid (ng·mL ⁻¹)	Extraction recovery	Matrix effect
501.5	80.96 $\pm$ 7.57	82.83 $\pm$ 9.49
3009	86.34 $\pm$ 3.48	83.90 $\pm$ 3.07
20 060	89.53 $\pm$ 1.55	88.40 $\pm$ 1.49

[§]: Plus-minus values are means $\pm$ SD.

Table 3. Stability of the method determined with salicylic acid sample solution at the three concentrations in rat plasma (%)

Actual concentration of salicylic acid (ng·mL ⁻¹)	At 4°C		At 25°C	
	RE	RSD	RE	RSD
501.5	2.66	7.12	-7.42	5.93
3009	8.24	1.71	2.67	2.52
20 060	-0.36	6.34	-4.74	3.64

Figure 2. Plasma concentration—time curves of salicylic acid in plasma obtained from rats after intragastric administration of drugs. Data were expressed as means $\pm$ SD ($n=6$).

Table 4. Comparisons of pharmacokinetic parameters of salicylic acid in the plasma of the two-group rats[§] ($n=6$)

Groups	AUC _(0-t) (ng·ml ⁻¹ ·h ⁻¹)	C _{max} (ng·ml ⁻¹)	MRT _(0-t) (h)	T _{max} (h)	t _{1/2z} (h)	CL _z /F (L·h ⁻¹ ·kg ⁻¹)
Aspirin group	93 204.13±24 873.73	17 339.63±2 738.64	3.69±0.71	1.17±0.68	1.86±0.83	0.11±0.04
Combined group	156 191.26±41 214.72**	25 999.77±3 679.22**	4.14±0.32	1.50±0.54	2.79±1.38	0.06±0.02*

§: Plus-minus values are means±SD. AUC: area under the curve; C_{max}: maximum concentration; MRT_{0-t}: mean residence time; T_{max}: peak time; t_{1/2z}: elimination half-time; CL_z/F: clearance rate.

* $P<0.05$, ** $P<0.01$ compared with the aspirin group.

viously (all $P<0.01$), while CL_z/F was decreased clearly ($P<0.05$).

DISCUSSION

Aspirin is widely used in the treatment of ischemic stroke, myocardial infarction, and the prevention of CCVD. During the treatment of these clinical illnesses, aspirin is often combined with other drugs.¹⁵⁻¹⁶ In recent years, some pharmacokinetic interactions between aspirin and other drugs have been investigated. Tian *et al*⁷ found that drug-drug interaction *in vivo* existed between aspirin and panax notoginseng saponins, the concentration of salicylic acid increased obviously when the two drugs were administered together, and the result was proved by observing the transport of aspirin and salicylic acid in MDCK-MDR₁ cell monolayer. In addition, Chen *et al*⁸ reported that single-dose of Mailing injection accelerated the clearance of aspirin in rats, but this effect of multiple-dose was not distinct.

Aspirin can be easily absorbed in the gastrointestinal tract, and then quickly decomposed into salicylic acid by aspirin esterases *in vivo*. It has been reported that aspirin can reach the peak plasma concentration in 30 minutes after oral administration, and salicylic acid can also reach the C_{max} after taking medicine for 1-2 hours. The elimination half-life of aspirin and salicylic acid are 20-30 minutes and 3-4 hours, respectively.^{17,18} These data suggests that aspirin has a fast absorption and metabolism in human body.

In this study, the effects of red ginseng extract on the pharmacokinetics of aspirin in rats were investigated. When comparing the results of the aspirin group, it can be found that AUC_(0-t) and C_{max} of the combined group increased clearly ($P<0.01$), and the calculated value of relative bioavailability of the combined group was 167.58%. These results indicated that red ginseng extract can improve relative bioavailability of salicylic acid. This study also found that CL_z/F of the combined group decreased obviously ($P<0.05$); MRT_(0-t), T_{max} and t_{1/2z} of the combined group increased, but the differ-

ences were not statistically significant ($P>0.05$). By analyzing the reduction of CL_z/F and the enhancement of MRT_(0-t) and t_{1/2z}, it suggests that red ginseng extract can improve the residence time of salicylic acid by reducing the speed of elimination of salicylic acid. To summarize, red ginseng extract can improve the absorption of salicylic acid and slow down its metabolism *in vivo*.

It can be seen from Figure 2, when plasma concentration of the aspirin group began decreasing after 30 minutes, that of the combined group still increased. It means that red ginseng extract can improve the absorption of aspirin. By consulting literature materials, it shows that saponins have cell membrane permeabilising properties by solubilizing cholesterol and can influence cellular transport and permeability as absorption enhancers.^{16, 19,20} It is supposed that the ginsenosides of red ginseng extract maybe act as absorption enhancers and make it easier for aspirin to penetrate cells in the gastrointestinal tract. Besides, this study also revealed that red ginseng extract can reduce the metabolism of salicylic. Because cytochrome P450 2C9 (CYP2C9) is generally thought to be related to the metabolism of aspirin,^{21,22} and ginsenosides can inhibit the activities of CYP2C9.²³ It is suspected that red ginseng extract may inhibit the activities of CYP2C9 and then retard the metabolism of salicylic acid. The specific mechanism of absorption and elimination will be further studied.

In clinical practice, low dose of aspirin (75-150 mg daily) is an effective antiplatelet regimen for long-term use, however, aspirin can also injury human gastric epithelial cells (GES-1) in a concentration-dependent and time-dependent manner, and long-term use of aspirin can increase the risk of upper gastrointestinal bleeding.²⁴⁻²⁶ It is indicated that red ginseng extract can apparently accelerate the absorption of aspirin and restrain the elimination of salicylic acid. The results showed red ginseng extract can help the curative effect of aspirin last longer. These findings have clinical significance in warning people to use aspirin safely and

modestly when aspirin and red ginseng are administered together. Although there is no obvious evidence suggesting reducing the dose of aspirin would reduce the incidence of gastrointestinal bleeding, the risk of gastrointestinal bleeding seems more strongly related to dose than duration of aspirin use.²⁶ According to these results, people should use aspirin cautiously and pay more attention to bleeding risk issues when combined with red ginseng.

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