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Interaction of baicalin and baicalein with antibiotics in the gastrointestinal tract

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Abstract

To clarify the absorption mechanism of baicalin and to investigate the interaction between baicalin and antibiotics, the pharmacokinetics of baicalin and its aglycone baicalein in normal rats and in antibiotic-treated (with a mixture of neomycin and streptomycin) rats were investigated. A method of liquid chromatography–tandem mass spectrometry with a selected reaction monitoring mode was used to determine the plasma concentrations of baicalin and baicalein. The plasma concentration of total baicalein was determined after treatment of β-glucuronidase/sulfatase. Unpaired Student's *t*-test was used for statistical comparison. After oral administration of baicalin, the absolute bioavailability of baicalin, based on the AUC of the baicalin parent form, was $2.2 \pm 0.2\%$ in normal rats, which decreased to $1.5 \pm 0.2\%$ in antibiotic-treated rats. Based on the AUC of total baicalein after enzymatic hydrolysis, the absorption of baicalin was $28.0 \pm 5.7\%$, which significantly decreased to $7.7 \pm 1.2\%$ in antibiotic-treated rats. After oral administration of baicalein, the glucuronides/sulfates of baicalein were predominant in plasma. Based on the AUC of total baicalein after enzymatic hydrolysis, the absorption of baicalein was $36.1 \pm 4.4\%$ in normal rats, which did not differ markedly from that in antibiotic-treated rats ($P > 0.05$). The presence of baicalin isomer in plasma after oral administration of baicalin indicated that baicalin was transformed, at least in part, to baicalein before absorption, then to its conjugated metabolites in rats. Aminoglycosides decreased the absorption of baicalin, but not of baicalein, which indicated that antibiotics decreased the decomposition of baicalin to baicalein by inhibiting intestinal flora, and further influenced the absorption, metabolism and efficacy of baicalin. These interactions should be paid attention to in clinical studies when baicalin is administered in combination with antibiotics.

Introduction

The flavones baicalin (baicalein glucuronide, BG) and its aglycone, baicalein (BL) (Figure 1) are bioactive components of *Scutellariae radix* and the main constituents of a number traditional Chinese medicines, including Huangqin-Tang. These preparations have been used since ancient times for the treatment of inflammation, fever and allergic disorders (Nakajima et al 2001; Shen et al 2003).

In traditional Chinese medicine, most of the remedies are administered orally as crude decoction and active components have to cross the intestinal barrier to reach the systemic circulation. Active components of their prescriptions are therefore brought into contact with bacterial flora, mostly composed of anaerobes, in the alimentary tract (Akao et al 1994). Because of the polar nature of its glucuronide, a small amount of baicalin is expected to reach the systemic circulation as unchanged compound. Following oral administration, most of the baicalin is absorbed as its aglycone, baicalein, which is thought to be formed after hydrolysis of the β-glucuronide bond by the bacterial flora present in the lower gastrointestinal tract (Akao et al 2000). Once in the circulation, baicalin and baicalein are further glucuronidated by various organs, including the liver (Abe et al 1990; Akao et al 2000).

Large-spectrum antibiotics have the potential to alter the content of intestinal bacteria and may interfere with the pharmacokinetics and eventually pharmacodynamics of compounds (Kitamura et al 2000). This has been shown in the co-administration of kampo medicines with antibiotics or bacterial preparations (Ishihara et al 2002). The

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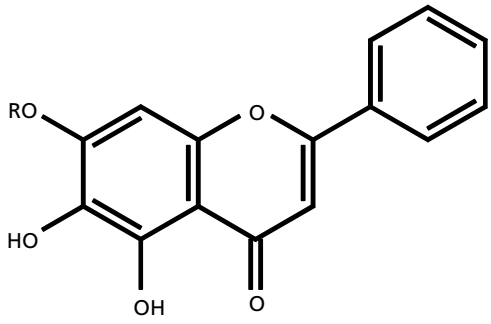

Figure 1 The structures of baicalin (BG) and baicalein (BL).

correlation of intestinal flora and activity of kampo ingredients has increasingly been recognized. As for baicalin, the intestinal bacteria, *Eubacterium* spp. and *Bifidobacterium* spp., are thought to participate in the absorption of baicalin (Ishihara et al 2002).

The metabolic pharmacokinetics of baicalin and baicalein in rats have been reported (Lai et al 2003). To evaluate the effect of antibiotic treatment on the absorption of baicalin, whether antibiotics affect the hydrolysis of baicalin to baicalein or of baicalein to the circulation, and to clarify the absorption mechanism of baicalin, we have attempted to characterize the metabolic pharmacokinetics of baicalin and baicalein when administered in combination with antibiotics.

Materials and Methods

Chemicals

Baicalin was purchased from Shifang Co. (purity > 98.3%, Sichuan, China). Daidzein was purchased from Xieli Co. (purity > 99.3%, Sichuan, China). Baicalein (purity > 99.5%) was obtained in our laboratory (a gift from the Department of Medicinal Chemistry, Shenyang Pharmaceutical University, China). Expected structures of all the standards were confirmed by MS and NMR spectroscopy. β-Glucuronidase (with arylsulfatase activity, partially purified, G-4259) was purchased from Sigma Chemical Co. (St Louis, MO, USA). Neomycin sulfate and streptomycin sulfate were purchased from Huabei Co. (purity > 99.0%, Hebei, China). Acetonitrile, methanol and ethyl acetate were HPLC grade and other chemicals used were of analytical reagent grade.

Instrumentation

Baicalin and baicalein were analysed using an HPLC system (Shimadzu 10-AT, Kyoto, Japan) coupled to a Thermo Finnigan TSQ Quantum Discovery triple quadrupole tandem mass spectrometer equipped with an electrospray interface and running in positive ion mode (San Jose, CA). The ionization was made possible by applying a spray voltage of 4.0 kV and keeping a capillary temperature of 320°C. Sheath gas and auxiliary gas (N_2) pressure were set at 35 and 3 Arb, respectively. The mass spectrometer was operated in selected reaction monitoring (SRM) mode using the transitions from the respective $[M + H]^+$ ions to the following product ions: baicalin m/z 447 $\rightarrow$ m/z 271, baicalein monitoring two product ions at m/z 271 $\rightarrow$ m/z 225 and 253, and daidzein m/z 255 $\rightarrow$ m/z 199. Argon was used as the collision gas at a pressure of 1.0 mTorr. Data were analysed by Xcalibur software (version 1.4, Thermo Finnigan).

A 20 μL aliquot was injected into a Diamonsil C_{18} column (particle size 5 μm, 200 × 4.6 mm ID, Dikma, China). The column temperature was kept constant at 25°C. The mobile phase consisted of a mixture of methanol/water/formic acid (60:40:0.5, v/v) (baicalin) or a mixture of acetonitrile/water/formic acid (50:50:0.5, v/v) (baicalein). Baicalin and baicalein were eluted isocratically using flow rates of 0.4 mL min^{-1} and 0.5 mL min^{-1}, respectively.

Drug administration and sample collection

Male Wistar rats (230–250 g) were supplied by the Lab Animal Center of Shenyang Pharmaceutical University (Grade II, Certificate No. SYXK 2003-0012). The experimental protocol was approved by the University Ethics Committee for the use of experimental animals and conformed to the Guide for Care and Use of Laboratory Animals. Rats were maintained at 22 ± 2°C and 55 ± 5% relative humidity on a 12 h light–dark cycle.

On the day of the experiment, baicalin was dissolved in phosphate solution (0.05 M, pH 7.4) (i.v., 37 μmol kg^{-1}) or suspended in 0.5% carboxymethyl cellulose (CMC)/Na solution (p.o., 224 μmol kg^{-1}) while baicalein was dissolved in a mixture containing DMSO:1,2-propanediol: water (0.2:6:4, v/v) (i.v., 37 μmol kg^{-1}) or suspended in 0.5% CMC/Na solution (p.o., 224 μmol kg^{-1}).

Before the beginning of the study, animals were fasted overnight and were allowed food only 3 h after dosing. Water was freely available. A first group of rats (n = 6) received an intravenous bolus of baicalin or baicalein (37 μmol kg^{-1}) while another group (n = 6) received a dose of 224 μmol kg^{-1} by oral gavage. Blood samples (250 μL) were withdrawn from the jugular vein before dosing and at the following time points after drug administration: 0.017 (only i.v.), 0.17 (only p.o., baicalein), 0.25 (only i.v.), 0.5, 1, 2 (not including p.o., baicalin), 3 (only p.o., baicalin), 4 (not including p.o., baicalin), 5 (only p.o., baicalin), 8, 12, 24, 36 (only p.o.) and 48 h (only p.o.). Blood samples were collected, transferred into heparin-containing tubes and gently mixed by inversion. Within 30 min of blood collection, plasma was obtained by centrifugation samples at 1860 *g* for 10 min at 4°C. Following centrifugation, plasma samples were divided into tubes and stored at −80°C until analysis.

Rats (n = 6/per group) were administered a cocktail of antibiotics according to the method of Kinouchi et al (1993). A mixture of neomycin sulfate and streptomycin sulfate (1:1, w/w; 200 mg kg^{-1}) dissolved in sterilized water was given orally to rats twice daily for 6 days. During that period, the animals had free access to diet and autoclaved

distilled water. Before the beginning of the study, animals were fasted overnight and were allowed food only 3 h after dosing. A group of rats (n = 6) received a dose of baicalin or baicalein (224 μmol kg^{-1}) by oral gavage. The antibiotic treatment was continued for two more days after administration of baicalin or baicalein. Blood samples (250 μL) were withdrawn from the jugular vein before dosing and at the following time points after drug administration: 0.17 (only baicalein), 0.5, 1, 2 (not including p.o., baicalin), 3 (only p.o., baicalin), 4 (not including p.o., baicalin), 5 (only p.o., baicalin), 8, 12, 24, 36 and 48 h. Blood samples were collected, transferred in heparin-containing tubes and gently mixed by inversion. Within 30 min of blood collection, plasma was obtained by centrifugation samples at 1860 *g* for 15 min at 4°C. Following centrifugation, plasma samples were divided into tubes and stored at −80°C until analysis.

Quantification of baicalin, its isomer and total baicalein in rat plasma

Plasma samples were subjected to a liquid–liquid extraction process. A 50 μL aliquot of rat plasma was acidified with 25 μL and partitioned with 1 mL ethyl acetate (containing 1.0 μg mL^{-1} of daidzein 50 μL as internal standard). The ethyl acetate layer was evaporated under N_2 gas to dryness and reconstituted with 100 μL of the mobile phase, then 20 μL of solution was subjected to LC/MS/MS analysis.

The concentration of total baicalein in plasma was determined in the form of baicalein after β-glucuronidase/sulfatase treatment according to Lai et al (2003). For enzymatic analysis, 50 μL of plasma was mixed with 50 μL of β-glucuronidase/sulfatase (1000 U mL^{-1} in pH 5 acetate buffer) and incubated at 37°C for 8 h under anaerobic conditions and protected from light. After hydrolysis, the plasma was subjected to the process described above. For calibrator preparation, 50 μL of plasma mixed with various concentrations of baicalin/baicalein was added. The calibration graph was plotted by least-squares linear regression of the peak area ratios (baicalin/baicalein to internal standard) against concentrations of baicalin/baicalein.

Method validation

The method was evaluated through linearity, intra-day and inter-day precision and accuracy, and recovery. The accuracy and precision of the method were assessed by determining quality control (QC) samples using six replicate preparations of plasma samples at three concentration levels (0.02, 0.50 and 8.00 μg mL^{-1}) on three separate days. The extraction recoveries of baicalin and baicalein at three QC levels were evaluated by calculating the mean value of the response of each concentration and dividing the extracted sample mean by the unextracted (spiked blank plasma extract with the standard solution) sample mean of the corresponding concentration. The lower limit of quantification (LLOQ) represents the lowest concentration of analysis in a sample that can be determined with acceptable precision and accuracy.

Data analysis

The peak plasma concentration (C_{max}) and the time-to-peak concentration (t_{max}) were obtained from experimental observation. The other pharmacokinetic parameters were analysed by a non-compartmental model with the aid of the program TOPFIT (version 2.0, Thomae GmbH, Germany). The area under the plasma concentration–time curve (AUC_{0-t}) was calculated using the linear trapezoidal rule to the last point. The mean residence time (MRT) was obtained by dividing the area under the first moment-time curve ($AUMC_{inf}$) by the area under the curve (AUC_{inf}). Unpaired Student's *t*-test was used for the statistical comparison of pharmacokinetic parameters between normal rats and antibiotic-treated rats. The other pharmacokinetic parameters were calculated from the following relationships:

$$\text{absolute bioavailability of baicalin} = AUC_{0-t\ (\text{baicalin or total baicalein after hydrolysis p.o.})} \times \text{dose}_{\text{i.v.}} / AUC_{0-t\ (\text{baicalin or total baicalein after hydrolysis i.v.})} \times \text{dose}_{\text{p.o.}}$$

$$\text{absorption of baicalein} = AUC_{0-t\ (\text{total baicalein after hydrolysis p.o.})} \times \text{dose}_{\text{i.v.}} / AUC_{0-t\ (\text{total baicalein after hydrolysis i.v.})} \times \text{dose}_{\text{p.o.}}$$

$$\text{relative absorption of baicalin in antibiotic-treated rats to normal rats} = AUC_{0-t\ (\text{total baicalein in antibiotic-treated rats p.o.})} / AUC_{0-t\ (\text{total baicalein in normal rats p.o.})}$$

$$\text{relative absorption of baicalin to baicalein} = AUC_{0-t\ (\text{total baicalein after p.o. baicalin})} / AUC_{0-t\ (\text{total baicalein after p.o. baicalein})}$$

Results

Validation of assay method for plasma

A good linear relationship was obtained for baicalin/baicalein in the concentration range of 0.01 – 10.00 μg mL^{-1} in plasma. Typical equations of the calibration curves using weighted ($1/x^2$) least squares linear regression were as follows:

$$\text{baicalin: } y = 1.020 \times 10^{-3} + 2.011 \times 10^{-1}x,\ r = 0.9940$$

$$\text{baicalein: } y = 1.652 \times 10^{-2} + 1.840 \times 10^{-1}x,\ r = 0.9905$$

where y represents the ratio of the analyte peak area to that of daidzein and x represents the plasma concentrations of baicalin or baicalein. The precision and accuracy of this method indicate that all coefficients of variation at each concentration level are below 15.0%. Table 1 summarizes the intra- and inter-day precision and accuracy for baicalin and baicalein evaluated by assaying the QC samples. The precision was calculated using one-way ANOVA. In this assay, the intra-day precision was 6.7% or less for each QC level of baicalin and 8.6% or less for each QC level of baicalein. The inter-day precision was 10.0% or less for baicalin and 11.2% or less for baicalein. The above results demonstrate that the values are within the acceptable range and the method is accurate and precise. Plasma samples were determined after proper dilution if the concentrations were higher than 10.00 μg mL^{-1}.

Table 1 Summary of precision and accuracy from QC samples of rat plasma extracts

Added concentration ($\mu g\,mL^{-1}$)	Found concentration ($\mu g\,mL^{-1}$)	Accuracy (%)	Precision (%)	
			Intra-day	Inter-day
0.020 (BG)	0.0195	97.5	6.7	8.9
0.50 (BG)	0.51	102.0	4.9	10.0
8.00 (BG)	7.88	98.5	5.6	4.4
0.020 (BL)	0.0194	97.0	7.5	8.3
0.50 (BL)	0.49	98.0	8.6	11.2
8.00 (BL)	8.01	100.1	6.3	10.1

n = 3 days; six replicates per day.

The LLOQ for baicalin/baicalein was $0.01\,\mu g\,mL^{-1}$. The recoveries of baicalin/baicalein from plasma were 65.2–76.5% for the concentration of 0.02, 0.50 and $8.00\,\mu g\,mL^{-1}$, respectively.

Plasma concentrations of baicalin and total baicalein after enzymatic hydrolysis

After baicalein/baicalin was administrated orally/intravenously to rats, baicalin and several metabolites were detected in plasma. However, baicalein was only detected in several plasma samples after i.v. administration of baicalein. Representative chromatograms of a blank plasma, a spiked blank plasma with baicalin, baicalein and internal standard, and a rat plasma at 5 h after oral administration of baicalin to normal rats are presented in Figure 2. As shown in Figure 2C for baicalin determination, the two peaks were observed in rat plasma after administration by monitoring the transition of m/z 447 → 271. Comparing the

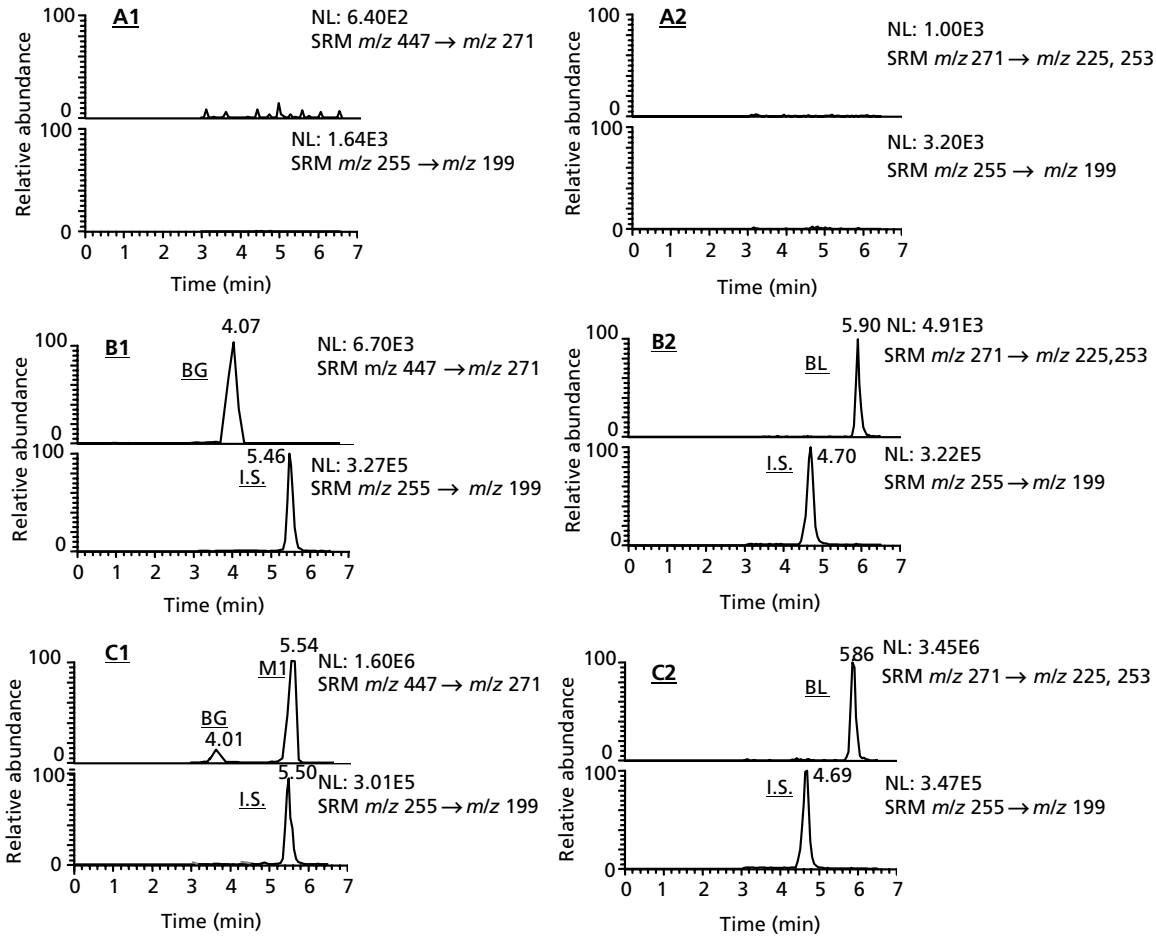

Figure 2 Representative SRM chromatograms of: (A1) a blank rat plasma sample of baicalin (BG); (A2) a blank rat plasma sample of baicalein (BL); (B1) a blank rat plasma sample spiked with BG ($0.02\,\mu g\,mL^{-1}$) and internal standard (I.S.) (daidzein, $1.0\,\mu g\,mL^{-1}$); (B2) a blank rat plasma sample spiked with BL ($0.02\,\mu g\,mL^{-1}$) and I.S. ($1.0\,\mu g\,mL^{-1}$); (C1) a rat plasma sample at 5 h after an oral dose of BG ($224\,\mu mol\,kg^{-1}$) to normal rats; (C2) a rat plasma sample at 5 h pretreated by enzymatic hydrolysis after an oral dose of BG ($224\,\mu mol\,kg^{-1}$) to normal rats.

LC retention time and full scan multistage mass spectra of the reference substance, the first peak (t_R 4.01 min) was identified as baicalin. The second peak (t_R 5.54 min, M1) has a similar pseudomolecular ion at m/z 447 and a fragment ion at m/z 271 (loss of a glucuronic acid) with baicalin. Treatment of plasma samples with β-glucuronidase before LC/MS/MS analysis led to the disappearance of both baicalin and M1. This implies that the second peak is an isomer of baicalin. Based on the method described by Jaeger et al (1998), its structure was further confirmed as baicalein 6-*O*-glucuronide. These results have been published by us (Xing et al 2004). The conjugate has been detected and characterized in rat bile (Abe et al 1990). Baicalin and its isomer in plasma were determined directly in this study, and total conjugates in plasma were determined in the form of baicalein after treatment with β-glucuronidase/sulfatase.

When baicalein was administered orally, the glucuronides/sulfates of baicalein were almost exclusively circulating in the bloodstream, whereas baicalein itself was negligible. Baicalin and its isomer M1 were exclusively present as baicalein glucuronides in plasma. After intravenous dosing of baicalein, the parent form of baicalein was only detected in several sampling points. Based on the AUC_{0-48} of baicalein after enzymatic hydrolysis, the bioavailability of baicalein was $36.1 \pm 4.4\%$ and $35.5 \pm 4.0\%$ in normal rats and antibiotic-treated rats, which suggests that the baicalein absorbed does not differ markedly between normal rats and antibiotic-treated rats. The AUC_{0-48} of baicalin (10.4 ± 0.9 nmol mL^{-1} h) and its isomer (302.9 ± 52.3 nmol mL^{-1} h) after an oral administration of baicalein to normal rats indicates that baicalin circulates at a relative low level in plasma compared with its isomer M1 (1.4% vs 39.8% of the total baicalein). The C_{max} of baicalin (5.0 ± 0.7 nmol mL^{-1}) was reached at 0.5 h and its isomer (50.7 ± 8.1 nmol mL^{-1}) was reached at 2.0 h. The C_{max}, t_{max} and AUC_{0-48} values of normal rats after oral administration of baicalein were not significantly different to those in antibiotic-treated rats. The mean plasma concentration–time profiles of baicalin, M1 and total baicalein after oral administration of baicalein to normal rats and antibiotic-treated rats are shown in Figure 3 and the pharmacokinetic parameters of baicalein conjugates are listed in Table 2. The MRT of isomer M1 or total baicalein in antibiotic-treated rats appears to be significantly different from that in normal rats ($P < 0.05$). Antibiotics delayed the clearance of baicalin isomer and total baicalein from circulation. The prolonged MRT after oral dosing of antibiotics has also been found in other reports (Fost et al 1999; Kuroda et al 2001). Such an effect has been attributed to drug interactions.

Table 2 Pharmacokinetic parameters of baicalin, its isomer and total baicalein after intravenous (i.v., 37 μmol kg^{-1}) or oral (p.o., 224 μmol kg^{-1}) administration of baicalein (BL) to normal rats (N, n = 6) and antibiotic-treated rats (A, n = 6)

Parameters	C_{max} (nmol mL^{-1})	t_{max} (h)	AUC_{0-48} (nmol mL^{-1} h)	MRT (h)
BG (N, i.v.)	na	na	23.06 ± 2.76	3.4 ± 0.8
BG isomer (N, i.v.)	na	na	176.59 ± 10.82	3.9 ± 0.5
Total baicalein (N, i.v.)	na	na	348.73 ± 19.66	3.2 ± 0.4
BG (N, p.o.)	5.03 ± 0.68	0.5	10.41 ± 0.92*	7.1 ± 1.9
BG isomer (N, p.o.)	50.70 ± 8.15	2.0	302.89 ± 52.31	5.4 ± 0.9*
Total baicalein (N, p.o.)	127.90 ± 7.01	2.0	761.77 ± 92.71	6.5 ± 0.6*
BG (A, p.o.)	7.00 ± 1.21	0.5	39.70 ± 12.91	7.9 ± 2.1
BG isomer (A, p.o.)	34.60 ± 15.78	3.0 ± 1.2	385.30 ± 43.38	10.3 ± 1.1
Total baicalein (A, p.o.)	101.95 ± 18.08	2.0	749.29 ± 84.14	10.1 ± 1.1

BG: baicalin; BL: baicalein; na, not applicable. *Denotes statistical difference compared with antibiotic-treated rats ($P < 0.05$, by unpaired Student's t-test).

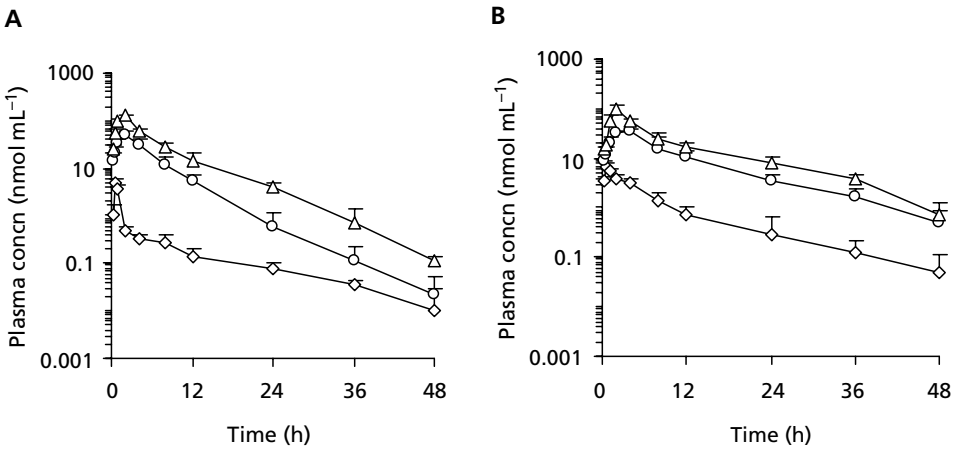

Figure 3 Mean (±s.d.) plasma concentration–time profiles of baicalin (—◊—), baicalin isomer (—○—) and total baicalein (—△—) after oral administration of baicalein (BL, 224 μmol kg^{-1}) to normal rats (A, n = 6) and antibiotic-treated rats (B, n = 6).

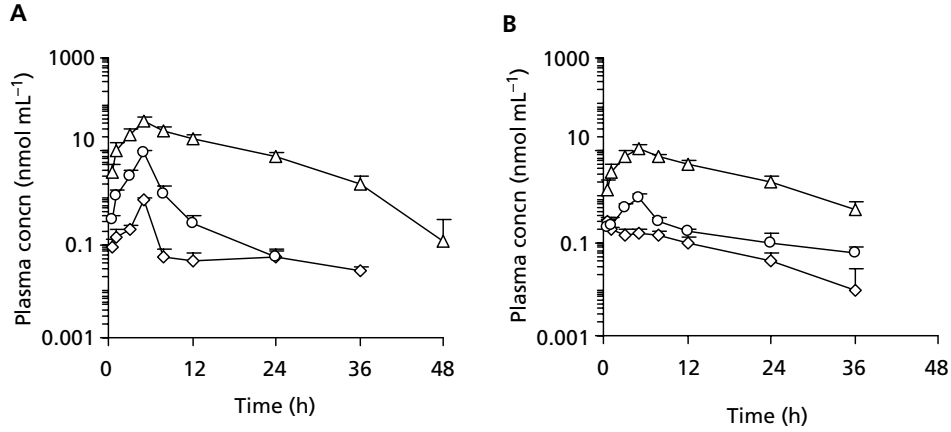

Figure 4 Mean (±s.d.) plasma concentration–time profiles of baicalin (—◊—), baicalin isomer (—○—) and total baicalein (—△—) after oral administration of baicalin (BG, 224 μmol·kg^{-1}) to normal rats (A, n = 6) and antibiotic-treated rats (B, n = 6).

The mean plasma concentration–time profiles of baicalin, M1 and total baicalein after oral administration of baicalin are shown in Figure 4, revealing that the conjugated metabolites of baicalin are predominant in plasma. The corresponding pharmacokinetic parameters are listed in Table 3. The AUC_{0-48} of baicalin (4.4 ± 0.4 nmol mL^{-1} h) and M1 (37.6 ± 6.0 nmol mL^{-1} h) after oral administration of baicalin indicates that baicalin circulates at a low level in plasma. Based on the AUC of baicalin, the bioavailability of baicalin was $2.2 \pm 0.2\%$ and $1.5 \pm 0.2\%$ in normal rats and antibiotic-treated rats, respectively. The AUC_{0-48} of M1 significantly decreased to 8.8 ± 1.5 nmol mL^{-1} h after oral administration of baicalin to antibiotic-treated rats ($P < 0.05$). The C_{max} of baicalin was 0.91 ± 0.13 nmol mL^{-1} at 5.0 h in normal rats, whereas the C_{max} (0.30 ± 0.05 nmol mL^{-1}) in antibiotic-treated rats was reached within a short time (the first sampling point, 0.5 h).

The AUC_{0-48} of total baicalein after enzymatic hydrolysis after oral dose (522.6 ± 106.2 nmol mL^{-1} h) of baicalin was compared with that after intravenous bolus (308.3 ± 19.6 nmol mL^{-1} h) to normal rats based on dose correction. The absorption of baicalin (calculated as total baicalein) was $28.0 \pm 5.7\%$. The percentage of baicalin absorbed in antibiotic-treated rats was 27.6% of that in normal rats.

The mean C_{max} of total baicalein after an oral administration of baicalein (127.9 ± 7.0 nmol mL^{-1}) was significantly higher than that (44.1 ± 10.9 nmol mL^{-1}) after an oral administration of baicalin. By comparing the AUC_{0-48} between oral baicalin and baicalein, the relative absorption of baicalin to baicalein was 68.6%.

Table 3 Pharmacokinetic parameters of baicalin, its isomer and total baicalein after intravenous (i.v., 37 μmol kg^{-1}) or oral (p.o., 224 μmol kg^{-1}) administration of baicalin (BG) to normal rats (N, n = 6) and antibiotic-treated rats (A, n = 6)

Parameters	C_{max} (nmol mL^{-1})	t_{max} (h)	AUC_{0-48}	MRT (h) (nmol mL^{-1} h)
BG (N, i.v.)	na	na	33.57 ± 1.80	0.5 ± 0.1
BG isomer (N, i.v.)	na	na	17.03 ± 1.87	6.7 ± 0.3
Total baicalein (N, i.v.)	na	na	308.28 ± 19.65	3.5 ± 0.3
BG (N, p.o.)	0.91 ± 0.13*	5.0	4.43 ± 0.40*	10.3 ± 1.5
BG isomer (N, p.o.)	9.14 ± 1.41*	5.0	37.64 ± 5.96*	5.6 ± 0.4*
Total baicalein (N, p.o.)	44.07 ± 10.92*	5.0	522.65 ± 106.19*	12.0 ± 1.2
BG (A, p.o.)	0.30 ± 0.05	0.5	3.12 ± 0.32	10.1 ± 1.1
BG isomer (A, p.o.)	0.95 ± 0.17	5.0	8.81 ± 1.50	13.1 ± 0.8
Total baicalein (A, p.o.)	10.68 ± 2.14	5.0	144.50 ± 22.10	11.9 ± 1.3

na, not applicable. *Denotes statistical difference compared with antibiotic-treated rats ($P < 0.05$, by unpaired Student's t-test).

Discussion

After baicalin and baicalein, a flavone glucuronide in *Scutellariae radix* and its aglycone, were administered orally to rats, baicalin, baicalein 6-*O*-glucuronide and several other conjugated metabolites were detected in plasma. Glucuronidation was the major metabolic pathway for both baicalin and baicalein. All plasma samples were determined before and after treatment with β-glucuronidase/sulfatase. From the AUC_{0-48} value of the baicalin parent form (Table 3) after its oral administration to normal rats, the absolute bioavailability of baicalin was calculated to be only $2.2 \pm 0.2\%$. The relative higher plasma level of baicalin isomer M1 than baicalin parent form (Table 3 or Figure 4) indicated that baicalin itself was

poorly absorbed from the gut, but was mainly hydrolysed to baicalein by intestinal bacteria, and then restored to its original form and isomer M1 from the absorbed baicalein in the body. The venous plasma concentration–time profile had a second peak (at 8.0 h) and the same phenomenon could be observed in oral plasma profiles (at 12.0 h or 24.0 h, Figure 4). The second peak in the plasma profile was supposed to be due to enterohepatic circulation. It confirmed that baicalin is probably subjected to the transformation to baicalein before absorption. This result is in agreement with that of Akao et al (2000).

When baicalein was administered orally, the glucuronides/sulfates of baicalein were almost exclusively circulating in the bloodstream (Figure 3). This indicates that the extensive conjugation metabolism of baicalein occurs during the first pass at gut and liver. This is consistent with the low K_m values for baicalein in rat hepatic and intestinal microsomes (Akao et al 2000). Accordingly, rat intestine, together with liver, is regarded as the primary site of first-pass glucuronidation of baicalein. There were marked differences in the metabolic pharmacokinetics between oral baicalin and baicalein when baicalin was orally administered at the equimolar dose with baicalein (Table 2 and 3). The absorption rate was slower and the C_{max} was lower for oral baicalin compared with oral baicalein. This confirmed that baicalin might be absorbed only after hydrolysis by enterobacteria in the intestine, whereas baicalein was directly absorbed through the small intestine. The AUC_{0-48} of baicalein glucuronides/sulfates after oral baicalin compared with that after the equimolar dose of baicalein indicated that the absorption extent of baicalin was lower than that of its aglycone baicalein. The relatively poor absorption of baicalin was probably due to the low lipophilicity, and the bacterial hydrolysis was the rate-limiting step for its absorption. Baicalin could serve as a sustained-release prodrug of baicalein.

As a flavone glucuronide, baicalin could be converted to its aglycone by β-glucuronidase produced by the intestinal bacteria, *Streptococcus* spp. and *E. coli* (Ishihara et al 2002). A mixture of the antibiotics bacitracin, neomycin and streptomycin was found to be most effective in reducing the various activities of intestinal microflora. They decreased the bacterial counts and activities of the β-glucuronidase derived from the intestinal microflora, while treatment with only one aminoglycoside antibiotic did not decrease the number of anaerobes in the intestine (Kinouchi et al 1993). Because of the high activity against these intestinal bacteria, aminoglycosides, neomycin and streptomycin were applied in this investigation. Co-administration of aminoglycosides with baicalin at a dose of 224 μmol kg^{-1} 1 resulted in the dramatically decreased baicalin levels in plasma. The plasma AUC of baicalin (70.4% of that in normal rats) and its isomer (23.4% of that in normal rats) were significantly reduced after antibiotic treatment (Table 3). The absorption of baicalin (calculated as total baicalein) reduced to 27.6% of that in normal rats (Table 3). The results suggest that the absorption of baicalin in rats could be inhibited by the treatment of the two antibiotics. It is obvious that aminoglycosides probably decrease the decomposition of baicalin to baicalein by inhibiting intestinal flora, and further influence the absorption, metabolism and efficacy of baicalin. The importance of intestinal bacteria in the absorption or metabolism of baicalin could be increasingly recognized. The presence of baicalin isomer in antibiotic-treated rat plasma from the first sampling point (0.5 h, Figure 4) indicates that the drug may be partially absorbed and metabolized in the stomach wall and in the upper regions of the intestine. The effect of antibiotics on the excretion of baicalin has been described in detail in another study. After oral administration of baicalin to normal rats, the urinary cumulative amount of baicalin corresponded to 1.5% of the dose, with no baicalin in faeces, and an appreciable amount of baicalein (14.1%) was recovered from faeces. In contrast, only a trace amount of baicalin (0.4%) was recovered in antibiotic-treated rat urine, with a small amount of baicalin (6.0%) in faeces, and a minor amount of baicalein (0.5%) was detected in antibiotic-treated rat faeces. When baicalein was orally administered to antibiotic-treated rats, the absorption rate and extent were not significantly different to those of antibiotic-treated rats (Table 2), which indicates that co-administration of antibiotics does not affect the absorption of baicalein. The pharmacokinetic results of baicalin and baicalein indicate that antibiotics decrease the absorption of baicalin by inhibiting the hydrolysis process in the gastrointestinal tract without affecting the absorption process of baicalein (presumed to be the absorped form of baicalin) to systemic circulation.

From the results of this investigation, we know that many antibiotics other than aminoglycosides do have activity against intestinal bacteria and antibiotics may inhibit the hydrolysis (then the further absorption or metabolism) of baicalin when administered in combination. On the other hand, it is also highly possible that bacteria preparations increase the number of many intestinal bacteria in humans (Ishihara et al 2002; e.g. bacteria preparations LAC-B could increase the bacteria *Bifidobacterium* spp. in human intestine and lower the enzymatic activity of β-glucuronidase), resulting in enhanced metabolism of baicalin when they are used concomitantly. The possible interactions should be confirmed in clinical studies when administered in combination.

References

Abe, K. I., Inoue, O., Yumioka, E. (1990) Biliary excretion of metabolites of baicalin in rats. *Chem. Pharm. Bull.*. **38**: 208–211

Akao, T., Hayashi, T., Kobashi, K. (1994) Intestinal bacterial hydrolysis is indispensable to absorption of 18 beta-glycyrrhetic acid after oral administration of glycyrrhizin in rats. *J. Pharm. Pharmacol.* **46**: 135–137

Akao, T., Kawabata, K., Yanagisawa, E. (2000) Baicalin, the predominant flavone glucuronide of scutellariae radix, is absorbed from the rat gastrointestinal tract as the aglycone and restored to its original form. *J. Pharm. Pharmacol.* **52**: 1563–1568

Fost, D. A., Leung, D. Y., Martin, R. J. (1999) Inhibition of methylprednisolone elimination in the presence of clarithromycin therapy. *J. Allergy Clin. Immunol.* **103**: 1031–1035

Ishihara, M., Homma, M., Kuno, E. (2002) Combination use of kampo-medicines and drugs affecting intestinal bacteria flora. *Yakugaku Zasshi*. **122**: 695–701

Jaeger, W., Zembsch, B., Wolschann, P. (1998) Metabolism of the anticancer drug flavopiridol, a new inhibitor of cyclin-dependent kinases, in rat liver. *Life Sci*. **62**: 1861–1873

Kinouchi, T., Kataoka, K., Miyanishi, K. (1993) Biological activities of the intestinal microflora in mice treated with antibiotics or untreated and the effects of the microflora on absorption and metabolic activation of orally administered glutathione conjugates of k-region epoxides of 1-nitropyrene. *Carcinogenesis* **14**: 869–874

Kitamura, S., Shiraishi, Y., Mita, M. (2000) Reductive metabolism of stibene oxide and styrene oxide to the olefins in rats. *Biol. Pharm. Bull*. **23**: 265–268

Kuroda, T., Namba, K., Torimaru, T. (2001) Variability of oral bioavailability for low dose methotrexate in rats. *Eur. J. Drug Metab. Pharmacokinet*. **26**: 227–234

Lai, M. Y., Hsiu, S. L., Tsai, S. Y. (2003) Comparison of metabolic pharmacokinetics of baicalin and baicalein in rats. *J. Pharm. Pharmacol*. **55**: 205–209

Nakajima, T., Inanishi, M., Yamamoto, K. (2001) Inhibitory effect of baicalein, a flavonoid in scutellaria root, on eotaxin production by human dermal fibroblasts. *Planta Med*. **67**: 132–135

Shen, Y. C., Chiou, W. F., Chou, Y. C. (2003) Mechanism in mediating the anti-inflammatory effects of baicalin and baicalein in human leukocytes. *Eur. J. Pharmacol*. **465**: 171–181

Xing, J., Chen, X. Y., Zhang, S. Q. (2004) LC/MS^n analysis of baicalin and its isomer in rats urine. *J. Chin. Mass Spectrom*. **25**: 129–133