Balicalin, the Predominant Flavone Glucuronide of Scutellariae Radix, is Absorbed from the Rat Gastrointestinal Tract as the Aglycone and Restored to its Original Form

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Abstract

When baicalin was orally administered to conventional rats, it was detected in their plasma for 24 h after administration, but baicalein, the aglycone of baicalin, was not detected. However, when baicalin was given to germ-free rats, only a small amount of baicalin was detected in their plasma within 2 h after the administration, its AUC_{0-lim} (the area under the concentration–time curve from 0 to last determination time) being 12.0% of that in conventional rats. Subsequently, a considerable amount ($55.1 \pm 6.2\%$) of baicalin was recovered from the gastrointestinal tract even 4 h after administration. When baicalein was orally administered to conventional rats, however, baicalin appeared rapidly in their plasma at an AUC_{0-lim} value similar to that obtained after oral administration of baicalin, despite the absence of baicalein in plasma. When intestinal absorption was evaluated by the rat jejunal loop method, baicalein was absorbed readily, but only traces of baicalin were absorbed. Moreover, in conventional rats a small amount ($13.4 \pm 3.1\%$) of baicalin and an appreciable amount ($21.9 \pm 3.4\%$) of baicalein were recovered from the gastrointestinal tract even 4 h after oral administration of baicalin, but only a small amount ($3.93 \pm 1.43\%$) of baicalein was detected in the intestinal tract 1 h after administration of baicalein.

Baicalin was transformed to baicalein readily by the rat gastric and caecal contents. When baicalin was administered orally to conventional rats, an appreciable amount of baicalein was recovered in their gastrointestinal tracts. Moreover, baicalein was efficiently conjugated to baicalin in rat intestinal and hepatic microsomes.

These results indicate that baicalin itself is poorly absorbed from the rat gut, but is hydrolysed to baicalein by intestinal bacteria and then restored to its original form from the absorbed baicalein in the body.

Scutellariae Radix, the root of *Scutellaria baicalensis* Georgi (Labiatae), is used in combination with other herbs in oriental (Kampo) medicines, and contains baicalin, 5,6,7-trihydroxyflavone-7-β-D-glucuronide (Figure 1), as the main active constituent. Baicalin and its aglycone, baicalein, show anti-allergic (Koda et al 1970), anti-inflammatory (Kubo et al 1984) activity, and also anti-oxidant action (Kimura et al 1981). Although it is necessary to study the disposition and pharmacokinetics of these drugs to elucidate their biological effects, few studies have been performed.

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After oral administration of baicalin to rats, baicalin has been detected in their plasma (Wakui et al 1992). The bioavailability of orally administered baicalin was calculated to be 62% from the area under the time–concentration curve (AUC) after its oral and intravenous administration, which suggests that it is absorbed directly from the rat gastrointestinal tract. However, the bile of rats administered baicalin orally contains the same five metabolites as that of rats treated with baicalein, but the biliary metabolites in rats treated with baicalein are excreted faster than those resulting from baicalin treatment (Abe et al 1990). These observations suggest that baicalin is absorbed as baicalein after hydrolysis in the gastrointestinal tract.
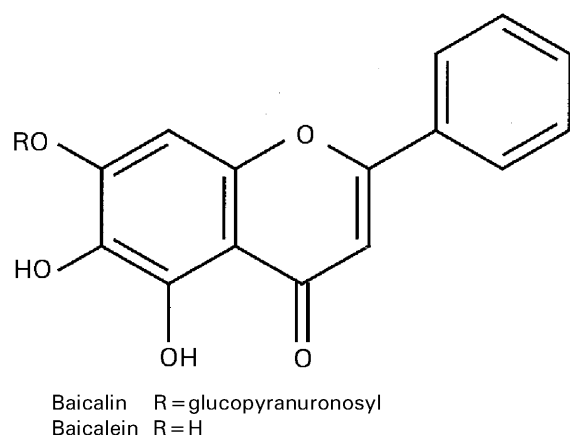
Baicalin   R = glucopyranuronosyl
Baicalein R = H

Figure 1. Structure of baicalin and baicalein.

Thus there is a discrepancy in the supposed fate of orally administered baicalin in rats.

No direct evidence for the metabolic processing of orally administered baicalin has been presented. In this paper, we report that baicalin is poorly absorbed from the rat gastrointestinal tract, but is absorbed as baicalein, which is formed by intestinal bacteria, and restored to the original form by glucuronidation in the body, intestine and liver.

Materials and Methods

Chemicals
Baicalin and baicalein as standards were products of Kishida Chemical Co. Ltd, Osaka, Japan. The internal standards used for HPLC were 2,3-hydroxynaphthalene and quercetin, which were purchased from Aldrich (Milwaukee, WI) and from Nacalai Tesque (Kyoto, Japan), respectively. Solvents for HPLC were of special HPLC grade. Uridine-5′-diphospho glucuronic acid (UDP-glucuronate) trisodium salt was purchased from Nacalai Tesque and D-saccharic acid 1,4-lactone from Sigma Chemical Co. (St Louis, MO). All other reagents were of the best quality available commercially.

Animals and treatment
Male Wistar rats (7 weeks old) and male germ-free Wistar rats (WA/Jic, 7 weeks old) were purchased from Clea Japan. Conventional rats (8 weeks old) that were fasted overnight received baicalin orally at a dose of $20\,\mathrm{mg\,kg^{-1}}$ or baicalein at a dose of $12.1\,\mathrm{mg\,kg^{-1}}$ (a dose equivalent to $20\,\mathrm{mg}$ baicalin/kg). Baicalin ($20\,\mathrm{mg\,mL^{-1}}$) was dissolved in sodium citrate solution for injection, and baicalein

($12.1\,\mathrm{mg\,mL^{-1}}$) was dissolved in dimethysulphoxide (DMSO) (final concn 6% v/v) and then diluted with saline just before administration. Sterilized baicalin or baicalein, at the same doses, were administered orally to germ-free rats in an isolator. Blood samples were withdrawn from the jugular vein of the conventional rats and the femoral artery of the germ-free rats at appropriate intervals after administration. Plasma was separated by centrifugation of the heparinized blood and stored at $-80°C$ until use. Gastrointestinal contents were taken under anaesthesia 4 h after drug administration and used immediately for measurement of baicalin and baicalein.

Intestinal absorption by the intestinal loop method
The small intestine of conventional rats (three male Wistar rats for each compound; 8 weeks old) that had been fasted overnight was exposed under pentobarbital anaesthesia. A 10-cm loop of empty, middle-tract jejunum was prepared by ligating both ends. Baicalin or baicalein at a concentration of $0.1\,\mathrm{mM}$, dissolved in $2.5\,\mathrm{mL}$ of saline containing $1\,\mathrm{mg\,mL^{-1}}$ bovine serum albumin, was injected into the jejunal loop through a cannulated polyethylene tube at one ligated end, and a 0.1-mL sample of the solution in the jejunal loop maintained in the body was withdrawn at 10 and 20 min after injection. At the end of the experiment (after 20 min), the solution remaining in the jejunal loop was collected and its volume measured. The amounts of baicalin and baicalein in the solution were determined as described below.

Determination of baicalin and baicalein in plasma and gastrointestinal contents
Sample preparation from rat plasma and the determination of baicalin and baicalein by HPLC with electrochemical detection were carried out as described by Wakui et al (1992). Pharmacokinetic evaluations were performed by non-compartmental analysis of the plasma concentration–time data on the statistical moment.

Suspensions of the contents of the stomach, small intestine, and caecal and colonic tracts in 3–4 volumes of water were treated with 4 volumes of methanol under acidic conditions, and then centrifuged at $10\,000\,g$ for 10 min to obtain a clear supernatant. Baicalin and baicalein were determined by HPLC, as above.

UDP-glucuronate glucuronosyltransferase activity
Microsomes from rat liver and small intestinal mucosa were prepared as described previously (Kuriyama et al 1969), except that $0.15\,\mathrm{M}$ KCl was

used for the homogenization of tissues instead of 0·25 M sucrose. The microsomes were washed once with 1·15% KCl containing 10 mM ethylenediaminetetraacetic acid tetrasodium salt (EDTA), suspended in 50 mM Tris-HCl buffer (pH 7·2) containing 20% (v/v) glycerol, 1 mM EDTA and 1 mM dithiothreitol, and stored at −80°C until use.

The hepatic glucuronosyltransferase activity toward 4-nitrophenol as an acceptor was measured according to the method reported by Yuasa (1977) with minor modification. The reaction mixture contained a suitable amount (50−100 μg) of microsomal protein, 1 μmol of 4-nitrophenol, 2 μmol of UDP-glucuronate trisodium salt, 2 μmol of $MgCl_2$, 0·2 μmol of D-saccharic acid 1,4-lactone, and 0·02% (w/v) (final concentration) of Triton X-100 in 0·2 mL of 50 mM Tris-HCl buffer (pH 7·4). After incubation for 10 min at 37°C, unreacted 4-nitrophenol was extracted with water-saturated ethyl acetate. The formed glucuronide in the solution was hydrolysed by heating in boiling water under alkaline conditions (after adding 1 mL of 1 N NaOH). The released p4-nitrophenol was determined photometrically at 400 nm. For intestinal microsomes, 0·02% (w/v) Triton X-100 was omitted from the reaction mixture and 100−300 μg of microsomal protein was used. Owing to the low activity, the formed glucuronide was extracted with water-saturated ethyl acetate under acidic conditions and determined using thin-layer chromatography (TLC), developed with a solvent system of acetic acid−n-butanol−1,2-dichloroethane−water (4 : 1 : 4 : 1) on Merck silica-gel type 60 plates and analysed at 305 nm using densitometric scanning (Shimadzu CS-9000).

The activity toward baicalein as an acceptor was measured as follows. The reaction mixture contained a suitable amount (100−200 μg) of micosomal protein, 2 μmol of UDP-glucuronate trisodium salt, 2 μmol of $MgCl_2$, 0·2 μmol D-saccharic acid 1,4-lactone, and 0·02% (w/v) (final concentration, omitted in the case of intestinal microsomes) Triton X-100 in 0·2 mL of 50 mM Tris-HCl buffer (pH 7·4). The reaction was started by addition of 20 nmol of baicalein dissolved in DMSO and stopped with 50 μL of 1 N HCl after incubation for 5 min at 37°C. The extract with water-saturated ethyl acetate was evaporated to dryness, dissolved with a small amount of chloroform–methanol solution, and spotted on silica-gel plates (Merck type $60F_{254}$). After developing with ethyl actate–methylethyl ketone−formic acid−water (5 : 3 : 1 : 1) as a solvent, baicalin on the plate was analysed densitometrically at 305 nm.

K_m values for 4-nitrophenol and baicalein were obtained by measuring the activities in varying concentrations of the respective acceptors as described above, and then by plotting 1/v versus 1/[acceptor] (Lineweaver-Burk plot).

Transformation of baicalin to baicalein by rat gastrointestinal content

Baicalin (1 mM) was incubated with gastric, small intestinal and caecal contents, which were suspended with 5 volumes of 50 mM potassium phosphate buffer (pH 6·8), from three male Wistar rats (7−8 weeks old) that had been fasted overnight. After incubating for 2 and 4 h at 37°C, the reaction mixture was extracted with water-saturated n-butanol under acidic conditions. A sample of the butanol layer was analysed for baicalin and baicalein by TLC as described above.

Results

Plasma baicalin and baicalein after their oral administration

When baicalin was orally administered to conventional rats, it was detected in their plasma (Figure 2), as reported by Wakui et al (1992), but baicalein was not (data not shown). The C_{max} was 1·34 ± 0·51 μg mL^{-1} at t_{max} 2·40 ± 0·89 h, with the second peak at 9 h, and the AUC_{0-lim} was 9·10 ± 2·91 μg h mL^{-1} (Table 1). However, when baicalin was orally administered to germ-free rats, only a small amount of baicalin was detected in their plasma (Figure 2) without the appearance of baicalein. The C_{max} was low (0·34 ± 0·10 μg mL^{-1}) and was reached within a short time (t_{max} = 0·38 ± 0·14 h), corresponding to the shoulder of the first peak in the conventional rats, and the AUC_{0-lim} value (1·09 ± 0·37 μg h mL^{-1}) was 12·0% of that in the conventional rats (Table 1). These results suggest that the absorption of baicalin itself was poor in the conventional and germ-free rats, but that the high plasma levels of baicalin in the conventional rats were due to the presence of intestinal bacteria in their gastrointestinal tract.

On the other hand, when baicalein was administered orally to conventional rats, baicalin appeared more rapidly in their plasma (Figure 3) than when baicalin itself was administered; the plasma levels of baicalein were extremely low (data not shown). Thus the C_{max} and AUC_{0-lim} values of baicalin were comparable with those measured after the administration of baicalin (Table 1). These observations indicate that baicalein was easily absorbed and then transformed to baicalin in the rat body.
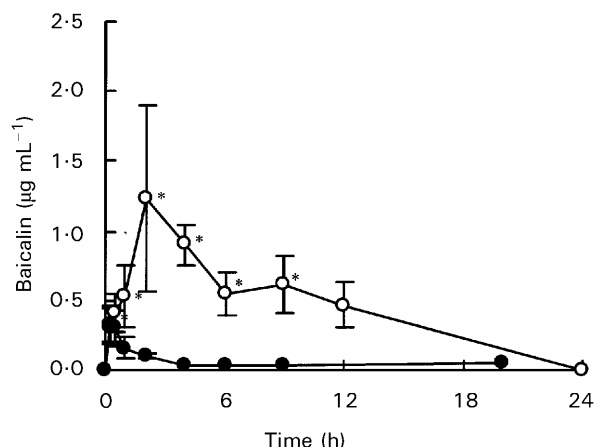
Figure 2. Plasma concentration profile of baicalin after its oral administration at a dose of $20 \, \text{mg kg}^{-1}$ to four germ-free (●) and five conventional (○) rats. Each point represents the mean ± s.d. *$P < 0.01$ vs germ-free group (Welch test).
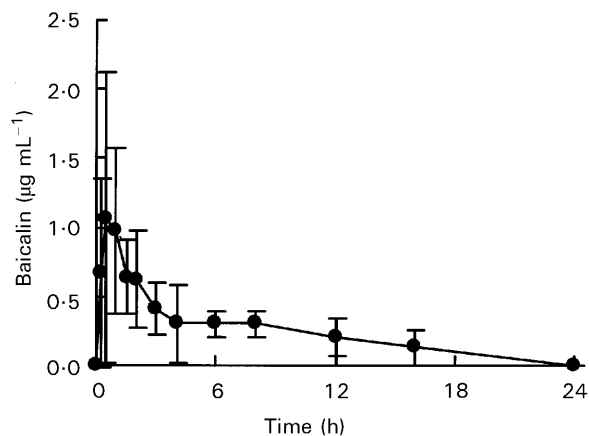

Figure 3. Plasma concentration profile of baicalin after oral administration of baicalein at a dose of $12.1 \, \text{mg kg}^{-1}$ (equivalent to $20 \, \text{mg kg}^{-1}$ baicalin) to rats. Each point represents the mean ± s.d. of six rats.

Table 1. Pharmacokinetic parameters of baicalin after oral administration of baicalin or baicalein to conventional and germ-free rats.

	t_{max} (h)	C_{max} ($\mu\text{g mL}^{-1}$)	AUC_{0-lim_l} ($\mu\text{g h mL}^{-1}$)
Conventional rats			
Baicalin (n = 5)	2.40 ± 0.89**	1.34 ± 0.51*	9.10 ± 2.91*
Baicalein (n = 6)	1.50 ± 1.34	1.69 ± 0.65	5.76 ± 1.63
Germ-free rats			
Baicalin (n = 4)	0.38 ± 0.14	0.34 ± 0.10	1.09 ± 0.37

Baicalin and baicalein were administered orally to rats at doses of $20 \, \text{mg kg}^{-1}$ and $12.1 \, \text{mg kg}^{-1}$ (equivalent to $20 \, \text{mg kg}^{-1}$ baicalin), respectively. Each value represents mean ± s.d. *$P < 0.05$, **$P < 0.01$ vs germ-free group (Welch test).

The pharmacokinetic parameters of baicalin after administration of baicalin or baicalein to conventional and germ-free rats are summarised in Table 1.

Intestinal absorption of baicalin and baicalein
Intestinal absorption of baicalin and baicalein in conventional rats was evaluated by the in-situ intestinal loop method. Baicalein ($100 \, \mu\text{M}$, $2.5 \, \text{mL}$) that was injected into the loop disappeared quickly

and time-dependently, and decreased to $14.2 \pm 4.1\%$ of the dose (to a concentration of $22.0 \pm 10.1 \, \mu\text{M}$ baicalein) $20 \, \text{min}$ after injection. When baicalin ($100 \, \mu\text{M}$, $2.5 \, \text{mL}$) was injected, $73.2 \pm 15.6\%$ of the dose ($113 \pm 18.4 \, \mu\text{M}$ baicalin) remained in the loop after $20 \, \text{min}$.

In-vivo absorption of baicalin and baicalein was also estimated. When baicalin was administered orally to conventional rats, a small amount ($13.4 \pm 3.1\%$) of baicalin and an appreciable amount of baicalein ($21.9 \pm 3.4\%$) was recovered from the gastrointestinal tract, mainly baicalin in the small intestine and baicalein in the caecum, even $4 \, \text{h}$ after administration. When baicalin was administered to germ-free rats, to remove any influence of intestinal bacteria, a substantial proportion of baicalin ($55.1 \pm 6.2\%$), but only a trace amount of baicalein, was recovered from the alimentary canal even $4 \, \text{h}$ after administration ($25.7 \pm 8.8\%$ and $29.2 \pm 13.2\%$ in the small intestinal and caecal tracts, respectively), indicating its poor absorption. Conversely, in conventional rats given baicalein orally, only a small amount ($3.93 \pm 1.43\%$) of baicalein was recovered in their intestinal tracts $1 \, \text{h}$ after administration, and $29.7 \pm 16.6\%$ was retained in their stomachs. Only

Table 2. UDP-glucuronosyltransferase activity and K_m value toward baicalein and 4-nitrophenol in rat jejunal and hepatic microsomes.

	Baicalein Activity (nmol min^{-1} mg)	K_m (μM)	4-Nitrophenol Activity (nmol min^{-1} mg)	K_m (μM)
Jejunum	29.2 ± 1.0	17	29.7 ± 0.4	1800
Liver	25.3 ± 1.0	68	94.1 ± 5.8	1600

Activity is expressed as mean ± s.d., n = 3 and K_m values are averages in duplicate.

a trace amount ($1.11 \pm 0.35\%$) of baicalein was detected 4 h after oral administration to germ-free rats, accompanied by the appearance of baicalin in plasma ($0.26 \pm 0.20\,\mu g\,mL^{-1}$).

Thus, it is clear that baicalin itself is poorly absorbed from rat gut, but baicalein is absorbed easily and restored to baicalin (its original form) in the body.

Transformation of baicalin to baicalein by intestinal bacteria and reverse reaction in rat intestinal and hepatic microsomes

Baicalin (1 mM) was transformed readily into baicalein by the rat gastric and caecal contents, which were suspended with 5 volumes of 50 mM posphate buffer (pH 6.8), with $74.1 \pm 24.8\%$ and complete conversion, respectively, during 2 h incubation at $37^{\circ}C$; conversion by small intestinal contents was low ($7.4 \pm 3.2\%$) under the same conditions. Moreover, when baicalin was administered orally to conventional rats, an appreciable amount of baicalein ($21.9 \pm 3.4\%$) was detected in the gastrointestinal tracts 4 h after the administration, compared with only a faint amount of baicalein in germ-free rats. These results indicate that baicalin is easily hydrolysed by intestinal bacteria in the rat gastrointestinal tract.

UDP-glucuronosyltransferase activity toward baicalein and 4-nitrophenol as acceptors was measured in microsomes of the rat small intestine and liver. In the liver, the UDP-glucuronosyltransferase activity toward baicalein was lower than that toward 4-nitrophenol; in the jejunum the activity was higher than in the liver, the activity towards baicalein and 4-nitrophenol being similar (Table 2). Ileal microsomes showed almost half of the jejunal activity toward both acceptors, and the caecal homogenate also showed about half of the activity in the jejunal homogenate (data not shown). The apparent K_m values for baicalein in the jejunal and hepatic microsomes were $17\,\mu M$ and $68\,\mu M$, respectively, which was much lower than the value for 4-nitrophenol (1800 and $1600\,\mu M$, respectively) in both microsomes (Table 2). Thus, absorbed baicalein is converted efficiently to baicalin in the rat intestine and liver.

Discussion

When baicalin, a flavone glucuronide in Scutellariae Radix, was administered orally to rats, baicalin (but not baicalein, the aglycone of baicalin) was detected in their plasma, as reported by Wakui et al (1992). Previously it had been thought that baicalin

itself was absorbed from rat gastrointestinal tract. However, our in-situ intestinal loop experiments and in-vivo absorption experiments confirmed that orally administered baicalin was poorly absorbed. Baicalin was poorly absorbed from the in-situ jejunal loop. In conventional rats given baicalin orally, a small amount of baicalin but an appreciable amount of baicalein were recovered in the intestinal tract even 4 h after administration. Also in germ-free rats given baicalin orally, a considerable amount (55.1%) of baicalin remained in the intestinal tract even 4 h after the administration but only a small amount of baicalin appeared in the plasma (Figure 2). From the AUC_{0-lim} value of plasma baicalin after its oral administration in the germ-free rats (Table 1), and that after its intravenous administration (Wakui et al 1992), the absolute bioavailability of baicalin is calculated to be 5.4%. Moreover, baicalin was transformed readily to baicalein by gastric and caecal contents in in-vitro experiments. Also in the conventional rats given baicalin orally, an appreciable amount (21.9%) of baicalein was recovered in the alimentary canal, compared with a trace amount of baicalein in the germ-free rats. These findings indicate that baicalin is converted to baicalein by intestinal bacteria. When baicalein was administered to conventional and germ-free rats, baicalein disappeared rapidly from the intestinal tract and baicalin appeared in the plasma (Figure 3) despite the very low plasma level of baicalein. Thus, baicalin is poorly absorbed from the rat gastrointestinal tract, but it is absorbed as baicalein formed by intestinal bacteria and converted back to baicalin in the body. Accordingly, the retarded and broad profile containing two peaks of plasma baicalin in the concentration–time curve for the conventional rats given baicalin orally (Figure 2) seems to be due to the time required for the hydrolysis of baicalin to baicalein by intestinal bacteria. It also may be partially due to the enterohepatic circulation of baicalin and its metabolites. This is compatible with findings that the biliary excretion of metabolites in rats administered baicalin orally is slower than that in rats given baicalein (Abe et al 1990). The low and broad profile of plasma baicalin 4–16 h after oral administration of baicalein (Figure 3) also seems to be caused by the enterohepatic circulation.

The findings that baicalin and baicalein were detected in the plasma after oral (Figure 3) and intravenous (Wakui et al 1992) administration of baicalein to rats indicate that first-pass glucuronidation of baicalein occurs. This was confirmed by the effective conversion of baicalein to baicalin and the low K_m values for baicalein in rat hepatic

and intestinal microsomes (Table 2). Accordingly, the rat intestine, together with the liver, is regarded as the primary site of first-pass glucuronidation of baicalein, as well as for significant first-pass gut metabolism of nifedipine by cytochrome P450s (Grundy et al 1997).

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