JPP Journal of Pharmacy and Pharmacology

JPP 2003, 55: 205–209

Received August 13, 2002
Accepted November 7, 2002
DOI 10.1211/002235702522
ISSN 0022-3573

Comparison of metabolic pharmacokinetics of baicalin and baicalein in rats

Miao-Ying Lai, Su-Lan Hsiu, Shang-Yuan Tsai, Yu-Chi Hou, Pei-Dawn Lee Chao

Abstract

Baicalin and baicalein, a flavone glucuronide and its aglycone, are bioactive constituents of Scutellariae Radix with various beneficial activities. We have characterized and compared the metabolic pharmacokinetics of baicalin and baicalein in rats. Baicalein was administered intravenously and orally to rats, and baicalin was orally administered. An HPLC method was used to determine the concentration of baicalein before and after hydrolysis using β-glucuronidase/sulfatase. The pharmacokinetic parameters were calculated by using WINNONLIN. Unpaired Student's t-test was used for statistical comparison. The result showed that after intravenous administration of baicalein, 75.7% of the dose was circulating as its conjugated metabolites. After oral administration of baicalein, absorption of baicalein itself was negligible, whereas the glucuronides/sulfates of baicalein were predominant in the plasma. When compared with intravenous bolus administration with dose correction, the absolute absorption was 40%. When baicalin was administered orally, glucuronides and sulfates of baicalein were exclusively circulating in the plasma. The relative absorption for baicalin was 65% when compared with baicalein. Profound differences of serum profile and pharmacokinetics were observed between oral baicalein and baicalin. Baicalin demonstrated significantly later time to peak concentration (t_{max}) and lower peak serum concentration (C_{max}) of baicalein conjugated metabolites than baicalein, indicating baicalin was absorbed more slowly and to a lesser extent than baicalein.

Introduction

In recent years, flavonoids have attracted increasing interest because of their various beneficial biological activities to human health. Baicalin and baicalein, a flavone glucuronide and its aglycone (Figure 1), are flavone constituents of Scutellariae Radix, which is widely used in clinical Chinese medicine as a treatment for inflammation, fever and allergic diseases. Anti-inflammatory (Nakajima et al 2001), anti-HIV (Kitamura et al 1998), anticancer (Matsuzaki et al 1996; Chan et al 2000; Ikemoto et al 2000), free radical scavenging and antioxidant (Gao et al 1999; Shieh et al 2000) activities were reported for baicalein and/or baicalin by in-vitro studies using cell lines. In addition, anti-inflammatory and vasodepressor effects were reported in rats (Lin & Shieh 1996; Takizawa et al 1998).

Many flavonoid glycosides are generally absorbed after being hydrolysed by bacterial enzymes to corresponding aglycones in the gut. Baicalin had been found to be transformed, in part, to baicalein before absorption, then formed its conjugated metabolites in man and rats (Muto et al 1997; Akao et al 2000). However, limited metabolic pharmacokinetic data of baicalin or baicalein is available in the literature. Therefore, in this study, we have attempted to characterize and compare the metabolic pharmacokinetics of equimolar baicalin and baicalein in rats (Akao et al 2000).

Materials and Methods

Chemicals

Baicalin, baicalein, β-glucuronidase/sulfatase (HP-2, from *Helix pomatia*), glycofurol and propyl paraben were purchased from Sigma Chemical Co. (St Louis, MO).

Graduate Institute of Chinese Pharmaceutical Sciences, China Medical College, Taichung, Taiwan 404, ROC

Miao-Ying Lai

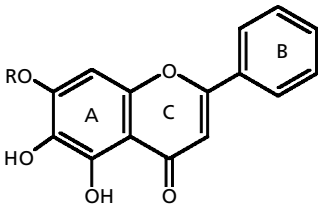
Department of Pharmacy, China Medical College, Taichung, Taiwan 404, ROC

Miao-Ying Lai, Su-Lan Hsiu, Shang-Yuan Tsai, Pei-Dawn Lee Chao

School of Chinese Medicine, China Medical College, Taichung, Taiwan 404, ROC

Yu-Chi Hou

Correspondence: P.-D. Lee Chao, Department of Pharmacy, China Medical College, Taichung, Taiwan 404, ROC.
E-mail: pdlee@mail.cmc.edu.tw



Baicalin: R = glucuronic acid
Baicalein: R = H

Figure 1 The structures of baicalin and baicalein.

Dimethyl sulfoxide (DMSO), PEG 400 and hydrochloric acid were obtained from Merck (Darmstadt, Germany). Acetonitrile, methanol and ethyl acetate were LC grade and purchased from Mallinckrodt Baker, Inc. (Phillipsburg, NJ). L-(+)-Ascorbic acid was obtained from Riedel-deHaen (Seelze, Germany). Milli-Q plus water (Millipore, Bedford, MA) was used throughout the study.

Instrumentation and HPLC conditions

The HPLC apparatus included one pump (LC-6AD, Shimadzu, Osaka, Japan), a UV spectrophotometric detector (SPD-6A, Shimadzu) and a chromatopac (C-R6A, Shimadzu) with an automatic injector (Series 200 Autosampler, Perkin Elmer, Norwalk). The Inertsil ODS-2 column (5 μm, 4.6 $\times$ 250 mm, GL Science Inc., Tokyo, Japan) was equipped with a guard column (4.6 $\times$ 50 mm, GL Science Inc.). The detection wavelength was set at 270 nm and flow rate was 1.0 mL min^{-1}. The mobile phase consisted of acetonitrile/0.05% phosphoric acid (40:60, v/v) for baicalein assay in serum.

Drug administration and blood collection

Male Sprague-Dawley rats (300$\sim$380 g) were fasted for 15 h before drug administration and for a further 3 h after dosing. Water was freely available. The animal study adhered to "The Guidebook for the Care And Use of Laboratory Animals (2002)" (Published by The Chinese Society for the Laboratory Animal Science, Taiwan, ROC). For intravenous (i.v.) administration, baicalein was dissolved in glycofurol and filtered through a 0.22-μm membrane. The intravenous bolus was given to rats (n = 6) via the tail vein at a dose of 37 μmol kg^{-1}. Blood samples were withdrawn via cardiac puncture before dosing and at 5, 10, 20, 30, 60, 120, 240 and 360 min post-dosing. For oral (p.o.) administration, baicalin was dissolved in a solvent mixture containing DMSO, dimethylacetamide, PEG 400 and H_2O (trace:1:5:4), and baicalein was dissolved in glycofurol. Baicalin and baicalein administrations were carried out via gastric gavage at an equimolar dose of 224 μmol kg^{-1}. For baicalin treatment, blood samples were withdrawn via cardiac puncture at 0, 10, 30, 60, 180, 300, 480, 1440 and 2160 min post-dosing. For baicalein treatment, blood samples withdrawn via cardiac puncture at 0, 10, 30, 60, 120, 240, 480, 1440 and 2160 min post-dosing. All blood samples were centrifuged at 9860 g for 15 min and the serum obtained was stored at $-30\,^{\circ}$C for later analysis.

Quantification of baicalein and its conjugated metabolites in serum

The concentration of conjugated metabolites of baicalein in serum was determined after β-glucuronidase/sulfatase treatment. For enzymolysis, 100 μL serum was mixed with 50 μL β-glucuronidase/sulfatase (121.9/4.1 and 6095/205 U mL^{-1} in pH 5 acetate buffer for oral and intravenous dosing, respectively), 10 μL ascorbic acid (200 mg mL^{-1}) and incubated at 37 °C for 8 h under anaerobic conditions and protected from light. After hydrolysis, the serum was acidified with 25 μL 0.1 M HCl and partitioned with 175 μL ethyl acetate (containing 0.5 μg mL^{-1} of propyl paraben as internal standard). The ethyl acetate layer was evaporated under N_2 gas to dryness and reconstituted with an appropriate volume of mobile phase, then 20 μL was subjected to HPLC analysis. For the assay of baicalein free form, 100 μL serum sample was subjected to the process described above except for the addition of 50 μL pH 5 buffer instead of β-glucuronidase/sulfatase. For calibrator preparation, 100 μL serum mixed with various concentrations of baicalein was added with 50 μL pH 5 buffer. The latter procedure was the same as that described above for serum samples. The calibration graph was plotted by linear regression of the peak area ratios (baicalein to internal standard) against concentrations of baicalein.

Validation of assay method for serum

The system suitability was evaluated through intraday and interday analysis of precision and accuracy. The accuracy of this method was further assessed with recovery studies by adding baicalein into blank serum and water in triplicates to afford 0.16, 0.63 and 1.25 μg mL^{-1}, respectively, and the concentrations obtained in blank serum to the corresponding ones in water were compared. The LOQ (limit of quantitation) represents the lowest concentration of analysis in a sample that can be determined with acceptable precision and accuracy, whereas LOD (limit of detection) represents the lowest concentration of analysis in a sample that can be detected with signal/noise greater than 3.

Data analysis

The peak serum concentration (C_{max}) and the time to peak concentration (t_{max}) were obtained from experimental observation. The pharmacokinetic parameters were analysed by a non-compartmental method with the aid of the program WINNOLIN (version 1.1 SCI software, Statistical Consulting, Inc., Apex, NC). The area under the serum concentration–time curve (AUC_{0-t}) was calculated using the trapezoidal rule to the last point. Unpaired Student's *t*-test was used for the statistical comparison

of pharmacokinetic parameters between treatments of oral baicalin and baicalein. The other pharmacokinetic parameters were calculated from the following relationships:

$AUC_{0-t\ (BE\ conjugates)}$ of baicalein (BE) i.v. = $AUC_{0-t\ (BE+BE\ conjugates)}$ of baicalein i.v. – $AUC_{0-t\ (BE)}$ of baicalein i.v.

absolute absorption of baicalein = $AUC_{0-t\ (conjugates)}$ of baicalein p.o. × 37/$AUC_{0-t\ (BE+BE\ conjugates)}$ of baicalein i.v. × 224

relative absorption of baicalin (BG) to baicalein = $AUC_{0-t\ (BE\ conjugates)}$ of baicalin p.o./$AUC_{0-t\ (BE\ conjugates)}$ of baicalein p.o.

Results

Typical chromatograms of baicalein in serum are shown in Figure 2. A good linear relationship was obtained for baicalein in the concentration range of 0.08–10.00 $\mu g\,mL^{-1}$ ($y = 1.74\ x + 0.07$, $r = 0.9999$) in serum. The precision and accuracy of this method indicated that all coefficients of variation (CVs) were below 6.3% and the relative errors were below 12.3%. The LOQ and LOD for baicalein were 0.08 and 0.04 $\mu g\,mL^{-1}$, respectively. The recoveries of baicalein from serum were 95.7, 92.7 and 93.1% for the concentrations of 0.16, 0.63 and 1.25 $\mu g\,mL^{-1}$, respectively.

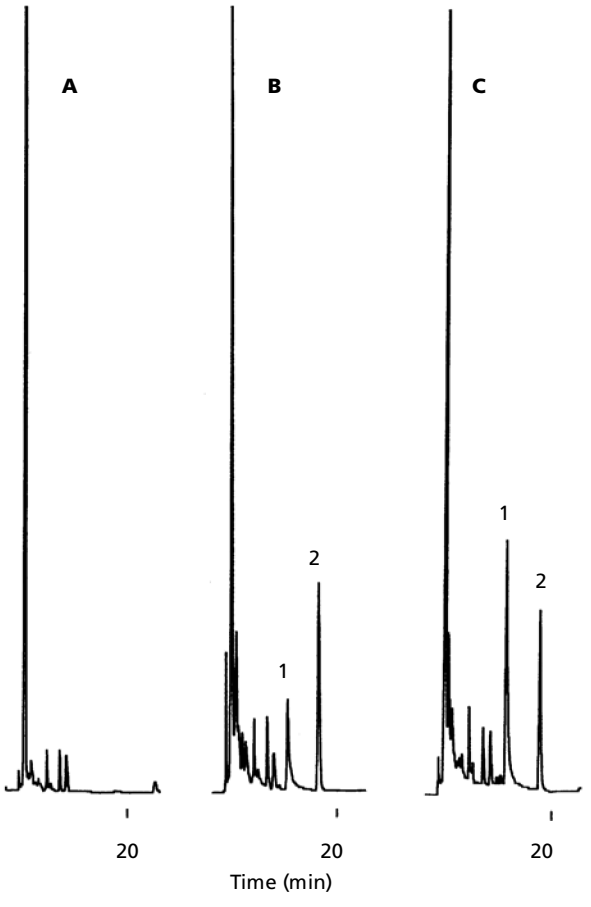


Figure 2 Chromatograms of (A) blank serum; (B) serum mixed with baicalein and internal standard; and (C) serum sample collected at 60 min after oral dose of baicalein and hydrolysed with enzymes. 1, baicalein; 2, internal standard (propyl paraben).

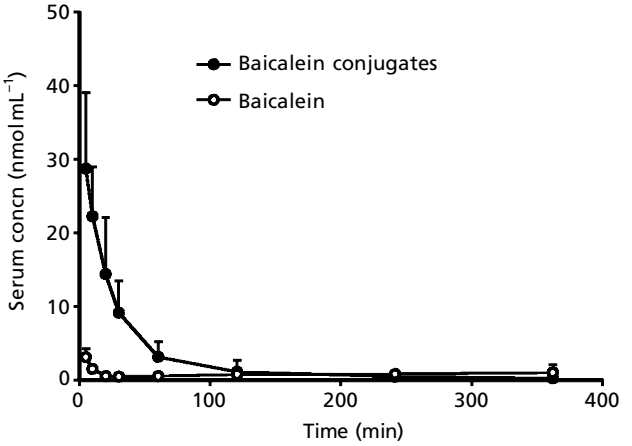


Figure 3 Mean (± s.d.) serum concentration–time profiles of baicalein and its conjugates after intravenous administration of baicalein (37 $\mu mol\ kg^{-1}$) to rats (n = 6).

Mean serum concentration–time profiles of baicalein and its conjugates after intravenous administration of baicalein are shown in Figure 3, revealing that the blood profile of baicalein conjugates was much higher than that of the baicalein parent form during the initial 120 min. The AUC_{0-360} of baicalein parent form and its conjugates after intravenous administration of baicalein are listed in Table 1, respectively, indicating that the conjugated metabolites accounted for 76% of the total baicalein including the parent form with its conjugates in the circulation.

After oral dosing of baicalein, the parent form of baicalein was only detected in a few samples during the early phase. The serum profiles of baicalein conjugates after oral doses of baicalin and baicalein (as shown in Figure 4) indicated that the patterns differed markedly between the two compounds. The pharmacokinetic parameters of

Table 1 The AUC_{0-360} ($nmol\,min\,mL^{-1}$) of baicalein and its conjugates after intravenous administration of baicalein (37 $\mu mol\,kg^{-1}$) to six rats.

	AUC_{0-360} (nmol min mL^{-1})
Total baicalein (BE + BE conjugates)	1224.8 ± 547.6
Baicalein parent form (BE)	297.6 ± 142.2
Baicalein conjugates (BE conjugates)	927.2 ± 462.8
Baicalein conjugates/total baicalein	75.7 ± 10.8%

Values are mean ± s.d.

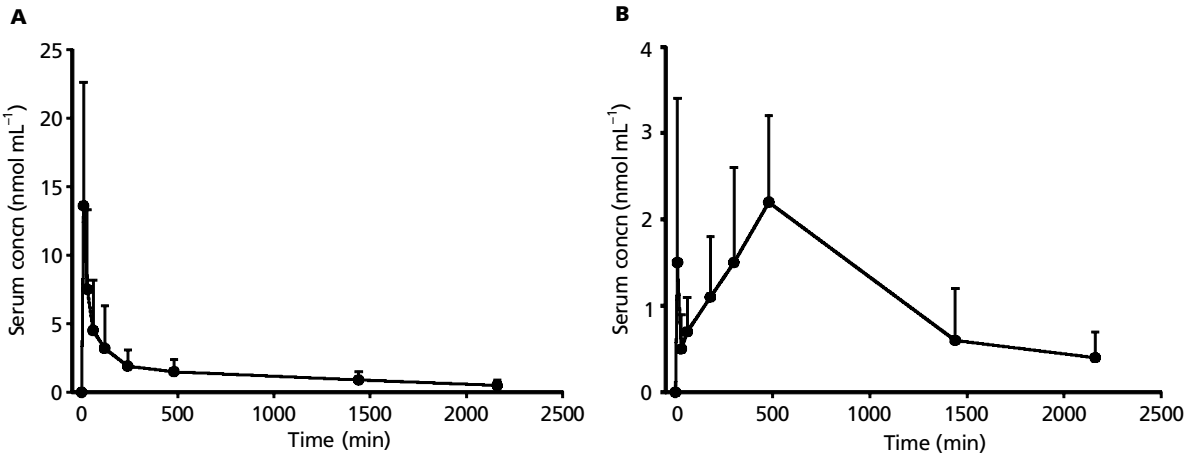


Figure 4 Mean (± s.d.) serum concentration–time profiles of conjugated metabolites of baicalein after oral administration of (A) baicalein (224 μmol kg^{-1}) and (B) baicalin (224 μmol kg^{-1}) to rats (n = 9 each).

Table 2 Pharmacokinetic parameters of baicalein conjugates after oral administration of equimolar dose of baicalin and baicalein (224 μmol kg^{-1}) to rats (n = 9 each).

Parameters	Baicalin	Baicalein	Difference (%)
C_{max} (nmol mL^{-1})	2.9 ± 1.3	13.6 ± 9.0	370.0 ± 349.0**
t_{max} (min)	395.6 ± 438.8	10.0 ± 0.0	−97.5 ± 42.5*
AUC_{0-2160} (nmol min mL^{-1})	1943.7 ± 810.6	2980.5 ± 1751.2	53.3 ± 173.1
MRT (min)	696.9 ± 320.8	651.7 ± 262.9	−6.5 ± 76.2

MRT, mean residence time. *$P < 0.05$, **$P < 0.01$ compared with baicalin. Values are mean ± s.d.

baicalein conjugates are listed in Table 2. The mean C_{max} of baicalein conjugates was significantly higher by 370.0% for oral baicalein than that of baicalin. The t_{max} of baicalein conjugates after oral baicalein was significantly earlier than that of baicalin. By comparing the AUC_{0-2160} of baicalein conjugates between oral baicalin and baicalein, the relative absorption of baicalin to baicalein was 65%.

The oral dose of baicalein given in this study was approximately six times that of the intravenous bolus, therefore, based upon dose correction, the comparison of AUC_{0-t} of baicalein conjugates after oral dose was compared with the total baicalein including baicalein and its conjugates after intravenous bolus. The absolute absorption of baicalein was 40%.

Discussion

Due to the fact that no authentic standard of baicalein glucuronides or sulfates was available, baicalein in serum sample was determined before and after treatment with β-glucuronidase/sulfatase to calculate the concentration of glucuronides/sulfates. The serum concentration of baicalein was assayed by a modified HPLC method (Wakui et al 1992). The serum profiles of the baicalein parent form and its conjugated metabolites after intravenous bolus of baicalein (shown in Figure 3) indicated that conjugation metabolism of baicalein occurred very rapidly and extensively. The profile of conjugated metabolites was much higher than that of baicalein, indicating that most baicalein was circulating as its conjugated metabolites. The AUC_{0-360} of conjugated metabolites constituted 76% of the total AUC_{0-360} of baicalein with its conjugates. In contrast with our previous study on intravenous dosing of a *Citrus* flavanone naringenin (50 mg kg^{-1}) to rabbit, which showed that only 18% was metabolized to its glucuronides/sulfates (Hsiu et al 2002), it clearly indicated that baicalein was more extensively metabolized by the liver than naringenin.

When baicalein was administered orally, the glucuronides/sulfates of baicalein were almost exclusively circulating in the bloodstream, whereas baicalein itself was negligibly absorbed. The C_{max} of baicalein conjugated metabolites occurred at the first sampling time (10 min), indicating very rapid absorption and simultaneous glucuronidation/sulfation.

Comparison of the AUC_{0-t} of baicalein parent form between oral and intravenous administrations of baicalein indicated that the absolute systemic bioavailability of baicalein parent form was almost zero. By contrast with the absolute systemic bioavailability of naringenin (4%) (Hsiu et al 2002), it corresponded well with the finding in the intravenous study that baicalein was more extensively

metabolized by the conjugation enzymes than naringenin. This indicated that extensive conjugation metabolism of baicalein occurred during the fist pass at gut and liver, which is in good agreement with a study by Spencer et al (1999). Those authors reported that glucuronidation/sulfation was central to the flavonoid metabolism and absorption. The absolute absorption of baicalein (40%) was much higher than that of naringenin (8%) (Hsiu et al 2002). This could be accounted for by the fact that the lipophilicity of baicalein was greater than naringenin from the observation of their retention times on the reversed HPLC chromatogram (data not shown).

When baicalin was orally administered at the equimolar dose with baicalein, the marked difference between their values of t_{max} showed that baicalin was absorbed more slowly than baicalein. This indicated that baicalin might be absorbed only after hydrolysis by enterobacteria in the colon, whereas baicalein was directly absorbed through the small intestine. The much lower mean C_{max} and AUC_{0-2160} of baicalein glucuronides/sulfates after oral baicalin compared with that after the equimolar dose of baicalein indicated that the absorption rate and extent of baicalin were lower than those of its aglycone baicalein. As a natural glycoside, baicalin exhibited better water solubility than its aglycone baicalein. However, unlike digoxin and glycyrrhizin, the lipophilicity of baicalin was too poor to be absorbed as its parent form. In contrast to the t_{max} (10 min) of baicalein conjugated metabolites after oral baicalein, it took approximately 396 min for oral baicalin to reach C_{max}, indicating that bacterial hydrolysis was the rate-limiting step for its absorption.

Baicalin served as a sustained-release prodrug of baicalein. Oral administration of baicalin and baicalein exclusively presented in the plasma as baicalein glucuronides/sulfates, therefore the conjugated metabolites of baicalein were in fact responsible for the in-vivo effects of baicalin and baicalein. Baicalin itself is one of the conjugated metabolites after administration of oral baicalin (Akao et al 2000) or baicalein. The activity of baicalin reported in in-vitro studies could only explain in part the in-vivo effects of oral baicalin and baicalein.

In conclusion, our results showed that there were marked differences in the metabolic pharmacokinetics between oral baicalin and baicalein. The absorption rate was slower and the C_{max} was lower for oral baicalin compared with oral baicalein. It is suggested that biologists focus less on the biological activities of baicalein and more on baicalin as well as other glucuronides/sulfates of baicalein for in-vitro studies. The previous reports using baicalein in in-vitro pharmacological investigations can not be extrapolated to the effects of oral baicalein and need further evaluation.

References

Akao, T., Kawabata, K., Yanagisawa, E., Ishihara, K., Mizuhara, Y., Wakui, Y., Sakashita, Y., Kobashi, K. (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

Chan, F. L., Choi, H. L., Chen, Z. Y., Chan, P. S. F., Huang, Y. (2000) Induction of apoptosis in prostate cancer cell lines by a flavonoid, baicalin. *Cancer Lett.* **160**: 219–228

Gao, Z., Huang, K., Yang, X., Xu, H. (1999) Free radical scavenging and antioxidant activies of flavonoids extracted from the radix of *Scutellarias baicalensis* Georgi. *Biochim. Biophys. Acta* **1472**: 643–650

Hsiu, S. L., Huang, T. Y., Hou, Y. C., Chin, D. H., Chao, P. D. (2002) Comparison of metabolic pharmacokinetics of naringin and naringenin in rabbits. *Life Sci.* **70**: 1481–1489

Ikemoto, S., Sugimura, K., Yoshida, N., Yasumoto, R., Wada, S., Yamamoto, K., Kishmoto, T. (2000) Antitumor effects of Scutellarias radix and its components baicalein, baicalin and wogonin on bladder cancer cell lines. *Urology* **55**: 951–955

Kitamura, K., Honda, M., Yoshizaki, H., Yamamoto, S., Nakane, H., Fukushima, M., Ono, K., Tokunaga, T. (1998) Baicalin, an inhibitor of HIV-1 production *in vitro*. *Antiviral Res.* **37**: 131–140

Lin, C. C., Shieh, D. E. (1996) The anti-inflammatory activity of *Scutellaria rivularis* extracts and its active components, baicalin, baicalein and wogonin. *Am. J. Chin. Med.* **24**: 31–36

Matsuzaki, Y., Kurokawa, N., Terai, S., Matsumura, Y., Kobayashi, N., Okita, K. (1996) Cell death induced by baicalein in human hepatocellular carcinoma cell lines. *Jpn. J. Cancer Res.* **87**: 170–177

Muto, R., Motozuka, T., Nakano, M., Tatsumi, Y., Sakamoto, F., Kosaka, N. (1997) The chemical structure of new substance as the metabolite of baicalin and time profiles for the plasma concentration after oral administration of Sho-Saiko-To in human. *Yakugaku Zasshi* **118**: 79–87

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

Spencer, J. P. E., Chowrimootoo, G., Choudhury, R., Debnam, E. S., Srai, S. K., Rice-Evans C. (1999) The small intestine can both absorb and glucuronidate luminal flavonoids. *FEBS Lett.* **458**: 224–230

Shieh, D. E., Liu, L. T., Lin, C. C. (2000) Antioxidant and free radical scavenging effects of baicalein, baicalin and wogonin. *Anticancer Res.* **20**: 2861–2865

Takizawa, H., DelliPizzi, A. M., Nasjletti, A. (1998) Prostaglandin I_2 contributes to the vasodepressor effect of baicalein in hypertensive rat. *Hypertension* **31**: 866–887

Wakui, Y., Yanagisawa, E., Ishibashi, E., Matsuzaki, Y., Takeda, S., Sasaki, H., Aburada, M., Oyama, T. (1992) Determination of baicalin and baicalein in rat plasma by high-performance liquid chromatography with electrochemical detection. *J. Chromatogr.* **575**: 131–136