Pharmaceutical Research, Vol. 22, No. 7, July 2005 (© 2005)
DOI: 10.1007/s11095-005-5303-7

Research Paper

Role of Intestinal First-Pass Metabolism of Baicalein in its Absorption Process

Li Zhang,[1] Ge Lin,[2] Qi Chang,[1,3] and Zhong Zuo[1,4]

Received December 8, 2004; accepted April 14, 2005

Purpose. The aim of the present study was to investigate the role of intestinal first-pass metabolism of baicalein (B) in its absorption process.
Methods. The intestinal absorption of B was characterized using Caco-2 cell monolayer model and rat *in situ* single-pass intestinal perfusion model. In addition, preliminary metabolic kinetics of B was evaluated in both rat and human intestinal S9 fractions.
Results. B was well absorbed and extensively metabolized to baicalin (BG), baicalein-7-*O*-β-glucuronide, in rat intestinal perfusion model, whereas less extent of metabolism was observed in the Caco-2 cell monolayer model. Moreover, BG generated in the intestinal epithelium during the absorption of B also rapidly transported to both the apical side (the apical chamber of Caco-2 model and the perfusate of the intestinal perfusion model) as well as the basolateral side of the small intestine (the basal chamber of Caco-2 model and the mesenteric vein of the intestinal perfusion model). From the preliminary metabolic studies, it was found that a higher loading dose of B resulted in a less extent of metabolism in intestine. In addition, the extent of metabolism of B was similar in jejunum and ileum when 50 μM of B was perfused through different sections of rat small intestine.
Conclusion. The first-pass metabolism of B in small intestine may play an important role in its low oral bioavailability.

KEY WORDS: baicalein; baicalin; first-pass metabolism; small intestine.

INTRODUCTION

Baicalein (B) (Fig. 1) is a bioactive flavone isolated from Scutellariae Radix, a traditional Chinese medicinal herb derived from the root of *Scutellaria baicalensis Georgi*. This herb has been used since ancient times to treat bacterial infections (1). A variety of pharmacologic effects of B have been demonstrated, including anti-inflammatory activity (2,3), antiallergic activity (4), antioxidative effect (5), antiviral effect (6), and antigenotoxic effect (7). After oral administration of B to rats, B could hardly be detected in the plasma, whereas baicalin (BG) (Fig. 1), the baicalein 7-*O*-β-glucopyranuronoside, appeared rapidly in systemic circulation (8,9). Further pharmacokinetic study of B in human after oral administration of Scutellariae Radix extract found that the glucuronides and sulfates of B were the major metabolites excreted in urine (10). All available data indicated that B underwent extensive biotransformation, mainly glucuronidation or sulfation, and the extensive first-pass metabolism through the small intestine was suggested to be one of the reasons responsible for the low bioavailability of B (9). Recently, a number of studies had addressed the contribution of small intestine to the first-pass metabolism of other flavonoids. Apigenin and genistein, the two representative naturally occurring flavonoids, were rapidly absorbed across the Caco-2 cell monolayer and then immediately form the glucuronide and sulfate conjugate metabolites, which excreted to both apical and basolateral sides (11,12). Similar result was also observed during the study of another flavonoid chrysin (13). However, to date, there are no publications reporting evidence of the extensive first-pass metabolism of B in the gut.

Caco-2 cell model is a well-established model to study the absorption and related mechanism of drugs. However, one of the major limitations of Caco-2 model is that its expressing level of metabolizing enzyme as well as the transporters may be different from the *in vivo* situation (14). Caution should be taken when attempting to extrapolate the *in vitro* data to *in vivo* situation. Therefore, an *in situ* intestinal perfusion model was adopted to study the absorption of various drugs including genistein and apigenin (11,15). The results indicated that these two flavonoids were readily

[1] School of Pharmacy, Faculty of Medicine, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong, People's Republic of China.
[2] Department of Pharmacology, Faculty of Medicine, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong, People's Republic of China.
[3] Institute of Medicinal Plant Development, Peking Union Medical College, Chinese Academy of Medical Sciences, Beijing 100094, People's Republic of China.
[4] To whom correspondence should be addressed. (e-mail: joanzuo@cuhk.edu.hk)

0724-8741/05/0700-1050/0 © 2005 Springer Science + Business Media, Inc.

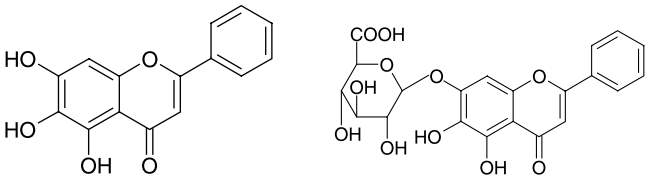

Fig. 1. Chemical structure of baicalein (B) and baicalin (BG).

absorbed and metabolized as indicated by the disappearance of the parent compound and the appearance of phase II conjugated metabolites in the perfusate, respectively. However, although such measurements in the perfusate can provide useful information on the extent of absorption and metabolism taking place at the small intestine, the measurement of the parent compounds and metabolites in the mesenteric blood should be a more direct and convincing approach to demonstrate drug absorption and metabolism in the small intestine.

Therefore, the purpose of the present study was to investigate the contribution of small intestine to the first-pass metabolism of B using both human Caco-2 cell monolayer model and rat single-pass intestinal perfusion model with analyses of the parent compounds and metabolites in both perfusate and mesenteric blood.

MATERIALS AND METHODS

Chemicals

Baicalein and baicalin were purchased from Aldrich Chem. Co. (Milwaukee, WI, USA). 6-Hydroxyflavanone (6-HF) used as an internal standard was from Indofine Chemical Co. (Hillsborough, NJ, USA). Waters Oasis hydrophilic–lipophilic-balanced (HLB, 1 ml) copolymer extraction cartridges were purchased from Waters (Milford, MA, USA). Acetonitrile (ACN) [high-performance liquid chromatography (HPLC) grade] and methanol (analytic grade) were obtained from Labscan (Labscan Asia, Bangkok, Thailand). All reagents were of analytic grade and used without further purification. Distilled and deionized water was used for the preparation of all solutions. For cell culture, Dulbecco's modified Eagle's medium, fetal bovine serum, 0.05% trypsin–ethylenediaminetetraacetic acid, penicillin–streptomycin, and nonessential amino acids were obtained from Gibco BRL (Carlsbad, CA, USA) and Life Technologies (Grand Island, NY, USA). Phosphate-buffered saline tablets were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Studies Using Caco-2 Cell Monolayer Model

Cell Culture

Caco-2 cells from the American Type Culture Collection (Rockville, MD, USA) were cultured and seeded in the Transwell® as described previously (16). Transepithelial electrical resistance (TEER) was used to monitor the integrity of the monolayer. Monolayer with TEER above 600 Ω cm^2 was employed in the present study. Caco-2 cells grown in Transwell® at passage 32–45 were used for the experiment.

Transport Studies of B and BG in Caco-2 Cell Model

The transport buffer employed in the transport studies was 0.01 M of phosphate-buffered saline, which was supplemented with 0.45 M calcium chloride and 0.4 M potassium chloride, then adjusted to pH 6.0. Transwell®s with Caco-2 cells grown on them for 21 days were rinsed twice and equilibrated with transport buffer at 37°C for 15 min before the transport experiment. The transport buffer (1.5 ml) containing 17 μM of B was loaded to the apical side, and 0.5 ml of samples was taken in the basolateral side at different time intervals (15, 30, 45, 60, and 90 min) to determine the amount of compounds absorbed with time. Same volume of blank transport buffer was replaced to the basolateral chamber after each sampling.

In the bidirectional transport study of BG, 17 μM of BG in transport buffer was loaded into the apical or basolateral side (the donor side). Aliquots of 0.5-ml samples were taken from the other side (the receiver side) at different time intervals (30, 60, 90, 120, 150, and 180 min) during the experiment.

Samples taken from the transport study in Caco-2 cell model were acidified with a solution containing 1% ascorbic acid, 0.28% H_3PO_4, and methanol (v/v/v; 6:1:6) and then stored at −80°C until analysis.

Perfusion Studies Using Rat Single-Pass Intestinal Perfusion Model

Animals and Surgical Procedures

Male Sprague–Dawley rats (body weight, 280–320 g) were fasted overnight with free access to water. The rats were anesthetized with an intramuscular injection of a mixture containing 60 mg/kg ketamine and 6 mg/kg xylazine. During the surgical process, the body temperature was maintained at 37°C by a heating pad as well as a heating lamp. Fresh heparinized blood was collected from donor rats by cardiac puncture. Right jugular vein for infusion of donor blood was cannulated with a polyethylene tubing (0.5 mm ID, 1 mm OD, Portex Ltd., Hythe, Kent, England). The infusion rate of donor blood via right jugular vein was set at 0.3 ml/min. The small intestine was then exposed by midline incision, and jejunum and ileum were identified as described previously by Chen *et al.* (11). A 7- to 11-cm-long segment of jejunum or ileum was cannulated with silicon tubing (2.4 mm ID, 4.0 mm OD) connected to a peristaltic pump. The segment was then flushed with warm saline to remove intestinal contents. The mesenteric vein for collecting blood from the specified segment of intestine was cannulated with a 6-cm-long polyethylene tubing (0.86 mm ID, 1.27 mm OD, Portex Ltd.). The blood was collected into the tubes at appropriate time intervals.

Perfusion Solution

The perfusion buffer was isotonic and composed of 2.7 mM KCl, 1.3 mM KH_2PO_4, 8.1 mM Na_2HPO_4, 0.9 mM

$CaCl_2$, 0.4 mM $MgCl_2$, and 7% PEG 400 (v/v) with pH 5.0 at 37°C. In addition, 10 μg/ml of phenol red was added to the perfusate as a nonabsorbable marker. The flow rate of perfusate applied to the lumen was set at 0.3 ml/min.

Perfusion Study of B and BG

The perfusate with 50 μM of B or 200 μM of BG was applied to the rat jejunum; samples obtained from the outlet of the intestine and the mesenteric vein were collected into the preweighted microtubes every 5 min. All samples were kept on ice until the end of the experiment. The plasma samples were obtained by centrifuging blood samples at 13,000 rpm for 10 min. To examine dose-dependent effect on the absorption and metabolism of B, 200 μM of B was also examined using the same rat perfusion model. To discover whether there is any discrepant absorption and metabolism property of B at between jejunum and ileum, perfusion study of B at the dose of 50 μM at ileum was also performed.

Sample Preparation

Aliquot (0.5 ml) of each perfusate sample was mixed with 200 μl of methanol and 100 μl of 20% ascorbic acid. To determine the concentration of the B and BG in perfusate, 100 μl of the above perfusate mixture was spiked with 20 μl of 6-HF (50 μg/ml) as an internal standard, and 50 μl of the resultant solution was injected into the HPLC column for analysis.

Solid phase extraction was employed for the plasma sample preparation as described in our previous study (17). Briefly, 10 μl of 50% methanol in phosphate buffer (pH 2.5) containing 1% ascorbic acid and 50 μl of internal standard (6-HF, 10 μg/ml) were added into 100 μl of plasma sample. The sample was then diluted with 1 ml of 35% methanol in 25 mM sodium dihydrogen phosphate buffer (pH 2.5) containing 1% ascorbic acid. After vortexing for 5 min and centrifuging at 16,000 × *g* for 10 min, the supernatant was loaded on the preconditioned HLB cartridge. The cartridge was flushed subsequently, and the analytes were eluted from the cartridge by 1-ml methanol. The dried residue of the eluent was reconstituted with 100 μl of 35% methanol in 25 mM sodium dihydrogen phosphate buffer (pH 2.5) containing 1% ascorbic acid. An aliquot (50 μl) of the resultant solution was injected into the HPLC system for analysis.

Comparison of the Biotransformation of B Between Rat and Human Intestinal S9 Fractions

B (1.1, 5.6, and 11 μM) was preincubated with rat jejunum S9 prepared by the standard method (18) and human jejunum S9 (Tissue Transformation Technologies, Edison, NJ, USA) with a final concentration of 0.8 mg of protein/ml in 50 mM Tris–HCl (pH 7.5) buffer containing 8 mM of $MgCl_2$ and 25 μg/ml of alamethicin for 10 min at 37°C. The glucuronidation was initiated by addition of 2 mM of uridine diphosphate glucuronic acid. The reaction mixture was incubated at 37°C for 10 min, and the reaction was stopped by the addition of 40 μl of ACN/acetic acid (9:1, v/v) containing 20 μg/ml of 6-HF as an internal standard. The reaction mixture was centrifuged at 16,000 × *g* for 5 min, and the supernatant was directly subjected to the HPLC analysis.

HPLC Analysis of B and BG

HPLC–ultraviolet method was employed for the simultaneous determination of B and BG in the plasma as described previously (17). Briefly, the chromatographic separation of B, BG, and the internal standard was achieved by using a reversed-phase HPLC column. The mobile phase consists of a mixture of methanol, acetonitrile, and 20 mM sodium dihydrogen phosphate buffer (pH 4.6) with a flow rate of 1 ml/min for 25 min. The autosampler was set at 10°C. The detection wavelength was set at 320 nm for both B and BG.

For the analysis of perfusate samples, the HPLC mobile phase was similar to that for plasma samples with a slight modification as follows. The gradient began with 80% of 20 mM phosphate buffer (pH 4.6) (A), 5% methanol (B), and 15% ACN (C), changed linearly to 57% A, 8% B, and 35% C in 10 min; 40% A, 0% B, and 60% C in 2 min; and then back to the initial composition in 6 min followed by 7 min after equilibrium.

The standard curves for B and BG were linear with R^2 of about 0.999. The CV was within 10%.

Data Analysis

The permeability coefficient (P_{app}) of B and BG for Caco-2 cell model was calculated as described previously (19).

The concentration of the analytes in the perfusate was corrected for the water flux as given in Eq. (1) before other calculations were performed:

$$\mathrm{Conc}_{\mathrm{corrected}} = \mathrm{Conc}_{\mathrm{measured}} \times \frac{(\mathrm{Phenol\,red})_{\mathrm{in}}}{(\mathrm{Phenol\,red})_{\mathrm{out}}} \qquad (1)$$

Hematocrit value of rat was reported to be 0.45 (20). The amount of the compound in the blood was calculated as Eq. (2):

$$\mathrm{Amount}_{\mathrm{Drug(blood)}} = C_{\mathrm{Drug(plasma)}} \times V_{\mathrm{(blood)}} \times 0.55 + D \times C_{\mathrm{Drug(plasma)}} \times V_{\mathrm{(blood)}} \times 0.45 \qquad (2)$$

Were $\mathrm{Amount}_{\mathrm{Drug(blood)}}$, $C_{\mathrm{Drug(plasma)}}$ and $V_{\mathrm{(blood)}}$ represented the amount of the compounds in the blood, plasma concentrations of the compounds and the volume of the blood, respectively. $D = C_{\mathrm{(drug)(red\ blood\ cell)}}/C_{\mathrm{Drug(plasma)}}$ was the partition coefficient of the drug between red blood cells (RBC) and plasma. The erythrocytes uptake of BG and B was estimated at their related blood concentration ranges from the present study. It was found that there was no BG (blood concentration range: 0.1–5 μg/ml) and 25.4% of B (blood concentration range: 0.1–1 μg/ml) uptake by the RBC, respectively. *D* was calculated to be 0 and 0.42 for BG and B, respectively.

The permeability coefficients of compounds based on the appearance of the compounds in the mesenteric blood

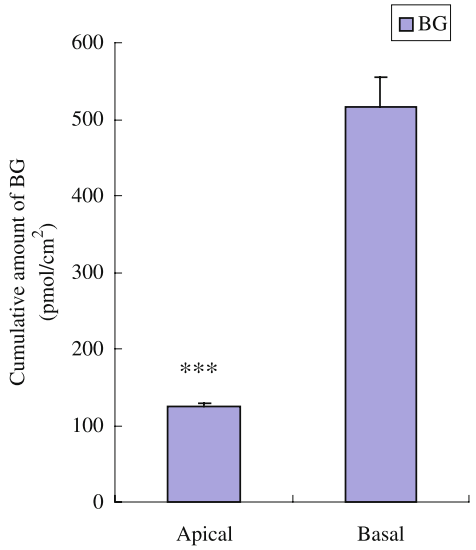

Fig. 2. Cumulative amount of BG formed at apical and basal side in transport study using Caco-2 cell model ($n = 3$). ***$p < 0.001$.

(P_{Blood}) and the disappearance of the compounds in the perfusate (P_{Lumen}) were calculated according to Eq. (3) (21,22) and Eq. (4) (23), respectively.

$$P_{Blood} = \frac{dX/dt}{AC_0} \tag{3}$$

where dX/dt is the rate of drug appearance in mesenteric blood, A is the area of the intestine segment, and C_0 is the initial drug concentration in the perfusate.

$$P_{Lumen} = -\frac{Q}{2\pi rl} \ln \frac{C_{out}}{C_{in}} \tag{4}$$

where r is the radius of the perfused intestinal segment and l is the length of the perfused intestinal segment, Q is the perfusate flow rate, C_{in} is the drug concentration in the inlet of the perfusate entering the intestinal segment, and C_{out} is the drug concentration in the exiting perfusate at the steady state.

To evaluate the extent of metabolism of B, Cummins's extraction ratio (ER) was employed (22). As described in Eq. (5), Cummins's ER was the ratio of the total amount of

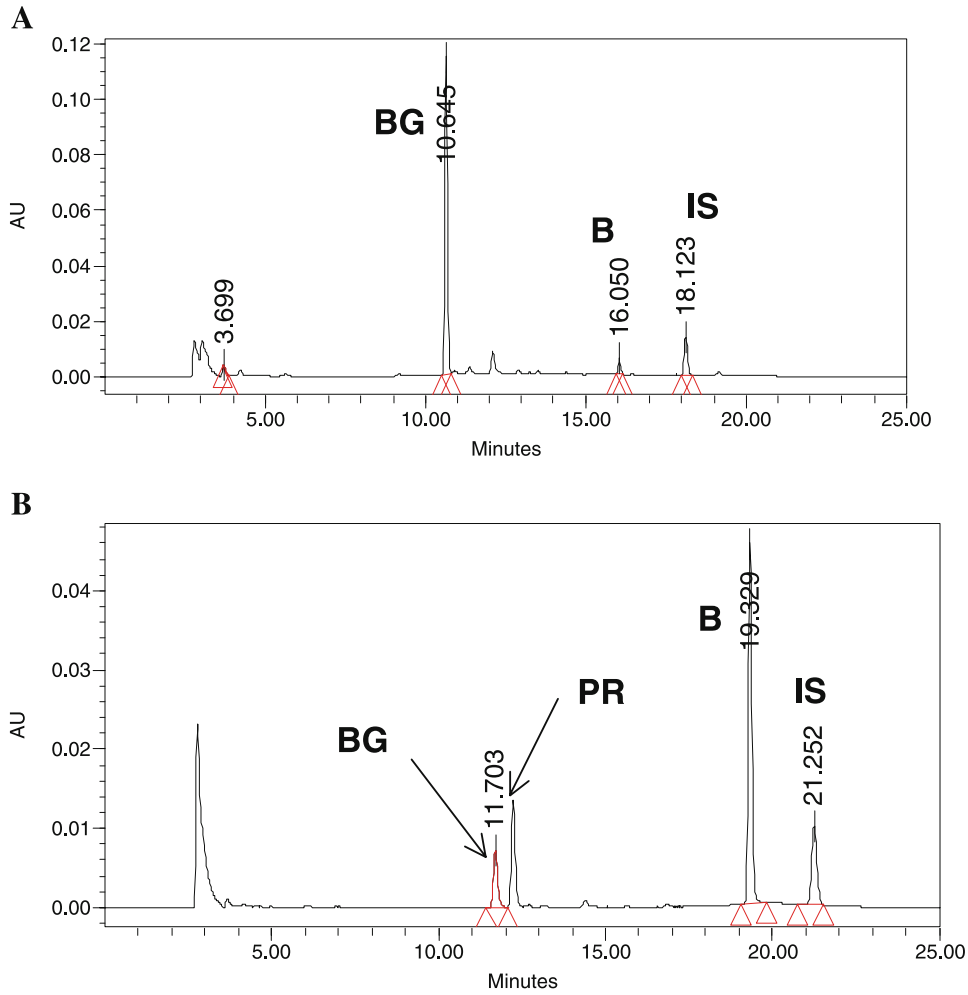

Fig. 3. High-performance liquid chromatogram of mesenteric plasma sample (A) and perfusate sample (B) obtained after perfusing 50 μM of B into rat jejunum. PR: phenol red. B: Baicalein. BG: Baicalin. IS: Internal Standard.

metabolites found during the perfusion process divided by a sum of the total amount of metabolites formed and the parent compound present in both the perfusate and mesenteric blood. As the drug and its metabolites appearing in the mesenteric blood are regarded as to be absorbed, the percentage of metabolite accounting for a total amount of the parent drug and metabolites in the mesenteric blood was also a meaningful parameter to evaluate the extent of metabolism of B during its absorption [Eq. (6)]. In addition, the amounts of both metabolite and parent drug were normalized by the area of the intestine (A) in both Eqs. (5) and (6).

$$\text{Cummins's ER} = \frac{\sum \text{metabolites}_{(\text{perfusate + blood})}/A}{\sum B_{(\text{blood})}/A + \sum \text{metabolites}_{(\text{perfusate + blood})}/A} \quad (5)$$

$$\text{Metabolites \% (in mesenteric blood)} = \frac{\sum \text{metabolites}_{(\text{blood})}/A}{\sum B_{(\text{blood})}/A + \sum \text{metabolites}_{(\text{blood})}/A} \quad (6)$$

The ER in the Caco-2 cell model was also calculated according to the equation used for rat intestinal perfusion model. The metabolite and the parent compound in apical chamber and basal chamber were treated as those in perfusate and mesenteric blood in rat intestinal perfusion model, respectively. Similarly, the metabolites % found in the basal chamber was treated the same as the metabolites % in mesenteric blood.

Statistically significant difference between two groups was evaluated by Student's t test. A $p < 0.05$ was considered significant for all tests.

RESULTS

Transport of B and BG in Caco-2 Cell Model

The absorptive P_{app} of B in the Caco-2 cell model was $1.691 \pm 0.135 \times 10^{-5}$ cm/s, which is comparable to that from the well-absorbed compounds (24). During its transport in Caco-2 cells, B was metabolized to BG, which was then transported to both apical and basal chambers (Fig. 2). Comparing the amount of BG formed at both chambers of Caco-2 model, BG was preferentially transported to the basal side. The calculated ER of B and BG% (in the basolateral chamber of Caco-2 cell model) [Eq. (5)] was 0.199 ± 0.01 and $17.7 \pm 1.3\%$, respectively. In addition, the bidirectional transport of BG showed that no significant absorption took place in the absorptive transport, whereas a significant secretion transport was observed with a P_{app} of $1.278 \pm 0.035 \times 10^{-5}$ cm/s ($n = 3$).

Absorption and Metabolism of B in Rat Single-Pass Jejunum Perfusion Model

Permeability coefficient (P_{Lumen}) of B determined based on the disappearance of B (50 μM) in the intestine lumen was calculated to be $1.810 \pm 0.517 \times 10^{-4}$ cm/s, which was comparable to antipyrine ($P_{Lumen} = 1.867 \times 10^{-4}$ cm/s), a well-absorbed marker (25). It implied that the permeability of B was favorable for absorption. However, fairly low amount of B was found in the mesenteric blood, and the major form of B existing in the mesenteric blood was BG (Fig. 3). This indicated that significant glucuronidation might take place during the absorption of B across intestinal epithelium. The cumulative amount of B and BG in the mesenteric blood increased linearly with the perfusion period (Fig. 4). The determined P_{Blood}(B) was $0.035 \pm 0.023 \times 10^{-4}$ cm/s, which was only 1.9% of P_{Lumen}(B). As summarized in the Table I, in 1-h perfusion study of B (50 μM), the cumulative amount of BG in the mesenteric blood was above 10-fold of that of B, and BG% (in the mesenteric blood) [Eq. (6)] was $91.4 \pm 3.2\%$. Both results indicated that extensive glucuronidation might occur in jejunum during the absorption of B.

As shown in Figs. 3 and 4, BG was also unequivocally identified and monitored in the perfusate. Comparing the cumulative amount of BG transported to the mesenteric blood and perfusate in 1-h perfusion study, about 45% of BG was transported to the mesenteric blood and 55% of BG was transported to the perfusate (Table I). The calculated Cummins's ER of B was 0.96 ± 0.02, suggesting that extensive metabolism of B may take place during its absorption.

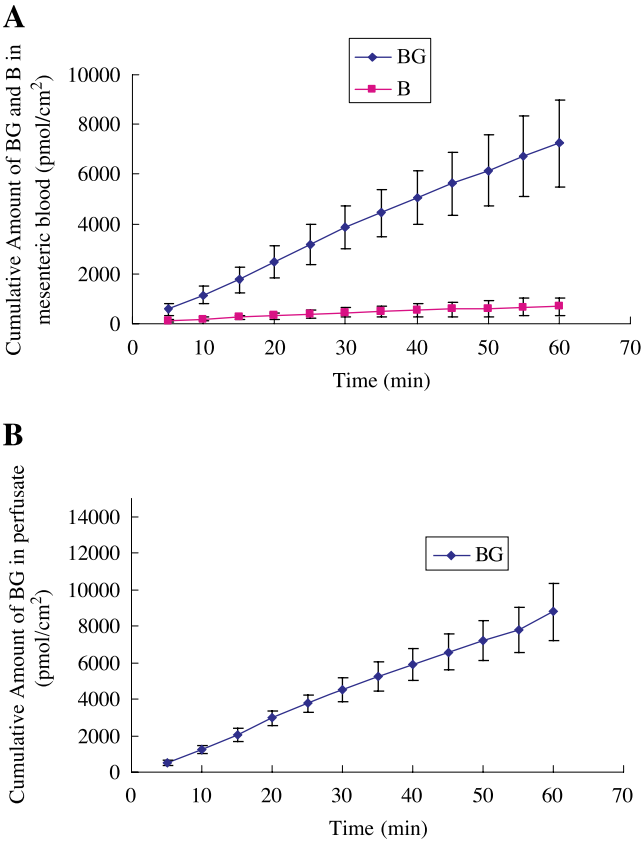

Fig. 4. Cumulative amount of BG and B appearing in the mesenteric blood over time in the rat jejunum perfusion study (A). Cumulative amount of BG appearing in the perfusate over time in the rat jejunum perfusion study (B) ($n = 6$). Data represent mean ± SD. B: Baicalein. BG: Baicalin.

Table I. Dose-Dependent Absorption and Metabolism of B in Rat Single-Pass Jejunum Perfusion

Conc. of B in the perfusate (μM)	In the mesenteric blood		BG% (in mesenteric blood)	Cumulative amount of BG in perfusate ($pmol/cm^2$)	ER
	Cumulative amount of B ($pmol/cm^2$)	Cumulative amount of BG ($pmol/cm^2$)			
50	682 ± 364	7,242 ± 1,736	91.4 ± 3.2	8,796 ± 1,574	0.96 ± 0.02
200	6,350 ± 2,354	19,204 ± 3,020	75.2 ± 3.8***	13,376 ± 3,270	0.84 ± 0.03***

Data represent mean ± SD, $n = 6$.
***$p < 0.001$ (compared with 50 μM).

Dose-Dependent Absorption and Metabolism of B in Rat Jejunum Single-Pass Perfusion Model

As summarized in Table I, with perfusion dose of B increased, the Cummins's ER ($p < 0.001$) and BG% (in mesenteric blood; $p < 0.001$) significantly decreased, suggesting that a less extensive metabolism occurred at a higher dose of B. In addition, the total amount of BG, appearing in both mesenteric blood and perfusate, only enhanced by about 2-fold despite increase in a dose by 4-fold. Moreover, about 10-fold higher amount of B was absorbed at higher dose than that at lower dose, suggesting that there might be a saturation of intestinal metabolism of B at the higher dose.

Absorption and Metabolism of B (50 μM) in Rat Ileum Single-Pass Perfusion Model

It was found that there was no significant difference in total cumulative amount of BG generated in mesenteric blood and perfusate between jejunum (16,038 ± 3,063 $pmol/cm^2$) and ileum (14,556 ± 2,611 $pmol/cm^2$). However, discrepancy was observed in terms of the distribution of BG between mesenteric blood and perfusate. As shown in Fig. 5, BG was preferentially transported to the mesenteric blood than the perfusate in ileum. Furthermore, Cummins's ER and BG% (in mesenteric blood) from ileum perfusion study were 0.95 ± 0.02 and 93.3 ± 2.6%, respectively, which were similar to those found in jejunum.

Absorption and Metabolism of BG (200 μM) in Rat Jejunum Single-Pass Perfusion Model

As shown in Fig. 6, no BG was found in the mesenteric blood after perfusion of BG at a dose of 200 μM. There were 99.3 ± 2.0% ($n = 3$) of BG recovered in the perfusate after 1-h perfusion, indicating that there was no absorption of BG in the small intestine. The calculated P_{Lumen}(BG) was 0.031 ± 0.007 × 10^{-4} cm/s.

Comparison of the Biotransformation of B Between Rat and Human Intestinal S9 Fractions

To further investigate whether extensive metabolism of B to form BG also takes place in human intestine, the velocity of BG formation was compared between human and rat intestine S9 fractions. From Fig. 7, the reaction rates

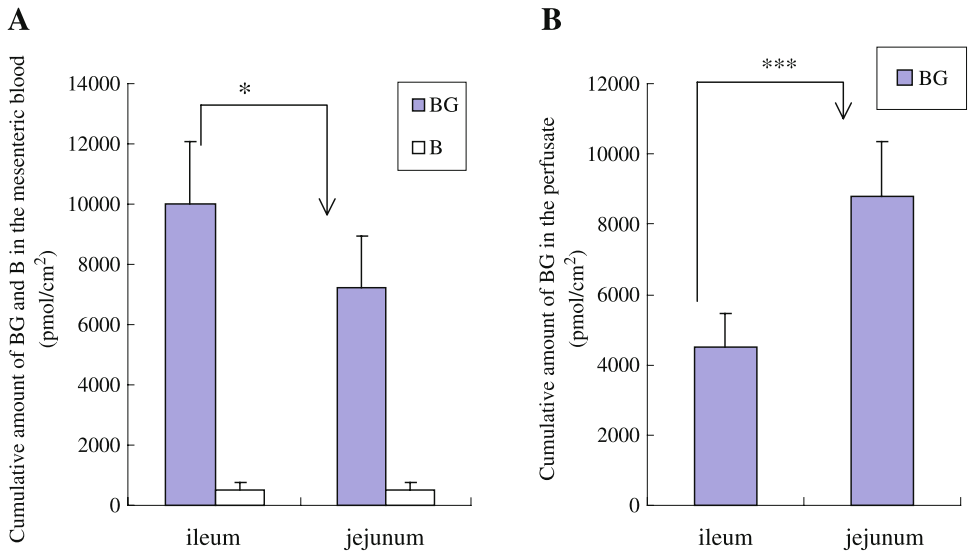

Fig. 5. Comparison of cumulative amount of B and BG in the mesenteric blood (A) and cumulative amount of BG in the perfusate (B) after perfusing 50 μM of B into rat jejunum ($n = 6$) and rat ileum ($n = 5$) for 60 min. Data represent mean ± SD. *$p < 0.05$, ***$p < 0.001$.

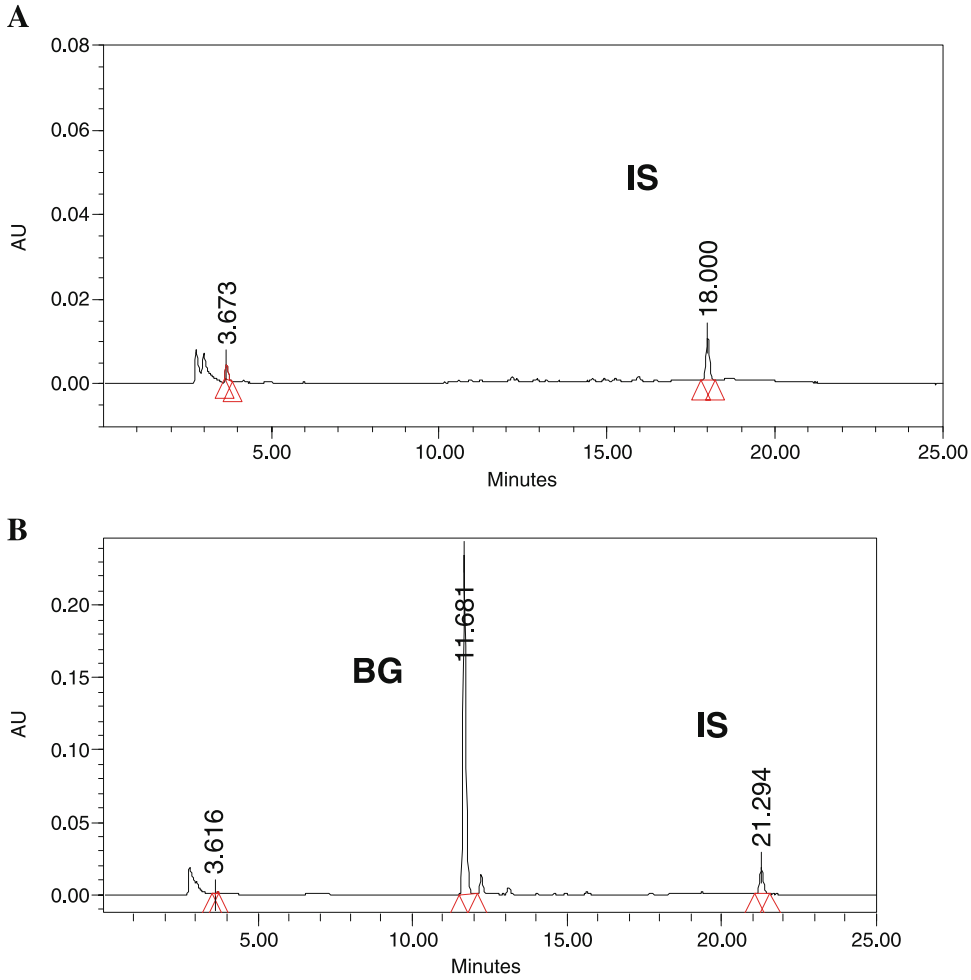

Fig. 6. High-performance liquid chromatogram of mesenteric plasma sample (A) and perfusate sample (B) obtained after perfusing 200 μM of BG into rat jejunum. BG: Baicalin. IS: Internal Standard.

obtained from the two species at concentrations of 1.1 and 11 μM were similar. Although at the concentration of 5.6 μM statistical difference was observed between the two species, the difference was not substantial.

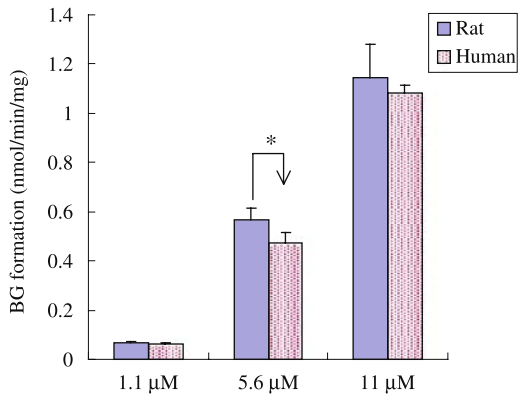

Fig. 7. Comparison of the formation rate of BG between rat and human intestine S9 at three concentrations of B. Data represent mean ± SD. BG: Baicalin. B: Baicalein. *$p < 0.05$.

DISCUSSION

As systemic blood sampling cannot completely elucidate the contribution of intestinal first-pass metabolism during the absorption process of a drug, the rat intestinal perfusion model with mesenteric blood sampling and human Caco-2 cell model was employed in the present study. Both Caco-2 and rat intestinal perfusion models suggested that B was well absorbed with both P_{app} and P_{Lumen} values comparable to those of the well-absorbed markers. However, good permeability of B did not result in a rapid and high-level appearance of B in the mesenteric blood. On the contrary, over 90% of B was rapidly converted to BG in the mesenteric blood when 50 μM of B was perfused into the jejunum and ileum. These results strongly supported that extensive first-pass metabolism of B in the intestinal wall during its absorption could play an important role in the low oral bioavailability of B. The biotransformation of B to BG was also observed in the Caco-2 cell model, and the extent of metabolism is substantially lower than that in the *in situ* perfusion model. These findings are consistent with the previous observation in that the expression of UGT in the Caco-2 cells was much lower than that in the *in vivo* situation

(14). Therefore, the data generated from the Caco-2 cell model may underestimate the extent of metabolism of B in the real situation. On the other hand, *in situ* model with only evaluation of the parent compounds in the perfusate could also overestimate the bioavailability because of the potential lack of assessment of the biotransformation across the small intestine. Therefore, for the study of a compound with extensive first-pass metabolism in gut, both models should be used for the accurate prediction.

Although BG formed during the absorption of B could be effectively transported to both the mesenteric blood and perfusate, no direct absorption of BG was detected during the direct perfusion of BG (200 μM) and no significant absorptive transport of BG was found in Caco-2 cell model. Flavonoid glycosides were suggested to be absorbed after releasing the aglycone by hydrolysis at brush border (15,26,27). However, in the present study, a high recovery of BG obtained in the perfusate from the present perfusion study of BG suggested that the hydrolysis by bush border is not significant during the BG absorption. As indicated from the previous study by Akao *et al.* (8), absorption of BG in germ-free rats was much less than that in conventional rats. It was suggested that the intestinal microflora, which might be absent in the current *in situ* intestinal perfusion model, might play an important role in the hydrolysis of BG in colon.

The poor intestinal absorption of BG implied that the capacity of BG to passively transport across the lipid bilayer was inferior. Therefore, the effective transport of BG generated in the epithelium cells may depend on the carrier-mediated transport. The members of multi-drug resistant associate proteins (MRPs), especially MRP1, MRP2, and MRP3, possess similar substrate selectivity and prefer to transport organic anion and phase II metabolites, including glutathione, glucuronide, and sulfate conjugates (28). In the present study, despite poor absorptive transport of BG, extensive secretion transport of BG was found in the Caco-2 cell model when loading BG itself in the basal chambers. These results suggested that there might be some apical transporters involved in the efflux transport of BG. MRP2 located at the apical side was proposed to be responsible for the apical efflux of chrysin (a flavonoid) glucuronide and chrysin sulfate (13). On the other hand, BG formed inside the intestinal epithelium was transported to the basal side, which implied that the transporters located at the basal side might also be involve in the transport of BG to the mesenteric blood. Based on their basal side location, MRP1 or MRP3 or both may be responsible for the basal efflux of BG. Further studies are definitely needed to confirm the involvement of specific transporters.

It was shown in the previous study that the cumulative amount of genistein glucuronide in perfusate in ileum perfusion model was significant lower than that in the jejunum perfusion model (15). Similar observation was also obtained in the present study when comparing jejunum and ileum perfusion of B. In addition to the reduced amount of BG transported to the perfusate at ileum, no significant difference was found for Cummins's ER at two different segments suggesting that the decreased apical efflux of BG might not be due to the reduced extent of metabolism of B. As the enhanced cumulative amount of BG in the mesenteric blood was found, it is proposed that despite the similar extent of metabolism of B at two segments, higher amount of BG was transported to the mesenteric blood at ileum resulting in less BG transported to the lumen. Different expression levels of MRP2 (duodenum ~ jejunum > ileum) and MRP3 (ileum > jejunum > duodenum) were found along the GI tract (29). Therefore, the different expression level of MRPs at jejunum and ileum may determine the distribution of BG at blood (basal) and perfusate (apical).

Moreover, our study indicated that there was no substantial discrepancy in the biotransformation of B in rat and human intestine, suggesting that the glucuronidation of B may also play an important role in the first-pass metabolism of B in human small intestine. Therefore, these animal models can be utilized for the prediction of human oral absorption of B and probably other flavonoids as well.

Previous studies have shown that BG is the major form in the general circulation after the intake of B (8,9) and our present study further indicated that extensive glucuronidation of B took place when passing through intestine. Therefore, the pharmacological properties of BG itself became crucial for the overall beneficial effect of B. Numerous investigations have already demonstrated that BG also exhibited various biological activities including anti-carcinogenesis (30–33) anti-oxidative and radical scavenging (34–36), anti-bacterial (37) and anti-inflammatory effects (38–40). It implies that part of the pharmacological effect of B after oral administration may be due to the effect from BG.

CONCLUSIONS

The present study demonstrated that B was well absorbed, whereas extensive glucuronidation of B took place during its abortion in the small intestine. BG, the predominant metabolite of B, was effectively transported to both mesenteric blood and perfusate. Thus, first-pass metabolism of B in small intestine may play an important role in the low oral bioavailability of B.

ACKNOWLEDGMENTS

This study was supported by CUHK Direct Grants (CUHK 2040830 and CUHK 2041012).

REFERENCES

1. D. Yang, H. Hu, S. Huang, J. P. Chaumont, and J. Millet. Study on the inhibitory activity, *in vitro*, of baicalein and baicalin against skin fungi and bacteria. *Zhongyaocai* **23**:272–274 (2000).
2. C. J. Chen, S. L. Raung, S. L. Liao, and S. Y. Chen. Inhibition of inducible nitric oxide synthase expression by baicalein in endotoxin/cytokine-stimulated microglia. *Biochem. Pharmacol.* **5**:957–965 (2004).
3. T. Hong, G. B. Jin, S. Cho, and J. C. Cyong. Evaluation of the anti-inflammatory effect of baicalein on dextran sulfate sodium-induced colitis in mice. *Planta Med.* **68**:268–271 (2002).
4. M. Kimata, M. Shichijo, T. Miura, I. Serizawa, N. Inagaki, and H. Nagai. Effects of luteolin, quercetin and baicalein on immunoglobulin E-mediated mediator release from human cultured mast cells. *Clin. Exp. Allergy* **30**:501–508 (2000).
5. Z. H. Shao, T. L. Vanden Hoek, Y. Qin, L. B. Becker, P. T. Schumacker, C. Q. Li, L. Dey, E. Barth, H. Halpern, G. M. Rosen, and C. S. Yuan. Baicalein attenuates oxidant stress in cardiomyocytes. *Am. J. Physiol.* **282**:H999–H1006 (2002).

6. K. Ono and H. Nakane. Mechanisms of inhibition of various cellular DNA and RNA polymerases by several flavonoids. *J. Biochem.* **108**:609–613 (1990).
7. B. H. Lee, S. J. Lee, T. H. Kang, D. H. Kim, D. H. Sohn, G. Ko, and Y. C. Kim. Baicalein, an *in vitro* antigenotoxic compound from *Scutellaria baicalensis*. *Planta Med.* **66**:70–71 (2000).
8. T. Akao, K. Kawabata, E. Yanagisawa, K. Ishihara, Y. Mizuhara, Y. Wakui, Y. Sakashita, and K. Kobashi. 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 (2000).
9. M. Y. Lai, S. L. Hsiu, S. Y. Tsai, Y. C. Hou, and P. D. Chao. Comparison of metabolic pharmacokinetics of baicalin and baicalein in rats. *J. Pharm. Pharmacol.* **55**:205–209 (2003).
10. M. Y. Lai, S. L. Hsiu, C. C. Chen, Y. C. Hou, and P. D. Chao. Urinary pharmacokinetics of baicalein, wogonin and their glycosides after oral administration of scutellariae radix in humans. *Biol. Pharm. Bull.* **26**:79–83 (2003).
11. J. Chen, H. Lin, and M. Hu. Metabolism of flavonoids via enteric recycling: role of intestinal disposition. *J. Pharmacol. Exp. Ther.* **304**:1228–1235 (2003).
12. M. Hu, J. Chen, and H. Lin. Metabolism of flavonoids via enteric recycling: mechanistic studies of disposition of apigenin in the Caco-2 cell culture model. *J. Pharmacol. Exp. Ther.* **307**:314–321 (2003).
13. U. K. Walle, A. Galijatovic, and T. Walle. Transport of the flavonoid chrysin and its conjugated metabolites by the human intestinal cell line Caco-2. *Biochem. Pharmacol.* **58**:431–438 (1999).
14. D. Sun, H. Lennernas, L. S. Welage, J. L. Barnett, C. P. Landowski, D. Foster, D. Fleisher, K. D. Lee, and G. L. Amidon. Comparison of human duodenum and Caco-2 gene expression profiles for 12,000 gene sequences tags and correlation with permeability of 26 drugs. *Pharm. Res.* **19**:1400–1416 (2002).
15. Y. Liu and M. Hu. Absorption and metabolism of flavonoids in the Caco-2 cell culture model and a perused rat intestinal model. *Drug Metab. Dispos.* **30**:370–377 (2002).
16. L. Zhang, Y. Zheng, M. Chow, and Z. Zuo. Investigation of intestinal absorption and disposition of green tea catechins by Caco-2 monolayer model. *Int. J. Pharm.* **287**:1–12 (2004).
17. L. Zhang, G. Lin, and Z. Zuo. High-performance liquid chromatographic method for simultaneous determination of baicalein and baicalein 7-glucuronide in rat plasma. *J. Pharm. Biomed. Anal.* **36**:637–641 (2004).
18. D. E. Williams, R. L. Reed, B. Kedzierski, G. A. Dannan, F. P. Guengerich, and D. R. Buhler. Bioactivation and detoxication of the pyrrolizidine alkaloid senecionine by cytochrome P-450 enzymes in rat liver. *Drug Metab. Dispos.* **17**:387–392 (1989).
19. P. Artursson and J. Karlsson. Correlation between oral drug absorption in humans and apparent drug permeability coefficients in human intestinal epithelial (Caco-2) cells. *Biochem. Biophys. Res. Commun.* **175**:880–885 (1991).
20. N. Okudaira, T. Tatebayashi, G. C. Speirs, I. Komiya, and Y. Sugiyama. A study of the intestinal absorption of an ester-type prodrug, ME3229, in rats: active efflux transport as a cause of poor bioavailability of the active drug. *J. Pharmacol. Exp. Ther.* **294**:580–587 (2000).
21. G. L. Amidon, G. D. Leesman, and R. L. Elliott. Improving intestinal absorption of water-insoluble compounds: a membrane metabolism strategy. *J. Pharm. Sci.* **69**:1363–1368 (1980).
22. B. M. Johnson, W. Q. Chen, R. T. Borchardt, W. N. Charman, and C. H. Porter. A kinetic evaluation of the absorption, efflux, and metabolism of verapamil in the autoperfused rat jejunum. *J. Pharmacol. Exp. Ther.* **305**:151–158 (2003).
23. C. L. Cummins, L. Salphati, M. J. Reid, and L. Z. Benet. *In vivo* modulation of intestinal CYP3A metabolism by P-glycoprotein: studies using the rat single-pass intestinal perfusion model. *J. Pharmacol. Exp. Ther.* **305**:306–314 (2003).
24. S. Yee. *In vitro* permeability across Caco-2 cells (colonic) can predict *in vivo* (small intestinal) absorption in man—fact or myth. *Pharm. Res.* **14**:763–766 (1997).
25. Y. Wang, R. Aun, and F. L. Tse. Absorption of D-glucose in the rat studied using *in situ* intestinal perfusion: a permeability-index approach. *Pharm. Res.* **14**:1563–1567 (1997).
26. A. J. Day, M. S. DuPont, S. Ridley, M. Rhodes, M. J. Rhodes, M. R. Morgan, and G. Williamson. Deglycosylation of flavonoid and isoflavonoid glycosides by human small intestine and liver beta-glucosidase activity. *FEBS Lett.* **436**:71–75 (1998).
27. A. J. Day, F. J. Canada, J. C. Diaz, P. A. Kroon, R. Mclauchlan, C. B. Faulds, G. W. Plumb, M. R. Morgan, and G. Williamson. Dietary flavonoid and isoflavone glycosides are hydrolysed by the lactase site of lactase phlorizin hydrolase. *FEBS Lett.* **468**:166–170 (2000).
28. P. Borst, R. Evers, M. Kool, and J. Wijnholds. A family of drug transporters: the multidrug resistance-associated proteins. *J. Natl. Cancer Inst.* **92**:1295–1302 (2000).
29. K. S. Pang. Modeling of intestinal drug absorption: roles of transporters and metabolic enzymes. *Drug Metab. Dispos.* **31**:1507–1519 (2003).
30. N. W. Baylor, T. Fu, Y. D. Yan, and F. W. Ruscetti. Inhibition T cell leukemia virus by the plant flavonoid baicalin (7-glucuronic acid, 5,6-dihydroxyflavone). *J. Infect. Dis.* **165**:433–437 (1992).
31. W. H. Chang, C. H. Chen, and F. J. Lu. Different effects of baicalein, baicalin and wogonin on mitochondrial function, glutathione content and cell cycle progression in human hepatoma cell lines. *Planta Med.* **68**:128–132 (2000).
32. S. Chen, Q. Ruan, E. Bedner, A. Deptala, X. Wang, T. C. Hsieh, F. Traganos, and Z Darzynkiewicz. Effects of the flavonoid baicalin and its metabolite baicalein on androgen receptor expression, cell cycle progression and apoptosis of prostate cancer cell lines. *Cell Prolif.* **34**:293–304 (2001).
33. S. Ikemoto, K Sugimura, N. Yoshida, R. Yasumoto, S. Wada, K. Yamamoto, and T. Kishimoto. Antitumor effects of scutellariae radix and its componets baicalein, baicalin, and wogonin on bladder cancer cell lines. *Urology* **55**:951–955 (2000).
34. C. Zhongli, H. Yongke, and W. Jilan. Study of baicalin scavenging hydroxyethyl peroxyl radicals by radiolysis of aerated ethanol-baicalin system. *Radiat. Phys. Chem.* **47**:869–871 (1996).
35. E. Ciesielska, A. Gwardys, and D. Metodiewa. Anticancer, antiradical and antioxidative actions of novel Antoksyd S and its major components, baicalin and baicalein. *Anticancer Res.* **22**:2885–2891 (2002).
36. Z. Gao, K. Huang, X. Yang, and H. Xu. Free radical scavenging and antioxidant activities of flavonoids extracted from the radix of scutellaria baicalensis georgi. *Bichim. Biophys. Acta.* **1472**: 643–650 (1999).
37. I. X. Liu, D. G. Durham, and R. M. Richards. Baicalin synergy with beta-lactam antibiotics against methicillin-resistant Staphylococcus aureus and other beta-lactam-resistant strains of S. aureus. *J. Pharm. Pharmacol.* **52**:361–366 (2000).
38. B. Q. Li, T. Fu, W. H. Gong, N. Dunlop, H. Kung, Y. Yan, J. Kang, and J. M. Wang. The flavonoid baicalin exhibits anti-inflammatory activity by binding to chemokines. *Immunopharmacology* **49**:295–306 (2000).
39. T. C. Chou, L. P. Chang, C. Y. Li, C. S. Wong, and S. P. Yang. The anti-inflammatory and analgesic effects of baicalin in carrageenan-evoked thermal hyperalgesia. *Anesth. Analg.* **97**: 1724–1729 (2003).
40. Y. C. Shen, W. F. Chiou, Y. C. Chou, and C. F. Chen. Mechanisms in mediating the anti-inflammatory effects of baicalin in human leukocytes. *Eur. J. Pharmacol.* **465**:171–181 (2003).