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Research Paper

Involvement of UDP-Glucuronosyltransferases in the Extensive Liver and Intestinal First-Pass Metabolism of Flavonoid Baicalein

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Purpose. The present study aims to investigate the involvement of UDP-glucuronosyltranferase (UGT) in the extensive liver and intestinal first-pass glucuronidation of baicalein (B) in both rats and humans and also to study sulfation and P450 mediated hydroxylation of B.
Materials and Methods. B was incubated with liver and intestine microsome, cytosol, S9 fractions from human, rat and various human recombinant UGT isozymes, respectively. The generated metabolites were identified by HPLC/MS/MS and quantified by HPLC/UV.
Results. Three metabolites of B namely baicalein 7-O-glucuronide (BG), the isomer of baicalein 7-O-glucuronide (BG'), and baicalein sulfate were found. BG, the predominant metabolite of B, was extensively generated in liver and jejunum microsomes in both humans and rats. Its formation was mainly catalyzed by UGT 1A9 and also mediated by UGT 1A1, 1A3, 1A8, 1A7 and 2B15 with different kinetic profiles. UGT 1A8 mediated formation of BG' was mainly found in human intestine and rat liver microsomes. Sulfation and P450 mediated hydroxylation of B were much less significant than glucuronidation.
Conclusions. Extensive liver and intestinal first-pass glucuronidation of B were found in both humans and rats. Under the current experimental conditions, UGT 1A9 and UGT 1A8 demonstrated the fastest formation rate of BG in human liver preparations and BG' in human intestine preparations, respectively.

KEY WORDS: baicalein; baicalin; glucuronidation; sulfation; UDP-glucuronosyltransferase.

INTRODUCTION

Flavonoids, characterized as polyphenolics, are widely distributed in edible plants and various foods. Epidemiological studies concerning polyphenol consumption and human cancer risk suggest the protective effects of certain food items and polyphenols (1,2). However, the oral bioavailabilities of flavonoids were reported to be low and extensive first-pass metabolism was suggested to be the major cause (3,4). Baicalein (B) (Fig. 1), a bioactive flavonoid, is isolated from the root of a traditional Chinese medicinal herb *Scutellaria baicalensis*. The demonstrated pharmacological effects of B include anti-inflammatory (5), anti-allergic (6) and anti-oxidative activities (7).

Previous pharmacokinetic studies of B demonstrated its extensive oral first-pass metabolism in rats with baicalin (BG) (Fig. 1), the baicalein 7-O-glucuronide, appeared rapidly in systemic circulation (8,9). Our recent study discovered that extensive glucuronidation of B may occur in the small intestinal wall since over 90% of B was rapidly metabolized to form BG and transported to the mesenteric blood when perfusing B through rat jejunum (10). Although extensive glucuronidation of B was observed in both intestine and liver of rats, limited information is available on metabolism of B in humans. Whether extensive oral first-pass metabolism by glucuronidation would take place in human is still unknown. Furthermore, other metabolic pathways such as sulfation and P450 mediated hydroxylation of B are rarely reported in human or rat, which warrant further investigation.

The present study was designed to: (1) investigate and compare the glucuronidation activity of B in intestine and liver of both rats and humans; (2) identify the specific human UDP-glucuronosyltransferase (UGT) isoforms mediating the glucuronidation of B; (3) characterize other metabolic pathways of B such as sulfation and P450 mediated hydroxylation in liver and intestine.

MATERIALS AND METHODS

Chemicals

Baicalein and baicalin were purchased from Aldrich Chem. Co (Milwaukee, WI, USA). 6-Hydroxyflavanone used as an internal standard (IS) was from Indofine Chemical Co (Hillsborough, NJ, USA). Uridine 5'-diphosphoglucuronic

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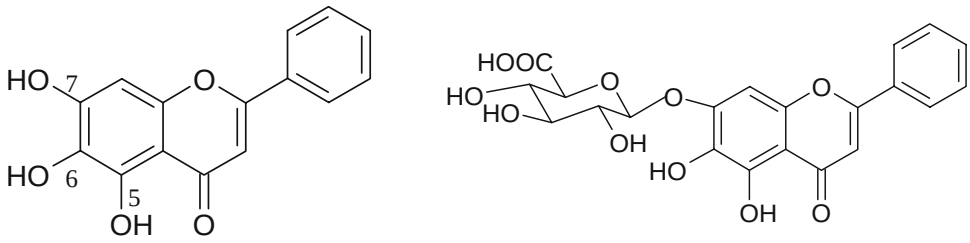

Baicalein (B) Baicalin (BG)

Fig. 1. Chemical structures of Baicalein (*B*) and Baicalin (*BG*).

acid (UDPGA), adenosine 3'-phosphate 5'-phosphosulfate (PAPS) and alamethicin were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Pooled human liver microsomes, recombinant human UGT 1A1, UGT 1A3, UGT 1A8, UGT 1A7, UGT 1A9 and UGT 2B15 were purchased from BD Gentest Corp. (Woburn, MA, USA). Pooled human jejunum S9/microsomes and ileum S9 were purchased from Tissue Transformation Technologies (New Jersey, USA). Other reagents were of analytical grade and used without further purification.

In Vitro Metabolism Incubation Studies

Glucuronidation Activity Assay

To determine the glucuronidation of B by liver and intestine, B (1.1–111 μM) was pre-incubated with pooled liver or jejunum microsomes of human or rat at a final protein concentration of 0.2 mg/ml in 50 mM Tris-HCl buffer (pH 7.4) containing 8 mM of $MgCl_2$ and 25 μg/ml of alamethicin for 10 min at 37°C. The reaction was initiated by the addition of 2 mM of UDPGA and last for 10 min.

To investigate the involvement of human UGTs in the glucuronidation of B, human recombinant UGTs at final protein concentrations of 0.2 mg/ml for UGT1A1, 1A3, 1A8, 1A9, 2B15 or 0.5 mg/ml for UGT 1A7 were used to catalyze the glucuronidation of B. The incubation conditions were the same as described above except for that the incubation time was 5 min for UGT 1A8.

In order to compare the glucuronidation activities from different segments of small intestine, pooled human jejunum and ileum S9 at a final protein concentration of 0.8 mg/ml were used to mediate the glucuronidation of B using the same incubation procedure described for liver and intestine microsome.

The protein concentrations and incubation times were optimized to ensure linearity for metabolite formation. All experiments were performed in triplicate, except that the UGT 1A1 incubation was performed in four replicates at all concentrations with additional triplicate (seven replicates in total) at three highest concentrations.

In Vitro Sulfation Activity Assay

B (1.1–111 μM) was pre-incubated with pooled jejunum or liver cytosol from both humans and rats, or human jejunum or ileum S9 at a final protein concentration of 0.8 mg/ml at 37°C for 10 min in Tris buffer (pH 7.4) containing 5 mM $MgCl_2$, 8 mM dithiothreitol and 0.0625% BSA. The reaction was initiated by addition of 200 μM of PAPS and lasted for 10 min. All the experiments were performed in triplicate.

In Vitro P450 Mediated Hydroxylation Activity Assay

B at concentrations of 1.5, 22.2 and 111 μM were incubated with human jejunum or liver microsomes (0.5 mg/ml of final protein concentration) at 37°C in Tris buffer (pH 7.4) containing 8 mM of $MgCl_2$, 25 μg/ml of alamethicin, 1.3 mM $NADP^+$, 3.3 mM glucose-6-phosphate and 0.4 U/ml glucose-6-phosphate dehydrogenase. The reaction lasted for 10 min. All the experiments were performed in triplicate.

Sample Preparation

All the metabolic reactions described above were terminated by adding 40 μl of ice-cold ACN/acetic acid (9:1 v/v) containing 20 μg/ml of the internal standard (6-hydroxyflavanone). The reaction mixture was centrifuged at 16,000 g for 5 min and the supernatant was directly analyzed by HPLC/UV or LC/MS/MS.

Quantitative Analysis of B and its Metabolites by HPLC/UV

HPLC coupled with UV detector was used for quantitative analysis of B and its identified metabolites. The HPLC system is composed of Waters 600 controller (pump), Waters 717 auto sampler and Waters 2487 dual wavelength detector. The chromatographic separation of B, BG and internal standard was achieved by using a reversed-phase HPLC column (BDS reversed phase column, 25 cm×4.6 mm I.D., 5 μm particle size, Thermo Hypersil) connected with a protective guard column (Delta-Pak C_{18} Guard-Pak, Waters). The mobile phase, consisting of a mixture of 20 mM sodium dihydrogen phosphate buffer (pH 4.6) (A), methanol (B) and acetonitrile (C) was run using a linear gradient elution program. The gradient began with 80% A, 5% B and 15% C, changed linearly to 57% A, 8% B and 35% C in the first 10 min; then 40% A, 0% B and 60% C in the next 2 min; and then back to the initial composition in 6 min followed by 7 min to allow the column to re-equilibrate. The flow rate of the mobile phase was set at 1 ml/min and the analytes were detected at 320 nm. The calibration curves for B and BG were linear with r^2 of about 0.999 and CV of less than 10%.

Qualitative Analysis of LC/MS/MS

To identify the metabolites of B, an API 2000 Triple Quadruple LC/MS/MS spectrometer equipped with two Perkin-Elmer PE-200 series micro-pumps and auto-sampler (Perkin-Elmer, Norwalk, CT, USA) was used to perform the analysis. HPLC reversed-phase column (BDS, 25 cm×4.6 mm I.D., 5 μm particle size, Thermo Hypersil) was eluted with the following gradient at a flow rate of 1 ml/min. The gradient began with 20% eluent A (acetonitrile) and 80% eluent B (0.04% formic acid), and was changed linearly to 40% eluent A and 60% eluent B in 5 min and remained in this percentage for 11 min. Then the gradient was changed back to the initial condition in 2 min. Twenty percent of eluent was introduced into mass spectrometer and the other 80% was splitted off. Negative ion mode of the mass spectrometer was utilized for the analysis. Other working mass spectrometer parameters were: orifice voltage, −71V; ring voltage, −80V; nebulization gas, 45 psi; auxiliary gas, 80 psi; nebulizer temperature, 400°C. To perform MS/MS analysis, the selected molecular ions were subjected to fragmentation at the following condition: collision energy 27 eV; cell entrance potential, 22 eV; cell exit potential, 16 eV.

Data Analysis

Reported values are presented as mean±SE. The kinetic parameters of glucuronidation were obtained by fitting the data to the typical Michaelis–Menten equation (Eq. 1) or

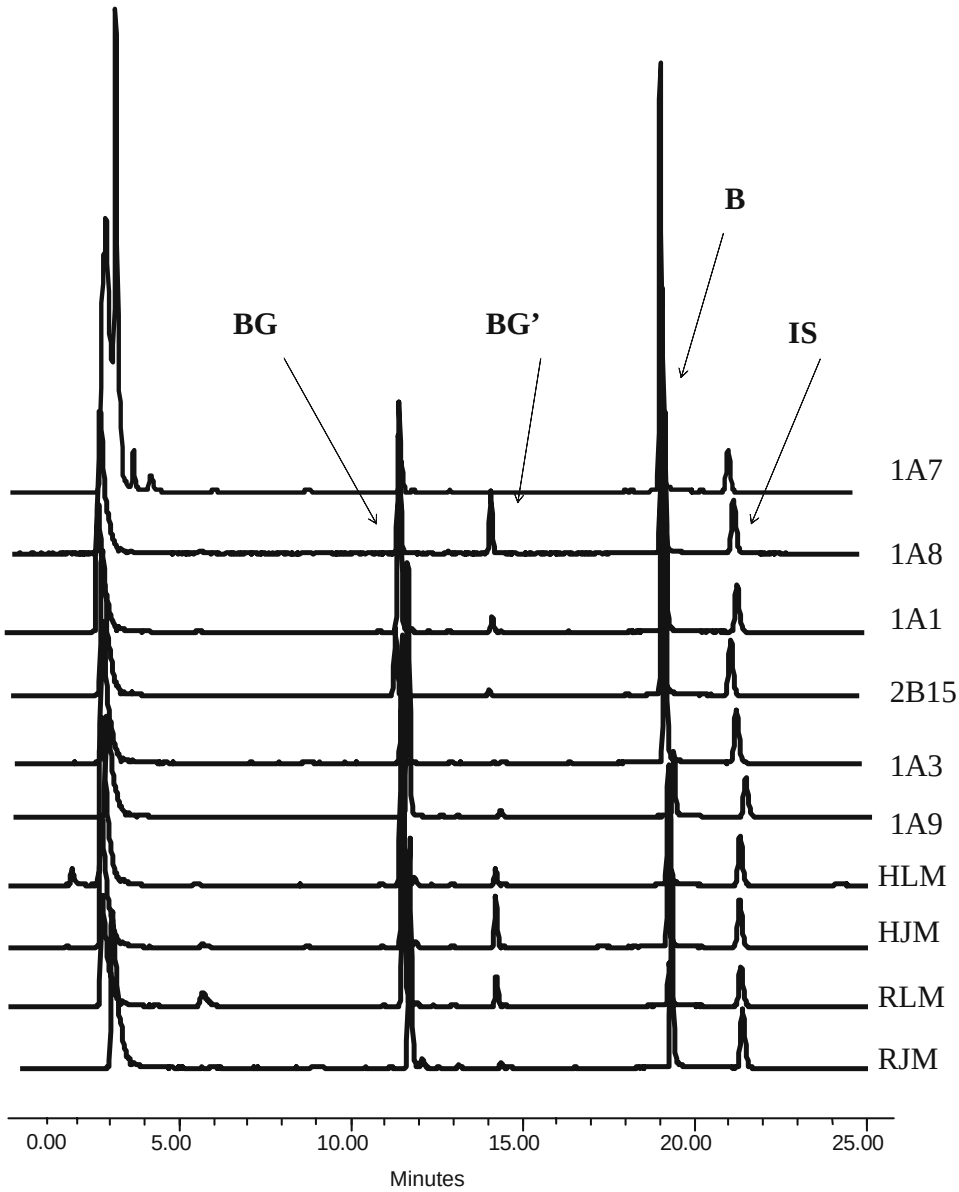

Fig. 2. HPLC/UV chromatograms of samples obtained after glucuronidation of B with various types of microsomes and UGTs.

substrate inhibition equation (Eq. 2) with Prism (GraphPad Software, Inc.).

$$V = V_{\max} \times C/(K_m + C) \quad (1)$$

$$V = V_{\max}/(1 + K_m/C + C/K_i) \quad (2)$$

Where $V_{\max}$ is the maximal velocity; K_m is the substrate concentration at half maximal velocity; K_i is the inhibition constant.

RESULTS

Glucuronidation Activity of B

Identification of Glucuronides of B

As shown in the representative HPLC/UV chromatograms (Fig. 2), B was rapidly bio-transformed into BG, the major glucuronide of B, after 10 min of incubation with liver and intestine microsome from both rats and humans. In addition to the verification of the chromatographic retention

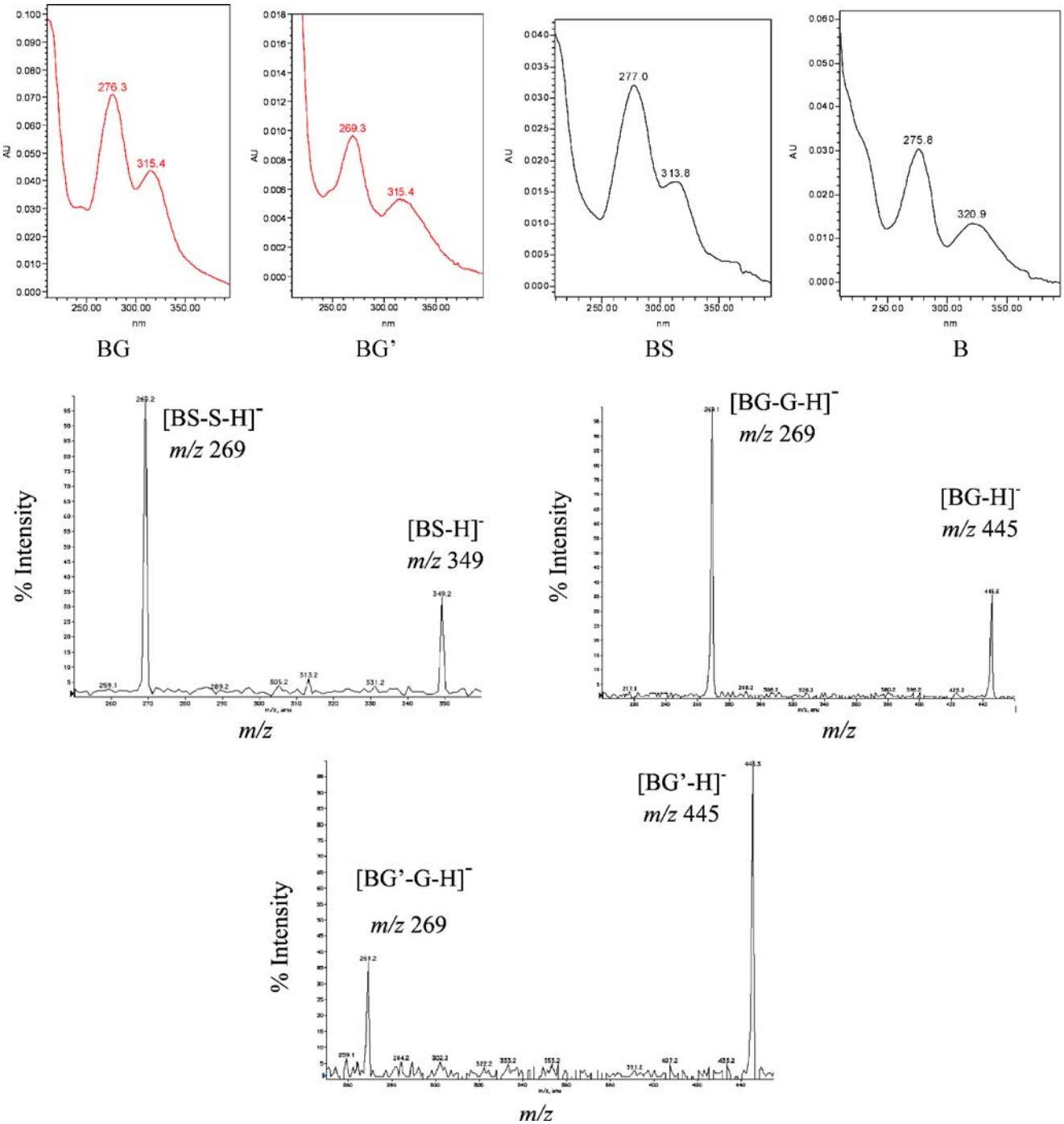

Fig. 3. Characteristic UV and mass spectra of metabolites of B. *BG*: baicalin, *BG*': isomer of baicalin, *B*: baicalein, *BS*: sulfate of baicalein.

time with that from the authentic standard, the formation of BG was further confirmed by its UV and mass spectrum (Fig. 3). The HPLC/MS/MS analysis at negative ion mode demonstrated that the quasi-molecular ion $[M\text{-}H]^-$ of the glucuronide of B was at *m*/*z* 445, and its major fragment ion at *m*/*z* 269 was formed by losing the glucuronic acid moiety (176 amu). In addition to BG, another glucuronide of B was found in the incubated samples, which had identical UV and mass spectrum but different chromatographic retention time of BG (Fig. 2). Therefore, this unknown glucuronide of B was identified as the isomer of BG, denoted as BG'. Moreover, it was found that the formation of both BG and BG' could not been observed in the absence of microsome, S9 or UDPGA.

Kinetic Profiles of Glucuronidation of B by Intestinal and Liver Microsome

The enzyme kinetic profiles of the glucuronidation of B by jejunum and liver microsomes from both rats and humans were obtained and compared. The formation rates of BG at various concentrations followed the Michaelis–Menten equation and the calculated K_m, V_{max} and Cl_{int} (V_{max}/K_m) are listed in Table I. Human jejunum displayed the highest V_{max}, whereas human liver exhibited the lowest K_m value. Despite of various kinetic profiles from different organs and species, the efficient biotransformation of B to BG by liver and intestine microsome from both rats and humans was clearly demonstrated from its high intrinsic clearance (Cl_{int}) values ranging from 298 to 618 μl min^{-1} mg^{-1}.

Comparison of Glucuronidation of B between Human Jejunum and Ileum

The glucuronidation kinetics of B by human jejunum and ileum S9 were compared. As shown in Table I, the metabolic capacity was higher in human jejunum, but higher affinity was observed in human ileum. No significant difference was observed in Cl_{int} between human jejunum and ileum S9, which implied the similar glucuronidation activity in these two human intestinal segments.

Glucuronidation of B by Human Recombinant UGT

Six UGTs, namely UGT 1A1, 1A3, 1A8, 1A7, 1A9 and 2B15, which were most frequently reported to be involved in the glucuronidation of flavonoids (11–15), were employed in the present study. All six UGTs exhibited different enzymatic kinetic properties in the glucuronidation of B. Among the six tested UGTs, UGT 1A9 displayed greatest capacity in the glucuronidation of B with highest V_{max}, whereas UGT1A3 had the highest affinity to B with the lowest K_m value. Under the current experimental condition, most efficient glucuronidation of B was found to be mediated by UGT 1A9 with a Cl_{int} value that was similar to the glucuronidation of B by human pooled liver microsome. Unlike the other UGTs tested, the metabolite formation rate *versus* substrate concentration plot of UGT 1A1 displayed a substrate inhibition kinetic profile under the studied concentration ranges of B. As shown in Fig. 4, the formation rate of BG by UGT 1A1

Table I. Enzyme Kinetic Properties for the Glucuronidation of B

	V_{max} (nmol min^{-1} mg^{-1})	K_m (μM)	Clint (nmol min^{-1} mg^{-1})
UGT 1A1[a]	N.A.	N.A.	329*
UGT 1A3	1.82±0.04	6.45±0.61	282
UGT 1A7	0.40±0.03	9.96±2.38	40.5
UGT 1A8	3.17±0.27	19.46±4.80	163
UGT 1A9	6.81±0.22	10.71±1.22	636
UGT 2B15	0.74±0.03	7.09±1.22	104
Human jejunum microsome	41.89±1.92	93.93±7.71	446
Human liver microsome	14.38±0.57	23.25±2.58	618
Rat jejunum microsome	12.50±0.74	41.92±5.80	298
Rat liver microsome	33.65±3.03	77.12±13.23	436
Human jejunum S9	2.35±0.12	18.75±2.86	125
Human ileum S9	1.38±0.04	10.76±1.22	128

N.A. Not available
*Cl_{int} was calculated based on the slope of the initial linear portion (from 1.1 μM to 11.1 μM of B) of the metabolite formation rate *versus* substrate concentrations plot for UGT 1A1.
[a] Significant substrate inhibition was observed

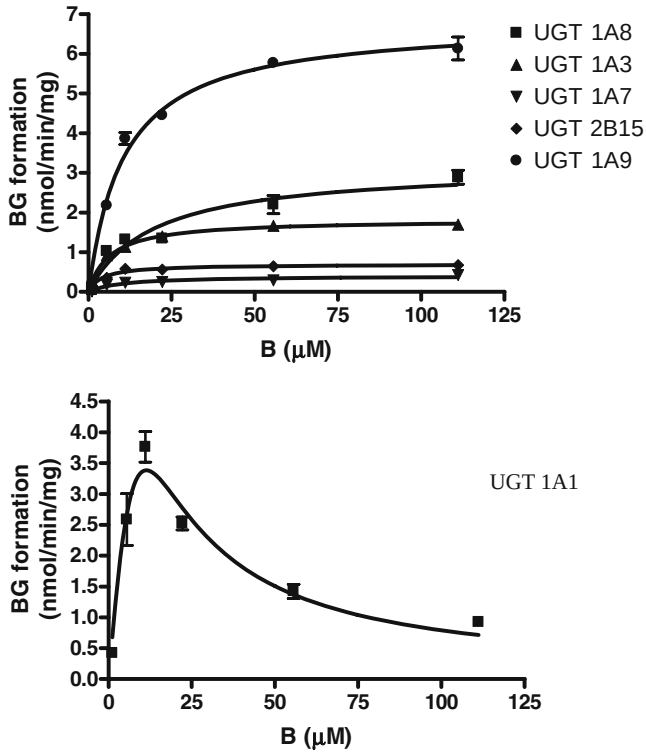

Fig. 4. Formation rate of BG versus substrate concentration plots for human UGTs.

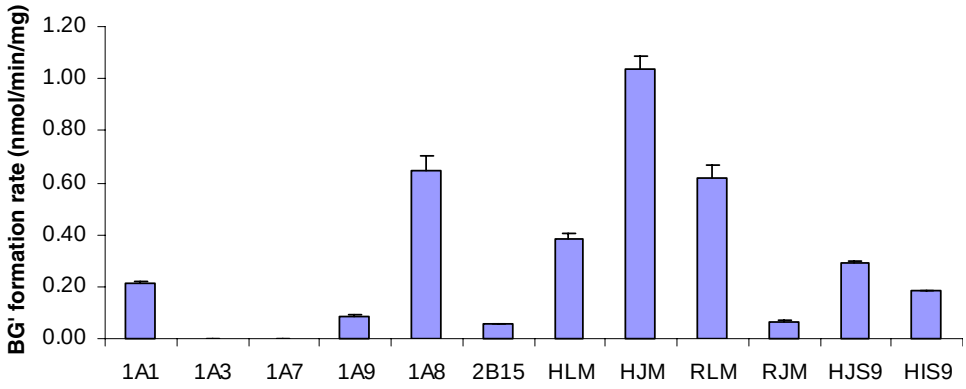

Fig. 5. Transformation of B (at 11.1 μM) to BG' by various microsomes, intestine S9 and UGT isoforms. *HLM*: human liver microsome; *HJM*: human jejunum microsome; *RLM*: rat liver microsome; *RJM*: rat jejunum microsome. *HJS9*: human jejunum S9; *HIS9*: human ileum S9.

increased with the concentrations of B at its low concentration range. The maximal formation rate of BG was reached at about 10 μM of B, and then substantial decrease of reaction rate appeared with further increase of concentrations of B in its higher concentrations range.

Formation of BG' by Liver, Intestine and UGT Isozymes

Figure 5 demonstrated significant variations in the formation rate of BG' in incubations of B (11.1 μM) with various sub-cellular fractions and UGT isoforms. Formation of BG' was most significant in human jejunum microsome, followed by human and rat liver microsome. However, it seemed less significant in rat jejunum microsome. Comparing the different segments of human intestine, the formation of BG' in human jejunum S9 seemed to be a bit higher than that in human ileum S9. Under the current experimental conditions, among the six UGTs, the production of BG' was the most efficient in UGT 1A8 and least significant in UGT 1A3 and 1A7.

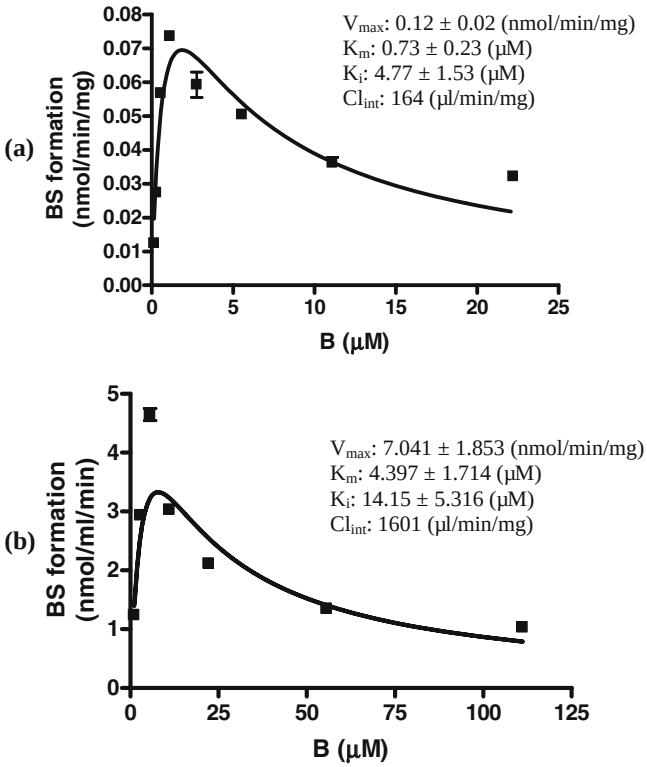

Fig. 6. Formation rate of sulfate of B *versus* substrate concentration plots for human liver cytosol (a) and rat liver cytosol (b).

Sulfation of B by Human and Rat Liver and Intestine Cytosol

A sulfate of B (BS) was formed during incubation of B with human and rat liver cytosol in the presence of PAPS. The formation of BS was confirmed by its UV spectrum, which was similar to that of B (the aglycone of BS), as well as its characteristic mass spectrum that demonstrated the quasi-molecular ion at m/z 349 and the major fragment ion at m/z 269 (Fig. 3). Substrate inhibition kinetics was also observed for the formation of BS by human and rat liver cytosol. Comparing with glucuronidation, the relative lower K_m and V_{max} value for the sulfation of B indicated a higher affinity and lower capacity for the sulfation of B in human and rat liver cytosol (Fig. 6). In addition, the low K_i value implied a high potency of inhibition of B towards the sulfotransferase in human and rat liver. Although glucuronidation by human jejunum and ileum S9 occurred readily as shown above, no sulfation of B mediated by these sub-cellular fractions was observed. In addition, sulfation of B was also not demonstrated in human and rat intestinal cytosol.

P450-mediated Hydroxylation of B

After incubating B with human liver and jejunum microsome in the presence of NADPH-generating system, no hydroxylation metabolite of B was detected by the developed assay methods. Moreover, comparing with the negative control, there are 100±1.8%–102±8% and 96±4%–103±7% of B remained in the human liver microsome and human jejunum reaction systems, respectively, which further indicated that no significant phase I metabolism occurred.

DISCUSSIONS

Previous pharmacokinetic studies in rats discovered an extensive first-pass metabolism of B with BG as the major metabolite found in circulation after oral administration of B (8–10). These studies indicated that extensive glucuronidation might occur in rat liver and intestine. However, species differences in metabolism of drugs do exist between humans and rats. Whether results from animal studies could be applied to human *in vivo* situation is always a concern. In order to identify the specific organ in charge of the first-pass metabolism, organ perfusion techniques are often used in animals. However, such technique would be difficult to perform in humans. Therefore, the *in vitro* metabolism kinetic studies of B by various organ sub-cellular fractions from both humans and rats were conducted for the present study aiming to reveal possible similarities of the metabolism in these two species.

The results demonstrated that three metabolites of B namely baicalein 7-O-glucuronide (BG), the isomer of baicalein 7-O-glucuronide (BG'), and baicalein sulfate (BS) were found. Among them, biotransformation of B to BG is the most predominant metabolic pathway for the clearance of B by liver and intestinal microsomes of both humans and rats. The Cl_{ints} of B by liver and intestine microsome of humans and rats ranged from 298 to 618 μl min^{-1} mg^{-1}, which are several folds faster than that of bilirubin, the endogenic UGT substrate, and two to three orders of magnitude greater than those of some typical glucuronidation substrates such as acetaminophen, morphine and androstanediol, etc. (16). In both liver and jejunum microsome, the clearances via transforming B to BG in human are comparable or even more extensive than those in rat. The results implied that extensive oral first-pass glucuronidation might also occur in human. Based on Cl_{int}, it was noticed that formation of BG in the liver was more rapid than that in intestine in both species, suggesting that hepatic first-pass effect for B may be more extensive than the intestinal one.

The six selected UGT isozymes in the present study have all been reported to catalyze the glucuronidation of various flavonoids. UGT 1A9, 1A1 and 2B15 were reported to catalyze the glucuronidation of galangin (11). UGT 1A1, 1A8 and 1A9 were involved in the glucuronidation of luteolin and quercetin (12). UGT 1A3 was reported to mediate the glucuronidation of flavonoids including naringenin, apigenin, galangin, fisetin, 7-hydroxyflavone, genestein and quercetin (13). Kaempferol and quercetin were demonstrated to be the substrate of UGT 1A9 (14). Moreover, UGT 1A7 displayed differential activities towards flavonoids such as chrysin, apigenin, galangin, fisetin kaempferol, morin, quercetin etc. (15). In our study, all six human UGT isoforms examined demonstrated various activities in the biotransformation from B to BG. Among the six UGTs, UGT 1A1, 1A3, 1A9 and 2B15 are expressed mainly but not exclusively in liver, whereas 1A7 and 1A8, the extrahepatic UGTs, are found mainly in intestine (17,18). UGT 1A9 was most active in the transformation of B to BG with greatest Cl_{int} and V_{max} among all UGTs tested. The significantly higher formation rate of UGT 1A9 that is mainly expressed in human liver but not significant in intestine further confirmed the primary role of UGTs (especially UGT 1A9) in the first-pass glucuronidation of B in liver. On the other hand, UGT 1A3, 1A7 and 2B15 have much lower K_m than that of the rest of the studied UGTs implying their higher affinity towards B. Since the absolute amount of specific recombinant UGT in each microsomal preparation is hard to quantify and may vary in different preparations, the enzyme activities of various UGTs normalized by their total protein contents may not truly reflect the real situation (19). Comparison among the different UGTs from the present study is only based on the results obtained under the current experimental conditions.

Metabolic kinetics of B by UGT 1A1 was characterized as substrate inhibition by Eadie-Hofstee and Lineweaver-Burke plots. Based on the Akaike's information criterion values, *F*-test and correlation coefficient obtained from fitting with various models including substrate inhibition, Michealis-Menten equation and Hill equation etc., the substrate inhibition model lead to the most reasonable results and was thus considered to be the best-fitted model for current sets of data for UGT 1A1. However, the estimation of V_{max} (74.03±296.1 nmol min^{-1} mg^{-1}) and K_m (118.9±498 μM) value for UGT 1A1 still had high standard errors. Therefore, the Cl_{int} for UGT 1A1 was estimated according to the slope of the linear portion of the metabolite formation rate *versus* concentration plot (i.e., at the lower substrate concentration range) to provide a relative comparison with the other UGTs. Further studies with more appropriate model fitting to provide meaningful V_{max}, K_m and Cl_{int} are definitely necessary. During the *in vitro* metabolism studies, the protein concentration and incubation time were optimized to ensure the linear product formation, and excessive amount of the cofactor UDPGA was used. In addition, the decline of reaction rate at the higher substrate concentration was found not to be due to the substantial substrate depletion since there were high concentrations of B remaining in the incubation system. In order to further confirm the finding, the metabolic kinetics study with UGT 1A1 had been repeated several times especially at high concentrations that have exhibited substrate inhibition. In summary, the observed substrate inhibition by UGT1A1 was unlikely caused by any artificial factors mentioned above. Although substrate inhibition by UGT1A1 was not investigated in detail in the current study, previous studies on other enzymes have suggested the possible mechanisms for such phenomenon. It was proposed that there are two binding regions within the enzyme active site, and the binding of second substrate would elicit conformational change in the enzyme active site resulting in the decrease in reaction rates (20). Further investigation into the mechanism of substrate inhibition on UGT1A1 is warranted.

Formation of BG' was found in both humans and rats. The possible site for the glucuronic acid of BG' may be at 6 or 5-OH. Previous studies on the glucuronidation of flavonoids from others and us indicated that the glucuronidation rate at 5-OH was about one to two order slower than those at the other positions (21,22). Moreover, our previous study indicated that the steric hindrance of carbonyl group at the C-4, intra-molecular hydrogen binding of OH at C-5 as well as unfavorable neucleophilicity of 5-OH towards C-1 of pyranose acid ring of UDPGA might be the reasons for the relative inert glucuronidation activity at 5-OH. In addition, a previous *in vivo* study found that it was the baicalein 6-O-glucucronide instead of baicalein 5-O-glucucronide excreted

in the urine of rats after oral administration of BG (23). Therefore, the BG' formed in the present study was likely to be baicalein 6-O-glucucronide.

Although formation rate of BG' seems to be less significant than that of BG, the important contribution of the human small intestine to the first-pass glucuronidation of B is noteworthy (Fig. 5). The significant formation rate of BG' by UGT 1A8, which is mainly express in intestine, further confirmed the important role of UGTs (especially UGT 1A8) in the intestinal first-pass glucuronidation of B. Furthermore, species difference was observed in the contribution of liver and intestine to the first-pass transformation of B to BG'. It was found that BG' was formed more rapidly in intestine of human and liver of rats. Such discrepancy may be due to the different tissue distribution of UGTs responsible for the formation of BG' in these two species. The current finding once again demonstrated the limitation of using animal model to predict human data.

In addition to the glucuronides of B, baicalein 6-O-sulfate was the sulfate of B found in human plasma after oral dosing with BG-containing herbal medicine Sho-Saiko-To (24). It was suggested that the BS formed in our study might be Baicalein 6-O-sulfate. Sulfation was usually regarded as a competitive metabolic pathway with glucuronidation (25). Direct comparison between sulfation and glucuronidation was conducted with human jejunum and ileum S9. The results demonstrated that glucuronidation occurred rapidly while no significant sulfation took place in intestine. It was further proved that sulfation of B was only mediated by human and rat liver cytosol. Comparing sulfation *versus* glucuronidation in liver, the lower values of both K_m and V_{max} for sulfation of B indicated that B has higher affinity but substantial lower capacity towards SULTs than UGTs. Furthermore, substrate inhibition of sulfation was also observed at higher concentrations of B. Based on the Akaike information criterion values, *F*-test and correlation coefficients from various model fitting, substrate inhibition model was chosen as the best-fitted model. On the other hand, such inhibition profile could also be due to an overall effect of various sulfatransferase in the pooled human or liver cytosol. Further investigations for the clarification are warranted. It is expected that sulfation mainly contribute to the hepatic first-pass metabolism at relatively low concentrations of the substrate, whereas glucuronidation is the major contributor to the first-pass effect in both liver and intestine at a broad concentration range of the substrate.

Unlike glucuronidation and sulfation, P450 mediated hydroxylation metabolites of B were not found in the present study. Our finding is consistent with the previous studies in which no P 450 mediated hydroxylation metabolites of B was found in plasma after either oral or intraperitoneal administration in rats (8,9). Moreover, in the present study, we also demonstrated that no significant P450 mediated hydroxylation occurred in both human liver and intestine microsomes, that is also consistent with previous study for another flavonoid galangin, whose glucuronidation was 30-fold more efficient than P450 mediated hydroxylation (12). Therefore, it could be concluded that the P450 mediated metabolism do not significantly contribute to the first-pass metabolisms of B.

CONCLUSIONS

The current study demonstrated an extensive first-pass glucuronidation of B in both liver and intestine. Under the current experimental conditions with the six UGT isoforms investigated, UGT 1A9 and UGT 1A8 demonstrated the fastest formation rate of BG in human liver and BG' in human intestine, respectively. Comparing with glucuronidation, sulfation was less extensive with relative higher affinity but lower capacity in human and rat liver. The P450 mediated hydroxylation of B was not significant in neither human liver nor intestine.

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