Pharmacokinetics of Baicalein, Baicalin and Wogonin after Oral Administration of a Standardized Extract of *Scutellaria baicalensis*, PF-2405 in Rats

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The pharmacokinetics of active components such as baicalein, wogonin and oroxylin A were evaluated after oral administration of a purified extract of *Scutellaria baicalensis* GEORGI (PF-2405) containing the high contents of baicalein, wogonin and oroxylin A to rats. Following oral administration of PF-2405 at 10, 20 and 40 mg/kg dose (equivalent to 4.5, 9.0 and 18 mg/kg baicalein), a major constituent baicalein and its active metabolite baicalin showed dose-linear pharmacokinetics as evidenced by unaltered dose-normalized AUC, dose-normalized \( C_{\text{max}} \) and \( \text{Ae}_{0-30h} \) values. Following oral administration of PF-2405 at three doses (equivalent to 0.4, 0.8 and 1.6 mg/kg wogonin), dose-normalized \( C_{\text{max}} \) and dose-normalized AUC were comparable between the 20 and 40 mg/kg PF2405 doses, but plasma concentrations of wogonin at 10 mg/kg of PF-2405 were not measurable as they were below limit of quantitation (LOQ; 18 pmol/mL). Following oral administration of PF-2405 at the three doses (equivalent to 1.5, 3.0 and 6.0 mg/kg oroxylin A), the concentrations of oroxylin A in plasma, urine and gastrointestinal samples were below the assay LOQ (18 pmol/mL). Significant differences in AUCs, \( \text{Ae}_{0-30h} \) and \( \text{GI}_{30h} \) values for baicalein and baicalin were observed after oral administration of pure baicalein (18 mg/kg) and PF-2405 (40 mg/kg). The increases in AUCs of baicalein and baicalin after oral administration of PF-2405 may have been due to the significant decrease in \( \text{GI}_{30h} \) values for baicalein.

Key words: Standardized extract of *Scutellaria baicalensis* (PF-2405), Pharmacokinetics, Baicalein, Baicalin, Wogonin, Rats

INTRODUCTION

The roots of *Scutellaria baicalensis* have been used in traditional oriental medicine for the treatment of various ailments including fevers, ulcers, inflammation and cancers. *Scutellaria baicalensis* contains a variety of flavones, phenylethanoids, amino acids, sterols and essential oils. Baicalein, baicalin, oroxylin A and wogonin are the main active components in *Scutellaria baicalensis*. These flavonoids possess antioxidant (Shieh et al., 2000; Gao et al., 1999), anti-HIV (Kitamura et al., 1998), anti-inflammatory (Chen et al., 2001), anti-tumor (Chan et al., 2000; Ikemoto et al., 2000), antigenotoxic (Lee et al., 2000), anxiolytic (Hui et al., 2002), and anti-hepatitis B virus (Huang et al., 2000) activity. A methanol extract of *Scutellaria baicalensis* root at a dose of 150 mg/kg inhibited fibrosis and lipid peroxidation in rat liver induced by bile duct ligation and scission (BDL) or carbon tetrachloride (Nan et al., 2002). Ye et al. (2004) reported the significant variation in chemical composition and biological activities of the commercial *Scutellaria baicalensis* extract.

A purified extract isolated from *Scutellaria baicalensis* GEORGI (PF-2405) was developed to enhance and control the contents of the active components such as baicalein, wogonin and oroxylin A (Sohn et al., 2006). PF-2405 inhibited glycochenodeoxycholate (GCDC)-induced apoptosis in freshly isolated hepatocytes, thereby demonstrating its protective effect on cholestatic liver diseases. PF-2405 inhibited the proliferation of rat hepatic stellate cells transformed by SV-40 (t-HSC/C1-6). Oral administration of PF-2405 at doses of 20 and 40 mg/kg inhibited BDL-
induced liver fibrosis (our unpublished data). Currently, PF-2405 is being developed as a hepatoprotective agent against cholestatic liver cirrhosis.

The absorption, metabolism and excretion studies of pure baicalein and baicalin (Abe et al., 1990; Akao et al., 2000; Lai et al., 2003a, b; Xing et al., 2005; Zhang et al., 2005; Tsai et al., 2002), or of wogonin (Chen et al., 2002) in rats and humans were evaluated. Several pharmacokinetic data of baicalein, baicalin, wogonin and/or oroxylin A after oral administration of Scutellaria radix or its medicinal preparations are available (Homma et al., 1997; Li et al., 1998a, 1998b; Muto et al., 1998; Lai et al., 2003b; Zuo et al., 2002, 2003).

A better understanding of the pharmacokinetics of herbal medicinal products will link data from pharmacological assays to clinical effects and also help in designing rational dosage regimens (Bhattaram et al., 2002). The purpose of this study was to characterize the pharmacokinetics of baicalein, wogonin and oroxylin A, the major constituents of PF-2405 and baicalin, a major active metabolite of baicalein, after oral administration of PF-2405 at various doses in rats and to compare the pharmacokinetics of equivalent baicalein between oral administration of pure baicalein and PF-2405 to rats.

**MATERIALS AND METHODS**

**Materials**

Baicalein, wogonin and oroxylin A were separated by our previous method (Lee et al., 2000) to purities of 99.0%. Baicalin was obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Methanol and ethyl acetate (HPLC grade) were obtained from Burdick & Jackson Inc. (Muskegon, MI, U.S.A.) and the other chemicals were of the highest quality available.

PF-2405 was prepared from Scutellaria baicalensis GEORGI by our previous method (Sohn et al., 2006). The contents of baicalein, oroxylin A and wogonin in PF-2405 were 45.0%, 15.0% and 4.0% by LC-MS/MS method, respectively, but baicalin was not detected in PF-2405.

**Animals**

Male Sprague-Dawley rats (8 week old, weighing 230-270 g) were purchased from Charles River Company Korea (Biogenomics, Seoul, Korea). Rats were housed in an air conditioned room at a temperature of 23 ± 2°C, with a relative humidity of 55 ± 10%, an illumination intensity of 150-300 lux, an air ventilation frequency of 15-20 times/h, and a 12 h illumination (07:00-19:00). Rats were fasted for 12 h before oral administration with the exception of free access to water. All procedures involving rat care were approved by the Wonkwang University Animal Care and Use Committee.

**Oral administration of PF-2405 or baicalein to rats**

The rats were cannulated with polyethylene tubing (PE-50, Clay Adams, Parsippany, NJ, U.S.A.) in the carotid artery under light ether anesthesia. Each rat was housed individually in a rat metabolic cage and allowed to recover from anesthesia for 1 day before the study began. The rats were not restrained at any time during the study. PF-2405 and baicalein were dissolved in a mixture of N,N-dimethylacetamide:PEG400:water (1:2:1, v/v). PF-2405 was administered at doses of 10 (n = 7), 20 (n = 9) and 40 (n = 11) mg/kg and baicalein was administered at 18 mg/kg (n = 8) by oral gavage in rats. Blood samples (150 mL) were collected at 0 (to serve as a control), 5, 15, 30, and 45 min, and at 1, 1.5, 2, 4, 6, 8, 10, 24 and 30 h after drug administration. Approximately 0.3 mL of heparinized 0.9% NaCl-injectable solution (20 units/mL) was used to flush the cannula immediately after each blood sampling to prevent blood clotting. After centrifugation of blood samples, 50 µL aliquots of plasma samples were collected and stored at -70°C. At the end of the experiment (30 h), the metabolic cage was rinsed with 10 mL distilled water, and the rinsed solutions were combined with the pooled urine samples collected for 30 h. After measuring the exact volume of the combined urine samples, two 50 µL aliquots of each sample were taken and kept at -70°C. At the end of experiment (30 h), each rat was sacrificed by cervical dislocation and the abdomen was opened. The entire gastrointestinal (GI) tract (including its contents and feces) was removed, transferred into a beaker containing 50 mL of methanol and homogenized. After centrifugation of the homogenate, two 50 µL aliquots of the supernatant were collected and stored at -70°C until drug analysis.

**LC-MS/MS analysis**

The concentrations (or amounts) of baicalein, baicalin, oroxylin A and wogonin were determined by our previous LC-MS/MS method (Kim et al., 2006). Plasma and urine samples (50 µL aliquots) were mixed with 10 µL of 10% ascorbic acid, 350 µL of 0.05 M hydrochloric acid, 5 µL of 2-(3,4-dimethoxy-phenyl)-5,7-dihydroxy-chromen-4-one (DPDC, internal standard) in acetonitrile solution and 1000 µL of ethyl acetate in 1.5 mL-polypropylene tubes. The mixtures were centrifuged at 13000 g for 5 min. The organic layer was pipette-transferred and evaporated to dryness using a vacuum concentrator. The residues were dissolved in 40 µL of 50% methanol by sonication for 3 min and centrifuged. The aliquots (10 µL) were injected into the LC-MS/MS system.

The chromatographic system consisted of a Nanospace SI-2 pump, a SI-2 autosampler and an S-MC system controller (Shiseido, Tokyo, Japan). The separation was performed on an Atlantis dC18 column (5 mm, 2.1 mm i.d.
×100 mm, Waters Co, Milford, MA, U.S.A.) using a mixture of methanol and 0.1% formic acid (60:40, v/v) at a flow rate of 0.2 mL/min. The column and autosampler were maintained at 50°C and 4°C, respectively. The analytical run time was 7.0 min. The eluent was introduced directly into the positive ionization electrospray source of a tandem quadrupole mass spectrometer (Quattro LC, Micromass U.K. Ltd.). The ion source and desolvation temperature were held at 120°C and 350°C, respectively. The optimum cone voltages were 35V for baicalein, 28V for baicalin, 33V for oroxylin A and wogonin, and 55V for DPDC. The molecular ions of baicalein, baicalin, oroxylin A, wogonin and DPDC were fragmented at collision energies of 31, 16, 25, 25 and 32 eV using argon as collision gas. The ions were detected by monitoring the transitions: m/z 271.2→123.3 for baicalein, m/z 447.3→271.1 for baicalin, m/z 285.3→270.1 for oroxylin A and wogonin, and m/z 315.0→299.0 for DPDC. Peak areas for all components were automatically integrated using MassLynx version 3.5 software (Micromass U.K. Ltd.).

Pharmacokinetic and statistical analysis

The plasma concentration vs. time data of baicalein and baicalin were analyzed by a non-compartmental method using the nonlinear least squares regression program WinNonlin (Scientific Consulting Inc., Cary, NC, U.S.A.). The area under the plasma concentration-time curve (AUC) was calculated using the trapezoidal rule. The peak plasma concentration (C_{max}) and the time to reach C_{max} (T_{max}) after oral administration were obtained directly from the experimental data.

All data were expressed as the mean ± standard deviation (SD). Three means for unpaired data were analyzed by the Duncan's multiple range test of the Social Package of Statistical Sciences (SPSS) posteriori analysis of variance (ANOVA). A p value of less than 0.05 was considered to be statistically significant.

RESULTS AND DISCUSSION

After oral administration of PF-2405 at doses of 10, 20 and 40 mg/kg (containing 4.5, 9.0 and 18.0 mg/kg baicalein) and pure baicalein at 18 mg/kg dose, the mean plasma concentration-time curves of baicalein and its major active metabolite, baicalin, are shown in Fig. 1; the relevant pharmacokinetic parameters of baicalein and baicalin are listed in Table I.

After oral administration of PF-2405 at 10, 20 and 40 mg/kg doses, baicalein and its active metabolite, baicalin, were detected at the first sampling time (5 min) (Fig. 1), supporting that absorbed baicalein is extensively metabolized to baicalin via glucuronidation in the rat intestine and liver (Zhang et al., 2005; Akao et al., 2000). The multiple peaks of baicalein and baicalin in plasma and the large inter-individual variability were also observed after oral administration of PF-2405 containing baicalein, supporting the enterohepatic recirculation of baicalein and baicalin (Abe et al., 1990; Akao et al., 2000; Xing et al., 2005).

The dose-normalized (based on 10 mg/kg of PF-2405) AUCs of baicalein were unaltered as a function of the doses and were 1050 ± 880, 427 ± 252 and 1152 ± 1157 pmol·h/mL at 10, 20 and 40 mg/kg PF-2405 doses, respectively. As expected from the AUC results, the dose-normalized C_{max} values of baicalein were also independent of oral doses studied and were 317 ± 311, 135 ± 105 and 1000 ± 1105 pmol·h/mL at 10, 20 and 40 mg/kg PF-2405 doses, respectively.
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Table I. Pharmacokinetic parameters of baicalein and its active metabolite, baicalin, after oral administration of PF-2405 at doses of 10, 20 and 40 mg/kg (equivalent to 4.5, 9.0 and 18.0 mg/kg of baicalein) and baicalein at 18 mg/kg dose to male SD rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Baicalein (10 mg/kg)</th>
<th>Baicalein (20 mg/kg)</th>
<th>Baicalein (40 mg/kg)</th>
<th>Baicalein (18 mg/kg)</th>
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<tr>
<td>Tmax (min)</td>
<td>187 ± 226</td>
<td>192 ± 236</td>
<td>214 ± 227</td>
<td>65.6 ± 120</td>
</tr>
<tr>
<td>Cmax (pmol/mL)</td>
<td>317 ± 311</td>
<td>270 ± 209</td>
<td>515 ± 278</td>
<td>374 ± 218</td>
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<td>AUC 0-30h (pmol h/mL)</td>
<td>1050 ± 880</td>
<td>855 ± 504</td>
<td>4606 ± 4630</td>
<td>548 ± 260</td>
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<td>Ae0-30h (% of dose)</td>
<td>26.1 ± 13.8</td>
<td>30.3 ± 9.9</td>
<td>20.7 ± 6.5</td>
<td>9.4 ± 8.0</td>
</tr>
<tr>
<td>GI30h (% of dose)</td>
<td>10.8 ± 4.1</td>
<td>27.4 ± 24.7</td>
<td>20.8 ± 6.5</td>
<td>53.0 ± 18.4</td>
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<table>
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<tr>
<th>Parameters</th>
<th>Baicalin (10 mg/kg)</th>
<th>Baicalin (20 mg/kg)</th>
<th>Baicalin (40 mg/kg)</th>
<th>Baicalin (18 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tmax (min)</td>
<td>7.9 ± 4.9</td>
<td>13.3 ± 7.9</td>
<td>15.0 ± 8.7</td>
<td>20.6 ± 7.8</td>
</tr>
<tr>
<td>Cmax (pmol/mL)</td>
<td>4217 ± 2899</td>
<td>6148 ± 2271</td>
<td>8841 ± 3154</td>
<td>6366 ± 3728</td>
</tr>
<tr>
<td>AUC 0-30h (pmol h/mL)</td>
<td>8627 ± 6405</td>
<td>20395 ± 3663</td>
<td>39875 ± 15395</td>
<td>21067 ± 10306</td>
</tr>
<tr>
<td>Ae0-30h (% of dose)</td>
<td>2.0 ± 2.3</td>
<td>0.7 ± 0.7</td>
<td>1.8 ± 3.1</td>
<td>3.4 ± 5.6</td>
</tr>
<tr>
<td>GI30h (% of dose)</td>
<td>0.8 ± 0.8</td>
<td>1.1 ± 1.8</td>
<td>0.6 ± 0.6</td>
<td>0.8 ± 0.6</td>
</tr>
</tbody>
</table>

Values expressed as mean ± SD.

*Significantly different from 10 mg/kg of PF-2405 (equivalent to 18 mg/kg baicalein) (p<0.05).

129 ± 69 pmol/mL at 10, 20 and 40 mg/kg of PF-2405, respectively (Table I). The percentages of the oral baicalein dose excreted in 30 h urine (Ae0-30h) as baicalein were 26.1, 30.3 and 20.7% for 10, 20 and 40 mg/kg PF-2405 doses, respectively. The percentages of the oral baicalein dose recovered from the entire GI tract at 30 h (GI30h) as baicalein were 10.8, 27.4 and 20.8% for 10, 20 and 40 mg/kg PF-2405 doses, respectively (Table I).

The dose-normalized (based on 10 mg/kg of PF-2405) AUCs of baicalin, an active metabolite of baicalein, were also independent of the three oral PF-2405 doses and were 8627 ± 6405, 10198 ± 1832, and 9969 ± 3849 pmol-h/mL for 10, 20 and 40 mg/kg PF-2405 doses, respectively (Table I). The dose-normalized (based on 10 mg/kg of PF-2405) Cmax values of baicalein were also comparable (i.e., not significantly different) among the three oral doses and were 4217 ± 2899, 6148 ± 2271, and 8841 ± 3154 pmol/mL for 10, 20 and 40 mg/kg PF-2405 doses, respectively (Table I). The dose-normalized (based on 10 mg/kg of PF-2405) Cmax values of baicalein were also comparable (i.e., not significantly different) among the three oral doses and were 4217 ± 2899, 6148 ± 2271, and 8841 ± 3154 pmol/mL for 10, 20 and 40 mg/kg PF-2405 doses, respectively (Table I). The GI30h and Ae0-30h values for baicalein were less than 1.1% and 2.0% for all three PF-2405 doses, respectively. These results indicate that the pharmacokinetic parameters of baicalein and its active metabolite, baicalin, were independent of the three oral PF-2405 doses studied.

After oral administration of pure baicalein at 18 mg/kg dosage, for baicalein and its metabolite, baicalin, the AUC values were 548 ± 260 and 21067 ± 10306 pmol-h/mL, while the Cmax values were 374 ± 214 and 6366 ± 3728 pmol/mL, respectively (Table I). The Ae0-30h and GI30h values after dosing with 18 mg/kg of pure baicalein were 9.4 ± 8.0 and 53.0 ± 18.4% for baicalein and were 3.4 ± 5.6% and 0.8 ± 0.6% for its metabolite, baicalin, respectively.

Significant differences in the pharmacokinetic parameters of baicalein and baicalin were observed between pure baicalein- and PF-2405-treated groups (Table I). The AUCs of baicalein and baicalin after dosing with 18 mg/kg of pure baicalein were significantly lower by 88% and 47%, respectively, than those after dosing with 40 mg/kg of PF-2405 (equivalent to 18 mg/kg of baicalein). The GI30h for baicalein after dosing with 18 mg/kg of pure baicalein was significantly higher by 155% than that obtained after dosing with 40 mg/kg of PF-2405. The Ae0-30h for baicalein after dosing with 18 mg/kg of pure baicalein was significantly lower by 55% than that after dosing with 40 mg/kg of PF-2405. These results suggest that the higher AUCs of baicalein and baicalin after oral administration of PF-2405 may have been due to increased baicalein absorption.

The mean plasma concentration-time curves of wogonin after oral administration of PF-2405 at the three doses (equivalent to 0.4, 0.8 and 1.6 mg/kg as the content of wogonin) are shown in Fig. 2 and the pharmacokinetic parameters are listed in Table II. Plasma concentrations of wogonin at 10 mg/kg PF-2405 dose were below the assay limit of quantitation (LOQ; 5 ng/mL). There were linear increases in Cmax (250 ± 136 vs. 394 ± 225 pmol/mL) and AUC (409 ± 140 vs. 973 ± 462 pmol-h/mL) of wogonin as the PF-2405 dose was increased from 20 to 40 mg/kg. Wogonin glucuronide, a metabolite of wogonin, was detected in the plasma but was not determined devoid of the authentic standard. The percentages of the oral wogonin dose recovered from the entire GI tract at 30 h (GI30h) as wogonin were 16.8 ± 12.8, 18.4 ± 13.3 and 9.3 ± 6.0% for 10, 20 and 40 mg/kg PF-2405 doses, respectively (Table I).
Fig. 2. Plasma concentration vs. time plots of wogonin after oral administration of PF-2405 at doses of 20 (\(\gamma\), \(n = 9\)) and 40 mg/kg (\(\delta\), \(n = 11\)) (equivalent to 0.8 and 1.6 mg/kg of wogonin) to male SD rats. Each point represents mean \(\pm\) SD.

Table II. Pharmacokinetic parameters of wogonin after oral administration of PF-2405 at doses of 10, 20 and 40 mg/kg (equivalent to 0.4, 0.8 and 1.6 mg/kg of wogonin) to male SD rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>10 mg/kg ((n = 7))</th>
<th>20 mg/kg ((n = 9))</th>
<th>40 mg/kg ((n = 11))</th>
</tr>
</thead>
<tbody>
<tr>
<td>(T_{\text{max}}) (min)</td>
<td>N.C.</td>
<td>13.3 (\pm) 7.9</td>
<td>51.8 (\pm) 72.9</td>
</tr>
<tr>
<td>(C_{\text{max}}) (pmol/mL)</td>
<td>N.C.</td>
<td>250 (\pm) 136</td>
<td>394 (\pm) 225</td>
</tr>
<tr>
<td>AUC (pmol.h/mL)</td>
<td>N.C.</td>
<td>409 (\pm) 140</td>
<td>973 (\pm) 462</td>
</tr>
<tr>
<td>(A_{\text{Eo-30h}}) (% of dose)</td>
<td>58.9 (\pm) 16.0</td>
<td>69.6 (\pm) 22.6</td>
<td>41.3 (\pm) 16.0</td>
</tr>
<tr>
<td>(G_{\text{I30h}}) (% of dose)</td>
<td>16.8 (\pm) 12.8</td>
<td>18.4 (\pm) 13.3</td>
<td>9.3 (\pm) 6.0</td>
</tr>
</tbody>
</table>

N.C.: not calculable

Values expressed as mean \(\pm\) SD.

dose-normalized (20 mg/kg) values were compared between PF-2405-treated groups by statistical analysis.

2). The percentages of the oral wogonin dose excreted in 30 h urine (\(A_{\text{Eo-30h}}\)) as wogonin were 58.9 \(\pm\) 16.0, 69.6 \(\pm\) 22.6 and 41.3 \(\pm\) 16.0% for 10, 20 and 40 mg/kg PF-2405 doses, respectively.

After oral administration of PF-2405 at 10, 20 and 40 mg/kg doses (containing 1.5, 3.0, and 6.0 mg/kg oroxylin A), the concentrations of oroxylin A in plasma, urine and GI samples were below the assay LOQ (18 pmol/mL). Its metabolite, oroxylin A glucuronide, was detected in plasma, urine and GI samples, but could not be quantified devoid of the authentic standard.

In summary, the dose-independent pharmacokinetics of baicalein and its active metabolite, baicalin, as well as wogonin were observed after oral administration of a purified extract isolated from *Scutellaria baicalensis* GEORGi (PF-2405) in rats over the dose range from 10 to 40 mg/kg. Significant differences in AUCs, \(A_{\text{Eo-30h}}\) and \(G_{\text{I30h}}\), values for baicalein and baicalin were observed between pure baicalein- and PF-2405-treated groups, indicating that the higher AUCs of baicalein and baicalin after oral administration of PF-2405, compared to the pure baicalein-treated group, may have been due to increased baicalein absorption.

ACKNOWLEDGEMENTS

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REFERENCES


Akao, T., Kawabata, K., Yanagisawa, E., Ishihara, K., Mizuhara, Y., Yakui, Y., Sakashita, Y., and Kobashi, K., 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).


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