http://dx.doi.org/10.5012/bkcs.2013.34.5.1559

Comparative Pharmacokinetics of Berberine After Oral Administration of Pure Berberine, Coptidis Rhizoma Extract, and Decoctions of Two Different Complex Herbal Formulas to Rats

Jianbo Chen,[†,‡] Xitao Yan,[†] Kyung Tae Kim,[†] Sang Hyuck Kim,[†] Eun-Sil Lee,[†] Young Keun Lee,[§] Chang Gyu Son,[#] Eun Hee Lee,[‡] Yong Ki Park,[¶] Cheong Weon Cho,[†] Young Ho Kim,[†] Youngjoo Byun,[‡,*] and Jong Seong Kang[†,*]

[†]*College of Pharmacy, Chungnam National University, Daejeon 305-764, Korea. [*]E-mail: kangjss@cnu.ac.kr*
[‡]*College of Pharmacy, Korea University, Chungnam 339-700, Korea. [*]E-mail: yjbyun1@korea.ac.kr*
[§]*Daejeon Health Sciences College, Daejeon 300-711, Korea*
[#]*Daejeon Oriental Hospital, Daejeon University, Daejeon 301-724, Korea*
[¶]*College of Oriental Medicine, Dongguk University, Gyeongju, Republic of Korea*
Received October 31, 2012, Accepted February 21, 2013

Key Words : Samgiumgagambang, Wen-Pi-Tang-Hab-Wu-Ling-San, Berberine, Pharmacokinetics, HPLC

The effects of traditional herbal medicines are usually attributed to synergism among the multiple herbs and constituents because the individual components interact with the other ingredients. Pharmacokinetic studies are useful in helping to explain and predict the various medical activities related to the efficacy and toxicity of drugs, as well as to evaluate the rationality and compatibility of herbs or herbal medications. Given the complex chemicals in herbal medications, one major active ingredient is generally selected as an indicative or marker compound and the interactions of ingredients in the herb or herbal medication are clarified based on the pharmacokinetic behavior of the selected compound.[1-3]

"Samgiumgagambang" (SGMX), a new herbal medication, was developed by altering the herbal composition of Samgium, and has been used at the Daejeon University Oriental Hospital since 2001 for treating cerebral vascular damage, hypertension, and hyperlipidemia.[4,5] The efficacy of SGMX has been evaluated clinically and experimentally.[6,7] Many recent reports have described new biological activities of certain SGMX constituents based on modern monitoring methods.[8-12] Wen-Pi-Tang-Hab-Wu-Ling-San (WHW), a decoction of 15 herbs, was developed by combining Wen-Pi-Tang and Wu-Ling-San. This decoction inhibits renal fibrosis in kidney cells[13] and has been shown to protect the kidney against ischemia/reperfusion injury in mice. Coptidis Rhizoma, a key herbal ingredient in both SGMX and WHW, has been widely used for centuries to treat dysentery, hypertension, inflammation, and liver diseases.[14,15] Berberine, an isoquinoline alkaloid that is the main active ingredient of Coptidis Rhizoma, has multiple bioactivities, including immunosuppressive,[16] *anti*-diarrheal,[17] *anti*-neoplastic,[18] *anti*-inflammatory,[19] *anti*-arrhythmic,[20] *anti*-proliferative,[21] and *anti*-biotic [22] properties. The pharmacokinetics of berberine in plasma, bile, and urine, as well as in the tissues of mice, rats, dogs, rabbits, and humans have been reported using UV spectrophotometry, fluorometry, tritium-labeled berberine, and high-performance liquid chromatography (HPLC)-MS/MS methods.[23-29] However, there are few reports on the comparative pharmacokinetics of pure berberine and berberine contained in single-herbs or complex herbal medications.

Here we successfully developed a simple HPLC method to determine the concentration of berberine in rat plasma after the administration of the SGMX, WHW, and single-herb extracts. The plasma concentration of berberine was determined separately after the oral administration of the SGMX, WHW, and Coptidis Rhizoma extracts, and pure berberine. The berberine peaks were clearly separated from the matrix and the mean concentration-time curve was plotted. The distribution processes of berberine after the oral administration to rats could be adapted into the one-compartment model. The mean concentration-time curves are shown in Figure 3 and the pharmacokinetic parameters are presented in Table 2. Calibration curves were generated by plotting chromatographic peak area as a function of marker compound concentration. The peak area of berberine in rat plasma showed a good linear relationship with the following regression lines: $y = 2.1x - 0.4$ in SGMX conditions, and $y = 3.5x - 0.7$ in WHW conditions (where y is peak area and x is the marker compound concentration in μg/mL). The LOQ value of berberine determined from the pooled plasma samples was 0.8 μg/mL. Table 1 shows that the intra- and inter-day variations as determined by duplicate analysis of the QC samples were less than 2.93% and 4.98%, respectively. The accuracy of the method ranged from 96.2% to 104.0%. The recovery of berberine in the QC samples (1.0, 5.0, and 20.0 μg/mL) ranged from 79.0% to 85.5%. Stability tests indicated that the analytes were stable in rat plasma at room temperature for 24 h under each freeze–thaw cycle as well as at –20 °C for 14 days (Tables 3 and 4). After oral administration of pure berberine alone, berberine was absorbed at a lower absorption rate with a T_{max} value of 60.8 min, and reached a maximum plasma concentration (C_{max}) value of 2.74 μg/mL. The plasma berberine concentration decreased to a $T_{1/2}$ value of 14.8 ± 2.1

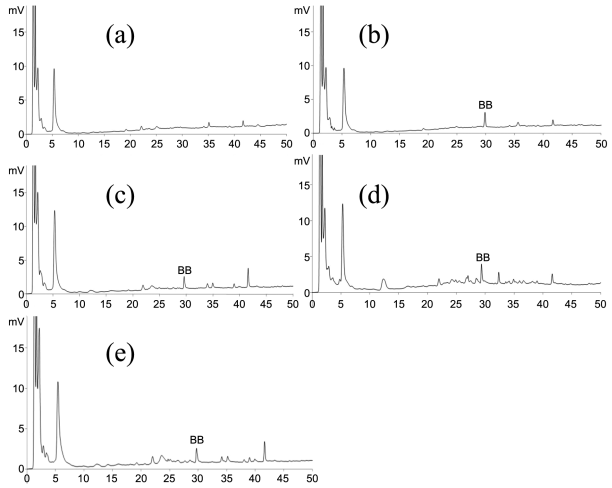

Figure 1. Representative HPLC-DAD chromatograms (a) blank plasma; (b) blank plasma spiked with berberine; (c) plasma sample obtained 60 min after oral administration of SGMX; (d) plasma sample obtained 60 min after oral administration of Coptidis Rhizoma extract; (e) plasma sample obtained 60 min after oral administration of pure berberine.

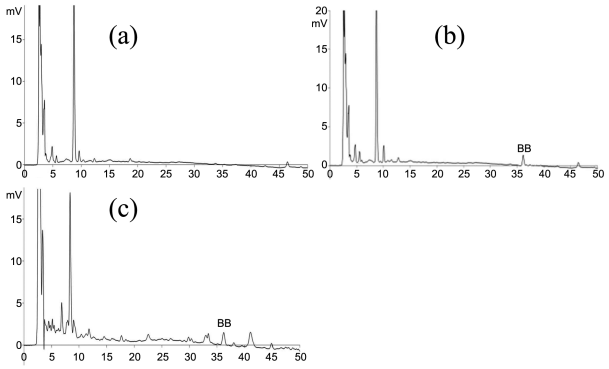

Figure 2. Representative HPLC-DAD chromatograms (a) blank plasma; (b) blank plasma spiked with berberine; (c) plasma sample obtained 60 min after oral administration of WHW.

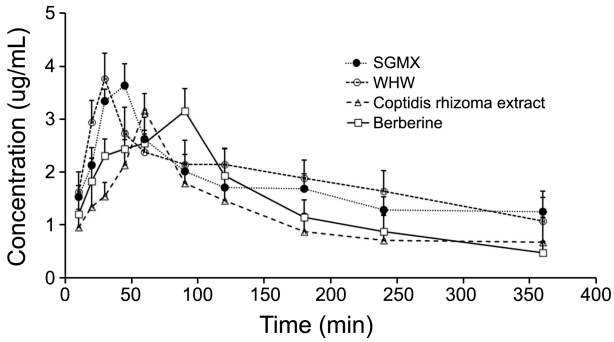

Figure 3. Plasma concentration-time curves of berberine in rat plasma after oral administration of WHW, SGMX, Coptidis Rhizoma extract, and pure berberine.

min. After the oral administration of WHW and SGMX, berberine was absorbed at a higher absorption rate with T_{max} values of 34.1 ± 5.2 and 42.2 ± 3.9 min, respectively, and reached similar C_{max} values of 2.9 ± 1.1 μg/mL and 2.8 ± 0.82 μg/mL, respectively. The C_{max} of berberine was not significantly different among the 3 different groups, indicating that all groups were administered the same amount of marker compounds. After the oral administration of WHW and SGMX, the AUC values of berberine were 893.9 μg·min/mL and 946.3 μg·min/mL, respectively. The AUC values of WHW and SGMX were higher than that of orally administrated pure berberine alone (537.4 μg·min/mL). The AUC value of berberine was significantly increased after the oral administration of the SGMX and WHW decoction extracts compared with the oral administration of pure berberine alone, indicating that a relatively larger amount of berberine was absorbed after the oral administration of SGMX and WHW. Regarding the pharmacokinetics of berberine, the AUC values of SGMX and WHW were increased approximated 39%-43% compared with that of pure berberine, indicating that the excretion of berberine in SGMX and WHW may be more retardant than in the Coptidis Rhizoma extract and pure berberine. In addition, a relatively short T_{max} was observed, suggesting the fast absorption of berberine after the oral administration of the SGMX and WHW extracts.

Although the mechanisms accounting for the different pharmacokinetic behaviors of berberine in the SGMX and WHW *versus* the Coptidis Rhizoma extracts are not clear, the present study is the first to report the differences in the pharmacokinetic parameters of berberine administered in SGMX, WHW, and single-herb extracts in rats. It is likely that potential interactions occur between berberine and the

Table 1. Intra- and inter-day precision and accuracy values for berberine in blank plasma

Comp. (μg/mL)	spiked	Intra-day (n=5)			Inter-day (n=5)		
		Measured (μg/mL)	Accuracy (%)	Precision (RSD%)	Measured (μg/mL)	Accuracy (%)	Precision (RSD%)
BB[a]	1.0	1.02	102.0	2.13	1.04	104.0	3.44
	5.0	4.89	97.8	1.72	4.81	96.2	2.16
	20	20.12	100.6	2.44	19.7	98.5	1.47
BB[b]	1.0	1.04	104.0	0.96	0.98	98.0	3.93
	5.0	4.98	99.6	2.93	5.12	102.4	4.86
	20	20.21	101.5	1.12	20.29	101.5	4.98

[a]HPLC condition for WHW. [b]HPLC condition for SGMX. BB: berberine

Table 2. Mean pharmacokinetic parameters of berberine in rat plasma

Parameter	BB	CR	WHW	SGMX
T_{max} (min)	60.77 ± 6.8	58.21 ± 9.9	34.13 ± 5.2*	42.21 ± 3.9**
C_{max} (μg/mL)	2.74 ± 1.4	2.23 ± 1.1	2.92 ± 1.1	2.89 ± 0.82*
AUC (μg min/mL)	537.4 ± 95.2	433.4 ± 33.2	946.3 ± 56.1**	893.9 ± 98.5**
$T_{1/2}$ (min)	14.77 ± 2.1	12.69 ± 5.9	6.75 ± 5.8*	9.55 ± 7.5*
CL/F	8.41 ± 7.4	10.42 ± 10.2*	4.76 ± 0.4**	5.66 ± 0.7**

BB: berberine, CR: Coptidis Rhizoma extract, *$P < 0.05$, **$P < 0.01$ compared with BB

other components in the formulations because this phenomenon was not observed in the single-herb extract. Possible compound-compound interactions should be studied to further elucidate the underlying mechanism of the pharmacokinetic differences. These differences in the pharmacokinetic properties observed between SGMX and WHW indicate the importance of investigating the pharmacological characteristics of herbal formulations for clinical applications.

Experimental

Preparation of the Samples and Standard Solutions. SGMX is an extract from a mixture of 14 natural products. The herbal mixtures were coarsely ground and then extracted with 2 L of boiling water for 3 h. The extracts were then filtered using a 2-layer mesh and concentrated under vacuum. Finally, the extract was recovered by freeze-drying. The same amount of Coptidis Rhizoma was extracted individually using the same method. All extracts were stored at 4 °C before use.

The stock solution of the berberine reference standard was prepared in methanol at a concentration of 1 mg/mL and stored at –20 °C. To construct calibration curves of berberine in rat plasma, berberine reference standard solutions were prepared at 6 different concentrations (1.0, 1.25, 2.5, 5.0, 10.0, and 20.0 μg/mL) by a serial dilution of the stock solution. High-, mid-, and low-level quality control (QC) samples containing 1.0, 5.0, and 20.0 μg/mL of the reference standard were also prepared in a manner similar to that used to prepare the reference standards.

Chromatography. The berberine in the 2 different herbal formulas and rat plasma was analyzed by HPLC with different separation conditions to achieve the best separation (Figures 1, 2). The berberine concentration in the extracts from SGMX and Coptidis Rhizoma, and plasma from rats administered with SGMX was determined using an HPLC system equipped with a C18 column (2.1 × 150 mm, 5 μm; Phenomenex, CA, USA) at 25 °C. The mobile phases consisted of 10% methanol in water containing 5% formic acid (A) and 90% methanol in water (B). The eluent was increased linearly from 0% to 40% B for the first 30 min, followed by up to 75% B from 30 to 60 min, and maintained at 75% B for 5 min with a flow rate of 0.4 mL/min.

The berberine concentration in WHW and plasma from rats administered with WHW was determined using a C_{18} column (4.6 × 250 mm, Optimapack, Korea). The mobile phases consisted of 5% methanol in water containing 5% acetic acid (A) and 95% methanol (B) with a flow rate of 1 mL/min. A gradient program was used as follows: from 0% to 25% B for the first 40 min, a linear increase up to 40% B from 40 to 80 min, a linear increase to 100% B, and then maintained at this level for a further 10 min. The eluent was monitored at 250 nm and UV spectra were recorded from 190 to 400 nm using a diode array detector. The separated peaks were identified by comparing retention times (R_t) with standard compounds using LC-MS spectra. To identify the marker compound, LC-MS analysis was performed using an LC-MS-2010EV system (Shimadzu, Japan) linked to an electrospray ionization source operating in both negative and positive modes.

Table 3. Stability of berberine in rat plasma under different conditions

Stability	Concentration		
	1.0 μg/mL	5.0 μg/mL	20.0 μg/mL
Short-term	101.1 ± 5.3	94.7 ± 4.1	98.5 ± 4.1
Long-term	93.2 ± 3.2	95.9 ± 3.3	104.6 ± 3.1
Free-thaw	99.3 ± 4.6	98.3 ± 3.4	96.2 ± 1.4
Post-preparative	103.4 ± 4.7	103.2 ± 2.5	103.4 ± 4.4

Data are represented as accuracy (mean ± S.D.)

Table 4. Recovery of berberine

Added (μg/mL)	Found (μg/mL) mean ± S.D.	Recovery (%)
1.0	0.79 ± 0.12	79.0
5.0	4.13 ± 1.45	82.6
20.0	17.11 ± 2.6	85.5

Pharmacokinetic Study. Animal experiments were performed following the approved procedure by the Animal Ethics Board of Chungnam National University. A total of 24 rats were randomly divided into 4 groups and a different extract was orally administered to each group by gavage with a syringe under the following conditions: WHW, 1.8 g/100 g body weight, containing 0.25% berberine; SGMX, 2.4 g/100 g body weight, containing 0.19% berberine; Coptidis Rhizoma extract, 0.15 g/100 g body weight, containing 3.1% berberine; and pure berberine (4.5 mg/100 g body weight). Blood samples (approximately 1 mL) were collected from the orbital vein at 0, 10, 20, 30, 45, 60, 90, 120, 180, 240, and 360 min after administration. The plasma was separated

by centrifugation (17,000 rpm for 10 min) at 4 °C, and 0.1 mL of plasma was then diluted with 0.4 mL of methanol. After 30 min, the mixture was centrifuged (17,000 rpm for 10 min) at 4 °C. The supernatant was dried under N_2 gas and the residue was dissolved in 0.1 mL of methanol and stored at 20 °C for analysis.

Acknowledgments. This work was supported by the Priority Research Centers Program through the National Research Foundation of Korea (NRF), funded by the Ministry of Education, Science, and Technology (2009-0093815). The publication cost of this article was supported by the Korean Chemical Society.

References

1. Yang, X. G.; Peng, B.; Zhang, G. H.; Wei, L.; Nie, S. F.; Pan, W. S. *J. Pharm. Biomed. Anal.* **2006**, *41*, 320.
2. Chen, L. C.; Chou, M. H.; Lin, M.; Yang, L. L. *Jpn. J. Pharmacol.* **2002**, *88*, 250.
3. Wang, C. H.; Wang, R.; Cheng, X. M.; He, Y. Q.; Wang, Z. T.; Wu, C.; Cao, J. *J. Ethnopharmacol.* **2008**, *117*, 467.
4. Kim, S. H.; Seo, S. H.; Choi, I. C.; Woo, Y. S.; Yu, Y. G.; Kim, J. Y.; Kim, J. H.; Hong, S. H. *Kor. J. Orient. Int. Med.* **2003**, *Autumn*, 42.
5. An, J. J.; Lim, S. M.; Cho, H. G.; Kim, M. S.; You, B. C.; Kim, Y. J.; Kim, Y. S.; Seol, I. C. *Kor. J. Orient. Int. Med.* **2002**, *23*, 604.
6. Jo, H. K.; Oh, Y. S.; Kim, Y. J.; Seol, I. C. *Kor. J. Orient. Pathol.* **2003**, *17*, 1092.
7. Lee, K. T.; Kim, Y. S.; Seol, I. C. *Kor. J. Orient. Med.* **2003**, *24*, 11.
8. Lee, H. C.; Kim, K. H. *J. Orient. Med.* **1999**, *4*, 30.
9. Luo, J. Y.; Tian, C. R.; Xu, J. G.; Sun, Y. *J. Enzym. Inhib. Med. Ch.* **2009**, *24*, 1280.
10. Kim, B. H.; Park, K. S.; Chang, I. M. *J. Med. Food* **2009**, *12*, 764.
11. Chen, X. P.; Tang, Q. C.; Chen, Y.; Wang, W. X.; Li, S. B. *Carbohydr. Polym.* **2010**, *79*, 409.
12. Kwon, T. H.; Jeong, S.; Kwon, J. W.; Kim, Y. C.; Jang, S. *Arch. Pharm. Res.* **2008**, *31*, 1172.
13. Lee, S. I.; Kim, H. J.; Baek, M. C.; Park, K. M.; Park, Y.; Yoon, C. H.; Boo, Y. C. *Phytother Res.* **2007**, *21*, 548.
14. Bova, S.; Padrini, R.; Goldman, W. F.; Berman, D. M.; Cargnelli, G. *J. Pharmacol. Exp. Ther.* **1992**, *261*, 318.
15. Chiou, W. F.; Yen, M. H.; Chen, C. F. *Eur. J. Pharmacol.* **1991**, *204*, 35.
16. Marinova, E. K.; Nikolova, D. B.; Popova, D. N.; Gallacher, G. B.; Ivanovska, N. D. *Immunopharmacol.* **2000**, *48*, 9.
17. Yamamoto, K.; Takase, H.; Abe, K.; Saito, Y.; Suzuki, A. *Nippo Yakurigaku Zasshi* **1993**, *101*, 169.
18. Kuo, C. L.; Chou, C. C.; Benjamin, Y. M. *Cancer Lett.* **1995**, *9*, 193.
19. Zhou, H.; Mineshita, S. *J. Pharmacol. Exp. Ther.* **2000**, *294*, 822.
20. Wang, Y. X.; Yao, X. J.; Tan, Y. H. J. *Cardiovasc. Pharmacol.* **1994**, *23*, 716.
21. Ko, W. H.; Yao, X. Q.; Lau, C. W.; Law, W. I.; Chen, Z. Y.; Kwok, W.; Ho, K.; Huang, Y. *Eur. J. Pharmacol.* **2000**, *399*, 187.
22. Mirska, I.; Kedzia, H.; Kowalewski, Z.; Kedzia, W. *Arch. Immunol. Ther. Exp.***1972**, *20*, 921.
23. Bhide, M. B.; Chavan, S. R.; Dutta, N. K. *Indian. J. Med. Res.* **1969**, *57*, 2128.
24. Chen, C. M.; Chang, H. C. *J. Chromatogr. B* **1995**, *665*, 117.
25. Ozaki, Y.; Suzuki, H.; Satake, M. *Yakugaku Zasshi* **1993**, *113*, 63.
26. Schein, F. T.; Hanna, C. *Arch. Int. Pharmacodyn. Ther.* **1960**, *124*, 317.
27. Sheng, M. P.; Sun, Q.; Wang, H. *Chin. Pharmacol. Bull.* **1993**, *9*, 6.
28. Tsai, P. L.; Tsai, T. H. *J. Chromatogr. A* **2002**, *961*, 125.
29. Cai, F.; Xu, W.; Wei, H.; Sun, L. N.; Gao, S. H.; Yang, Q.; Feng, J.; Zhang, F.; Chen, W. S. *J. Chromatogr. B* **2010**, *878*, 184.