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Steady-state pharmacokinetics and tissue distribution of anthraquinones of Rhei Rhizoma in rats

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ABSTRACT

Aim of the study: Rhei Rhizoma, the rhizome of *Rheum palmatum* L. (RP), is a popular herb in clinical Chinese medicine. RP is abundant in polyphenolic anthraquinones, which have been reported to show various beneficial bioactivities. This study investigated the pharmacokinetics and tissue distribution of anthraquinones following seven-dose administration of RP decoction to rats.
Materials and methods: Six Sprague-Dawley rats were given 2.0 g/kg of RP twice daily for seven doses and blood samples were collected at designated time after the 7th dose. Another six rats were sacrificed at 30 min after the 7th dose and organs including liver, kidney, lung and brain were collected. Serum and tissue specimens were assayed by HPLC before and after hydrolysis with β-glucuronidase and sulfatase, respectively.
Results: Pharmacokinetic analysis indicated that the anthraquinones in serum mainly presented as glucuronides/sulfates and contained higher ratio of sulfates when compared with single-dose administration of RP. Contrary to the finding in serum, tissue analysis discovered mainly free form of anthraquinone in most organs assayed, such as aloe-emodin and rhein in kidney, liver, lung; emodin in liver, lung; trace of chrysophanol in kidney and liver. In all brains, neither free forms nor their glucuronides/sulfates have been detected.
Conclusions: The glucuronides/sulfates of anthraquinones were the major forms in bloodstream, whereas the free forms of most anthraquinones were predominant in kidney and liver.

1. Introduction

Anthraquinones are a group of polyphenolic constituents in Rhei Rhizoma (the rhizome of *Rheum palmatum* L., RP), which is commonly used in clinical Chinese medicine as potent laxative, antibacterial, anti-inflammatory, anticancer, antispasmodic and choleretic agents (Shen and Li, 1997; Shia et al., 2011). The anthraquinones of RP include aloe-emodin, rhein, emodin, chrysophanol and physcion (chemical structures shown in Fig. 1) and their glycosides (Li, 2002). Numerous studies reported that these anthraquinones exhibited beneficial bioactivities such as anticancer (Shi et al., 2001; Pecere et al., 2003; Kuo et al., 2004; Guo et al., 2007; Huang et al., 2007), antivirus (Shuangsuo et al., 2006), antioxidation (Cai et al., 2004) and anti-diabetes (Zheng et al., 2008). Despite these positive results, it remains unclear whether those activities found *in vitro* can exist *in vivo*.

Our previous study documenting the pharmacokinetics of anthraquinones after single dosing of RP has pointed out that the free forms of aloe-emodin, emodin and chrysophanol were not detected in blood by HPLC/UV detection, whereas their glucuronides/sulfates were predominant. The only free form found in the circulation was rhein, which also presented mainly as its glucuronides/sulfates (Shia et al., 2009). Later, another our study reporting the tissue distribution of flavonoids of Scutellariae Radix (root of *Scutellariae baicalensis*) disclosed that the free forms of baicalein and wogonin were the major forms in many organs, in contradiction to the finding in blood (Hou et al., 2011). Therefore, in order to elucidate the real molecular forms and concentrations of rhubarb anthraquinones in the circulation and in various organs at the steady state, pharmacokinetic and tissue distribution studies following repeated dosing of RP decoction were conducted in rats.

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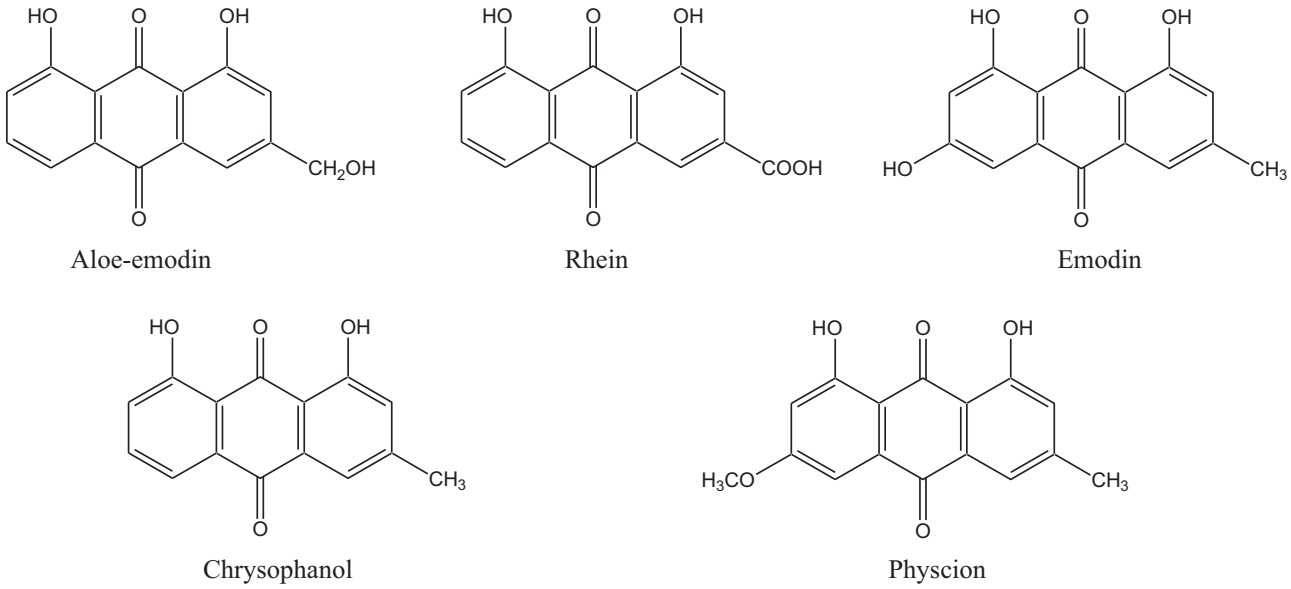

Fig. 1. Chemical structures of anthraquinones.

2. Materials and methods

2.1. Chemicals

Aloe-emodin (purity 95%), rhein (purity 95%), emodin (purity 90%), chrysophanol (purity 98%), physcion (purity 95%), β-glucuronidase (type B 1,666,400 units/g, from bovine liver), sulfatase (type H 1, 14,400 units/g, from *Helix pomatia*, containing 300,000 units/g of β-glucuronidase) and 2-methlylanthraquinone (purity 95%) were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Acetonitrile, methanol and ethyl acetate were LC grade and purchased from Mallinckrodt Baker, Inc. (Phillipsburg, NJ, U.S.A.). L(+)-Ascorbic acid was obtained from RdH Laborchemikalien GmbH & Co. KG (Seelze, Germany). Other reagents were HPLC grade or analytical reagent grade. Milli-Q plus water (Millipore, Bedford, MA, U.S.A.) was used throughout this study.

2.2. Instrumentation

The HPLC apparatus included a pump (LC-10AT, Shimadzu, Japan), an UV spectrophotometric detector (SPD-10AVP, Shimadzu, Japan), a fluorescence detector (RF-10A, Shimadzu, Japan) and chromatopac (C-R6A, Shimadzu, Japan) with an automatic injector (SIL-10A, Shimadzu, Japan) and an Alltech Apollo C18 column (5 μm, 4.6 mm × 150 mm, Alltech, Deerfield, IL, USA).

2.3. Preparation of RP decoction

The rhizomes of *Rheum palmatum* was purchased from a Chinese drugstore in Taichung, Taiwan. The origin of the crude drug was identified microscopically by the corresponding author Dr. Yu-Chi Hou. Water (5 l) was added to 250 g crude drugs and heating on a gas stove. After boiling, gentle heating was continued until the volume reduced to about 2.5 l and then the mixture was filtered while hot. The filtrate was gently boiled until the volume reduced to less than 500 ml and sufficient water was added to make 500 ml, which was immediately divided into aliquots and frozen at −20 °C for later use.

2.4. Quantitation of RP decoction and its acid hydrolysate

Rhubarb decoction (3.0 ml) was mixed with 7.0 ml of methanol and centrifuged to remove polysaccharide. Then the properly diluted rhubarb decoction (100 μl) was added with 100 μl of 2-methylanthraquinone solution (50.0 μg/ml in methanol) as internal standard and 20 μl were subjected to HPLC analysis. The mobile phase consisted of acetonitrile (A)–0.1% phosphoric acid (B) and programed in a gradient manner as follows: A/B: 50/50 (0–10 min), 85/15 (15–22 min) and 50/50 (27 min). The detection wavelength was set at 250 nm and the flow rate was 1.0 ml/min.

Acid hydrolysis of 1 ml of the decoction was conducted by adding 1 ml of 1.2 N HCl and the hydrolysis was conducted at 80 °C in a water bath for 6 h. The hydrolysate was then added with sufficient methanol to make 2.0 ml before HPLC analysis. The contents of glycosides of aloe-emodin, rhein, emodin, chrysophanol and physcion were determined by subtracting the concentrations of each aglycone in the decoction from those of correspondent aglycone in the decoction hydrolysate.

2.5. Animals

Male Sprague-Dawley rats were supplied by National Laboratory Animal Center (Taipei, Taiwan) and kept in Animal Center of Chinese Medical University. The animal study adhered to "The Guidebook for the Care and Use of Laboratory Animals (2002)" (Published by the Chinese Society for the Animal Science, Taiwan, ROC). The animal protocol was approved by Institutional Animal Care and Use Committee (IACUC) of China Medical University. Rats were housed in a 12-h light–dark cycle, constant temperature environment prior to study.

2.6. Drug administration, blood and tissue collection

For pharmacokinetic study, six rats weighing 390–440 g were fasted for 12 h before drug administration. After fasting, rats were administered orally with 2.0 g/kg of RP decoction twice daily for 7 doses via gastric gavage in order to reach steady-state. In the 4th day morning, blood samples (0.6 ml) were withdrawn from rats under anesthesia with isoflurane at 15, 30, 60, 120, 240, 480, 720 and 1440 min after the 7th dose via cardiopuncture. The blood samples were collected in microtubes and centrifuged at 10,000 × *g*

for 15 min to obtain the serum, which was stored at −20 °C until analysis.

For tissue distribution study, six rats weighing 350–450 g were administered RP decoction with the same dosage regimen described above in pharmacokinetic study. In the 4th day morning, rats were sacrificed at 30 min after the 7th dose and a blood sample was collected via cadiopuncture. Following immediate systemic perfusion with cold saline, organs including liver, lung, kidney and brain were removed, washed with cold saline, blotted dry with filter paper and then accurately weighed. The tissues were homogenized with cold saline (3 g/5 ml) and the homogenates were stored at −30 °C until analysis.

2.7. Quantitation of anthraquinones and their glucuronides/sulfates in serum and tissues

The determination of anthraquinone concentrations in serum and tissue homogenates followed a previous method with minor modification (Shia et al., 2009). For the assay of anthraquinone aglycones, 100 μl of serum or tissue homogenate was mixed with 50 μl of pH 5 acetate buffer and 50 μl of ascorbic acid (100 mg/ml). The mixture was acidified with 50 μl of 0.1 N HCl and partitioned with 250 μl of ethyl acetate (containing 1 μg/ml of 2-methylantraquinone as internal standard). The ethyl acetate layer was evaporated under N_2 gas to dryness and reconstituted with 50 μl of methanol, then 20 μl was subjected to HPLC analysis. For the quantitation of glucuronides and sulfates, 100 μl of serum or tissue was mixed with 50 μl of β-glucuronidase and sulfatase, respectively, in pH 5 acetate buffer (1000 units/ml) and 50 μl of ascorbic acid (100 mg/ml), then incubated at 37 °C for 10 min, which had been determined by a preliminary time study. After hydrolysis, the serum or tissue homogenate was acidified with 50 μl of 0.1 N HCl and the later processes followed those described above. The mobile phase consisted of acetonitrile (A) and 0.1% phosphoric acid (B), and a gradient elution program was run as follows: A/B: 50/50 (0–10 min), 85/15 (15 min), 80/20 (20 min) and 50/50 (25 min). For serum assay, the wavelength of UV detector was set at 250 nm. For tissue assay, fluorescence detection was performed with wavelengths of 435 and 515 nm for excitation and emission, respectively. The flow rate was 1.0 ml/min.

For calibrator preparation, 100 μl of serum or tissue homogenate each spiked with various concentrations of anthraquinones was added with 50 μl of pH 5 buffer. The later procedure was the same as that described above for serum sample. The calibration graph was plotted by linear regression of the peak area ratios (anthraquinones to internal standard) against concentrations of anthraquinones.

The system suitability was evaluated through intra-day and inter-day analysis of precision and accuracy. Recovery was assessed by comparing the peak area obtained from extracted sample spiked with standards to that obtained from unextracted standards in extracted sample matrix, which represents 100% recovery. The lower limit of quantitation (LLOQ) represents the lowest concentration of analysis in a sample that can be determined with acceptable precision and accuracy, whereas limit of detection (LOD) represents the lowest concentration of analysis in a sample that can be detected (with S/N > 3).

2.8. Data analysis

The peak serum or tissue concentration (C_{max}) was obtained from experimental observation. The pharmacokinetic parameters were analyzed by noncompartment model with the aid of the program WinNonlin (version 1.1 SCI software, Statistical Consulting, Inc., Apex, NC, U.S.A.). The area under the serum or tissue concentration–time curve (AUC_{0-t}) was calculated using trapezoidal rule to the last point. The concentrations of anthraquinone sulfates in tissue were obtained by subtracting the concentration of anthraquinone glucuronides from that of correspondent anthraquinone glucuronides/sulfates. Unpaired Student's *t*-test was used for statistical comparison taking $p < 0.05$ as significant.

Table 1
Contents (μg/ml) of aloe-emodin, rhein, emodin, chrysophanol and physcion in RP decoction before and after acid hydrolysis.

Constituents	Before hydrolysis	After hydrolysis
Aloe-emodin	119.5	613.6
Rhein	577.9	910.1
Emodin	114.4	552.6
Chrysophanol	90.5	471.1
Physcion	38.9	199.5

3. Results

3.1. Quantitation of anthraquinones in RP decoction

The quantitation of anthraquinones in RP decoction was performed by HPLC analysis before and after acid hydrolysis. The concentrations of each anthraquinone in the RP decoction before and after acid hydrolysis were shown in Table 1. The most abundant anthraquinone in RP decoction was rhein and the least was physcion either before or after acid hydrolysis. Aloe-emodin, emodin, chrysophanol and physcion were predominantly present in glycoside forms, whereas rhein mainly existed in free form.

3.2. Quantitation of anthraquinones and their glucuronides/sulfates in serum and tissues

The quantitation of anthraquinones and their glucuronides/sulfates in serum essentially employed a method published previously (Shia et al., 2009). The serum analysis revealed that rhein free form was present in all specimens, and emodin free form existed only transiently in the early phase after dosing, whereas the free forms of aloe-emodin, chrysophanol and physcion were not detected. The glucuronides/sulfates of aloe-emodin, rhein, emodin and chrysophanol predominantly existed in bloodstream, while no trace of physcion glucuronides/sulfates had been detected. The mean serum concentration–time profiles of each anthraquinone after the 7th dose of RP decoction in six rats are shown in Fig. 2. All profiles revealed a second peak, a clear indication of enterohepatic circulation for these anthraquinones. The pharmacokinetic parameters of rhein free form, glucuronides/sulfates (G/S) and glucuronides (G) of each anthraquinone are listed in Table 2. The systemic exposures (area under the concentration–time curve, AUC) of anthraquinones ranked as follows: rhein G/S > rhein > emodin G/S > aloe-emodin G/S > chrysophanol G/S.

The assay method of tissue homogenates was established and validated in this study. The calibration ranges of aloe-emodin (0.04–4.0 μg/ml), rhein (0.01–20.0 μg/ml), emodin and chrysophanol (0.01–2.0 μg/ml) in various tissue homogenates were with good linearities. The method validation indicated that all coefficients of variation in intra-run and inter-run analysis of various tissue homogenates were below 9.9%, and the relative errors were below 19.2%. For liver homogenate, the LLOQs and LODs were 0.04 and 0.01 μg/ml for aloe-emodin and chrysophanol, 0.08 and 0.02 μg/ml for rhein, 0.06 and 0.01 μg/ml for emodin, respectively. For kidney homogenate, the LLOQs and LODs were 0.01 and 0.006 μg/ml for aloe-emodin, 0.06 and 0.04 μg/ml for rhein, 0.08 and 0.005 μg/ml for emodin, 0.06 and 0.01 μg/ml for chrysophanol. For lung homogenate, the LLOQs and LODs were 0.008 and

Table 2
Pharmacokinetic parameters of anthraquinones and their glucuronides/sulfates (G/S) and glucuronide (G) after 7th dose of RP (2 g/kg) in six rats.

Constituents	Metabolites	C_{max} (nmol/ml)	AUC_{0-720} (nmol min/ml)	MRT_{0-720} (min)
Aloe-emodin	G/S	2.1 ± 0.3	288.0 ± 63.4	147.5 ± 19.4
	G	1.5 ± 0.2	151.7 ± 30.9	105.5 ± 20.1
Rhein	Free form	5.8 ± 0.7	1250.2 ± 167.0	243.1 ± 23.2
	G/S	17.1 ± 1.7	3209.4 ± 398.0	236.8 ± 13.1
	G	9.3 ± 1.3	1725.1 ± 294.3	215.5 ± 28.2
Emodin	G/S	3.7 ± 0.3	717.0 ± 62.8	235.9 ± 15.2
	G	2.1 ± 0.3	391.5 ± 70.7	203.6 ± 24.1
Chrysophanol	G/S	1.8 ± 0.2	161.6 ± 47.6	93.5 ± 22.4
	G	1.4 ± 0.2	117.6 ± 27.6	136.5 ± 44.2

C_{max}, the maximum concentration (nmol/ml).
AUC_{0-t}, the area under concentration–time curve to the last time (nmol min/ml).
MRT_{0-t}, the mean residence time (min).
Data expressed as mean ± S.E.

0.002 μg/ml for aloe-emodin, 0.04 and 0.01 μg/ml for rhein, 0.006 and 0.002 μg/ml for emodin, 0.004 and 0.001 μg/ml for chrysophanol, respectively. The recoveries of anthraquinones from liver, kidney and lung were 68.6–84.2%, 78.0–111.1% and 76.9–109.4%, respectively, at tested concentrations.

Tissue analysis had detected the free forms of aloe-emodin, rhein, emodin and chrysophanol in many organs as shown in Fig. 3. The tissue contents of each anthraquinone free form were ranked as follows: kidney, liver > lung for aloe-emodin; kidney > liver > lung for rhein; liver > lung for emodin; only traces of chrysophanol in kidney and liver. Among the assayed organs, the concentration of rhein free form in kidney was the highest (24 μg/g) and even much higher than serum concentration (14 μg/ml). With regard to the concentrations of glucuronides/sulfates, no traces of them were found in liver. Only traces of S of aloe-emodin, rhein, emodin were found in lung. Both G and S of aloe-emodin, rhein, emodin and chrysophanol were present in kidney, but in much lower concentrations than their free forms. Neither physcion nor its G/S had been detected in all tissues assayed. In addition, the free forms and the G/S of each anthraquinone were not detected in all brains (Fig. 4).

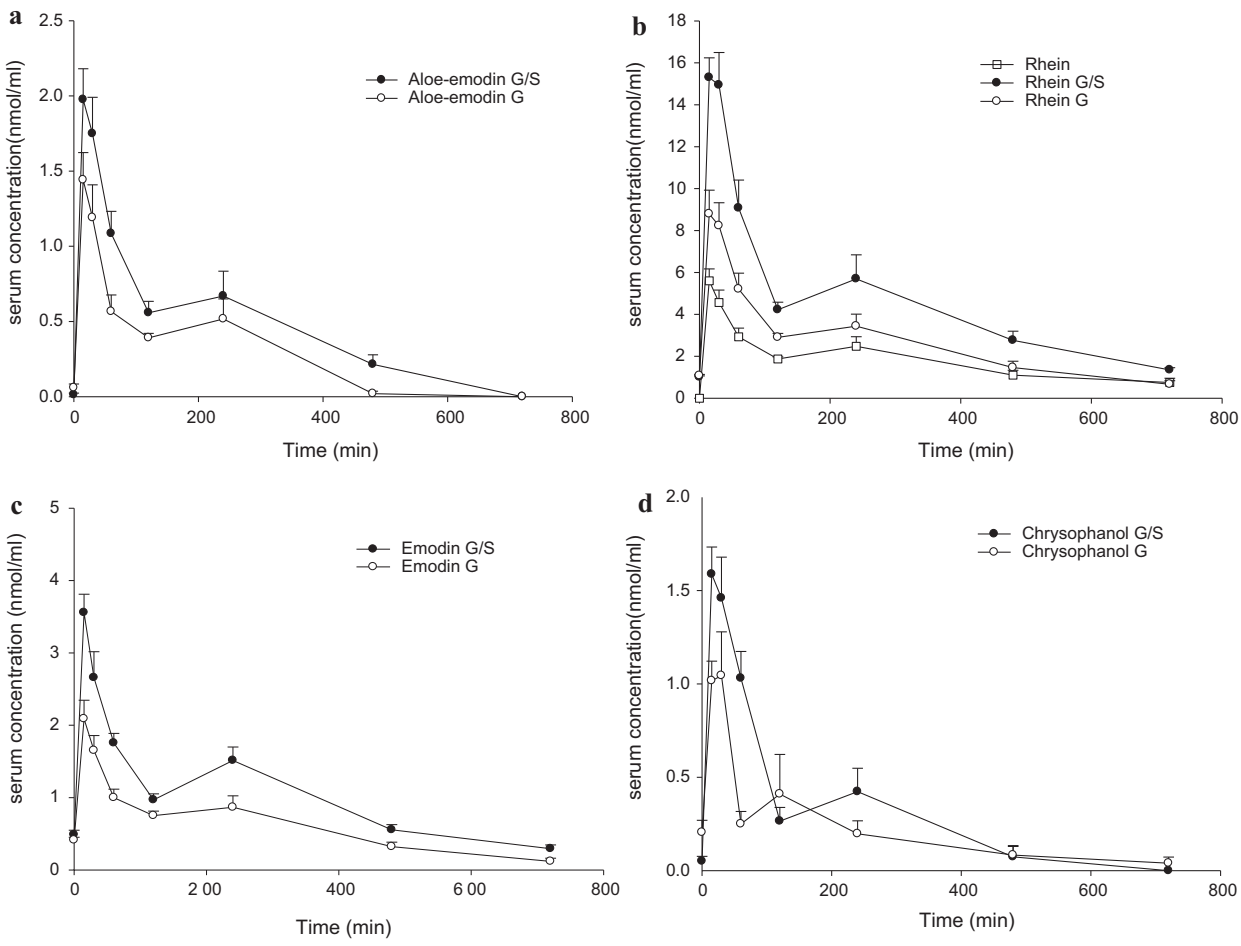

Fig. 2. Mean (±S.E.) serum concentration–time profiles of anthraquinones and their glucuronides/sulfates (G/S) and glucuronides (G) after the 7th dose of RP (2 g/kg) in six rats. (a) Aloe-emodin, (b) rhein, (c) emodin, and (d) chrysophanol.

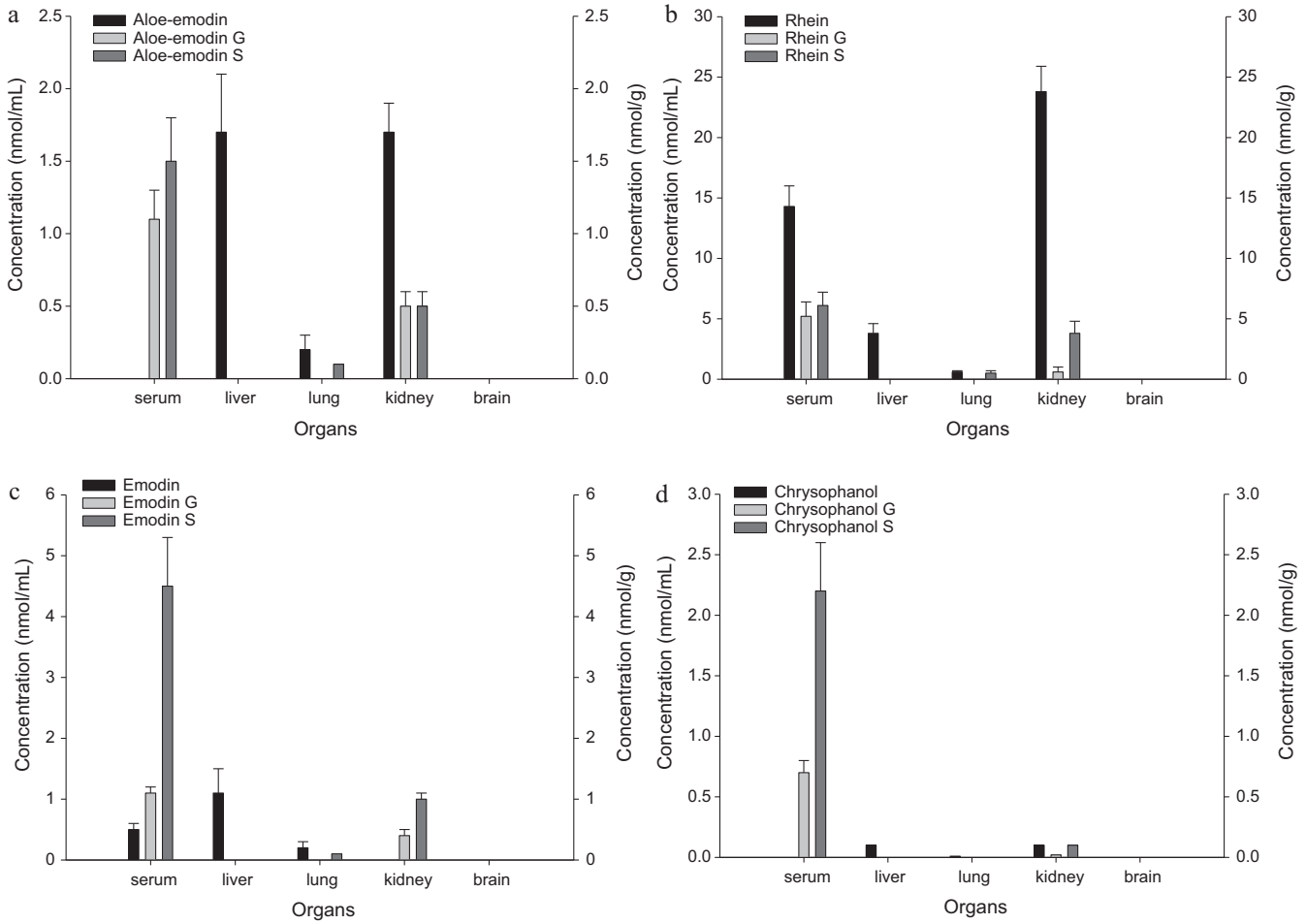

Fig. 3. Concentrations of anthraquinones and their sulfates (S) and glucuronide (G) in serum (nmol/ml) and various tissues (nmol/g) after the 7th dose of RP (2 g/kg) in six rats. (a) Aloe-emodin, (b) rhein, (c) emodin, and (d) chrysophanol.

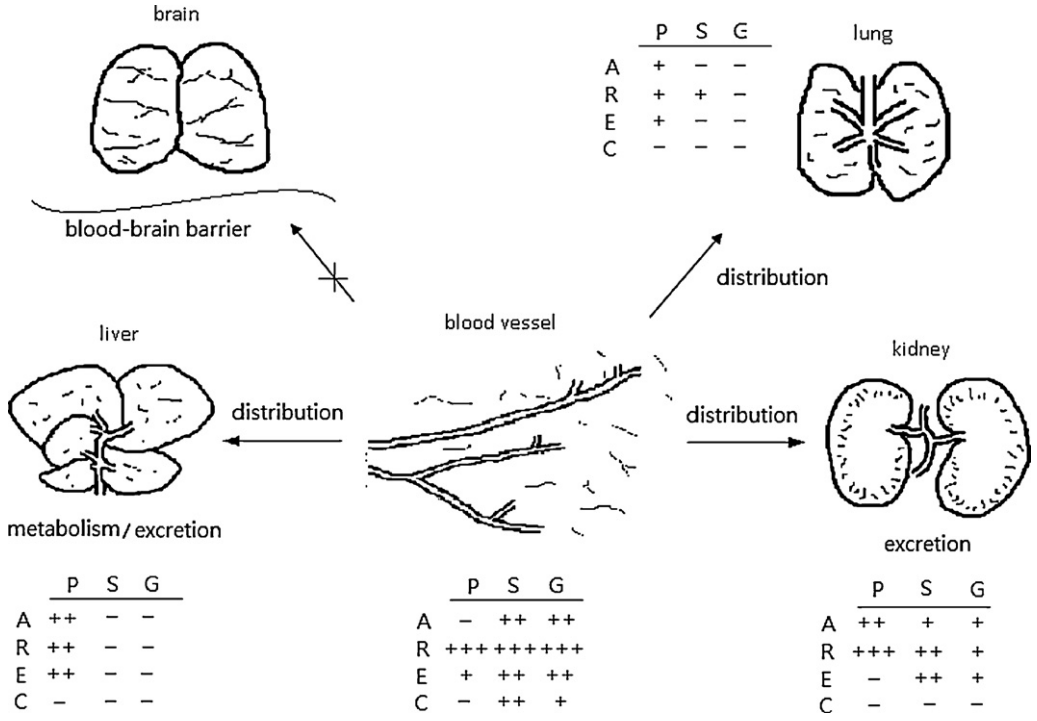

Fig. 4. Tissue distributions of anthraquinones after the 7th dose of RP in rats (P, parent form; S, sulfates; G, glucuronides; A, aloe-emodin; R, rhein; E, emodin; and C, chrysophanol).

4. Discussion

In order to reach the steady state of pharmacokinetics, a total of seven doses of RP decoction have been administered twice daily prior to the pharmacokinetic study (Shargel et al., 2005). For the purpose to have enough blood to define the blood profiles of the G/S and G of various anthraquinones, we have collected 18.8% of the circulating blood within 24 h from each rat, which was below the maximum blood sample volumes (20%) allowed for multiple sampling in rats (Diehl et al., 2001). Quantitation results indicated that the major molecules found in the bloodstream were the glucuronids/sulfates of aloe-emodin, rhein, emodin and chrysophanol. Like the result of our previous single-dose study, the free form of rhein was still present in the steady-state, which could be explained by the chemical feature of rhein possessing a functionality of carboxylic acid with low affinity toward glucuronyl-transferase (Tukey and Strassburg, 2000). The systemic exposure of rhein free form was 39.0% of rhein G/S. The absence of the free forms of aloe-emodin, emodin and chrysophanol in bloodstream suggested their feasibility for metabolism during the first pass. In regard to the null bioavailability of physcion within 6 h after dosing, this phenomenon might be arisen from its low dose and high lipophilicity which had been observed from the highest retention time than other anthraquinones in reversed-phase HPLC, which probably led to poor dissolution and the resultant negligible absorption. Besides, extensive demethylation of physcion may also explain the null bioavailability of physcion (Song et al., 2009).

The total AUC of rhein free form with its G/S was 14.5-, 6.2- and 27.6-folds of aloe-emodin G/S, emodin G/S and chrysophanol G/S, respectively, indicating that rhein G/S and rhein were major molecules, followed by emodin G/S. However, our quantitaion of RP decoction used in this study showed that the content of rhein with its glycosides was only 1–2 folds of aloe-emodin, emodin and chrysophanol with their glycosides. The unusual higher bioavailability of rhein may be accounted for by the presence of many prodrugs of rhien coexisting in RP, such as sennoside A and B, aloe-emodin and chrysophanol, which could be metabolized to rhein and rhein G/S (Mueller et al., 1998; Song et al., 2009). When AUC_{0-720}/dose of aloe-emodin G/S, emodin G/S and chrysophanol G/S were calculated (data not shown), the comparison among them indicated that the bioavailabilities of aloe-emodin and chrysophanol were much lower than that of emodin. An explanation for this is that aloe-emodin and chrysophanol may be metabolized to rhein and rhein G/S (Mueller et al., 1998; Song et al., 2009), which resulted in lower systemic exposure of aloe-emodin G/S and chrysophanol G/S.

Owing to considerable amount of glucuronidase in the sulfatase (type H-1) used in this study, treatment with sulfatase resulted in the hydrolysis of both sulfates and glucuronides. The profiles of G/S of each anthraquinone were higher than correspondent profile of G as shown in Fig. 2, indicating that their sulfates are present in the circulation. Through subtracting the AUC_{0-720} of G from that of correspondent G/S, the AUC_{0-720} of S of each anthraquinone can be estimated. The ratios of AUC of anthraquinone S to those of correspondent G/S following seven-dose administration were compared with those following single dose (Shia et al., 2009) and indicated that the ratios of S of aloe-emodin, rhein and emodin after seven-dose administration of RP were increased by 744%, 144% and 460%. This phenomenon may stem from the saturated glucuronidation following multiple-dose administration like diflunisal (Verbeeck et al., 1990).

In regard to tissue analysis, rats were sacrificed near the serum peak time of G/S of various anthraquinones based on our pharmacokinetic data. The quantitation methods of anthraquinones in various tissue homogenates by HPLC-fluorescence detection were established in this study (Yan and Ma, 2007; He et al., 2009). Validation of the methods showed that the precision and accuracy of intra-run and inter-run analyses as well as the recoveries were satisfactory. The lower limits of quantification (LOQs) for anthraquinones were comparable to those reported by LC/MS method (Fuh and Lin, 2003), while LC/MS/MS methods offered higher sensitivity (Zan et al., 2011). The emergence of aloe-emodin free form in liver, kidney, lung and chrysophanol free form in liver, kidney should have been biotransformed from their G/S in the circulation, which is probably due to the presence of deconjugation enzymes such as glucuronidase and sulfatase on the cell membranes of these organs (O'Leary et al., 2001; Pasqualini and Chetrite, 2005; Reed et al., 2005). The distribution of free forms of aloe-emodin, rhein and chrysophanol was highest in kidney, followed by liver and the least in lung. All these findings point to the likelihood that the deconjugation fate of anthraquinone G/S varied from organ to organ. The significantly lower concentrations of anthraquinone G/S in kidney, liver and lung than those in serum implied that they were not permeable between vascular compartment and these organs. Neither free forms nor the G/S of various anthraquinones were detected in all brains of six rats, suggesting that these anthraquinones could not cross the blood-brain barrier after administration of RP decoction.

Thus far there have been numerous *in vitro* assays investigating the bioactivities of commercially available polyphenols. For instance, various beneficial activities of rhein, aloe-emodin, emodin and chrysophanol have been reported (Shi et al., 2001; Pecere et al., 2003; Sanchez et al., 2003; Jing et al., 2006; Park et al., 2009; Lu et al., 2010). Based on our pharmacokinetic study showing that G/S of aloe-emodin, rhein, emodin and chrysophanol were the major forms in the blood, the *in vitro* bioactivities of anthraquinone free forms, except rhein, might not properly predict the effects in vascular system. On the other hand, our tissue analysis showing that the free forms of aloe-emodin, rhein, emodin and chrysophanol appeared as the major molecules in kidney and/or liver, suggesting that the *in vitro* bioactivities of them might predict *in vivo* effects in kidney and/or liver. However, the concentrations of anthraquinone free forms being used by *in vitro* investigators were too high to be achievable *in vivo* to exert pharmacological activity (Shi et al., 2001; Pecere et al., 2003; Cai et al., 2004; Kuo et al., 2004; Shuangsuo et al., 2006; Guo et al., 2007; Huang et al., 2007). Therefore, we suggest it is not appropriate to predict the *in vivo* effects of anthraquinones based merely on the previously published *in vitro* bioactivities.

In conclusion, anthraquinone G/S were the major forms in bloodstream, whereas the free forms of anthraquinones were mostly predominant in kidney and liver following repeated dosing of RP decoction to rats.

Acknowledgements

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References

Cai, Y., et al., 2004. Antioxidant phenolic constituents in roots of *Rheum officinale* and *Rubia cordifolia*: structure–radical scavenging activity relationships. Journal of Agricultural and Food Chemistry 52, 7884–7890.

Diehl, K.H., et al., 2001. A good practice guide to the administration of substances and removal of blood, including routes and volumes. Journal of Applied Toxicology 21, 15–23.

Fuh, M.R.S, Lin, H.J., 2003. Analysis of rhubarb by liquid chromatography–electrospray-mass spectrometry. Tamkang Journal of Science and Engineering 6, 31–36.

Guo, J., et al., 2007. Anticancer effect of aloe-emodin on cervical cancer cells involves G2/M arrest and induction of differentiation. Acta Pharmacologica Sinica 28, 1991–1995.
He, D., et al., 2009. Simultaneous determination of five anthraquinones in medicinal plants and pharmaceutical preparations by HPLC with fluorescence detection. Journal of Pharmaceutical and Biomedical Analysis 49, 1123–1127.
Hou, Y.C., et al., 2011. Flavonoid pharmacokinetics and tissue distribution after repeated dosing of the roots of *Scutellaria baicalensis* in rats. Planta Medica 77, 455–460.
Huang, Q., et al., 2007. Anti-cancer properties of anthraquinones from rhubarb. Medicinal Research Reviews 27, 609–630.
Jing, Y., et al., 2006. Alteration of subcellular redox equilibrium and the consequent oxidative modification of nuclear factor [kappa] B are critical for anticancer cytotoxicity by emodin, a reactive oxygen species-producing agent. Free Radical Biology and Medicine 40, 2183–2197.
Kuo, P.L., et al., 2004. Rhein inhibits the growth and induces the apoptosis of Hep G2 cells. Planta Medica 70, 12–16.
Li, T.S.C., 2002. Chinese and Related North American Herbs: Phytopharmacology and Therapeutic Values. CRC Press.
Lu, C.C., et al., 2010. Chrysophanol induces necrosis through the production of ROS and alteration of ATP levels in J5 human liver cancer cells. Molecular Nutrition & Food Research 54, 967–976.
Mueller, S.O., et al., 1998. Biotransformation of the anthraquinones emodin and chrysophanol by cytochrome P450 enzymes. Drug Metabolism and Disposition 26, 540–546.
O'Leary, K.A., et al., 2001. Flavonoid glucuronides are substrates for human liver [beta]-glucuronidase. FEBS Letters 503, 103–106.
Park, M.Y., et al., 2009. Evaluation of aloin and aloe-emodin as anti-inflammatory agents in aloe by using murine macrophages. Bioscience, Biotechnology, and Biochemistry 73, 828–832.
Pasqualini, J.R., Chetrite, G.S., 2005. Recent insight on the control of enzymes involved in estrogen formation and transformation in human breast cancer. The Journal of Steroid Biochemistry and Molecular Biology 93, 221–236.
Pecere, T., et al., 2003. Involvement of p53 in specific anti-neuroectodermal tumor activity of aloe-emodin. International Journal of Cancer 106, 836–847.
Reed, M.J., et al., 2005. Steroid sulfatase: molecular biology, regulation, and inhibition. Endocrine Reviews 26, 171.
Sanchez, C., et al., 2003. Effects of rhein on human articular chondrocytes in alginate beads. Biochemical Pharmacology 65, 377–388.
Shargel, L., et al., 2005. Applied Biopharmaceutics and Pharmacokinetics, 5th ed. McGraw-Hill, New York, p. 110.
Shen, Y.J., Li, Y.K., 1997. Pharmacology of Traditional Chinese Medicines. Shanghai Science and Technology Publishing House, Shanghai.
Shi, Y.Q., et al., 2001. Cytotoxic and DNA damage-inducing activities of low molecular weight phenols from rhubarb. Anticancer Research 21, 2847–2853.
Shia, C.S., et al., 2009. Metabolism and pharmacokinetics of anthraquinones in *Rheum palmatum* in rats and ex vivo antioxidant activity. Planta Medica 75, 1386–1392.
Shia, C.S., et al., 2011. Suppression on metastasis by rhubarb through modulation on MMP-2 and u-PA in human A549 lung adenocarcinoma: an ex-vivo approach. Journal of Ethnopharmacology 133, 426–433.
Shuangsuo, D., et al., 2006. Inhibition of the replication of hepatitis B virus in vitro by emodin. Medical Science Monitor: International Medical Journal of Experimental and Clinical Research. 12, 302–306.
Song, R., et al., 2009. Profiling the metabolic differences of anthraquinone derivatives using liquid chromatography/tandem mass spectrometry with data-dependent acquisition. Rapid Communications in Mass Spectrometry 23, 537–547.
Tukey, R.H., Strassburg, C.P., 2000. Human UDP-glucuronosyltransferases: metabolism, expression, and disease. Annual Review of Pharmacology and Toxicology 40, 581–616.
Verbeeck, R.K., et al., 1990. The effect of multiple dosage on the kinetics of glucuronidation and sulphation of diflunisal in man. British Journal of Clinical Pharmacology 29, 381–389.
Yan, D., Ma, Y., 2007. Simultaneous quantification of five anthraquinones in rat plasma by high-performance liquid chromatography with fluorescence detection. Biomedical Chromatography 21, 502–507.
Zan, B., et al., 2011. Simultaneous quantification of multiple active components from Xiexin decoction in rat plasma by LC–ESI-MS/MS: application in pharmacokinetics. Biomedical Chromatography 25, 816–826.
Zheng, J.M., et al., 2008. Rhein reverses the diabetic phenotype of mesangial cells over-expressing the glucose transporter (GLUT1) by inhibiting the hexosamine pathway. British Journal of Pharmacology 153, 1456–1464.