Absorption and Disposition of Ginsenosides after Oral Administration of Panax notoginseng Extract to Rats

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ABSTRACT:

Panax notoginseng (Sanqi) is a cardiovascular herb containing ginsenosides that are believed to be responsible for the therapeutic effects of Sanqi. The aim of this study was to evaluate rat exposure to ginsenosides after oral administration of Sanqi extract and to identify the key factors affecting their absorption and disposition. Ginsenosides were administered to rats, either in the form of Sanqi extract or as pure chemicals. The ginsenosides Ra3, Rb1, Rd, Re, Rg1, and notoginsoside R1 were the major saponins present in the herbal extract. Systemic exposure to ginsenosides Ra3, Rb1, and Rd after oral administration of the extract was significantly greater than that of the other compounds. Considerable colonic deglycosylation of the ginsenosides occurred, but the plasma levels of deglycosylated metabolites were low in rats. Poor membrane permeability and active biliary excretion are the two primary factors limiting systemic exposure to most ginsenosides and their deglycosylated metabolites. In contrast with other ginsenosides, biliary excretion of ginsenosides Ra3 and Rb1 was passive. Meanwhile, the active biliary excretion of ginsenoside Rd was significantly slower than that of other saponins. Slow biliary excretion, inefficient metabolism, and slow renal excretion resulted in long-circulating and thus relatively high exposure levels for these three ginsenosides. For these reasons, plasma ginsenosides Ra3, Rb1, and Rd were identified as pharmacokinetic markers for indicating rat systemic exposure to Sanqi extract. This is a systematic investigation of the absorption and disposition of ginsenosides from an herb, the information gained from which is important for linking Sanqi administration to its medicinal effects.

Herbs have been used for medicinal purposes in China for millennia, and traditional Chinese medicine still plays an important role in Chinese health care. The dried root of Panax notoginseng (family Araliaceae) is an important Chinese medicinal herb known as Sanqi. In traditional Chinese medicine, Sanqi is indicated for analgesia and hemostasis (Chinese Pharmacopoeia Commission, 2005). The herb is also used to treat patients with angina and coronary artery disease (Mashour et al., 1998); the pharmacological mechanism may be that it functions as a selective calcium ion antagonist that may interact with the receptor-operated calcium ion channel (Kwan, 1995). Sanqi also exerts protective effects on the cardiovascular system (Ng, 2006). Most of the bioactivities of Sanqi are believed to be associated with triterpene saponins derived mainly from the tetracyclic dammarane. These compounds, known as ginsenosides, can be classified according to their structures as 20(S)-protopanaxadiol type (ppd-type) and 20(S)-protopanaxatriol type (ppt-type). The ppd-type ginsenosides possess sugar moieties at the C-3 and/or C-20 positions, whereas the ppt-type ginsenosides have a hydroxyl group at C-3 and sugar moieties at C-6 and/or C-20. The major saponins present in Sanqi include the ppd-type ginsenosides Ra3, Rb1, and Rd and the ppt-type ginsenosides Re and Rg1 and notoginsenoside R1. These ginsenosides contain two to five sugars. Although ginsenosides are also prominent constituents of other Panax species, the relative amounts of the saponin constituents present in Sanqi differ from those in Asian ginseng (Panax ginseng) (Chinese Pharmacopoeia Commission, 2005).

ABBRVIATIONS: ppd-type, 20(S)-protopanaxadiol type; ppt-type, 20(S)-protopanaxatriol type; PK, pharmacokinetic; GRb1, ginsenoside Rb1; GRd, ginsenoside Rd; GRg1, ginsenoside Rg1; GF1, ginsenoside F1; GRh1, ginsenoside Rh1; GRg2, ginsenoside Rg2; GRf, ginsenoside F; GF2, ginsenoside F2; NGR1, notoginsenoside R1; C-K, compound-K; Ppd, protopanaxadiol; Ppt, protopanaxatriol; GRa3, ginsenoside Ra3; GRc, ginsenoside Rc; 20gRf, 20-glucosyl-ginsenoside Rf; GRh2, ginsenoside Rh2; GRg3, ginsenoside Rg3; MK571, 3-[[3-[2-(7-chloroquinolin-2-yl)vinyl]phenyl]-2-dimethylcarbamoyethylsulfanyl][methyl]sulfanyl] propionic acid; AUC, area under concentration-time curve; CLb, biliary clearance; CLp, renal clearance; Cum.A, cumulative amount excreted during sampling period; Papp, apparent permeability coefficient; P-gp, P-glycoprotein; MR2, multidrug resistance-associated protein 2; S, aqueous solubility at a given pH; TPSA, topological polar surface area; LC, liquid chromatography; t1/2, elimination half-life; CLtot, total plasma clearance; QSPKR, quantitative structure-PK relationships.
and American ginseng (*Panax quinquefolius*). Sanqi contains approximately equivalent amounts of ginsenosides Rb₁ and Rg₁, whereas the ginsenoside Rb₁ is often more abundant than ginsenoside Rg₁ in Asian and American ginsengs. In addition, Sanqi contains a substantial amount of notoginsenoside R₁, which is also different from Asian and American ginsengs.

The pharmacological effects of ginsenosides vary and can even be oppositional. In contrast to the antiangiogenic effects of the ppd-type ginsenoside Rb₁, the ppt-type ginsenoside Rg₁ has angiogenic properties (Sengupta et al., 2004). However, the lack of quantitative data regarding the absorption, distribution, metabolism, and excretion of ginsenosides has hindered investigation of the pharmacological activities. Earlier PK studies have focused on the development of bioanalytical assays for ginsenosides and their application to preliminary PK assessments (Xu et al., 2003; Li et al., 2004a,b, 2006, 2007a,b). The PK profiles and disposition of ppd-type and ppt-type ginsenosides after administration of Sanqi extracts remain largely unknown. Some studies suggested low oral bioavailability for ginsenoside Rb₁ and Rg₁, only 0.1 and 2%, respectively (Odani et al., 1983a,b), whereas these values were reported to be 4 and 18%, respectively, by others (Xu et al., 2003). The intestinal microflora can degrade ginsenosides via cleavage of the sugar moieties (Hasegawa et al., 1996; Akao et al., 1998; Tawab et al., 2003), however, the effects of deglycosylation on the systemic exposure to ginsenosides are inconclusive.

The objective of this study was to gain understanding about comparative systemic exposure and PK properties of various ginsenosides from Sanqi and about the key factors that affect their absorption and disposition. We also investigated the influence of colonic deglycosylation on systemic exposure to the ginsenosides. The information gained from this study is indispensable for evaluating the contributions of the ginsenosides to the medicinal effects of Sanqi, for assessing potential herb-drug or herb-herb interactions, and for developing Sanqi-based pharmacotherapeutics. To this end, ginsenosides were administered to rats, either in the form of Sanqi extract or as pure compounds. Multiple in vivo, in vitro, and in silico approaches were integrated to determine ginsenoside exposure, absorption, and disposition.

**Materials and Methods**

**Chemicals and Reagents.** Ginsenosides Rb₁ (GRb₁; molecular mass 1108), Rd (GRd; 946), Rg₁ (GRg₁; 784), F₂ (GF₂; 784), Rf₁ (GRf₁; 622), Re (GRé; 946), Rg₂ (GRg₂; 800), Rf (GRf; 800), F₁ (GF₁; 638), notoginsenoside R₁ (NGR₁; 932), compound-K (C-K; 622), protopanaxadiol (Ppd; 460), and protopanaxatriol (Ppt; 476) were obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Ginsenosides Ra₁ (GRa₁; 1240), Rc (GRc; 1078), and 20-glucosides of Rf (20gRf; 962) were obtained from Fengshanjian Co., Ltd. (Kunning, China). Ginsenoside Rb₂ (GRb₂; 638) was obtained from Wuhu Delta Co., Ltd. (Wuhu, China). Ginsenoside Rg₂ (GRg₂; 784) was purchased from Shanghai Tauto Biotech Co., Ltd. (Shanghai, China). The compound purity exceeded 98%.

Hydroxypropylmethyl cellulose was obtained from Colorcon (Shanghai, China). N,N-Dimethylacetamide, Cremophor, and taurocholic acid were purchased from Sigma-Aldrich (St. Louis, MO). Dulbecco’s modified Eagle’s medium, penicillin-streptomycin, and minimal essential medium nonessential amino acids were modified with hydroxypropylmethyl cellulose at 0.3% (grams per milliliter) and stored at −15°C pending use. For intravenous administration, GRa₁, GRb₁, GRc, GRd, GRf₂, GRg₁, C-K, GRh₁, Ppd, 20gRf, GRé, NGR₁, GRg₂, GRf, GRg₂, GRh₁, GF₁, and Ppt were prepared individually as injectable 1.0 mM solutions in 0.5% N,N-dimethylethanolamide, 9.5% Cremophor, and 90% saline.

**Experimental Animals.** Rat studies were conducted according to protocols approved by the Review Committee of Animal Care and Use at the Shanghai Institute of Materia Medica (Shanghai, China). Male Sprague-Dawley rats (220–250 g; Shanghai SLAC Laboratory Animal Co., Shanghai, China) were housed in rat cages (48 × 29 × 18 cm³) in a unidirectional airflow room under controlled temperature (20–24°C), relative humidity (40–70%), and a 12-h light/dark cycle. Filtered tap water was available ad libitum, and the rodents were given commercial rat chow ad libitum except for the overnight period before dosing. Rats were acclimated to the facilities and environment for 7 days before the experiments.

**Plasma Pharmacokinetic Studies in Rats.** Rats were randomly assigned to different groups (three rats per group) to receive an oral dose of Sanqi extract at 4, 8, or 16 ml/kg (via gavage). In addition, bolus intravenous doses of the injectable solutions of individual ginsenosides, including GRa₁, GRb₁, GRc, GRd, GF₂, GRg₁, C-K, GRh₁, Ppd, 20gRf, GRé, NGR₁, GRg₂, GRf, GRg₂, GRh₁, GF₁, and Ppt, at 5 μmol/kg were given to rats (two or three rats per compound) through the tail vein. Serial blood samples (−60 μl; 0, 0.5, 15 oral only), 30 min, and 1 (intravenous only), 2, 4 (intravenous only), 6, 8 (oral only), 10, 12 (oral only), 15, 24, 36, and 48 h were collected in heparinized tubes from the orbital sinus under light ether anesthesia. The blood samples were centrifuged at 3000 rpm for 5 min, and the plasma fractions were decanted and frozen at −70°C until analysis.

In a multiple-dose study, rats received Sanqi extract at a daily oral dose of 4 ml/rat. Four rats per time points were chosen randomly for blood sampling on days 1, 8, 15, or 22. Serial blood samples (−60 μl; 0, 15, and 30 min and 2, 6, 8, 10, 12, 15, and 24 h after dosing) were collected and centrifuged to yield plasma fractions.

**Excretion Studies in Rats.** Rats were housed in Nalgene metabolic cages (one rat per cage) and received oral Sanqi extract at 8 ml/kg (via gavage). Urine and feces samples were collected from four rats at 0 to 4, 4 to 10, and 10 to 24 h on day 1 and every 24 h over the next 3 days. The urine and feces collection tubes were frozen at −15°C to keep collected samples stable. All excretory samples were weighed before storage at −70°C. The thawed feces samples were homogenized in 9 volumes of saline for analysis. Similar urinary/fecal excretion studies were performed for individual ginsenosides administered in intravenous boluses of 5 μmol/kg.

**Preparation of Sanqi Extract and Individual Ginsenosides.** The dried roots of *P. notoginseng* (Sanqi) were obtained from Shanghai Huayu Chinese Herbs Co., Ltd. (Shanghai, China). For oral administration, 400 g of Sanqi was pulverized, mixed, and steeped in 2400 ml of drinking water for 0.5 h at room temperature before a 1-h sonication-enhanced extraction. The extract was separated by filtration, and the residue was re-extracted with 1600 ml of water. The pooled extract was concentrated under reduced pressure at 40°C and was modified with hydroxypropylmethyl cellulose at 0.3% (grams per milliliter) before addition of water to 800 ml to yield Sanqi extract. The extract was stored at −15°C pending use. For intravenous administration, GRa₁, GRb₁, GRc, GRd, GRf₂, GRg₁, C-K, GRh₁, Ppd, 20gRf, GRé, NGR₁, GRg₂, GRf, GRg₂, GRh₁, GF₁, and Ppt were prepared individually as injectable 1.0 mM solutions in 0.5% N,N-dimethylethanolamide, 9.5% Cremophor, and 90% saline.

**Tissue Distribution Study in Rats.** Rats under ether anesthesia were sacrificed by bleeding (−10 ml of blood; the resulting plasma samples were used for analysis) the abdominal aorta at 0, 5, or 15 min or 1, 2, 4, 24, or 48 h (three rats per time point) after the intravenous bolus of GRb₁, GRg₂, or C-K at 5 μmol/kg (via tail vein). Samples of abdominal adipose tissue, testicle, bladder, spleen, stomach, small intestine, kidney, liver, lung, heart, and brain were excised and then rinsed in ice-cold saline before gently blotting on absorbent paper and weighing. The large tissues were homogenized in 4 volumes of ice-cold saline, whereas the small ones were prepared in 9 volumes. The homogenates were stored at −70°C.

**Pharmacokinetic Data Processing.** Plasma PK parameters were determined by noncompartmental and compartmental methods using the Kinetica 2000 software package (version 3.0; InnaPhase Corporation, Philadelphia, PA).
PA). The \( C_{\text{max}} \) and \( T_{\text{max}} \) were observed values without interpolation. The area under the concentration-time curve up to the last measured time point (AUC\(_{0-\infty} \)) was calculated using the trapezoidal rule. The value of AUC\(_{0-\infty} \) was generated by extrapolating AUC\(_{0-\infty} \) to infinity using the elimination rate constant and the last measured concentration. The biliary clearance (CL\(_B \)) and the renal clearance (CL\(_R \)) were calculated from the cumulative amount excreted (\( \text{Cum.A} \)) in the bile and in the urine, respectively, divided by the plasma AUC. Dose proportionality studies on AUC\(_{0-\infty} \) and \( C_{\text{max}} \) were conducted by the regression of log-transformed data (power model) (Smith et al., 2000).

**Cell Culture and Transport Study in Caco-2 Monolayers.** Caco-2 cells (American Type Culture Collection, Manassas, VA) were cultured as described previously (Dai et al., 2008). Bidirectional transport experiments were conducted in triplicate at 2 to 50 \( \mu \)M for the individual ginsenosides in Hanks’ balanced salt solution. Dimethyl sulfoxide was used as a cosolvent to improve the solubility of one- or two-sugar-containing ginsenosides, Ppd, and Ppt, but its final concentration did not exceed 1% (v/v). After incubation for 2 h at 37°C, 50-\( \mu \)l samples were collected from both the receiver and donor compartments, and the compound recovery was determined. The apparent permeability coefficient (\( P_{\text{app}} \)) was expressed in centimeters per second and an efflux ratio (\( P_{\text{app basolateral}} / P_{\text{app apical-basolateral}} > 3 \) was considered to be a positive result, suggesting that the tested compound was an in vitro substrate for the efflux transporter(s). The P-gp substrate rhodamine 123 exhibited substantial directional preference with an efflux ratio of 51.6, which was significantly reduced to 2.3 in the presence of the P-gp inhibitor verapamil. For salsalazine, a MR2 substrate, the presence of the MR2 inhibitor MK571 caused reduction of efflux ratios from 137 to 4.8. These data suggested the normal presence of the efflux transporters in the Caco-2 cell monolayers used.

**In Silico Assessment of Permeability and Solubility.** Chemoinformatic assessment of the physicochemical properties governing intestinal absorption was performed for ginsenosides and their aglycones. Aqueous solubility (\( S_a \)) was calculated with ACD/aqueous solubility version 8.02 via the ACD/Lab service (Advanced Chemistry Development Inc., Toronto, ON, Canada). The octanol-water partition coefficient (LogP) was calculated using Pallas software (Pallas 3.6; CompuDrug International, Sedona, AZ). The total hydrogen bond count (donors and acceptors), topological polar surface area (TPSA), and number of rotatable bonds were determined using the Molinspiration Property Calculator (Molinspiration Cheminformatics, Bratislava, Slovak Republic). Lipinski’s rule of 5 (Lipinski et al., 1997) and the molecular surface properties (total hydrogen bond count, TPSA, and number of rotatable bonds) (Veber et al., 2002) were used to predict the permeability of the tested compounds.

**LC-Tandem Mass Spectrometry Bioassays.** Validated bioanalytical methods were used to measure ginsenosides and their metabolites. Biological samples (20 \( \mu \)l), including plasma, tissue homogenate, excretory, and cell culture medium samples, were precipitated with 80 \% methanol followed by centrifugation at 21,885 \( \times \) g for 5 min before LC-tandem mass spectrometry analysis. A TSQ Quantum mass spectrometer (Thermo Fisher Scientific, Waltham, MA) was interfaced via an electrospray ionization probe with a liquid chromatograph (Agilent Technologies, Waldbronn, Germany). The LC separation was achieved on a Synergi 5 \( \mu \)m C18 column (Phenomenex, Torrance, CA). The LC mobile phases were CH\(_3\)OH/H\(_2\)O (2:98, v/v, containing 0.08 mM HC\(_2\)O\(_4\)Na for A and CH\(_2\)O/H\(_2\)O (98:2, v/v, containing 0.08 mM HC\(_3\)O\(_4\)Na for B, which were used for pulse gradient chromatography (Wang et al., 2007). The gradient parameters including the start proportion, the elution proportion, the elution proportion segment, and the column equilibration segment were 15% B, 100% B, 5.4, and 4.4 min, respectively, except for excretory samples requiring a 5-min start proportion segment. Electrospray ionization and collision energy were optimized to maximize generation of the sodiated or protonated molecules and to produce the characteristic ions, respectively. The precursor-to-product ion pairs selected for specific reaction monitoring of GRa3, GRb1, GRc, GRd, GF2, GRg1, C-K, GRh2, Ppd, 20gRf, GRe, NGR1, GRg3, GRf, GRg2, GRh1, GF1, and Ppt were m/z: 1263 \( \rightarrow \) 497, 1131 \( \rightarrow \) 365, 1101 \( \rightarrow \) 355, 969 \( \rightarrow \) 789, 807 \( \rightarrow \) 627, 807 \( \rightarrow \) 655, 645 \( \rightarrow \) 203, 623 \( \rightarrow \) 407, 461 \( \rightarrow \) 443, 985 \( \rightarrow \) 365, 969 \( \rightarrow \) 789, 955 \( \rightarrow \) 775, 823 \( \rightarrow \) 643, 823 \( \rightarrow \) 365, 807 \( \rightarrow \) 349, 639 \( \rightarrow \) 621, 661 \( \rightarrow \) 203, and 477 \( \rightarrow \) 109, respectively. An online motorized six-port divert valve was used to introduce the LC eluent flow to the ion source for data acquisition over the period of 3 to 9 min, except for excretory samples (8-14 min). Matrix-matched calibration curves were constructed for the analytes (1.37 or 12.3-3000 ng/ml) using weighted linear regressions of the analytic peak area against the corresponding nominal concentrations of the analyte (nanograms per milliliter), which showed good linearity (\( r^2 > 0.99 \)).

**Results**

**Rat Systemic Exposure to Ginsenosides from Oral Sanqi Extract.** Major ginsenosides in Sanqi extract were the ppd-type GRa3 (4.6 mM), GRb1 (23.2 mM), and GRd (6.9 mM) and the ppt-type GRe (5.2 mM), NGR1 (7.9 mM), and GRg3 (33.1 mM). 20gRf and GRh1 were present at lower levels (2.2 mM for both). In addition, several minor ginsenosides, including GRc, GF2, GRg3, GRf, GRG2, and GF1, were at the levels of 0.1 to 0.5 mM. No other ginsenosides or aglycones were detected in Sanqi extract.

As shown in Fig. 1, the ppd-type GRa3, GRb1, and GRd were measurable in rat plasma up to 48 h after oral administration of Sanqi extract (4–16 ml/kg), whereas the ppt-type GRe, NGR1, and GRg3 were measurable only within 24 h. Of the other ginsenosides, the ppd-type GRc, GF2, and GRG3 were only measured in nanomolar plasma levels at 6 to 10 h after dosing and/or 16 ml/kg. The ppt-type GRe, GRG2, GRh1, and GF1, except for 20gRf, were not detected in plasma. C-K (ppd-type) was also measured in rat plasma with delayed occurrence, i.e., 6 h after dosing. C-K was not contained in the administered Sanqi extract; it was a colonic deglycosylated product of the bulky ppd-type ginsenosides (Hasegawa et al., 1998; Akao et al., 1998). We found that C-K could be measured in rat feces after oral administration of purified GRb1 or GRd.

The maximal plasma concentrations of the ppd-type GRa3, GRb1, or GRd occurred 6 to 10 h after dosing. The exposure levels of GRa3, GRb1, and GRd in AUC\(_{0-\infty} \) and \( C_{\text{max}} \) increased directly with Sanqi extract dose from 4 to 16 ml/kg but nonlinearly. However, no significant dose-exposure relationship was observed for C-K. In addition, we also observed a strong linear relationship from a scatter plot of logAUC\(_{0-\infty} \) versus GRa3, GRb1, and GRd versus their corresponding logarithmic compound doses from the ingested extract (\( r^2 = 0.96; n = 27 \)) (Fig. 1). The mean oral bioavailability of GRa3, GRb1, and GRd after administration of the extract was quite low: 0.1 to 0.2%. Because of their long \( t_{1/2} \), i.e., 13, 18, and 13 h for GRa3, GRb1, and GRd, respectively, significant increases in systemic exposure were observed in the multiple-dose study (Fig. 1). The plasma AUC\(_{0-\infty} \) and \( C_{\text{max}} \) of these ppd-type ginsenosides increased from day 1 to day 15, and the values (corrected for rat body weight) on day 15 were 2.1 to 2.6 times higher than those on day 1. However, the systemic exposure levels on day 22 increased only 1.3- to 1.9-fold compared with those on day 1. The reason for these decreases remains to be explored.

Double or triple peaks occurred in the plasma-concentration time courses of the ppt-type GRe, NGR1, and GRg3 after an oral dose of Sanqi extract (Fig. 1). Despite the interanimal variability, the peak appearance patterns for GRe, NGR1, and GRg3 for a given rat were almost identical. The \( F \) values of these ppd-type ginsenosides ranged from 0.2 to 0.6%. Unlike the preceding ppd-type compounds, the systemic exposure levels of GRe, NGR1, and GRG3 were poorly correlated with the oral extract dosage. In addition, the AUC\(_{0-\infty} \) values of GRe, NGR1, and GRG3, normalized according to the corresponding compound doses from the extract, were approximately 1/10 of those of the ppd-type compounds.

Collectively, although their total amounts present in Sanqi extract were comparable, the systemic exposure levels of the ppd-type ginsenosides GRa3, GRb1, and GRd were significantly greater than those of the ppd-type ginsenosides GRe, NGR1, and GRg3. The long \( t_{1/2} \) of these ppd-type compounds counteracted the unfavorable effects of their poor \( F \) on the systemic exposure levels.
Permeability and Solubility of Ginsenosides and Their Deglycosylated Products. Because of the poor \( F \), the permeability and solubility of individual ginsenosides were assessed in vitro and in silico to gain understanding of the mechanisms governing intestinal absorption of ginsenosides. Because it had been reported that ginsenosides could be stripped of their sugar moieties by the colonic microflora (Hasegawa et al., 1996; Akao et al., 1998; Tawab et al., 2003), some monoglucosides and aglycones were also included in the assessment. As shown in Fig. 2, the \( P_{app} \) values of ginsenosides measured in Caco-2 monolayers suggested that the membrane permeability of most ginsenosides was poor with \( P_{app} \) values < 3 \( \times 10^{-7} \) cm/s. GF2, C-K, GRh2, GRh1, and GF1 possessed slightly increased \( P_{app} \) values (approximately 1 \( \times 10^{-6} \) cm/s); the \( P_{app} \) values of Ppd and Ppt were approximately 2 \( \times 10^{-6} \) cm/s. The comparable bidirectional \( P_{app} \) values of most of the ginsenosides and their aglycones suggested passive diffusion as the transport mechanism, except for GF2 and GF1, which exhibited efflux ratios of 10.0 and 48.7, respectively. The efflux ratio of GF2 was significantly reduced to 2.1 and 2.2, in the presence of verapamil and MK571, respectively, suggesting that this ginsenoside might be the substrate of both P-gp and MRP2. A similar situation occurred for GF1; i.e., the efflux ratio was reduced to 12.0 and 9.6 with verapamil and MK571, respectively.

In silico assessment suggested that many ginsenosides could be defined as soluble (Fig. 2). \( S \) values ranged from 160 \( \times 10^{-6} \) to 17 000 \( \times 10^{-6} \) M, which were significantly greater than the highest initial concentrations in the Caco-2 study (50 \( \times 10^{-6} \) M). However, the solubility significantly decreased for compounds with fewer or no sugar moieties attached. The \( S \) values of GF1 and GRh1 were 86 \( \times 10^{-6} \) and 75 \( \times 10^{-6} \) M, respectively, whereas those of GRg3, C-K, GRh2, Ppd, GRg2, and Ppt ranged from 0.08 \( \times 10^{-6} \) to 9 \( \times 10^{-6} \) M. The \( S \) values did not vary significantly as pH changed. On the other hand, most of the ginsenosides, except C-K, GRh2, Ppd, GF1, GRh1, and Ppt, had unfavorable traits underlying poor membrane permeability, including high total hydrogen bond counts ranging from 22 to 44 (donors and acceptors; favorable value \( \leq 12 \)), high TPSA from 219 to 436 Å (favorable value \( \leq 140 \) Å), and high flexibility ranging from 10
to 18 (favorable value $\leq 10$). Reducing the number of sugar moieties decreased the hydrogen-bonding capacity, molecular flexibility, and molecular mass but also reduced solubility significantly (Fig. 2). In addition, the lipophilicity of the Ppd and Ppt aglycones was significantly higher ($\log P$, 5.4 and 4.4, respectively) than that of the ginsenosides ($\log P$, −0.1 to 3.1).

Collectively, our in vitro observations and in silico calculations suggested that the poor intestinal absorption of the ginsenosides could be attributed to poor membrane permeability, which was influenced by the increased sugar number. Although these traits were improved for the deglycosylated products, significant increases in intestinal absorption appeared to be limited by their lowered solubility.

**Structure-Dependent Disposition of Intravenously Administered Ginsenosides in Rats.** To understand the variations in the systemic exposure to ginsenosides after oral administration of Sanqi extract, pure ginsenosides and their aglycones were administered as intravenous boluses. As shown in Fig. 3, the systemic exposure levels of the ginsenosides were directly related to their $t_{1/2}$: GRa3, GRb1, GRc, and GRd had significantly longer $t_{1/2}$ values (7.5–19.8 h) than the other ginsenosides (0.2–3.2 h). The wide variation in $\text{CL}_{\text{tot,p}}$ was the primary reason for the considerable differences in $t_{1/2}$. The ginsenosides tested could be divided into two groups according to $\text{CL}_{\text{tot,p}}$: 1) the three- to five-sugar-containing ppd-type ginsenosides GRa3, GRb1, GRc, and GRd ($\text{CL}_{\text{tot,p}}$, 4–20 ml/h/kg) and 2) the ppt-type (352–2718 ml/h/kg) and the minor ppd-type ginsenosides (505–2296 ml/h/kg, except for GF2 being 66 ml/h/kg). In contrast with $\text{CL}_{\text{tot,p}}$, the $V_{\text{ss}}$ values for most ginsenosides fell within the range of 93 ml/kg for GRg1 to 533 ml/kg for GRg3, which was between the rat plasma volume (31 ml/kg) and the rat total body water volume (670 ml/kg) (Davies and Morris, 1993). The $V_{\text{ss}}$ value of GF1 was 808 ml/kg. Ppd and Ppt had short $t_{1/2}$ values of 1.5 and 0.2 h, respectively, as well as high $\text{CL}_{\text{tot,p}}$ (1482 and 6960 ml/h/kg, respectively) and large $V_{\text{ss}}$ (2016 and 1716 ml/kg, respectively).

Collectively, the notable differences in systemic exposure among ginsenosides were primarily related to their variations in $\text{CL}_{\text{tot,p}}$, and the clearance mechanisms were most likely subject to structure-specific recognition. Analysis of the structural features of the compounds suggested that the attachment of four or more sugar moieties significantly reduced the rate of elimination. In addition, the sugar
attachment site appeared to be also relevant as demonstrated by the significant difference in \( \text{CL}_{\text{int, p}} \) between the ppd-type GRd and the ppt-type GRd, NGR1, and 20gRf (Fig. 3).

Elimination Pathways of Ginsenosides in Rats Receiving Sanqi Extract. To identify the primary elimination pathways that influenced systemic exposure to the ginsenosides, we evaluated the biliary and renal excretion profiles of the ginsenosides as well as the metabolism in rats. In addition, fecal samples were also analyzed to monitor intestinal nonabsorption, biliary excretion, and/or colonic deglycosylation of the ginsenosides. As shown in Fig. 4, the ppd-type ginsenosides (GRa3, GRb1, and GRd) and the ppt-type ginsenosides (NGR1, GRc, and GRg1) exhibited significant differences in excretion profiles. In contrast to the rat systemic exposure levels (as per AUC), the cumulative amounts of the ppt-type ginsenosides excreted intact into bile and urine, corrected according to the corresponding compound doses from Sanqi extract, were significantly greater than those of the ppd-type ginsenosides.

As shown in Fig. 4, the most abundant fecal compounds in rats were GRg1 (\( \text{Cum.A} \), 39.1 \( \mu \text{mol/kg} \)), C-K (20.9 \( \mu \text{mol/kg} \)), and GF1 (7.1 \( \mu \text{mol/kg} \)). We speculated that the high levels of these compounds in feces resulted from the colonic microflora stripping the sugar moieties from the ginsenosides in the extract. Their absence (C-K) or presence at a very low level (GF1) in Sanqi extract supports this hypothesis. The contribution of colonic deglycosylation of major ppt-type ginsenosides to the fecal GRg1 was supported by two lines of evidence: 1) fecal recovery of GRg1 after oral administration of pure GRg1 was significantly lower than that after oral administration of Sanqi extract (at the same GRg1 dose) in the same rats and 2) oral administration of pure 20gRf, GRc, and NGR1 generated fecal GRg1. Meanwhile, poor intestinal absorption and rapid biliary excretion accounted for the relatively low plasma levels of GRg1, C-K, and GF1. Although both of the aglycones (Ppd and Ppt) were detected in fecal samples after oral administration of Sanqi extract, their fecal \( \text{Cum.A} \) values were quite low, 0.4 and 0.2 \( \mu \text{mol/kg} \), respectively. Ppd and Ppt were not detected in plasma, bile, or urine, suggesting that they were two minor metabolites in negligible quantities.

To further elucidate the excretion profiles of ginsenosides, individual pure compounds were administered intravenously to rats at 5 \( \mu\text{mol/kg} \). We confirmed that biliary excretion was the major elimination route for most ginsenosides, demonstrating the percentage of the intravenous dose excreted intact into bile (43–100%). The ginsenosides were excreted rapidly via active biliary transport, indicative of high bile/plasma distribution ratios (AUC\text{bile}/AUC\text{plasma} = 22–1907). The AUC\text{bile}/AUC\text{plasma} ratio of GRd was 4.4. However, the biliary excretion of GRa3, GRb1, and GRc was slow, and their AUC\text{bile}/AUC\text{plasma} ratios ranged from 0.2 to 0.4. As to renal excretion, most ginsenosides were slowly and passively excreted into rat urine. GRg1, and monoglycosides were detected in negligible amounts in urine. However, when corrected for plasma protein binding, the unbound \( \text{CL}_{\text{ur}} \) of NGR1, 20gRf, and GRg1, 497 to 1007 \( \mu\text{L/h/kg} \), exceeded the rat glomerular filtration rate of 314 \( \mu\text{L/h/kg} \) (Davies and Morris, 1993), suggesting that some active tubular secretion occurred in rats. Figure 5 shows the excretion profiles of GRb1 and GRg1, which could represent the two types of typical situations among the ginsenosides tested. Approximately 10 and 40% of intravenously administered GRb1 was excreted intact into rat bile and urine, respectively, whereas the corresponding fractions for GRg1 were approximately 73 and 18%, respectively. The \( \text{CL}_{\text{ur}} \) and \( \text{CL}_{\text{ur}} \) were 1840 and 427 \( \mu\text{L/h/kg} \), respectively, for GRg1, but only 1.0 and 3.5 \( \mu\text{L/h/kg} \), respectively, for GRb1.

As to the metabolism of intravenously administered GRb1, the monooxidized metabolite, the deglycosylated metabolite (GRd), and the monooxidized metabolite of GRd were measured in rat bile and urine (Fig. 5). These metabolic reactions were catalyzed by enzymes...
in rat tissues such as the liver. Approximately 1.8 and 1.5% of intravenously administered GRb1 were eliminated very slowly as the tissue-deglycosylated metabolite GRd via bile and urine, respectively, and the values would continue to increase beyond 48 h for bile and 96 h for urine. The tissue oxidation of GRb1 appeared to take place faster than the tissue deglycosylation but to a lesser extent. We estimated that in rats approximately 10 to 15% of intravenously administered GRb1 might be ultimately eliminated via these metabolic pathways. As for intravenously administered GRg1, only one monooxidized metabolite was detected in rat bile and urine, which accounted for approximately 0.2 and 0.1% of the dose, respectively. The significantly minimal tissue metabolism observed for GRg1 could have resulted, at least in part, from the rapid biliary and renal excretions. More recently, Qian et al. (2006) also detected the oxidation and deglycosylation products of GRb1 in rat urine.

Collectively, rapid and extensive biliary excretion was a key factor limiting the systemic exposure to most ginsenosides from Sanqi extracts, which appeared to involve active secretion mechanisms. However, the ppd-type GRa3, GRb1, GRc, and GRd circumvented or limited the systemic exposure to most ginsenosides from Sanqi extract, which appeared to involve active secretion mechanisms. Renal excretion also contributed considerably to the elimination of some ginsenosides including GRa3, GRb1, GRc, GRd, NGR1, 20gRf, and GRg1, but via different transportation mechanisms. There was considerable deglycosylation of unabsorbed ginsenosides by the colonic microflora, but the colonic metabolism did not significantly improve overall systemic exposure to ginsenosides from orally administered Sanqi extract. The relative contributions of tissue deglycosylation and tissue oxidation to overall elimination of ginsenosides appeared to be poor.

**Comparative Tissue Exposure to Intravenously Administered GRb1, GRg1, and C-K.** To address whether the observed differences in plasma concentration among ginsenosides reflect the situation in tissues well, a comparative study of tissue distribution was performed with GRb1, C-K, and GRg1, which was based on measurements in whole rat tissue. These three compounds were selected as prototype compounds for the study, because GRb1 and GRg1 were the ppd-type and the ppt-type ginsenosides, respectively, present in the greatest amounts in Sanqi extract and because GRb1, C-K, and GRg1 were the compounds measured in large amounts in rat plasma or excretory samples (Fig. 4). To ease comparisons, each investigational compound was administered as an intravenous bolus of 5 μmol/kg. After dosing, all compounds transferred rapidly from blood to tissues with \( T_{max} \) values of 0.1 to 2.7 h for different tissues tested. Plasma and tissue concentrations of GRb1 at 5 min after dosing (\( C_{5\,min} \)) were in the rank order of plasma (50 μM) > liver (22 μM) > kidney (19 μM) > heart (16 μM) > lung (12 μM) > bladder (5.1 μM) > spleen (4.7 μM) > testicle (3.8 μM) > large intestine (3.2 μM) > small intestine (2.7 μM) > stomach (2.2 μM) > adipose (1.9 μM) > brain (0.8 μM). The tissue AUC values of GRb1 versus the corresponding plasma value are shown in Fig. 6.

Consistent with the differences observed for plasma, both the \( C_{5\,min} \) and AUC of GRb1 in the tissues were much greater than the corresponding levels for GRg1 or C-K. The tissue \( t_{1/2} \) values of GRb1 (14–31 h) were comparable with the corresponding plasma data (20 h), which were significantly longer than those of GRg1 (0.2–1 h;
except for the kidney 22 h) or C-K (0.3–6 h). Despite its generally low levels in tissue, GRg1 exhibited a relatively high C_{min} and AUC in the liver (70 μM and 28 μM · h, respectively) compared with the corresponding plasma data (11 μM and 2 μM · h, respectively).

Similar phenomena were observed for C-K (276 μM and 242 μM · h, respectively, in the liver versus 29 μM and 11 μM · h, respectively, in plasma). The hepatic uptake of GRg1 and C-K could be associated with the active biliary secretion mediated by the hepatic transporters. In addition, renal uptake of GRg1 was also extensive, demonstrating a relatively high C_{min} (86 μM) and AUC (99 μM · h) compared with the corresponding plasma data. In agreement with their unfavorable properties regarding membrane permeability, GRb1, GRg1, and C-K were poorly delivered to the central nervous system.

Collectively, the significant differences in the plasma levels among ginsenosides could reflect the situations in the tissues. In addition, the tissue distribution data for GRg1 and C-K support the possible involvement of active secretion mechanisms in the hepatic and/or renal elimination of the compounds.

Discussion

Bioactive constituents with favorable PK properties and existing in adequate abundance in a medicinal herb are most likely to account for the pharmacological effects of the herb and to form the basis of its therapeutic efficacy. Our current and recent studies (Lu et al., 2008) indicate that both animals and humans are exposed significantly to some but not all constituents of an herbal medicine after dosing and that the PK profiles, like the pharmacological activities, can be used as a "sieve" to assess the importance and usefulness of the individual herbal constituents. Such PK studies are essential for understanding the link between the herb consumption and the pharmacological effects and for identifying the medicinal principles from the chemical constituents in an herbal medicine.

In contrast with Western medicines that normally contain only one active ingredient, herbal medicines contain numerous chemical constituents. Multicomponent PK studies of an herbal medicine can be challenging. Such studies are complicated by the great diversity of the constituents with regard to both chemical structure and content. Variations in chemical structures result in different physiochemical properties and PK profiles among the constituents, whereas the variations in content result in dissimilarities in constituent dosages. Some important goals for PK studies of an herbal medicine are to 1) measure systemic exposure to the administered medicine, 2) identify suitable PK markers indicative of exposure to the medicine, 3) compare the PK properties of the constituents, 4) understand the pathways that influence their systemic exposure, 5) analyze the quantitative structure-PK relationships for the constituents and metabolites, and 6) assess differences in PK properties between the animal species and humans. It is worth mentioning that the constituents in an herbal medicine are often structurally related and may be divided into more than one class. Analysis of quantitative structure-PK relationships (QSPKR) of herbal chemicals may provide insight into the role of molecular properties and/or functional group presentation in the pharmacokinetics of compounds and may help understand the PK trends within the compound series and anticipate which homologs have favorable PK properties compatible with the pharmacological activities. The key role that structure-based differentiation can play in the absorption and disposition of herbal constituents highlights the fact that the QSPKR analysis is a vital component of the multicomponent PK study. In the current study, QSPKR analysis helped us identify key factors affecting systemic exposure to different ginsenosides and their deglycosylated products after administration of Sanqi extract.

The PK properties of constituents contained in an herbal medicine can be quite different. When bioactive constituents from an herb are measurable in a biosample, such as plasma or urine, and have favorable PK properties that could be used to substantiate systemic exposure to the herb, they are referred to as "PK markers" of the herb (Lu et al., 2008). Identification of PK markers for herbs could be helpful in the design and interpretation of toxicity and clinical studies, as well as in the evaluation of potential herb-drug or herb-herb interactions. Here, we measured rat systemic exposure to ginsenosides and their deglycosylated products after oral administration of Sanqi extract and compared their PK properties. We found that the ppt-type GRe, GRb1, and GRd measured in plasma were suitable PK markers of orally administered Sanqi extract in rats. This conclusion was based on the dose-dependent systemic exposures and PK properties of these ginsenosides. In contrast, the other plasma ginsenosides, including the ppt-type GRe, NGR1, and GRg1, and the metabolite ppt-type C-K, were not the PK markers for Sanqi extract because of their low exposure levels and poor dose proportionality. Although the ppt-type ginsenosides were excreted into the rat urine in substantial amounts, large interindividual variation prevented the use of the urinary compounds as surrogate PK markers. Meanwhile, renal excretion of GRA3, GRb1, GRd, and C-K was very slow.

Poor membrane permeability was a major factor limiting intestinal absorption of ginsenosides, which was attributed mainly to the sugar moieties increasing the hydrogen bond count, polar surface area, and molecular flexibility of the molecules to unfavorable levels. Furthermore, reducing the sugar moiety content seemed not to improve intestinal absorption significantly, because of the associated decrease in solubility. This finding is supported by our rat data that the fecal Cum.A values of the deglycosylated products exceeded substantially the corresponding sum of biliary and urinary Cum.A. In addition, the poor permeability could also partially explain our observations in the tissue distribution study. In most cases, the tissue concentrations of ginsenosides were lower than the corresponding plasma levels, and the compounds persisted in the organ capillaries and the organ interstitial fluid rather than in the organ cells. However, the liver concentrations of most ginsenosides were significantly higher than the plasma levels. This finding can most likely be attributed to some uptake transporters mediating active transport of the ginsenosides into the hepatic cells.

Most of the ginsenosides and their deglycosylated products were subject to rapid extensive biliary excretion through active transport, resulting in their short t_{1/2} values and low systemic exposure levels. Meanwhile, renal excretion of most ginsenosides is slow, and its role in the overall elimination could be minimized by rapid extensive biliary excretion. There were two exceptions, i.e., 1) slow but extensive renal excretion because of slow biliary excretion for GRA3, GRb1, and GRc and 2) rapid renal excretion involving active tubular secretion for NGR1, 20gRf, and GRG1. In addition, the contribution of ginsenoside metabolism to their overall elimination was also limited. The metabolism occurring in rat tissues, such as the liver, included deglycosylation and oxidation. The tissue deglycosylation was found to be quite slow, whereas the tissue oxidation was rapid. Compared with the minor tissue deglycosylation, the preabsorption deglycosylation of ginsenosides mediated by the gut microflora appeared to be relatively extensive. Our data (Fig. 4) suggested that the colonic deglycosylation of the ppt-type ginsenosides in rats occurred at C-20 until only one glucose remained, i.e., GRd, and then deglycosylation preferentially took place at C-3, followed by stripping of the last glucose moiety at C-20 to yield the aglycone Ppd. Meanwhile, the initial colonic deglycosylation of the ppt-type ginsenosides appeared to occur preferentially at C-6 to yield GRg1. Further deglycosylation...
occurred at either C-6 or C-20 to form GF1 or GRh1, respectively, which was followed by stripping of the last glucose moiety at C-20 or C-6, respectively, to yield the aglycone Ppt.

The attachment of four and five sugar moieties in the ppd-type GRa1 and GRb1, respectively, appeared to play a key role in blocking access to the biliary transporters responsible, resulting in slow biliary excretion of the two compounds (CLh1 1.5 and 1.0 ml/h/kg, respectively) in rats. GRd had a significantly slower CLh1 value (19 ml/h/kg) than the other three-sugar-containing ginsenosides 20gRf, GRe, and NGR1 (258–775 ml/h/kg), suggesting that the sugar attachment sites also influenced the active biliary excretion. In addition, GRa2, GRb1, and GRd underwent limited metabolism and slow renal excretion. Slow elimination of these bulky ppd-type ginsenosides made them long-circulating, which counteracted the unfavorable effect of poor intestinal absorption on their systemic exposure.

GRb1 and GRd have been reported to possess activities associated with the putative cardiovascular effects of Sanqi. GRb1 has estrogenic activity (Liu et al., 2003) via a mechanism independent of estrogen receptor binding (Cho et al., 2004). GRb1 also has antioxidant activity (Liu et al., 2003). GRd can dilate vascular muscle by blocking Ca2+ channels in the muscle cells (Guan et al., 2006). The pharmacological activities of GRa1 remain to be understood.

Drug exposure is a crucial determinant of drug response and therefore its efficacy and safety. In the current study, we compared the systemic exposure to putatively active ginsenosides from orally administered Sanqi extract and investigated the relevant mechanisms governing exposure to ginsenosides. In summary, our data indicate that poor membrane permeability and rapid and extensive active biliary excretion are two primary factors limiting systemic exposure to most Sanqi ginsenosides and their deglycosylated metabolites. The major ginsenosides measured in the plasma of rats were long-circulating GRa3, GRb1, and GRd, because of their slow biliary excretion. These plasma ginsenosides can be used as suitable PK markers for Sanqi extract. Considerable colonic deglycosylation occurred, but the systemic exposure to the metabolites was low. The PK profiles of the Sanqi ginsenosides in humans were comparable to those found in rats. Slight interspecies differences could have resulted from humans being relatively poor biliary excreters compared with rats. The details of the human study will be reported elsewhere.

References


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