DMD Fast Forward. Published on September 2, 2016 as DOI: 10.1124/dmd.116.071647
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Short Communication

Pharmacokinetics and Disposition of Circulating Iridoids and Organic Acids in Rats Intravenously Receiving ReDuNing Injection

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DMD Fast Forward. Published on September 2, 2016 as DOI: 10.1124/dmd.116.071647
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Running Title: Rat Pharmacokinetic Studies of ReDuNing Injection

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Number of Text Pages: 8

Number of Tables: 1

Number of Figures: 3

Number of References: 13

Number of Words in Abstract Section: 248

Number of Words in Introduction Section: 328

Number of Words in Results and Discussion Section: 1266

ABBREVIATIONS: $AUC_{0-\infty}$, area under plasma concentration-time curve to infinity; COMT, catechol-*O*-methyltransferase; CR, carbonyl reductase; $Cum.A_{e\text{-}B,0\text{-}24h}$, cumulative amount excreted into bile collected 0–24 h after dosing started; $Cum.A_{e\text{-}U,0\text{-}24h}$, cumulative amount excreted into urine collected 0–24 h after dosing started; $f_{e\text{-}B}$, fraction of dose excreted into bile; $f_{e\text{-}U}$, fraction of dose excreted into urine; GSH, glutathione; PAPS, 3'-phosphoadenosine-5'-phosphosulfate; SAM, *S*-adenosylmethionine; SULT, sulfotransferase; $t_{1/2}$, elimination half-life; UDPGA, uridine 5'-diphospho-glucuronic acid; UGT, uridine 5'-diphospho-glucuronosyltransferase.

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ABSTRACT

ReDuNing injection, prepared from a combination of *Gardenia Jasminoides* fruits, *Lonicera japonica* flower buds, and *Artemisia annua* aerial part, is extensively used for treatment of viral upper respiratory tract infection in China. Iridoids, organic acids, and flavonoids are probably important for the herbal injection because of their reported pharmacological properties. This study was designed to characterize pharmacokinetics and disposition of major circulating herbal compounds in rats intravenously receiving the injection. ReDuNing injection was found to contain 19 iridoids (content levels, 0.01–27.93 mM), 16 organic acids (0.04–19.06 mM), and 11 flavonoids (<0.08 mM). After dosing the injection, the iridoids geniposide, secologanic acid, secoxyloganin, genipin-1-*β*-gentiobioside, geniposidic acid, sweroside, and shanzhiside and the organic acids chlorogenic acid, quinic acid, cryptochlorogenic acid, and neochlorogenic acid were found to be the major circulating compounds with mean elimination half-lives of 0.2–0.9 hour, whereas other plasma compounds were at low exposure levels. These major circulating compounds exhibited small apparent volumes of distribution (0.03–0.34 l/kg). Most of the iridoids were eliminated predominantly via renal excretion of the unchanged compounds, whereas the organic acids were eliminated via methylation and sulfation and excreted into urine as the unchanged and metabolized compounds. The methylated metabolites also underwent subsequent conjugations before hepatobiliary and renal excretion. In vitro data suggested that the preceding metabolism of the organic acids in rats also occurred in humans. The current pharmacokinetic research could serve as a crucial step in identifying the chemical basis responsible for the therapeutic action of ReDuNing injection.

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Introduction

ReDuNing is an herbal injection, which was approved in 2005 by the China Food and Drug Administration (China FDA) for treatment of viral upper respiratory tract infection associated with high fever, chills, headache, myalgia, and cough with phlegm. Each milliliter of the injection is prepared from a combination of 0.60 g of *Gardenia jasminoides* fruits (Zhizi), 0.75 g of *Lonicera japonica* flower buds (Jinyinhua), and 1.25 g of *Artemisia annua* aerial part (Qinghao), yielding an herb-to-injection ratio of 2.6:1. The injection is available as a sterile and nonpyrogenic dosage form for intravenous administration at a dose of 20 ml once daily for three days. ReDuNing therapy appears to have low incidence of side effects (Xu et al., 2009). Recently, ReDuNing injection is also used as an add-on therapy in conventional treatment of hand, foot, and mouth disease in children infected with coxsackievirus type A16 or enterovirus type 71 (Li et al., 2014). Despite its extensive use in clinics, little is known about the chemical basis responsible for the therapeutic action of ReDuNing injection.

Pharmacologically active constituents existing in adequate abundance in an herbal medicine and exhibiting favorable pharmacokinetic profiles are most likely to form the chemical basis responsible for the medicine's therapeutic action. ReDuNing injection has been reported to contain iridoids, organic acids, and flavonoids (Li et al., 2015a). These compounds are important because of their reported antiviral, anti-inflammatory, and antioxidative properties as pure isolates (Shang et al., 2011; Liu et al., 2013). Although bioanalytical assays were developed for measurement of concentrations of some iridoids and organic acids in human and rat plasma after dosing ReDuNing injection (Ni et al., 2015; Wang et al., 2015), pharmacokinetic information about ReDuNing injection is still limited. This hinders understanding the

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chemical basis responsible for the therapeutic action of ReDuNing injection. The current study was designed to assess systemic exposure to ReDuNing compounds in rats intravenously receiving the herbal injection and to investigate pharmacokinetics and elimination of the major circulating herbal compounds.

Materials and Methods

A detailed description of materials and methods is provided in Supplemental Materials and Methods, which are available online.

ReDuNing Injection and Its Component Herbs. Samples of six lots of ReDuNing injection (China FDA drug ratification number, GuoYaoZhunZi-Z20050217) and samples of its component herbs *G. Jasminoides* fruits, *L. japonicae* flower buds, and *A. annua* aerial part were obtained from Jiangsu Kanion Pharmaceutical Corporation (Lianyungang, Jiangsu Province, China).

Chemicals and Reagents. Reference standards of iridoids, organic acids, and flavonoids (purity, ≥98% by HPLC) were obtained from Tauto Biotech (Shanghai, China), Shanghai Nature Standard R&D and Biotech (Shanghai, China), BioBioPha (Kunming, Yunnan Province, China) , and Sigma-Aldrich (St. Louis, MO).

Rat Studies. All animal care and experimental procedures were in compliance with the Guidance for Ethical Treatment of Laboratory Animals (The Ministry of Science and Technology of China, 2006) and approved by an Institutional Animal Care and Use Committee at Shanghai Institute of Materia Medica (Shanghai, China). Male Sprague Dawley rats (200–230 g) were obtained from the Sino-British SIPPR/BK Laboratory Animal (Shanghai, China). Three rat studies were performed by giving a single 30-minute intravenous infusion of ReDuNing injection at 2 ml/kg via the tail veins. The rat dose was derived from the label daily dose of ReDuNing injection (20 ml/person) according to dose normalization by body surface area

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(Reagan-Shaw et al., 2008). In the first rat study, blood samples (~80 μl) were collected from four rats, into heparinized tubes, before and 0.083, 0.25, 0.5, 0.58, 0.75, 1, 1.5, 2.5, 4.5, 6.5, 8.5, and 24 hours after starting infusion; the plasma fractions were prepared by centrifuging the blood samples. In the second rat study, urine samples were collected, from four rats, before and 0–4, 4–8, and 8–24 hours after starting infusion. In the third rat study, bile samples were collected, from four rats, before and 0–2, 2–4, 4–6, 6–8, and 8–24 hours after starting infusion.

In Vitro Metabolism Studies. To characterize metabolism of herbal compounds in rats and to facilitate prediction of the metabolism in humans, metabolic capacities of cytochrome P450 (P450), carbonyl reductase (CR), catechol-*O*-methyltransferase (COMT), UDP-glucuronosyltransferase (UGT), and sulfotransferase (SULT) for ReDuNing compounds were assessed using rat liver microsomes and cytosol, as well as human liver microsomes and cytosol. The preceding metabolic reactions were repeated by adding glutathione (GSH) into the incubations. Also, interplays of COMT with UGT and with SULT were also evaluated using methods by Li et al. (2015b).

Determination of Plasma Protein Binding. Fractions of unbound compounds to rat plasma proteins (f_u) were assessed by an ultrafiltration method (Guo et al., 2006).

Liquid Chromatography/Mass Spectrometry-Based Assays. A Waters Synapt G2 high definition time-of-flight mass spectrometer (Manchester, UK), interfaced via a Zspray/LockSpray ESI source with a Waters Acquity UPLC separation module (Milford, MA), was used for analysis of unchanged and metabolized herbal compounds in ReDuNing injection, the component herbs, rat biosamples, and in vitro metabolism study samples.

Data Processing. Pharmacokinetic parameters were estimated by noncompartmental analysis using Kinetica software (version 5.0; Thermo Scientific,

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Philadelphia, PA). All data are expressed as the mean ± standard deviation.

Results and Discussion

Analysis of the chemical composition of ReDuNing injection was the first step in pharmacokinetic research on the injection. The aims of the analysis were to understand which, and how much, herbal compounds were introduced into the bloodstream by dosing the injection and to understand the injection's lot-to-lot variability. A total of 46 compounds (19 iridoids, 16 organic acids, and 11 flavonoids) were detected in ReDuNing injection (Fig. 1). The compounds could be graded according to their dose levels from ReDuNing injection at the label daily dose 20 ml/person. The iridoids geniposide (**6**) and secologanic acid (**4**) and the organic acids chlorogenic acid (**30**), quinic acid (**25**), cryptochlorogenic acid (**31**), and neochlorogenic acid (**29**) exhibited dose levels of >100 μmol/person (135–592 μmol/person); the injection exhibited small lot-to-lot variability (3.1–7.9%) for these compounds, except for **4** being 26.4% (Supplemental Table 1). The sum of the dose levels of **6** and **4** was 78.2% of the total dose of iridoids present in ReDuNing injection, whereas the sum of the dose levels of **30**, **25**, **31**, and **29** was 89.6% of the total dose of organic acids in the injection. The dose levels of secoxyloganin (**12**), genipin-1-β-gentiobioside (**16**), geniposidic acid (**3**), sweroside (**2**), shanzhiside (**9**), isochlorogenic acid C (**35**), caffeic acid (**24**), isochlorogenic acid B (**33**), and isochlorogenic acid A (**34**) were in the range 10–100 μmol/person (13–69 μmol/person); the lot-to-lot variability for these compounds was 6.0–16.1%. The remaining ReDuNing compounds (including all the detected flavonoids) were in the range <10 μmol/person (0.0007–8 μmol/person); the lot-to-lot variability for these minor compounds was 6.3–61.7%. Most of the ReDuNing iridoids originated from the component herbs Zhizi and Jinyinhua. However, **6** originated predominantly from

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Zhizi, whereas **4** originated predominantly from Jinyinhua. Most of the ReDuNing organic acids originated from Zhizi, Jinyinhua, and Qinghao. However, **30** and **25** originated predominantly from Jinyinhua. Despite being minor constituents present in the component herbs, **31** and **29** were also major ReDuNing organic acids, which, in part, were transformed from **30** during the preparation of the injection. Dawidowicz et al. (2014) reported heating-induced conversion of chlorogenic acid into cryptochlorogenic acid and neochlorogenic acid in water.

To understand systemic exposure to ReDuNing compounds, rats received a 30-minute intravenous infusion of the herbal injection. As shown in Fig. 2, a total of 39 unchanged herbal compounds (19 iridoids, 16 organic acids, and 4 flavonoids) were detected in plasma samples of rats after starting infusion. The iridoids geniposide (**6**), secologanic acid (**4**), secoxyloganin (**12**), and geniposidic acid (**3**) and the organic acids chlorogenic acid (**30**), quinic acid (**25**), cryptochlorogenic acid (**31**), and neochlorogenic acid (**29**) exhibited high levels of systemic exposure; whereas the iridoids sweroside (**2**), genipin-1-β-gentiobioside (**16**), and shanzhiside (**9**) were at lower levels. Chemical structures of these major circulating ReDuNing compounds are shown in Fig. 2. The plasma flavonoids detected were at quite low levels. A total of 40 unchanged ReDuNing compounds were detected in rat urine samples, whereas only 24 such compounds were detected, all at low levels, in bile samples (Fig. 2). Renal excretion of unchanged compound was found to be the main elimination pathway for the major circulating iridoids **6**, **12**, **16**, **2**, and **9** with fractions of doses excreted into urine ($f_{e\text{-}U}$) of 64.0–87.5%, whereas the $f_{e\text{-}U}$ of the iridoid **3** was 334.8%. As shown in Supplemental Table 2B, an in vitro metabolism study using rat liver microsomes suggested that P450-mediated and NADPH-dependent oxidative ester cleavage of **6** probably took place in rats to yield **3**. Unlike for these iridoids, renal

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excretion was a minor elimination pathway for the iridoid **4** ($f_{e\text{-}U}$, 7.3%); so was biliary excretion (fraction of dose excreted into bile, $f_{e\text{-}B}$; 0.1%). A reduced metabolite of **4** (**M4$_R$**) was detected in rat plasma, urine, and bile samples (Supplemental Fig. 1 and Supplemental Table 2A); the metabolism was probably mediated by carbonyl reductase (CR) in rat liver cytosol fortified with NADPH (Supplemental Table 2B) and could be inhibited by quercetin. The organic acid **25** was also primarily eliminated unchanged via renal excretion ($f_{e\text{-}U}$, 89.7%). The $f_{e\text{-}U}$ values of the other organic acids **30**, **31**, and **29** (31.9–44.2%) were lower than that of **25**; several metabolites of these compounds were detected in rats (Supplemental Fig. 1 and Supplemental Table 2A). Because **30**, **31**, and **29** were isomers with the same molecular mass 354 daltons, characterization of the parent compound and metabolite relationship was achieved by in vitro metabolism study using purified compounds as substrates (Supplemental Table 2B). As a result, the detected plasma metabolites were methylated metabolites of **30** (**M30$_{M\text{-}1}$** and **M30$_{M\text{-}2}$**), **31** (**M31$_{M\text{-}1}$** and **M31$_{M\text{-}2}$**), and **29** (**M29$_{M\text{-}1}$** and **M29$_{M\text{-}2}$**) and their sulfated metabolites (**M30$_{S\text{-}2}$**, **M31$_S$**, and **M29$_S$**); these metabolites were excreted into urine and, all to less extent, into bile. In addition, the methylated metabolites were subsequently glucuronized to yield **M30$_{M\text{-}G}$**, **M31$_{M\text{-}G}$**, and **M29$_{M\text{-}G}$** (excreted into urine and bile) or conjugated with GSH to yield **M30$_{M\text{-}Gsh\text{-}1}$**, **M30$_{M\text{-}Gsh\text{-}2}$**, **M31$_{M\text{-}Gsh}$**, and **M29$_{M\text{-}Gsh}$** (all excreted only into bile). Figure 3 shows proposed metabolic pathways of **6**, **4**, **30**, **31**, and **29** in rats intravenously receiving ReDuNing injection. In vitro metabolism studies using human liver microsomes and cytosol suggested that the preceding metabolism of **6**, **4**, **30**, **31**, and **29** in rats probably also occurred in humans (Supplemental Table 2B).

Table 1 summarizes the pharmacokinetic parameters of major circulating herbal compounds in rats receiving ReDuNing injection; their plasma concentrations rose as

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the 30-minute infusion was continued with the maximum plasma concentrations (C_{max}) being measured just prior to completion of the infusions (Supplemental Fig. 2). The ReDuNing compounds exhibited short elimination half-lives ($t_{1/2}$), i.e. 0.3–0.9 hour for the iridoids geniposide (**6**), secologanic acid (**4**), secoxyloganin (**12**), genipin-1-β-gentiobioside (**16**), geniposidic acid (**3**), sweroside (**2**), and shanzhiside (**9**) and 0.2–0.5 hour for the organic acids chlorogenic acid (**30**), quinic acid (**25**), cryptochlorogenic acid (**31**), and neochlorogenic acid (**29**). The mean total plasma clearance ($CL_{tot,p}$) values of the iridoids and organic acids were 3–22% and 7–21% of rat cardiac plasma output (7.3 l/h/kg; Toutain et al., 2004), respectively, indicating the clearance of these compounds was not high. The mean distribution volumes at steady state (V_{SS}) of iridoids (0.2–0.4 l/kg) and organic acids (0.3–0.4 l/kg) were close to the rat extracellular volume (0.3 l/kg; Davies and Morris, 1993). The iridoids and organic acids were poorly bound to rat plasma protein with fractions of compounds unbound to rat plasma proteins (f_u) of 75.2–99.0% and 48.8–65.8%, respectively.

Chinese herbal medicines are often combinations of multiple herbs and have very complex chemical composition. Pharmacokinetic research, serving as compound sieve (Liu, et al. 2009), is a crucial step in identifying the chemical basis responsible for the therapeutic actions of herbal medicines. In the current study, 11 unchanged herbal compounds (seven iridoids and four organic acids) showed considerably high levels of systemic exposure with short $t_{1/2}$ (<1 hour) in rats intravenously receiving ReDuNing injection. Also, several methylated and sulfated metabolites of the organic acids exhibited high levels of systemic exposure. Further investigation of these major circulating herbal compounds, including assessing their pharmacodynamic activities related to the injection's therapeutic action, exploring the translation of the compounds' individual activities into the injection's overall action, and evaluating

their pharmacokinetic compatibility (i.e., exhibiting limited pharmacokinetic interaction problems that can counteract the compounds' synergistic or additive pharmacodynamic effects), will finally lead to understanding the chemical basis responsible for the therapeutic action of ReDuNing injection. The current pharmacokinetic study in rats also facilitates designing a future clinical pharmacokinetic study of ReDuNing injection and future systematic evaluation of potential pharmacokinetic herb-drug interactions related to the injection.

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Authorship Contributions

Participated in research design: C. Li, Cheng.

Conducted experiments: Cheng, Du, Yu, Xu, Wang, L. Li, Olaleye, Yang, Chen, Zhong, Liu, J. Li.

Contributed new reagents: Xiao, Wang.

Performed data analysis: C. Li, Cheng.

Wrote or contributed to the writing of the manuscript: C. Li, Cheng.

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References

Davies B and Morris T (1993) Physiological parameters in laboratory animals and humans. *Pharm Res* **10:**1093–1095.

Dawidowicz AL and Typek R (2014) Transformation of 5-*O*-caffeoylquinic acid in blueberries during high-temperature processing. *J Agric Food Chem* **62:**10889–10895.

Guo B, Li C, Wang G-J, and Chen L-S (2006) Rapid and direct measurement of free concentrations of highly protein-bound fluoxetine and its metabolite norfluoxetine in plasma. *Rapid Commun Mass Spectrom* **20:**39–47.

Li H-B, Yu Y, Wang Z-Z, Geng J-L, Dai Y, Xiao W, and Yao X-S (2015a) Chemical profiling of ReDuNing injection by ultra-performance liquid chromatography coupled with electrospray ionization tandem quadrupole time-of-flight mass spectrometry through the screening of diagnostic ions in MS^E mode. *PLoS One* **10:**e0121031.

Li M-J, Wang F-Q, Huang Y-H, Du F-F, Zhong C-C, Olaleye OE, Jia W-W, Li Y-F, Xu F, and Dong J-J et al. (2015b) Systemic exposure to and disposition of catechols, derived from *Salvia miltiorrhiza* roots (Danshen), after intravenous dosing DanHong injection in human subjects, rats, and dogs. *Drug Metab Dispos* **43:**679–690.

Li X-H, Zhang X, Ding J-B, Xu Y, Wei D, Tian Y-M, Chen W, Huang J-H, Wen T, and Li S-J (2014) Comparison between Chinese herbal medicines and conventional therapy in the treatment of severe hand, foot, and mouth disease: a randomized controlled trial. *Evid Based Complement Alternat Med* 2014:140764.

Liu H, Chen Y-F, Li F, and Zhang H-Y (2013) *Fructus Gardenia* (*Gardenia jasminoides* J. Ellis) phytochemistry, pharmacology of cardiovascular, and safety with the perspective of new drugs development. *J Asian Nat Prod Res* **15:**94–110.

Liu H-F, Yang J-L, Du F-F, Gao X-M, Ma X-T, Huang Y-H, Xu F, Niu W, Wang F-Q, Mao Y et al. (2009) Absorption and disposition of ginsenosides after oral administration of *Panax notoginseng* extract to rats. *Drug Metab Dispos* **37:**2290–2298.

Ni T-Q, Hu S-Y, Si D-Y, Wei G-L, and Xia Y-Y (2015) Experimental study on pharmacokinetics of ReDuNing injection in human body. *Chinese tradit herb drugs* **46:**2270–2274.

Reagan-Shaw S, Nihal M, and Ahmad N (2008) Dose translation from animal to human studies revisited. *FASEB J* **22:**659–661.

Shang X-F, Pan H, Li M-X, Miao X-L, and Ding H (2011) *Lonicera japonica* Thunb.: ethnopharmacology, phytochemistry and pharmacology of an important traditional Chinese medicine. *J Ethnopharmacol* **138:**1–21.

Toutain PL and Bousquet-Mélou A (2004) Plasma clearance. *J Vet Pharmacol Ther* **27:**427–439.

Wang Y, Wen J, Zheng W, Zhao L, Fu X, Wang Z, Xiong Z, Li F, and Xiao W (2015) Simultaneous determination of neochlorogenic acid, chlorogenic acid, cryptochlorogenic acid and geniposide in rat plasma by UPLC-MS/MS and its application to a pharmacokinetic study after administration of ReDuNing injection. *Biomed Chromatogr* **29:**68–74.

Xu H-M, Wang Y, and Liu N-F (2009) Safety of an injection with a mixture of extracts from *Herba Artemisiae annuae*, *Fructus Gardeniae* and *Flos Lonicerae*. *Pharm World Sci* **31:**458–463.

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Footnotes

This work was supported by the National Science Fund of China for Distinguished Young Scholars [Grant 30925044], the National Science and Technology Major Project of China "Key New Drug Creation and Manufacturing Program" [Grants 2009ZX09304-002], and the National Basic Research Program of China [Grant 2012CB518403].

This work was previously presented as a poster presentation at the following workshop: Cheng et al. (2016) Pharmacokinetic study of ReDuNing injection in rats. *The 13th Asia Pacific Federation of Pharmacologist Meeting "New Paradigms in Pharmacology for Global Health"*, 2016 February 1–3; Bangkok, Thailand.

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Legends for Figures

Fig. 1. Iridoids (both compound names and ID numbers, black), organic acids (purple), and flavonoids (brown) present in ReDuNing injection. (**A**) Stacked chromatograms of herbal compounds from a typical sample of ReDuNing injection by mass spectrometry-based monitoring of their ionized molecules; (**B**), (**C**), and (**D**) content levels (mg/g) of compounds in the component herbs Zhizi (*G. Jasminoides* fruits), Jinyinhua (*L. japonicae* flower buds), and Qinghao (*A. annua* aerial part), respectively; (**E**) content levels (mM) of the compounds in samples of six lots of ReDuNing injection; (**F**) compound dose levels (μmol/person) of the compounds from ReDuNing injection (lot number, 141106) at the label daily dose 20 ml/person; (**G**) percentage doses of iridoids in the total dose of iridoids and percentage doses of organic acids in the total dose of organic acids in ReDuNing injection (141106). See Supplemental Table 1 for the compounds' names and the associated detection and characterization information.

Fig. 2. Systemic exposure to and excretion of unchanged iridoids (compound ID numbers, black), organic acids (purple), and flavonoids (brown) in rats receiving a 30-minute intravenous infusion of ReDuNing injection (lot number, 141106) at 2 ml/kg and chemical structures of the major circulating herbal compounds. ReDuNing compounds (shown as compound ID) in (**A**–**C**) are ranked according to their content levels in ReDuNing injection (Fig. 1E). See Supplemental Table 1 for the compounds' names. The symbol "×" (in red) denotes that the compound was not detected.

Fig. 3. Proposed metabolic pathways for the iridoids (in black) geniposide (**6**) and secologanic acid (**4**) and the organic acids (in purple) chlorogenic acid (**30**), cryptochlorogenic acid (**31**), and neochlorogenic acid (**29**) in rats intravenously receiving ReDuNing injection. The metabolite ID is used to indicate the compound being a metabolite, showing its parent compounds, type of metabolism, and metabolite isomer. For instance, **M30** in $\mathbf{M30_{M\text{-}1}}$ denotes that the compound is a metabolite of chlorogenic acid (**30**). The subscript letter **M** denotes "methylation" and the subscript number **1** denotes the first eluted metabolite isomer. The subscript letters **R**, **G**, **S**, and **Gsh** in other metabolite IDs denote "reduction", "glucuronidation", "sulfation", and "glutathionylation", respectively. Glc, β-D-glucopyranosyl; Glu, glucuronosyl; Gsh, glutathione.

TABLE 1

Pharmacokinetics of major circulating iridoids and organic acids in rats receiving a 30-minute intravenous infusion of ReDuNing injection

C_{max}, maximum plasma concentration; $AUC_{0\text{-}24h}$, area under the plasma concentration-time curve from 0 to 24 hours after starting infusion; $t_{1/2}$, elimination half-life; MRT, mean residence time; $CL_{tot,p}$, total plasma clearance; V_{SS}, apparent volume of distribution at steady state; $f_{e\text{-}U}$, fraction of dose excreted into urine; CL_R, renal clearance; and f_u, fraction of compound unbound to rat plasma proteins. **6**, geniposide; **4**, secologanic acid; **12**, secoxyloganin; **16**, genipin-1-*β*-gentiobioside; **3**, geniposidic acid; **2**, sweroside; **9**, shanzhiside; **30**, chlorogenic acid; **25**, quinic acid; **31**; cryptochlorogenic acid; and **29**, neochlorogenic acid.

PK parameter	Iridoid							Organic acid			
	6	**4**	**12**	**16**	**3**	**2**	**9**	**30**	**25**	**31**	**29**
C_{max} (μM)	71.7 ± 6.4	30.8 ± 4.0	19.1 ± 1.1	6.7 ± 0.9	11.3 ± 2.4	6.3 ± 0.4	4.6 ± 0.2	55.7 ± 4.2	51.9 ± 5.3	24.5 ± 2.1	19.0 ± 2.2
$AUC_{0\text{-}24h}$ (μM·hour)	36.4 ± 3.4	16.2 ± 3.0	12.1 ± 1.8	4.4 ± 0.6	16.1 ± 4.2	4.0 ± 0.6	2.9 ± 0.6	28.1 ± 3.0	35.3 ± 7.0	12.3 ± 1.5	8.8 ± 1.1
$t_{1/2}$ (hour)	0.3 ± 0.0	0.3 ± 0.0	0.4 ± 0.1	0.4 ± 0.0	0.9 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.3 ± 0.0	0.5 ± 0.1	0.3 ± 0.0	0.2 ± 0.0
MRT (hour)	0.5 ± 0.0	0.6 ± 0.0	0.7 ± 0.1	0.7 ± 0.0	1.2 ± 0.1	0.7 ± 0.1	0.6 ± 0.1	0.5 ± 0.0	0.7 ± 0.1	0.5 ± 0.0	0.5 ± 0.0
$CL_{tot,\ p}$ (l/hour/kg)	1.6 ± 0.2	1.4 ± 0.2	0.6 ± 0.1	0.8 ± 0.1	0.2 ± 0.0	0.6 ± 0.1	0.7 ± 0.1	1.3 ± 0.1	0.5 ± 0.1	1.3 ± 0.2	1.6 ± 0.2
V_{SS} (l/kg)	0.4 ± 0.0	0.4 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.2 ± 0.0	0.4 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0
$f_{e\text{-}U}$ (%)	74.3 ± 15.4	7.3 ± 3.2	87.5 ± 20.8	71.3 ± 18.4	334.8 ± 90.3	80.3 ± 24.6	64.0 ± 28.5	35.2 ± 11.8	89.7 ± 15.0	31.9 ± 14.9	44.2 ± 11.2
$f_{e\text{-}B}$ (%)	0.3 ± 0.1	0.1 ± 0.0	1.0 ± 0.2	1.4 ± 0.3	38.4 ± 24.7	1.3 ± 0.2	0.3 ± 0.1	0.0 ± 0.0	0.8 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
f_u (%)	92.3	91.2	75.2	80.4	88.0	95.7	99.0	56.9	65.8	48.8	65.3

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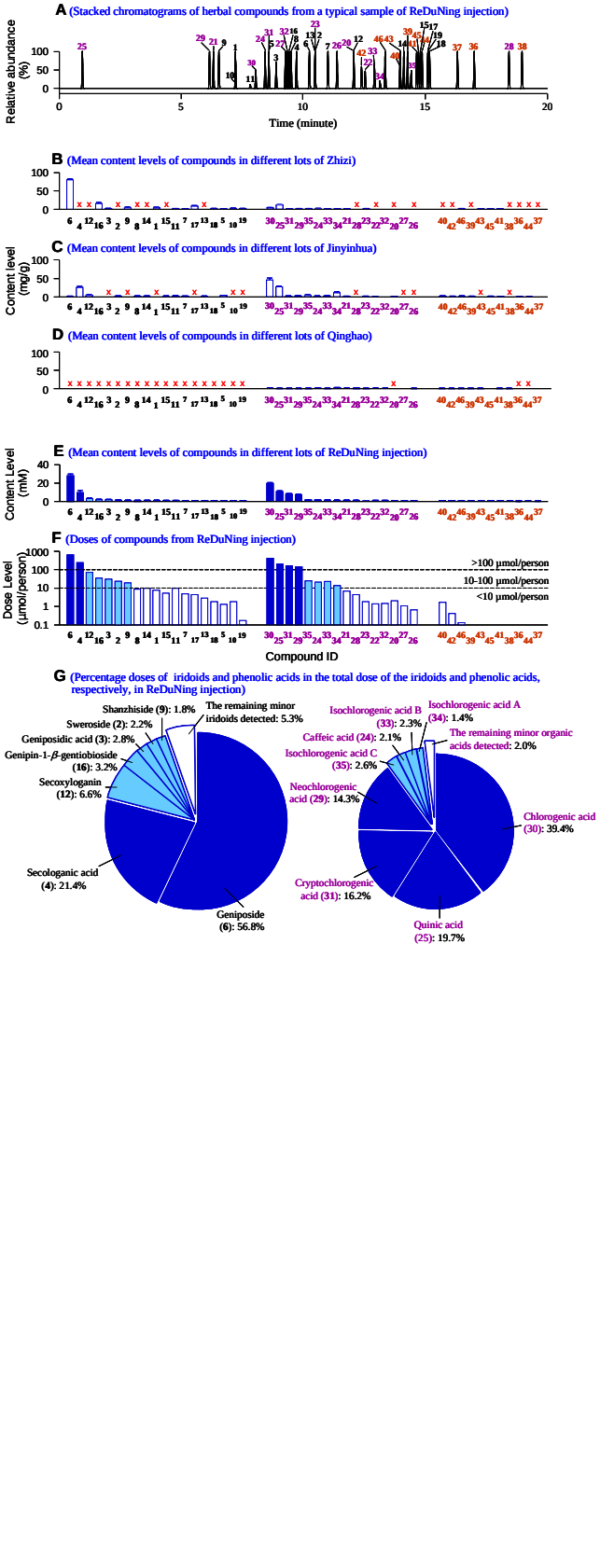
DMD #71647 - Figure 1



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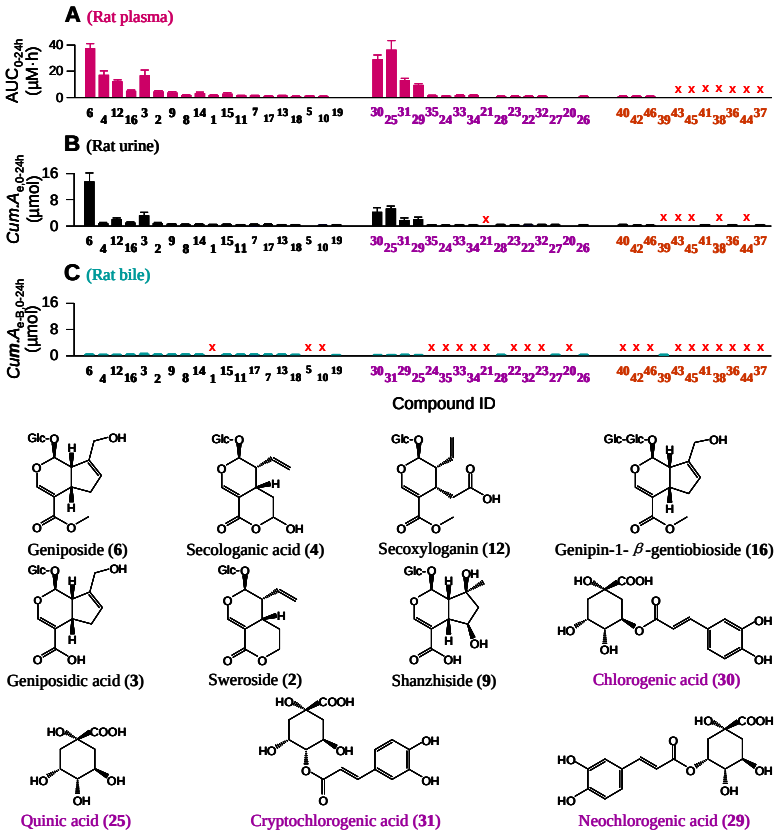

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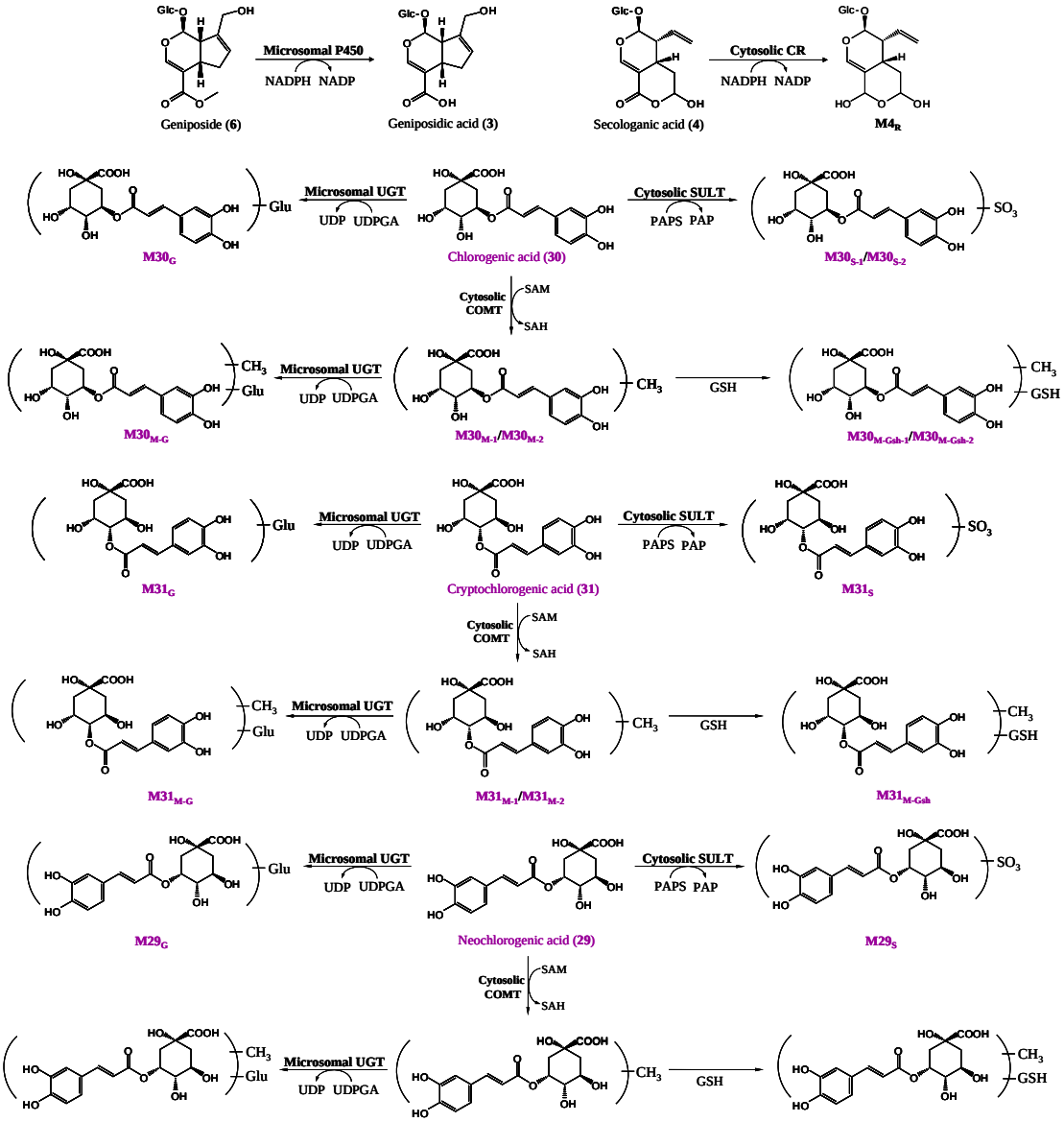
DMD #71647 - Figure 3



Pharmacokinetics and Disposition of Circulating Iridoids and Organic Acids in Rats Intravenously Receiving ReDuNing Injection

Chen Cheng, Feifei Du, Ke Yu, Fang Xu, Fengqing Wang, Li Li, Olajide E. Olaleye, Junling Yang, Feng Chen, Chenchun Zhong, Qiongwei Liu, Jing Li, Zhenzhong Wang, Chuan Li, and Wei Xiao

Drug Metabolism and Disposition

Supplemental Materials and Methods

ReDuNing Injection and Its Component Herbs. Samples of six lots (lot numbers: 140633, 140731, 140927, 140928, 141106, and 141108) of ReDuNing injection (China FDA ratification number, GuoYaoZhunZi-Z20050217) and samples of its component herbs *Gardenia Jasminoides* fruits (Zhizi), *Lonicera japonica* flower buds (Jinyinhua), and *Artemisia annua* aerial part (Qinghao) were obtained from Kanion Pharmaceutical Corporation (Lianyungang, Jiangsu Province, China). ReDuNing injection is standardized to contain geniposide at 7.2–12.6 mg/ml, secoxyloganin at 0.8–2.1 mg/ml, chlorogenic acid at 4.6–7.8 mg/ml, neochlorogenic acid at 1.8–3.7 mg/ml, and cryptochlorogenic acid at 1.7–3.6 mg/ml.

Chemicals and Reagents. Sweroside, geniposidic acid, loganic acid, geniposide, scandoside methyl ester, secoxyloganin, morroniside, genipin-1-*β*-gentiobioside, benzoic acid, protocatechuic aldehyde, *p*-hydroxycinnamic acid, caffeic acid, ferulic acid, syringic acid, neochlorogenic acid, chlorogenic acid, cryptochlorogenic acid, 1,5-dicaffeoylquinic acid, isochlorogenic acids A, B, and C, luteolin, quercetin, quercetin-7-*O*-glucopyranoside, quercetin-3-*O*-galactoside, rutin, kaempferol-7-*O*-glucopyranoside, isorhamnetin-3-*O*-glucopyranoside, kaempferol-3-*O*-rutinoside, apigenin-7-*O*-glucopyranoside, luteolin-7-*O*-glucoside, midazolam, dextromethorphan, 7-hydroxy-4-methylcoumarin, tanshinol, and 7-hydroxyflavone were obtained from Tauto Biotech (Shanghai, China). Secologanic acid was obtained from Shanghai Nature Standard R&D and Biotech (Shanghai, China). Loganin, shanzhiside, gardenoside, and chrysosplenol D were purchased from BioBioPha (Kunming, Yunnan Province, China). Salicylic acid and quinic acid were purchased from Sigma-Aldrich (St. Louis, MO). All the compounds had purity ≥98% by high performance liquid chromatography.

Nicotinamide adenine dinucleotide (NADH), glucose-6-phosphate monosodium salt, glucose-6-phosphate dehydrogenase, *S*-adenosylmethionine (SAM), uridine 5′-diphospho-glucuronic acid (UDPGA), 3′-phosphoadenosine-5′-phosphosulfate (PAPS), glutathione (GSH), alamethicin, *tris*-hydroxymethyl-aminomethane (Tris-base), taurocholic acid, HPLC-grade methanol, and HPLC-grade formic acid were also obtained from Sigma-Aldrich. Rat liver microsomes and cytosol were prepared in-house by differential centrifugation. Human liver microsomes and cytosol were obtained from Corning Gentest (Woburn, MA). Sodium heparin and isoflurane were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Pentobarbital was obtained from Shanghai Westang Biotechnology (Shanghai, China).

Rat Studies. All animal care and experimental procedures were in compliance with the *Guidance for Ethical Treatment of Laboratory Animals* (The Ministry of Science and Technology of China, 2006; www.most.gov.cn/fggw/zfwj/zfwj2006). The experimental protocols were approved by an Institutional

Animal Care and Use Committee at Shanghai Institute of Materia Medica (Shanghai, China). Animal studies are reported in accordance with the ARRIVE guidelines (Kilkenny et al., 2010). Male Sprague-Dawley rats (200–230 g) were obtained from the Sino-British SIPPR/BK Laboratory Animal (Shanghai, China); they were maintained in a unidirectional airflow room at 20–24ºC and relative humidity between 30% and 70% with a 12 hour light/dark cycle. Rats were allowed to acclimate to the facilities and environment for three days prior to receiving in-house femoral artery cannulation for blood sampling or bile duct cannulation for bile sampling (Chen et al., 2013). The operated rats were allowed to regain their preoperative body weight prior to the study and were euthanized with CO_2 gas after use. A total of 12 rats were used in the experiments described here.

Three rat studies were performed and rats received a single 30-minute intravenous infusion of ReDuNing injection (lot number, 141106) at 2 ml/kg via the tail veins using PHD 2000 infusion pumps (Harvard Apparatus, Holliston, MA). The rat dose was derived from the label daily dose of ReDuNing injection (20 ml/person, once daily) according to dose normalization by body surface area (Reagan-Shaw et al., 2008). In the first rat study, blood samples (~80 µl) were collected, from four rats, into heparinized tubes before and 0.083, 0.25, 0.5, 0.58, 0.75, 1, 1.5, 2.5, 4.5, 6.5, 8.5, and 24 hours after starting infusion; the plasma fractions were prepared by centrifugation of the blood samples. In the second rat study, urine samples were collected, from four rats, before and 0–4, 4–8, 8–24, and 24–48 hours after starting infusion and were weighed. For urine sampling, the rats were housed singly in metabolic cages and the urine collection tubes were frozen at −15°C. In the third rat study, bile samples were collected, from four rats, before and 0–2, 2–4, 4–6, 6–8, 8–24, and 24–48 hours after starting infusion and were weighed. A sodium taurocholate solution (pH 7.4) was infused, at 1.5 ml/hour, into the duodenum during the day of bile collection. All rat samples were stored at −70°C until analysis.

In Vitro Metabolism Studies. To characterize metabolism of herbal compounds in rats and to facilitate prediction of the metabolism in humans, metabolic capacities of the enzymes cytochrome P450 (P450), carbonyl reductase (CR), UDP-glucuronosyltransferase (UGT), catechol-*O*-methyltransferase (COMT), and sulfotransferase (SULT) for ReDuNing compounds (geniposide, secologanic acid, chlorogenic acid, quinic acid, cryptochlorogenic acid, and neochlorogenic acid) were assessed using rat liver microsomes and cytosol, as well as human liver microsomes and cytosol. The metabolites formed in vitro were analyzed and compared with the metabolites detected in rats receiving ReDuNing injection. The test compounds were those that exhibited dosed levels >100 µmol/person; they were geniposide, secologanic acid, chlorogenic acid, quinic acid, cryptochlorogenic acid, and neochlorogenic acid. Rat and human liver microsomes were fortified with NADPH for P450-mediated oxidation of test compounds and with UDPGA for UGT-mediated glucuronidation. Rat and human liver microsomes were unfortified or fortified with NADPH for P450-mediated oxidation of geniposide. Rat and human liver cytosol was fortified with SAM for COMT-mediated methylation, with PAPS for the SULT-mediated sulfation, and with NADPH for carbonyl reduction. The metabolic reactions were initiated by adding the test compound (90 µM) at 37ºC and were terminated, at 120 minutes, by adding two volumes of ice-cold methanol. The methanol-treated incubations were centrifuged at 1369 *g* for 10 minutes to yield the supernatants for analysis. The detailed incubation conditions were described in the earlier publications by Li et al. (2012) and Tian et al. (2015). Positive control reactions were implemented using the known substrates midazolam and dextromethorphan (for P450-mediated oxidation), 7-hydroxy-4-methylcoumarin (for UGT-mediated glucuronidation), tanshinol (for COMT-mediated methylation), and 7-hydroxyflavone

(for SULT-mediated sulfation), whereas negative control reactions were also implemented by incubation without liver microsomes or cytosol.

The preceding metabolic reactions were repeated for the test compounds by adding GSH and the incubations, after treating with two volumes of ice-cold methanol, were analyzed to determine the occurrence of GSH conjugates. In addition, the interplay of COMT with UGT and SULT were also evaluated using methods described by Li et al. (2015).

Determination of Plasma Protein Binding. A rapid ultrafiltration method by Guo et al. (2006) was used to assess fractions of compounds unbound to rat plasma proteins (f_u). The test compounds were geniposide, secologanic acid, secoxyloganin, genipin-1-β-gentiobioside, geniposidic acid, sweroside, shanzhiside, chlorogenic acid, quinic acid, cryptochlorogenic acid, and neochlorogenic acid. Microcon YM-30 centrifugal filter devices (Bedford, MA) were used for determination of f_u and nonspecific binding of the test compounds to the Microcon filter membrane was found to be negligible.

Liquid Chromatography/Mass Spectrometry-Based Assays. A Waters Synapt G2 high definition time-of-flight mass spectrometer (TOF-MS; Manchester, UK), interfaced via a Zspray/LockSpray ESI source with a Waters Acquity UPLC separation module (Milford, MA), was used for detection, characterization, and quantification of ReDuNing compounds and their metabolites. The mass spectrometer was operated in resolution mode with a resolving power of about 20,000 and the ESI source worked in the negative ion mode. The mass spectrometer was calibrated in a manner as described by Li et al. (2015). Initial detection of herbal compounds present in ReDuNing injection was achieved according to molecular masses of compounds reported for the component herbs of the injection and their electrospray ionization profiles. Detection of unchanged ReDuNing compounds in rat biosamples was achieved on the basis of the detection results of the herbal compounds present in the injection; detection of metabolites of major circulating ReDuNing compounds was achieved using an Accelrys metabolite database (version 2015.1; Accelrys, Inc., San Diego, CA) to collect prior knowledge of the likely metabolic pathways of the compounds (Williams et al., 2015). The subsequent characterization of unchanged herbal compounds detected in ReDuNing injection and in the biosamples was achieved by comparing with their corresponding reference standards with respect to accurate molecular mass, fragmentation profile by collision-induced dissociation, and chromatographic retention time. When the reference standard was not available, the detected compound was characterized by comparing with the compounds reported in the literature with respect to accurate molecular mass, fragmentation profile (if available), and elution order along with known compounds (if available). Chemical characterization of the detected herbal metabolites was performed according to the method by Anari et al. (2004). Oxidized metabolites formed via hydroxylation, desaturation, and oxidative methyl ester cleavage were characterized by mass changes of +15.9949, −2.0157, and −14.0157 Da, respectively, of the parent compound. Reduced metabolite formed via hydrogenation was characterized by mass changes of +2.0157 Da of the parent compound. Methylated metabolites were characterized by a mass change of +14.0157 Da of the parent compound. Glucuronides and sulfates were characterized by neutral losses of 176.0321 and 79.9568 Da, respectively. Protonated GSH conjugates were characterized by neutral loss of 129.0426 Da, whereas deprotonated GSH conjugates were characterized by detection of the fragment ion at *m/z* 272.0883 (Huang et al. 2015). For the compound detection and characterization, chromatographic separation was achieved on a Waters Acquity UPLC HSS T3 1.8-μm column (100 × 2.1 mm i.d.; Dublin, Ireland; kept at 45°C) using a mobile phase that consisted of solvent A (methanol/water, 1:99, v/v, containing 25 mM formic acid) and solvent B (methanol/water, 99:1, v/v, containing 25 mM formic acid). The mobile phase was delivered at 0.3

ml/minute. For detection of unchanged ReDuNing compounds, a gradient program was used as follows: 0–2 minutes, at 2% solvent B; 2–32 minutes, from 2% to 98% solvent B; 32–37 minutes, at 98% solvent B; and 37–42 minutes, at 2% solvent B. For detection of metabolized ReDuNing compounds, the gradient program was modified as: 0–1 minutes, 2% solvent B; 1–25 minutes, from 2% to 30% solvent B; 25–27 minutes, 98% solvent B; and 27–30 minutes, at 2% solvent B. Virtual quantification of metabolites was achieved by using the calibration curve of an available reference standard of an analog that bore close structural similarity to the analyte.

For quantification of ReDuNing compounds, the chromatographic method was modified by using a shortened gradient program (0–1 minute, 2% solvent B; 1–15 minutes, from 2% to 70% solvent B; 15–17 minutes, 98% solvent B; and 17–20 minutes, at 2% solvent B). No internal standard was used in the quantification of the multiple compounds. This is due to the difficulty in selecting an appropriate internal standard for a wide variety of compounds of interest; using a single stable isotope-labeled internal standard often does not adequately resolve matrix effect problems in multi-compound bioassays (Guo et al., 2006). Also, introducing multiple internal standards may limit the assay performance (Niessen et al., 2006; Li et al., 2007). Matrix effects were minimized by optimizing sample preparation and using small injection volume (5 μl). Matrix-matched calibration curves (37–9000 nM) were constructed using weighted ($1/X$ or $1/X^2$) linear regression of the peak areas (Y) of the major circulating ReDuNing compounds against the corresponding plasma nominal concentrations of the compounds (X, nM), which showed good linearity (r^2 >0.99). Assay validation was carried out according to the US FDA guidance on bioanalytical validation (2013; www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm368107.pdf) to prove that the bioanalytical assays were reliable for the intended applications. The bioassay lower limit of quantification (LLOQ) values were 37–111 nM for the major circulating iridoids (geniposide, secologanic acid, secoxyloganin, genipin-1-β-gentiobioside, geniposidic acid, sweroside, and shanzhiside) and 37–111 nM for the major circulating organic acids (chlorogenic acid, quinic acid, cryptochlorogenic acid, and neochlorogenic acid). The intra-day accuracy and precision values of the bioassay were 85–115% and 1–15% for the iridoids, respectively, and 87–108% and 3–12% for the organic acids, respectively. The inter-day accuracy and precision values of the bioassay were 94–112% and 4–15% for the iridoids, respectively, and 85–113% and 7–15% for the organic acids, respectively. The iridoids and organic acids were all stable in situations mimicking those encountered during sample storage, handling, and analysis, as indicated by the errors in the peak area values of the iridoids and organic acids for all the test rat plasma samples (freeze/thaw stability during and after three −70°C↔23°C cycles and short-term stability at room temperature for 5 h) and the supernatants resulting from the methanol-precipitated plasma samples (autosampler storage stability at 8°C for 18 h) were between +15% and −11% of nominal, and well within the limits of acceptability (not exceeding ±15%). For quantification of herbal compounds in the ReDuNing injection, the accuracy and precision values were 94–115% and 3–14% for the iridoids, respectively, and 100–108% and 1–10% for the organic acids, respectively.

Sample preparation method, supporting detection, characterization, and quantification of ReDuNing compounds, was as follows: ReDuNing injection was diluted in water before analysis. Pulverized Zhizi, Jinyinhua, and Qinghao were each extracted with 50% methanol; after centrifugation, the resulting supernatants were diluted with 50% methanol before analysis. Rat plasma, urine, and bile samples were precipitated with methanol at a volumetric sample-to-methanol ratio of 1:3; after centrifugation, the resulting supernatants were applied for analysis.

Data Processing. Pharmacokinetic parameters were estimated by noncompartmental analysis using Kinetica software (version 5.0; Thermo Scientific, Philadelphia, PA). The area under the concentration-time curve up to the last measured point in time ($AUC_{0\text{-}t}$) was calculated using the trapezoidal rule. The half-life ($t_{1/2}$) was calculated using the relationship $0.693/k_e$. The total plasma clearance ($CL_{tot,p}$) was estimated by dividing the compound dose by the $AUC_{0\text{-}\infty}$ and the distribution volume at steady state (V_{SS}) was estimated by multiplying the $CL_{tot,p}$ by the mean residence time (MRT). The renal excretory clearance (CL_R) was estimated by dividing the cumulative amount excreted into urine ($Cum.A_{e\text{-}U}$) by the plasma $AUC_{0\text{-}\infty}$. The fractions of dose excreted into urine ($f_{e\text{-}U}$) and the fractions of dose excreted into bile ($f_{e\text{-}B}$) were established using the relationship $Cum.A_{e\text{-}U}$/Dose and $Cum.A_{e\text{-}B}$/Dose, respectively. All data are expressed as the mean ± standard deviation.

Reference

Anari MR, Sanchez RI, Bakhtiar R, Franklin RB, and Baillie TA (2004) Integration of knowledge-based metabolic predictions with liquid chromatography data-dependent tandem mass spectrometry for drug metabolism studies: application to studies on the biotransformation of indinavir. *Anal Chem* **76:**823–832.

Chen F, Li L, Xu F, Sun Y, Du F-F, Ma X-T, Zhong C-C, Li X-X, Wang F-Q, and Zhang N-T et al. (2013) Systemic and cerebral exposure to and pharmacokinetics of flavonols and terpene lactones after dosing standardized *Ginkgo biloba* leaf extracts to rats via different administration routes. *Br J Pharmacol* **170:**440–457.

Guo B, Li C, Wang G-J, and Chen L-S (2006) Rapid and direct measurement of free concentrations of highly protein-bound fluoxetine and its metabolite norfluoxetine in plasma. *Rapid Commun Mass Spectrom* **20:**39–47.

Huang K, Huang L-Y, and van Breemen RB (2015) Detection of reactive metabolites using isotope-labeled glutathione trapping and simultaneous neutral loss and precursor ion scanning with ultra-high-pressure liquid chromatography triple quadruple mass spectrometry. *Anal Chem* **87:**3646−3654.

Kilkenny C, Browne W, Cuthill IC, Emerson M, and Altman DG (2010) Animal research: reporting in vivo experiments: the ARRIVE guidelines. *Br J Pharmacol* **160:**1577–1579.

Li L, Liang S-P, Du F-F, and Li C (2007) Simultaneous quantification of multiple licorice flavonoids in rat plasma. *J Am Soc Mass Spectrom* **18:**778–782.

Li L, Zhao Y-S, Du F-F, Yang J-L, Xu F, Niu W, Ren Y-H, and Li C (2012) Intestinal absorption and presystemic elimination of various chemical constituents present in GBE50 extract, a standardized extract of *Ginkgo biloba* leaves. *Curr Drug Metab* **13:**494–509.

Li M-J, Wang F-Q, Huang Y-H, Du F-F, Zhong C-C, Olaleye OE, Jia W-W, Li Y-F, Xu F, and Dong J-J et al. (2015) Systemic exposure to and disposition of catechols, derived from *Salvia miltiorrhiza* roots (Danshen), after intravenous dosing DanHong injection in human subjects, rats, and dogs. *Drug Metab Dispos* **43:**679–690.

Niessen WMA, Manini P, and Andreoli R (2006) Matrix effects in quantitative pesticide analysis using liquid chromatography-mass spectrometry. *Mass Spectrom Rev* **25:**881–899.

Reagan-Shaw S, Nihal M, and Ahmad N (2008) Dose translation from animal to human studies revisited. *FASEB J* **22:**659–661.

Tian D-D, Jia W-W, Liu X-W, Wang D-D, Liu J-H, Dong J-J, Li L, Du F-F, Xu F, and Wang F-Q et al. (2015) Methylation and its role in the disposition of tanshinol, a cardiovascular carboxylic catechol from *Salvia miltiorrhiza* roots (Danshen). *Acta Pharmacol Sin* **36:**627–643.

Williams AJ, Ekins S, Spjuth O, and Willighagen EL (2012) Accessing, using, and creating chemical property databases for computational toxicology modeling. *Methods Mol Biol* **929:**221–241.

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Drug Metabolism and Disposition

Supplemental Table 1

Detection of herbal compounds in ReDuNing injection

ID	Compound	LC/TOF-MS^E data			Molecular mass (Da)	Molecular formula	Log*P*[c]	Compound dose level[d] (μmol/person)	Lot-to-lot variability (%)
		t_R[a] (minute)	$[M-H]^-$ (*m/z*)	Fragmentation profile[b] (*m/z*)					
Iridoids									
1	Jasminoside	7.23	345.1544	165.0921, **337.0923**	346.1622	$C_{16}H_{26}O_8$	−1.01	6.8 ± 0.5	6.9
2	Sweroside	10.47	357.1186	81.0343, **125.0240**, 179.0550, 195.0656	358.1264	$C_{16}H_{22}O_9$	−1.53	20.9 ± 2.8	13.3
3	Geniposidic acid	8.86	373.1135	123.0449, **149.0603**, 167.0705, 211.0609	374.1213	$C_{16}H_{22}O_{10}$	−1.55	31.8 ± 2.1	6.6
4	Secologanic acid	9.73	373.1135	149.0606, 193.0499, 207.0657, **225.0762**	374.1213	$C_{16}H_{22}O_{10}$	−2.78	180.5 ± 47.7	26.4
5	Loganic acid	8.61	375.1291	113.0239, 151.0755, 169.0863, **213.0761**	376.1369	$C_{16}H_{24}O_{10}$	−1.89	1.4 ± 0.3	24.5
6	Geniposide	10.28	387.1291	123.0450, **163.0399**, 207.0651, 225.0764	388.1369	$C_{17}H_{24}O_{10}$	−1.62	558.6 ± 34.2	6.1
7	Loganin	10.98	389.1448	127.0399, **227.0929**	390.1526	$C_{17}H_{26}O_{10}$	−2.02	3.4 ± 1.0	30.2
8	Deacetylasperulosdic acid	9.50	389.1084	121.0658, **149.0610**, 167.0713, 345.1188	390.1162	$C_{17}H_{26}O_{10}$	−2.34	14.6 ± 10.7	73.2
9	Shanzhiside	6.52	391.1241	185.0814, 229.0713, **351.0714**	392.1319	$C_{16}H_{24}O_{11}$	−2.58	17.3 ± 1.0	6.0
10	Gardenoside	7.22	403.1241	191.0350, **241.0716**, 371.0971	404.1319	$C_{17}H_{24}O_{11}$	−3.44	1.3 ± 0.6	49.7
11	Scandoside methyl ester	7.83	403.1241	101.0245, 139.0399, **241.0714**	404.1319	$C_{17}H_{24}O_{11}$	−2.27	6.3 ± 3.4	54.1
12	Secoxyloganin	12.10	403.1241	121.0290, **219.0630**, 223.0604, 371.0982	404.1319	$C_{17}H_{24}O_{11}$	−0.81	61.6 ± 8.1	13.2

13	Morroniside	10.47	405.1397	95.0137, 141.0556, **155.0352**, 243.0873	406.1475	$C_{17}H_{26}O_{11}$	−2.66	1.7 ± 1.1	61.7
14	Lonijaposide J	14.12	456.1506	180.0663, 224.0560, **276.0880**, 381.1184	457.1584	$C_{20}H_{27}NO_{11}$	−2.30	8.5 ± 2.3	26.9
15	Xylostosidine acid	14.83	458.1121	234.0593, **335.0794**, 395.0978, 435.1289	459.1199	$C_{19}H_{25}NSO_{10}$	−2.06	6.3 ± 1.0	15.5
16	Genipin-1-*β*-gentiobioside	9.42	549.1820	101.0240, 123.0447, **225.0765**, 245.0813	550.1898	$C_{23}H_{34}O_{15}$	−1.99	31.2 ± 2.1	6.8
17	6"-*O*-Coumaroylgenipin gentiobioside	15.07	695.2187	145.0294, 469.1346, 493.1712, **525.1613**	696.2265	$C_{32}H_{40}O_{17}$	0.55	3.3 ± 0.6	19.4
18	6"-*O*-Feruloylgenipin gentiobioside	15.21	725.2293	**291.0904**, 446.0837, 461.1087	726.2371	$C_{33}H_{42}O_{18}$	0.04	1.4 ± 0.2	13.1
19	6"-*O*-Sinapoylgenipin gentiobioside	15.07	755.2399	291.0911, 469.1344, **532.1817**	756.2477	$C_{34}H_{44}O_{19}$	0.05	0.2 ± 0.0	10.3
Organic acids									
20	Benzoic acid	12.11	121.0290	**77.0422**	122.0368	$C_7H_6O_2$	1.87	1.4 ± 0.3	23.4
21	Protocatechuic aldehyde	6.35	137.0238	**108.0211**, 109.0279	138.0316	$C_7H_6O_3$	1.07	7.9 ± 1.1	13.6
22	Salicylic acid	12.54	137.0239	**93.0347**	138.0317	$C_7H_6O_3$	2.37	1.6 ± 0.3	19.2
23	*p*-Hydroxycinnamic acid	10.45	163.0395	**119.0503**, 123.0450	164.0473	$C_9H_8O_3$	1.69	1.7 ± 0.1	6.3
24	Caffeic acid	8.41	179.0345	134.0367, **135.0446**	180.0423	$C_9H_8O_4$	1.35	20.4 ± 1.8	8.8
25	Quinic acid	0.96	191.0556	**85.0292**, 111.0449, 127.0400, 171.0309	192.0634	$C_7H_{12}O_6$	−1.93	208.7 ± 15.6	7.5
26	Ferulic acid	11.41	193.0501	133.0292, **134.0373**, 178.0272	194.0579	$C_{10}H_{10}O_4$	1.68	0.7 ± 0.1	19.4
27	Syringic acid	9.30	197.0450	123.0084, 138.0322, **153.0556**, 182.0220	198.0528	$C_9H_{10}O_5$	1.25	1.2 ± 0.1	8.8
28	6-Carboxy-10-methyl-methylene-1-(1-oxobutyl)-cyclohexaneacetic acid	18.44	281.1389	177.1283, 193.1596, 219.1389, **237.1492**	282.1467	$C_{15}H_{22}O_5$	1.96	4.5 ± 0.4	9.9
29	Neochlorogenic acid	6.19	353.0873	135.0444, 179.0342, **191.0555**	354.0951	$C_{16}H_{18}O_9$	−0.33	136.9 ± 4.3	3.1
30	Chlorogenic acid	8.09	353.0873	135.0445,179.0341, **191.0556**	354.0951	$C_{16}H_{18}O_9$	−0.33	381.2 ± 30.1	7.9
31	Cryptochlorogenic acid	8.59	353.0873	135.0442, **173.0446**, 179.0342, 191.0553	354.0951	$C_{16}H_{18}O_9$	−0.74	153.0 ± 7.0	4.6
32	1,5-Dicaffeoylquinic acid	9.36	515.1190	**191.0556**, 335.0764, 353.0878, 357.0588	516.1268	$C_{25}H_{24}O_{12}$	1.23	1.5 ± 0.2	14.2
33	Isochlorogenic acid B	12.89	515.1190	191.0551, 201.0157, 335.0761, **375.0690**	516.1268	$C_{25}H_{24}O_{12}$	1.12	19.1 ± 2.2	11.5
34	Isochlorogenic acid A	13.10	515.1190	191.0549, 201.0154, 335.0783, **375.0694**	516.1268	$C_{25}H_{24}O_{12}$	1.16	10.8 ± 1.7	16.1
35	Isochlorogenic acid C	14.46	515.1190	173.0448, 285.0398, 353.0871, **447.0923**	516.1268	$C_{25}H_{24}O_{12}$	1.11	20.5 ± 2.8	13.5

Flavonoids									
36	Luteolin	16.99	285.0399	**133.0290**, 175.0394, 199.0389, 217.0500	286.0477	$C_{15}H_{10}O_6$	2.35	0.001 ± 0.000	26.3
37	Quercetin	16.35	301.0349	121.0286, 151.0029, **178.9980**, 273.0398	302.0427	$C_{15}H_{10}O_7$	1.95	0.0009 ± 0.0001	16.0
38	Chrysosplenol D	18.99	359.0767	286.0115, 314.0066, 329.0298, **344.0536**	360.0845	$C_{18}H_{16}O_8$	2.40	0.002 ± 0.001	37.2
39	Apigenin-7-*O*-glucopyranoside	14.31	431.0978	149.0240, 240.0436, **268.0381**, 311.0562	432.1056	$C_{21}H_{20}O_{10}$	0.18	0.09 ± 0.02	23.0
40	Luteolin-7-*O*-glucoside	13.97	447.0928	284.0324, **285.0399**, 286.0431	448.1006	$C_{21}H_{20}O_{11}$	0.13	1.6 ± 0.2	14.6
41	Kaempferol-7-*O*-glucopyranoside	14.67	448.0928	151.0033, 257.0450, **285.0397**, 307.0221	449.1006	$C_{21}H_{20}O_{11}$	1.44	0.006 ± 0.002	24.7
42	Quercetin-7-*O*-glucopyranoside	12.41	463.0877	**301.0349**	464.0955	$C_{21}H_{20}O_{12}$	1.33	0.4 ± 0.0	11.1
43	Quercetin-3-O-galactoside	13.96	463.0877	255.0289, 271.0243, **300.0270**, 323.0166	464.0955	$C_{21}H_{20}O_{11}$	0.46	0.05 ± 0.01	16.6
44	Isorhamnetin-3-*O*-glucopyronoside	14.91	477.1033	243.0295, 271.0247, 299.0193, **314.0428**	478.1111	$C_{22}H_{22}O_{12}$	0.67	0.001 ± 0.000	32.4
45	Kaempferol-3-*O*-rutinoside	14.73	593.1507	255.0291, **285.0398**, 327.0508	594.1585	$C_{27}H_{30}O_{15}$	0.10	0.02 ± 0.01	21.5
46	Rutin	13.36	609.1456	178.9973, **301.0335**, 343.0452	610.1534	$C_{27}H_{30}O_{16}$	−0.21	0.1 ± 0.0	27.9

[a] t_R, chromatographic retention time.

[b] Number in bold, product ion of base peak.

[c] Log*P* value was calculated using via the ACD (Advanced Chemistry Development Inc, Toronto, Canada).

[d] Compound dose levels of iridoids, organic acids, and flavonoids present in six lots of ReDuNing injections.

Pharmacokinetics and Disposition of Circulating Iridoids and Organic Acids in Rats Intravenously Receiving ReDuNing Injection

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Drug Metabolism and Disposition

Supplemental Table 2A

Detection of metabolites of geniposide (**6**), chlorogenic acid (**30**), cryptochlorogenic acid (**31**), and neochlorogenic acid (**29**) in rats intravenously receiving ReDuNing injection

Metabolite ID	Metabolite	Rat sample	LC/TOF-MS^E detection and characterization			Molecular Mass (dalton)
			t_R (minute)	Ionized molecule (*m/z*)	Characteristic fragment ion (CFI) or neutral loss (NL) (*m/z* or dalton)	
Geniposide (**6**, *molecular mass: 387.1291*)						
Geniposidic acid (**3**)	Demethylated metabolites	Plasma/Urine	9.65	$[M-H]^-$/373.1136	CFI: 149.0611, 193.0521	374.1213
Secologanic acid (**4**, *molecular mass: 374.1213*)						
M4$_R$	Hydrogenated metabolites	Plasma/Urine	15.65	$[M-H]^-$/375.1288	CFI: 151.0754, 195.0651	376.1369
Chlorogenic acid (**30**, *354.0951*)						
M30$_{M-1}$	Methylated metabolites	Plasma/Urine	20.03	$[M-H]^-$/367.1039	CFI: 173.0444, 191.0553	368.1108
M30$_{M-2}$			21.74	$[M-H]^-$/367.1027	CFI: 173.0452, 179.0549	
M30$_{S-1}$	Sulfates	Plasma/Urine	11.61	$[M-H]^-$/433.0436	NL: 79.9566	434.0519
M30$_{S-1}$			12.84	$[M-H]^-$/433.0445	NL: 79.9575	
M30$_G$	Glucuronides	Plasma/Urine	13.64	$[M-H]^-$/529.1196	NL: 176.0318	530.1272
M30$_{M-G}$	Glucuronides of methylated	Plasma/Urine	17.03	$[M-H]^-$/543.1348	NL: 176.0318 (compared with **M30$_M$**)	544.1428

	metabolites					
M30$_{M\text{-}Gsh\text{-}1}$	Glutathione conjugates of methylated metabolites	Bile	10.92	$[M-H]^-$/674.1863 $[M+H]^+$/676.2030	CFI: 272.0883 NL: 129.0441	675.1946
M30$_{M\text{-}Gsh\text{-}2}$			12.08	$[M-H]^-$/674.1870 $[M+H]^+$/676.2040	CFI: 272.0883 NL: 129.0449	
Cryptochlorogenic acid (**31**, *354.0951*)						
M31$_{M\text{-}1}$	Methylated metabolites	Plasma/Urine	20.27	$[M-H]^-$/367.1027	CFI: 173.0446, 193.0492	368.1108
M31$_{M\text{-}2}$			22.01	$[M-H]^-$/367.1027	CFI: 173.0448, 193.0496	
M31$_{S}$	Sulfates	Plasma/Urine	13.69	$[M-H]^-$/433.0439	NL: 79.9569	434.0519
M31$_{G}$	Glucuronides	Plasma/Urine	14.92	$[M-H]^-$/529.1189	NL: 176.0319	530.1272
M31$_{M\text{-}G}$	Glucuronides of methylated metabolites	Plasma/Urine	18.25	$[M-H]^-$/543.1337	NL:176.0310 (compared with **M31$_{M}$**)	544.1428
M31$_{M\text{-}Gsh}$	Glutathione conjugates of methylated metabolites	Bile	8.95	$[M-H]^-$/674.1856 $[M+H]^+$/676.2040	CFI: 272.0883 NL: 129.0433	675.1946
Neochlorogenic acid (**29**, *354.0951*)						
M29$_{M\text{-}1}$	Methylated metabolites	Plasma/Urine	14.30	$[M-H]^-$/367.1027	CFI: 193.0499	368.1108
M29$_{M\text{-}2}$			16.37	$[M-H]^-$/367.1027	CFI: 193.0497	
M29$_{S}$	Sulfates	Plasma/Urine	8.41	$[M-H]^-$/433.0441	NL: 79.9575	434.0519
M29$_{G}$	Glucuronides	Plasma/Urine	9.88	$[M-H]^-$/529.1187	NL: 176.0321	530.1272
M29$_{M\text{-}G}$	Glucuronides of methylated metabolites	Plasma/Urine	13.59	$[M-H]^-$/543.1352	NL: 176.0325 (compared with **M29$_{M}$**)	544.1428
M29$_{M\text{-}Gsh}$	Glutathione conjugates of methylated metabolites	Bile	11.29	$[M-H]^-$/674.1868 $[M+H]^+$/676.2032	CFI: 272.0884 NL: 129.0438	675.1946

The metabolite ID is used to indicate the compound being a metabolite, showing its parent compounds, type of metabolism, and metabolite isomer. For instance, **M30** in **M30$_{M\text{-}1}$** denotes that the compound is a metabolite of chlorogenic acid (**30**). The subscript letter **M** denotes "methylation" and the subscript number **1** denotes the first eluted metabolite isomer. The subscript letters **R**, **G**, **S**, and **Gsh** in other metabolite IDs denote "reduction", "glucuronidation", "sulfation", and "glutathionylation", respectively. t_R, chromatographic retention time.

Supplemental Table 2B

Detection of metabolites of geniposide (**6**), chlorogenic acid (**30**), cryptochlorogenic acid (**31**), and neochlorogenic acid (**29**) after incubation with rat and human liver cytosol and microsomes

Subcellular fraction	Cofactor	Metabolite ID	LC/TOF-MS^E detection and characterization			Molecular Mass (dalton)
			t_R (minute)	Ionized molecule (*m/z*)	Characteristic fragment ion (CFI) or neutral loss (NL) (*m/z* or dalton)	
Geniposide (**6**, *molecular mass: 387.1291*)						
RLM	NADPH	Geniposidic acid (**3**)	9.70	$[M-H]^-$/373.1130	CFI: 149.0594, 193.0484, 211.0615	374.1213
HLM			9.86	$[M-H]^-$/373.1134	CFI: 149.0596, 193.0474, 211.0615	
Secologanic acid (**4**, *molecular mass: 374.1213*)						
RLC	NADPH	**M4$_R$**	15.59	$[M-H]^-$/375.1289	CFI: 151.0762, 195.0657	376.1369
HLC			15.57	$[M-H]^-$/375.1285	CFI: 151.0768, 195.0643	
Chlorogenic acid (**30**, *354.0951*)						
RLC	SAM	**M30$_{M-1}$**	20.02	$[M-H]^-$/367.1028	CFI: 173.0444, 191.0553	368.1108
		M30$_{M-2}$	21.71	$[M-H]^-$/367.1024	CFI: 173.0452, 179.0549	
	PAPS	**M30$_{S-1}$**	11.61	$[M-H]^-$/433.0431	NL: 79.9561	434.0519
		M30$_{S-2}$	12.84	$[M-H]^-$/433.0431	NL: 79.9561	
	SAM + GSH	**M30$_{M-Gsh-1}$**	11.29	$[M-H]^-$/674.1868	CFI: 272.0885	675.1946
				$[M+H]^+$/676.2029	NL: 129.0444	
		M30$_{M-Gsh-2}$	12.08	$[M-H]^-$/674.1847	CFI: 272.0882	
				$[M+H]^+$/676.2040	NL: 129.0449	
RLM	UDPGA	**M30$_G$**	13.65	$[M-H]^-$/529.1188	NL: 176.0318	530.1272
RLC + RLM	SAM + UDPGA	**M30$_{M-G}$**	17.03	$[M-H]^-$/543.1348	NL: 176.0316 (compared with **M30$_M$**)	544.1428

HLC	SAM	**M30$_{M-1}$**	20.03	$[M-H]^-$/367.1029	CFI: 191.0558	368.1108
		M30$_{M-2}$	21.77	$[M-H]^-$/367.1030	CFI: 173.0447, 193.0501	
	PAPS	**M30$_{S-2}$**	13.16	$[M-H]^-$/433.0451	NL: 79.9581	434.0519
Cryptochlorogenic acid (**31**, *354.0951*)						
RLC	SAM	**M31$_{M-1}$**	20.26	$[M-H]^-$/367.1027	CFI: 173.0447, 193.0499	368.1108
		M31$_{M-2}$	22.00	$[M-H]^-$/367.1026	CFI: 173.0447, 193.0494	
	PAPS	**M31$_{S}$**	13.69	$[M-H]^-$/433.0436	NL: 79.9560	434.0519
	SAM + GSH	**M31$_{M-Gsh}$**	8.95	$[M-H]^-$/674.1866	CFI: 272.0883	675.1946
				$[M+H]^+$/676.2040	NL: 129.0433	
RLM	UDPGA	**M31$_{G}$**	14.91	$[M-H]^-$/529.1189	NL: 176.0313	530.1272
RLC + RLM	SAM + UDPGA	**M31$_{M-G}$**	18.23	$[M-H]^-$/543.1342	NL: 176.0316 (compared with **M31$_{M}$**)	544.1428
HLC	SAM	**M31$_{M-1}$**	20.39	$[M-H]^-$/367.1028	CFI: 173.0448, 193.0501	
		M31$_{M-2}$	22.14	$[M-H]^-$/367.1028	CFI: 173.0449, 193.0499	368.1108
	PAPS	**M31$_{S}$**	13.88	$[M-H]^-$/433.0441	NL: 79.9567	
Neochlorogenic acid (**29**, *354.0951*)						
RLC	SAM	**M29$_{M-1}$**	14.29	$[M-H]^-$/367.1031	CFI: 193.0500	368.1108
		M29$_{M-2}$	16.37	$[M-H]^-$/367.1029	CFI: 193.0499	
	PAPS	**M29$_{S}$**	8.41	$[M-H]^-$/433.0429	NL: 79.9555	434.0519
	SAM + GSH	**M29$_{M-Gsh}$**	10.92	$[M-H]^-$/674.1857	CFI: 272.0881	675.1946
				$[M+H]^+$/676.2040	NL: 129.0444	
RLM	UDPGA	**M29$_{G}$**	9.92	$[M-H]^-$/529.1147	NL: 176.0273	530.1272
RLC + RLM	SAM + UDPGA	**M29$_{M-G}$**	13.61	$[M-H]^-$/543.1342	NL: 176.0321 (compared with **M29$_{M}$**)	544.1428
HLC	SAM	**M29$_{M-1}$**	14.03	$[M-H]^-$/367.1029	CFI: 173.0438, 193.0499	368.1108
		M29$_{M-2}$	16.11	$[M-H]^-$/367.1026	CFI: 173.0445, 193.0497	

RLM, rat liver microsomes; HLM, human liver microsomes; RLC, rat liver cytosol; an NADPH-generating system, 3.3 mM magnesium chloride, 3.3 mM of glucose-6-phosphate, 0.5 $U \cdot ml^{-1}$ glucose-6-phosphate dehydrogenase and 1.3 mM nicotinamide adenine dinucleotide; SAM, *S*-adenosylmethionine; PAPS, 3′-phosphoadenosine-5′-phosphosulfate; UDPGA, uridine 5’-diphosphoglucuronic acid; GSH, glutathione. t_R, chromatographic retention time.

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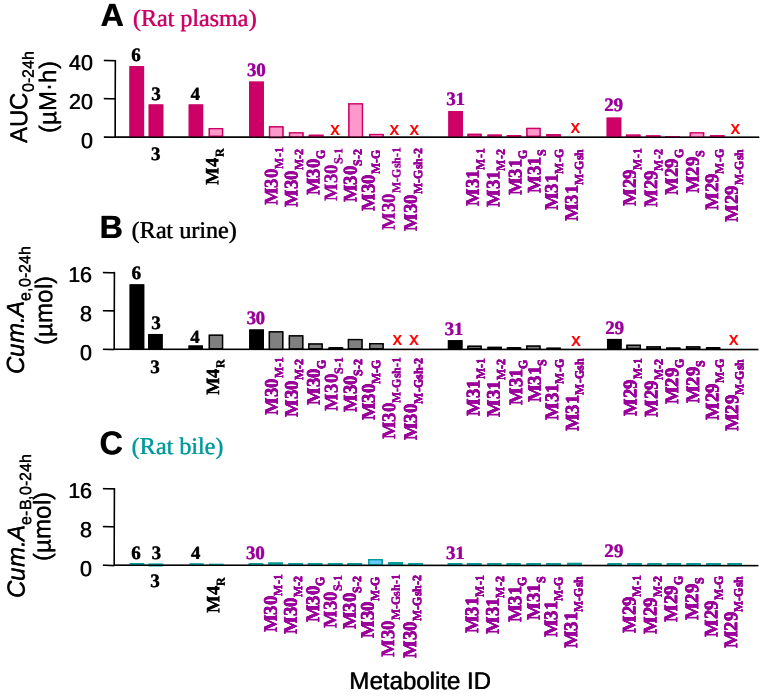
Drug Metabolism and Disposition

Supplemental Fig. 1



Systemic exposure to and excretion of metabolites of geniposide (**6**), secologanic acid (**4**), chlorogenic acid (**30**), cryptochlorogenic acid (**31**), and neochlorogenic acid (**29**) in rats receiving a 30-minute intravenous infusion of ReDuNing injection (lot number, 141106) at 2 ml/kg. The metabolite ID is used to indicate the compound being a metabolite, showing its parent compounds, type of metabolism, and metabolite isomer. For instance, **M30** in $\mathbf{M30_{M\text{-}1}}$ denotes that the compound is a metabolite of chlorogenic acid (**30**). The subscript letter **M** denotes "methylation" and the subscript number **1** denotes the first eluted metabolite isomer. The subscript letters **R**, **G**, **S**, and **Gsh** in other metabolite IDs denote "reduction", "glucuronidation", "sulfation", and "glutathionylation", respectively. The iridoid **3** detected in the rat biosamples resulted from dosed ReDuNing injection in part and from biotransformation of **6** in part. The metabolites of the organic acids **30**, **31**, and **29** are shown in a left-to-right ranking order of methylated metabolites, glucuronides, sulfates, glucuronides of methylated metabolites, and glutathione conjugates of methylated metabolites.

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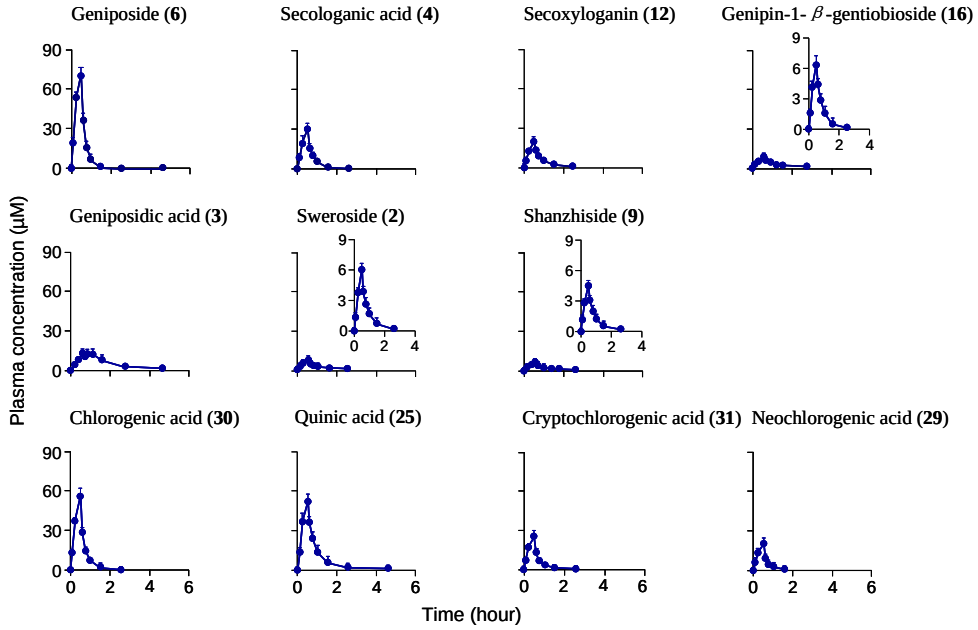
Drug Metabolism and Disposition

Supplemental Fig. 2



Plasma concentrations of major circulating iridoids and organic acids over time in rats receiving a 30-minute intravenous infusion of ReDuNing injection at 2 ml/kg.