Radix Polygoni Multiflori

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Contributor: Dan Li , Mengbi Yang , Zhong Zuo

Radix Polygoni Multiflori (RPM), the dry root of Polygonum multiflorum Thunb. (Fam. Polygonaceae), has been used as a tonic and an anti-aging remedy for centuries. However, its safe and effective application in clinical practice could be hindered by its liver injury potential and lack of investigations on its hepatotoxicity mechanism.

Radix Polygoni Multiflori | herb induced liver injury | pharmacokinetics | Mechanism

1. Introduction

Radix Polygoni Multiflori (RPM) is the dry root of Polygonum multiflorum Thunb. (Fam. Polygonaceae). It could be used as raw material (raw RPM) or after steaming with black bean juice (processed RPM) in traditional Chinese medicine since the Tang dynasty with different indications [1]. According to Chinese Pharmacopeia, raw RPM at 3–6 g/person/day is mainly used for detoxification, eliminating carbuncle, preventing malaria, relaxing the bowel [1], while processed RPM at 3–12 g/person/day is used for nourishing the liver and kidney, supplementing the essence and blood, blackening hair, strengthening bones and muscles, eliminating dampness, and reducing lipids [1]. In addition to the clinical indications stated in Pharmacopeia, RPM and its major components, including 2,3,5,4′-tetrahydroxystilbene-2-O-β-D-glucopyranoside (TSG), emodin, emodin-8-O-β-D-glucopyranoside (EMG), and polysaccharides have also demonstrated pharmacological activities for anti-aging [2][3], immunomodulating [4][5], hepatoprotective [6][7], anticancer [8], and anti-inflammatory [9] effects, etc., in various preclinical studies.

Despite the wide use of RPM as a medicine or health supplement, an increasing number of hepatic adverse effect reports of RPM or proprietary Chinese medicinal products containing it have been constantly received since the 1990s in China and other countries [10][11][12][13][14][15][16]. Since the occurrence of hepatotoxicity cases associated with RPM has raised serious concerns regarding its safety in clinical practice, drug regulatory agencies of Canada, Australia, the United Kingdom (UK), and China have conducted monitoring of the usage of RPM [17][18][19][20]. To explore the potential hepatotoxicity mechanisms of RPM, many preclinical studies on the pharmacokinetic characteristics and liver injury mechanisms associated with RPM and its major constituents, including TSG, emodin, and physcion, have been performed. Besides hepatotoxicity, it was found that emodin, the major component of RPM, also has carcinogenic activity and kidney toxicity [21]. Although the botany, phytochemistry, quality evaluation, traditional uses, pharmacological research, and toxicology of RPM have been well-reviewed [22][23][24], there is no comprehensive information about the pharmacokinetic characteristics of RPM and mechanisms of its induced liver injury.

Chemical Constituents in RPM

Major chemical constituents in RPM include stilbenes, anthraquinones, flavonoids, and phenolic acids, etc. [25][26], with stilbenes and anthraquinones as the two major phytochemical groups for these components. As shown in Figure 1, among the stilbenes, including TSG, resveratrol, and oxyresveratrol, TSG is the most abundant. Among the major anthraquinones, including emodin, physcion, aloe-emodin, rhein, chrysophanol, EMG, emodin-8-O-(6′-O-malonyl)-glucopyranoside, physcion-8-O-β-D-glucopyranoside, and physcion-8-O-(6′-O-malonyl)-glucopyranoside. etc., emodin and EMG are the two most abundant [25][27][28]. Processing of RPM could decrease the contents of EMG and physcion-8-O-β-D-glucopyranoside and increase that of their corresponding aglycones, emodin, and physcion [28]. Chinese Pharmacopeia suggests that contents of TSG should not be less than 1.0% and 0.7% in the raw material and processed herb of RPM, respectively, and the combined contents of emodin and physcion should be greater than 0.1% in both raw and processed RPM [1]. The Hong Kong Standard of Material of Medica requires that the content of TSG should not be less than 2.2% in raw RPM [29].

2. Pharmacokinetics

2.1. Pharmacokinetic studies of RPM extract

So far, pharmacokinetics properties of the major components in RPM extract have primarily been investigated in rats. TSG, emodin, EMG, aloe-emodin, physcion, oxyresveratrol and rhein could be detected after oral administrations of RPM extracts (equivalent to 40 g raw RPM/kg) to rats. With the doses decreasing from 40 g/kg to 10 g/kg and 20 g/kg, oxyresveratrol and rhein became undetectable in plasma [25]. The pharmacokinetic parameters indicated that the absorption and elimination of these major components were generally fast with time to maximum plasma concentration (T_{max}) less than 2 h for TSG, emodin and physcion, and half-lives of these three compounds ranged from 0.18 to 8.37 h after a single dose of RPM extracts orally administrated to SD rats [25][27][30][31][32]. Since the area under the concentration-time curve (AUC) and peak concentration (C_{max}) of TSG and emodin increased linearly along with the dose of RPM extract increasing from 10 g/kg (TSG: 327.9 mg/kg, emodin: 5.6 mg/kg) to 40 g/kg (TSG: 1312.0 mg/kg, emodin: 22.3 mg/kg), linear pharmacokinetics of TSG and after single oral administration of RPM extracts in rats were suggested [25].

The pharmacokinetics of TSG, emodin and EMG after multiple dosing of RPM extracts to SD rats [33] found that the AUC and C_{max} values of TSG and emodin could significantly increase after 11 days of treatment of RPM extracts, which could be attributed to the change in metabolic enzymes after repeated RPM extracts administrations [34]. As for EMG, it was only detectable at a few time points after prolonged treatment of RPM possibly due to its low content in RPM and low oral bioavailability *in vivo*.

2.2. Pharmacokinetic properties of TSG, emodin and physcion

Besides RPM extract, pharmacokinetics of pure compounds of TSG, emodin and physcion in beagle dogs and SD rats have also been studied. Among these three compounds, only TSG and emodin have been investigated for their oral bioavailabilities. The absolute oral bioavailabilities of TSG were reported to be 24.2% and 36.5% for 50 mg/kg and 100 mg/kg in SD rats, respectively [35]. Oral administered 8 mg/kg of emodin resulted in 6~9%

bioavailability in SD rats. In addition, gender specific pharmacokinetics of emodin was noticed with much higher C_{max}/AUC and shorter half-life observed in male rats [36]. The T_{max} values among these major RPM components indicated their fast absorption and comparison of the half-lives of these three compounds suggested the order of elimination rate as TSG > emodin > physcion [34][35][36][37][38][39][40].

2.3. Effect of co-occurring ingredients in RPM on the pharmacokinetics of emodin

In addition to the above-mentioned ADME of each individual component in RPM, there are a number of studies reported about the potential interaction between the co-occurring ingredients in RPM. After comparing the pharmacokinetic parameters of TSG and emodin after oral administration of RPM extracts versus that obtained from their pure components in SD rats, it was noticed that the half-life values of TSG and emodin after oral administration of RPM extracts (0.5~2 h for TSG, 1.5~3 h for emodin) are similar to that from their pure components (0.5 h for TSG, 3 h for emodin). However, the C_{max} and AUC values of emodin after oral administration of RPM extracts (dose of emodin: 11.17 mg/kg; AUC: 683.0 ± 268.9 ng h/mL, C_{max}: 224.5 ± 131.1 ng/mL) were higher than that from pure emodin (10 mg/kg, AUC: 420.3 ± 48.1 ng h/mL, C_{max}: 74.9 ± 17.4 ng/mL) to SD rats, suggesting that systemic exposure of emodin could be affected by other co-occurring ingredients in RPM extract. It was found that the presence of TSG could significantly increase the C_{max} and AUC values of emodin via inhibiting its metabolism [34][41]. Moreover, the *in vitro* study also confirmed that TSG could increase the absorption of emodin via inhibiting its MRP mediated transport in Caco-2 cells and UDP glycosyltransferase (UGT) mediated glucuronidation in human liver microsomes at a dose dependent manner [42]. In addition to the influence of TSG, it was reported that the co-occurring anthraquinones components including aloe-emodin, rhein, chrysophanol and physcion may lead to decrease of emodin AUC in cerebral ischemia-reperfusion model rats [58]. Overall, since the contents of these components are much lower than TSG in RPM [1][25], TSG may have the most significant influence on the change of emodin pharmacokinetics, which warrants further experimental verification.

In summary, the ADME characteristics of the major components in RPM including TSG, emodin and physcion were well studied in rats and beagle dogs with fast absorption and the elimination of TSG was faster than emodin followed by physcion. The transporters such as SLTC1, P-glycoprotein and MRP2 were involved in the absorption of TSG and emodin with glucuronidation as their major metabolic pathway. Additionally, TSG could increase the systemic exposure of emodin via increasing its absorption and inhibiting its metabolism at a dose dependent manner. However, there is no information about the biodistribution of the major components of RPM after its extract treatment which can offer better understanding of the toxicity of RPM, especially hepatotoxicity.

3. Hepatotoxicity

3.1. Case Reports on Liver Injury of RPM

So far, there are several retrospective analysis studies investigating the clinical cases on RPM induced liver injury [43][44][45]. According to these studies, the common reasons for consuming RPM products included treating grey hair, hair loss, using it as a health supplement, or for the treatment of hypertension, coronary heart disease,

hyperlipidemia, etc. In addition to proprietary products of RPM, decoction pieces processed with water, alcohol, or ground into powder were commonly used for oral administration in clinics. For all the patients from the above-mentioned case reports, the onset time for liver toxicity ranged from 1 to 240 days with a median of 30 days after oral administrations of RPM at doses ranging from 1 g/person/day to 100 g/person/day.

According to the Roussel Uclaf Causality Assessment Method, based on the type of damaged target cells, liver injury can be classified into three types, including hepatocyte liver injury, cholestatic liver injury, and their mixture type [46]. Most of the liver injuries induced by RPM were diagnosed as hepatocellular injury followed by mixed liver injury and cholestatic liver injury with jaundice, fatigue, anorexia as the major symptoms of RPM induced liver injury. Although RPM can induce liver injury in different degrees and even lead to death, the majority of RPM associated liver damage was found to be reversible after discontinuing RPM products and conservative care [43][44][45].

3.2. Mechanistic Studies on Liver Injury Induced by RPM Extract and Its Major Components

Since hepatic adverse effect reports on RPM had raised much concern for its safe use in clinics, a series of studies have been conducted to investigate the mechanisms of RPM associated liver injury. The findings are summarized in Table 1 with the major mechanisms highlighted below.

Table 1. Summary of reported clinical and preclinical liver injury mechanisms of RPM and its components

Mechanisms	Model	Substance	Dose/Duration	Findings
Metabolic enzymes alteration and genetic polymorphism	SD rats [31]	RPM extract	6 g raw RPM/kg/bolus	Protein expression: CYP3A4, CYP2C19, CYP2E1, UGT1A1 and UGT1A8 ↓; ALT and AST ↑.
	SD rats [47]	RPM aqueous extract	40 g raw RPM/kg/3 weeks	CYP1A2 or CYP2E1 inhibitors + RPM: ALT and AST↑; moderate liver injury.
	Human (43 cases) [48]	RPM	NR	CYP1A2*1C frequency: 46.5%: RPM induced liver injury patients; 27.9%: healthy controls.
	Human (87 cases) [49]	RPM	4 weeks	HLA-B*35:01 allele: 45.4%: RPM induced liver injury patients; 2.7%: Han Chinese population.
Hepatocytes apoptosis	SD rats [50]	Emodin	1500 mg/kg/7 days	Emodin: ↑caspase-9, caspase-3, and Cyt c → mitochondrial apoptosis and liver injury
	L02 cells [51]	Emodin	50 μM	Emodin: ↑caspase-3 and ROS, ↓mitochondrial membrane potential, disrupting ATP

Mechanisms	Model	Substance	Dose/Duration	Findings
				synthesis → mitochondrial damage and hepatocyte apoptosis.
	HepaRG cells [52]	Emodin	20–80 μM	Emodin: cell cycle arrest and ROS generation → mitochondrial apoptosis → cell apoptosis.
Bile acids homeostasis disruption	SD rats [53]	Extracts of raw RPM (75% EtOH)	1 and 20g extract/kg/90 days	Protein expression of 3-hydroxy-3-methylglutaryl CoA reductase and CYP7A1 ↑ in a dose-dependent manner.
	SD rats [54]	RPM concentrated powder (1:10)	30 and 60g extract/kg/28 days	mRNA and protein expression of MRP2/*Mrp2*, MRP3/*Mrp3*, BSEP/*Bsep*, FXR/*Fxr*, CYP7A1/*Cyp7a1* ↑.
	SD rats [55]	Extracts of raw RPM (75% EtOH)	1 and 20 g extract/kg/ 3, 6, 7 weeks	• HDCA, CA, TUDCA, and DCA in serum, TβMCA, TCA, CA, and βMCA in urine ↑ in a dose- and time-dependent manner; • HDCA in serum and TβMCA in urine were identified as potential biomarkers for RPM induced liver injury; • The mRNA expression of *Bsep* ↑ and *Ntcp* ↓ in liver.
	SD rats [56]	Extracts of raw and processed RPM (75% EtOH)	50 g extract/kg/42 days	GDCA in bile, as well as HDCA in serum, could be selected as potential biomarkers for RPM induced liver injury.
	C57BL/6J mice [57]	Extracts of processed RPM (60% EtOH)	1.275 and 3.825g extract/kg/7 days	• Total bile acids↓ in liver and serum, unconjugated BAs ↑ in intestines; • mRNA expression: *Nctp* and *Bsep*↑; protein expression of CYP7A1 ↓.

Mechanisms	Model	Substance	Dose/Duration	Findings
	Sandwich cultured rat hepatocytes [58]	Emodin, Physcion, Chrysophanol	1–50 μM	All compounds could alter bile acids disposition through direct ↓BA transporters as well as regulated expression of bile acids transporters and metabolic enzymes.
Inflammatory damage	SD rats [59]	Emodin	20, 40, 80 mg/kg	Emodin + lipopolysaccharide: ↑ proinflammatory cytokines (TNF-α, IL-1β and IL-6) → ALT and AST ↑.
	L02 cells [60]	Emodin	10.93, 54.09, 267.7 μM	Emodin: ↑ p-NF-κB and IL-6 → inflammatory damage.

NR: not reported, ↑: increase, ↓: decrease.

3.3. Correlations between Pharmacokinetics of RPM and Its Induced Liver Injury

Similar to western drugs, RPM could exhibit therapeutic windows with toxicities identified at higher doses [61]. It was reported that RPM extract could attenuate liver cirrhosis induced by dimethylnitrosamine in mice at the dose of 20–100 mg/kg/day (equal to 0.093–0.465 g raw RPM/kg/day), while such therapeutic effect decreased and toxic effects were observed with the dose increasing to 500 mg/kg/day (equal to 2.326 g raw RPM/kg/day) [7]. A similar trend was also observed for emodin in rats with liver protection from CCl_4-induced fibrogenesis after its oral administration at 40 mg/kg/day [62] and liver damage induced after its oral administration at 1500 mg/kg [50]. Moreover, Ma et al. indicated that the gradual increased in vivo exposure of emodin after oral administration of RPM extract (20 g raw RPM/kg) for 21 days might contribute to the RPM-induced hepatic lesions [33]. Therefore, it is speculated that the therapeutic and toxic effects of RPM could be correlated with the dose and in vivo level of emodin.

It is noticed that the maximum concentration of emodin in the rat plasma ranged from 61.29 ng/mL to 348.10 ng/mL after oral administration of RPM extract at doses ranging from 6 g/kg to 40 g/kg. Such concentration is far below 20 μM (or 54 μg/mL), the minimum concentration of emodin observed in the in vitro liver toxicity study in human L02 and HepaRG cells [51][52] and rat sandwich cultured hepatocyte [58]. Shi et al. reported a much higher concentration of emodin in the liver (940.12 ng/g) than that in the plasma (120.98 ng/ml) after oral administration of 10 mg/kg emodin loaded nanoemulsion in rats [38]. Although its human liver concentration remains unknown, emodin is expected to have a higher accumulation in the liver than in plasma, leading to potential liver toxicity.

In addition to the above-mentioned liver toxicity of emodin itself, the co-occurring components in RPM could also affect its in vivo levels leading to enhanced liver toxicity. Although both CYP1A2 and UGT1A8 were involved in the metabolism of emodin in rats, UGT mediated phase II metabolism is the dominant metabolic pathway of emodin. After consecutively treating with TSG (117 mg/kg) for 7 days, a decrease in the mRNA expression of Ugt1a8 in rat liver and intestine led to increased C_{max} and AUC of emodin in rats [34], and the metabolism of emodin could be

inhibited by TSG in the human liver microsome in a dose-dependent manner [42]. Moreover, the absorption of emodin could be increased in the Caco-2 cell in the presence of TSG. Such increased systemic exposure of emodin by TSG may further contribute to the RPM induced liver injury. According to the existing Pharmacopeia, only the lower limits for the contents of TSG and emodin in RPM were required. However, the impact of RPM with different contents of TSG on the in vivo concentrations of emodin and its related liver damage is not clear so far. Therefore, the relevant upper limits of the content of TSG and emodin in RPM, and the relationship with its induced liver toxicity need further clarification.

Toxicokinetics is usually adopted to determine the relationship between the systemic exposure of a compound and its toxicity in animals and humans. To achieve the toxicokinetics of herbal medicines, such as RPM, we need to determine the exposure of its major bioavailable components in blood and major organs and the relationship with its induced liver toxicity. Since there is no information about the concentrations of the major components of RPM in the liver after oral administration of its extract, detailed biodistributions (especially liver concentrations) of RPM major components in preclinical animal studies should first be obtained to better understand the in vivo levels of these components, including TSG and emodin, and how they correlate with the RPM induced liver injury.

3.4. Role of Herb–Drug/Herb Interactions in RPM Induced Liver Injury

Herb–drug/herb interactions are of great concern when patients concomitantly take drugs and herbs, especially taking herbal and western medicines at the same time. Since emodin was the major component contributing to liver toxicity of RPM, the interaction of emodin in RPM with other drugs/herbs could be critical to the safe use of RPM in the clinic.

It was noted that emodin induced hepatotoxicity at 150mg/kg could be further enhanced by probenecid (100mg/kg) due to increased systemic exposure of emodin resulted from its inhibition on UGTs and MRP2 in rats [63]. In addition, piperine, the bioactive compound of Piper nigrum L. and Piper longum L., could significantly increase the AUC and C_{max} of emodin via the inhibition of its glucuronidation [64]. Therefore, people should pay more attention to hepatotoxicity when they take emodin-containing herbal medicine together with drugs/herbs that could inhibit the expression or activity of UGT or MRP2. On the other hand, the herb–herb interaction may attenuate the RPM induced liver injury. A recent study found that combined use of Poria and RPM could significantly ameliorate the RPM-induced liver injury and systemic inflammation in LPS treated rats [65]. Since emodin could also induce liver injury in LPS treated rats with significantly increased proinflammatory cytokines [52], the above-mentioned detoxification effects of Poria could be related to its influence on emodin leading to a reduction in corresponding inflammatory cytokines, which warrants further verification.

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