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Pharmacokinetic mechanisms underlying the detoxification effect of *Glycyrrhizae Radix et Rhizoma* (*Gancao*): drug metabolizing enzymes, transporters, and beyond

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Abstract

Introduction: Glycyrrhizae Radix et Rhizoma (Gancao in Chinese) is the most frequently used traditional Chinese medicine (TCM) owing to its various pharmacological effects and, more importantly, the synergistic effects that enhance the efficacy and reduce the toxicity of other TCMs.

Areas covered: We reviewed publications, predominantly between 1990 and 2018, that examined pharmacokinetic interactions between Gancao and other TCMs, or the bioactive constituents of these TCMs. This review focuses on the underlying mechanisms and the components responsible for the pharmacokinetic modulation by Gancao.

Expert opinion: In general, the pharmacokinetic effects of Gancao are a result of its constituents such as macromolecules, like proteins, and small molecules, such as saponins and flavonoids. The mechanisms are related to formation of complexes and the influence of these on drug solubility, permeability, distribution, and metabolism. The detoxification effect of a single dose of Gancao is mainly mediated by the suppression of the intestinal absorption of toxic constituents of the co-administered TCMs and is attributable to constituents that form complexes with the toxic compounds and cause them to sediment. In contrast, the detoxification effects of repeated doses of Gancao are mediated mainly via the induction of drug metabolizing enzymes and efflux transporters.
Keywords: drug metabolizing enzymes, drug transporters, Gancao protein, Glycyrrhiza Radix et Rhizoma, pharmacokinetic interaction, synergistic function.

Article highlights

1. Glycyrrhiza Radix et Rhizoma (Gancao) exerts synergistic effects with other TCMs, increasing their efficacy and/or decreasing their toxicity.

2. The detoxification effect of Gancao is related to its strong impact on the pharmacokinetics of some of the toxic constituents of co-administered TCMs.

3. The detoxification effect of a single dose of Gancao is mainly mediated by the suppression of the intestinal absorption of toxic constituents of the co-administered TCMs and is attributable to constituents that form complexes with the toxic compounds and cause them to sediment.

4. The detoxification effect of repeated doses of Gancao is mediated mainly via the induction of drug metabolizing enzymes and efflux transporters.

5. The formation of sediment and, consequently, the decrease in diester-diterpene alkaloids contents contribute significantly to the detoxification of Aconiti Kusnezoffii Radix (Caowu) and Aconiti Lateralis Radix Praeparata (Fuzi) by Gancao.

6. The detoxification effects of Gancao on Tripterygium wilfordii (Leigongteng) are associated with the induction of drug metabolizing enzymes as well as efflux drug transporters.

7. The formation of complexes between constituents of Gancao, such as saponins, and toxic constituents of Coptidis Rhizoma (Huanglian) reduces the risk of acute toxicity of Huanglian.
1 Introduction

Glycyrrhizae Radix et Rhizoma, also known as Gancao in Chinese or licorice in English, is produced from the dried root and stem of medicinal plants of the family Leguminosae, including G. uralensis Fisch. (GU), G. inflata Bat. (GI), or G. glabra L. (GG) [1]. It is the most frequently used traditional Chinese medicine (TCM) [2]. In a Chinese Formulae Database containing 96,592 prescriptions, Gancao is present in 26,185 of these prescriptions [2].

Gancao is widely used partly owing to its various pharmacological effects including antitumor, antibacterial, antiviral, anti-inflammatory, and immune-regulatory activities [3]. Although the qualities of clinical trials of some TCMs need to be improved [4], the clinical efficiencies of Gancao had been well proved [5]. In several randomized controlled clinical trials, Gancao was effective in decreasing transaminase activities in non-alcoholic fatty liver disease [6], relieving oral mucositis in head and neck cancer patients undergoing radiotherapy [7], reducing the body weight and the body mass index of patients [8], managing functional dyspepsia [9], and reducing the incidence of postoperative sore throat [10]. Furthermore, Gancao is usually used as an “Envoy” TCM herb. Hence, in many TCM formulae, Gancao shows “harmonic function”, i.e., the critical synergistic effect that enhances the efficacy and/or reduces the toxic reactions of TCMs used in combination [11]. It is the “harmonic function” that makes a major contribution to the varied use of Gancao in TCM formulae [11].

At least 83 toxic herbs are recorded in the China Pharmacopeia as components of TCMs and therefore used clinically [2]. The most frequently used toxic TCMs, i.e., Aconiti Lateralis Radix Praeparata (Fuzi), Pinelliae Rhizoma (Banxia), Cinnabaris (Zhusha), are also the three
TCMs that are most frequently used with *Gancao* [2]. For example, *Fuzi* is included in 8,111 prescriptions in the Chinese Formulae Database, of which 2420 prescriptions (29.8%) also include *Gancao* [2]. The detoxification effect of *Gancao* on some toxic TCMs, including *Fuzi* [12], *Aconiti Kusnezoffii Radix (Caowu)* [13], *Sophorae Flavescentis Radix (Kushen)* [14], and *Semen Strychni (Maqianzi)* [15], has been verified in animals. The beneficial property of *Gancao* was usually assessed based on its influences on the mortality rate, behavior, biochemical markers, and histopathological changes of the toxic TCMs treated animals. For example, the co-administration of *Gancao* relieved high-dose *Maqianzi* induced deterioration in the renal function markers as well as histopathological examination of the tested rats [15]. Metabonomics is able to understand the global change of an organism at the metabolic level. The method was recently used to evaluate the detoxification effects of *Gancao* on *Fuzi* [12] and *Caowu* [13].

The detoxification effect of *Gancao* is related to its marked effect on systemic exposure to the toxic constituents of the TCMs. The effect of *Gancao* on many pharmacokinetic interactions has been revealed, but not systematically summarized. Here, we reviewed studies published mainly between 1990 and 2018 that examined the pharmacokinetic interactions between *Gancao* and TCMs or their bioactive constituents and aimed to emphasize the underlying mechanisms and components of *Gancao* responsible for the effects on TCM pharmacokinetics. This review was based on Medline and Web of Science searches for keywords including *Glycyrrhizae Radix et Rhizoma*, *Glycyrrhizae*, *Gancao*, licorice, specific names of constituents in *Gancao*, and specific names of the involved TCMs. We hope this review will assist in the understanding of synergistic effects of *Gancao* with other TCMs, which result in
altered pharmacokinetic behavior and, in particular, detoxification.

2 Pharmacokinetic interactions of Gancao extracts and mechanisms of action

Gancao extracts alter the activity as well as the expression of drug metabolizing enzymes, including cytochrome p450 enzyme isoforms (CYPs) and UDP-glucuronosyltransferase isoforms (UGTs), and drug transporters, such as p-glycoprotein (P-gp). Both these enzymes and transporters are extensively involved in the intracorporal process of xenobiotics [16-18] and mediate a majority of pharmacokinetic interactions [19].

Multiple doses of Gancao extracts induce the expression as well as the bioactivities of some key drug metabolizing enzymes. For example, treatment with an oral aqueous extract of GU (daily gavage of 900 mg/kg) time-dependently increased the total CYP expression in rats (from 0.7 nmol/mg protein to more than 0.9 and 1.3 nmol/mg protein after 3 and 6 days of administration, respectively) and increased the metabolism of co-administered warfarin [20]. Treatment with an oral aqueous decoction of GU once daily for 6 days consecutively dose-dependently increased CYP expression in rats (1 and 3 g/kg led to increases of 62% and 91%, respectively) [21]. After treatment with 3 g/kg of the GU decoction, the area under the concentration time curve (AUC) for lidocaine in the rats decreased by 41%, the elimination half-life was shortened by 39%, and total clearance increased by 59% [21] When the dosage was increased to 10 g/kg/day, the oral administration of the GU aqueous extract for 7 days led to a greater increase in the activity and expression of CYP1A1, CYP2B1, and CYP2C11 (1.8, 1.3, and 3.2-fold increases, respectively) [22]. When cyclosporin A (CsA) was orally administered to rats as a single dose or with several doses of Gancao extract (containing 150
mg/kg of glycyrrhizic acid), the maximum concentration (C_max) of CsA significantly decreased by 81.3% and 91.4%, respectively, whereas the AUC_0-t of CsA significantly decreased by 78.2% and 89.9%, respectively [23]. The elimination half-life of CsA in rats pretreated with seven doses of Gancao extract was significantly prolonged by 80.9% [23]. The activation of P-gp and CYP3A contributed to the decrease of the oral bioavailability of CsA [23].

In terms of GG, daily doses of its extract (3,138, or 6,276 mg/kg, p.o.) for 4 or 10 days consecutively induced several-fold increase in the content, as well as activities of murine liver CYPs, including CYP3A and, to a lesser extent, 2Bl and 1A2 [24]. The induction of the CYP3A isozyme was due to regulation at the mRNA level [24] Furthermore, a methanol extract of GG activated glucuronidation in the liver of rats [25]. The oral administration of the extract (1 g/kg) for 6 days specifically increased the activities of UGT1A by 111% [25] and, consequently, the concentration of UDP-glucuronic acid increased by 257% [25]. Gancao extract (species unknown) induced UGT1A1 in HepG2 cells, which was associated with modulation of the nuclear factor (erythroid-derived 2)-like 2 (Nrf2) signaling pathway [26].

In contrast, single doses of Gancao extracts inhibited the activities of drug metabolizing enzymes. For example, an aqueous extract of Gancao (species unknown) inhibited the activities of CYP1A2, CYP2C9, and CYP2C19 in a dose-dependent manner with half maximal inhibitory concentration (IC_{50}) values of 201.7, 188.8, and 374.1 μg/mL, respectively [27], but did not affect the activity of CYP2D6, CYP2E1, or CYP3A4 [27]. Additionally, a methanol extract of GU inhibited half of the CYP3A4 activity at concentrations as low as 0.022 mg/mL [28]. There are differences among Gancao species
with respect to the inhibitory effects on CYPs. GG extract exhibited moderate inhibitory effects against CYPs 2B6, 2C8, 2C9, and 2C19, and weak inhibition against CYP3A4 when testosterone was used as a substrate [29]. GG only weakly inhibited CYP2D6 and CYP3A4 [30]. In contrast, GU extract strongly inhibited CYP2B6 and moderately inhibited CYPs 2C8, 2C9 and 2C19, whereas GI extract strongly inhibited CYP2C family enzymes and moderately inhibited CYPs 1A2, 2B6, 2D6, and 3A4 with midazolam as a substrate [29].

_Gancao_ influences drug transporters. GU extract (containing 150 mg/kg of glycyrrhizin) activated intestinal P-gp and consequently decreased the absorption of oral CsA in rats [23]. _Gancao_ (species unknown) induced multi-drug resistance related proteins 2 (MRP2) in HepG2 cells, which was associated with modulation of the Nrf2 signaling pathway [26].

In summary, _Gancao_ extracts modify important drug metabolizing enzymes and drug transporters, which may be the source of its pharmacokinetic interaction potential.

### 3 Pharmacokinetic interactions and mechanisms of constituents of _Gancao_ extracts

_Gancao_ extracts contain large molecules including polysaccharides [31] and proteins [32, 33]. The polysaccharides in GU include fractions I to V (GUP I–V), of which GUP II is the main fraction, and contains rhamnose, arabinose, galactose, and glucose monosaccharide [31]. However, the pharmacokinetic interactions between _Gancao_ polysaccharides and TCMs used in combination have not yet been reported. The protein content of _Gancao_ is in the range 0.3–35.0 μg/mg powder of crude herbal pieces [32]. The molecular weight of _Gancao_ proteins was approximately 33 or 62 kDa [33]. In another study, the molecular weight of _Gancao_
proteins was between 14.0 and 66.2 kDa; proteins with a molecular weight of approximately 31.0 kDa were the most abundant [34].

In addition, *Gancao* extracts contain more than 250 small molecules, including saponins, flavonoid glycosides, and various types of free phenolic compounds [1]. Recently, 151 compounds (including 17 flavonoid glycosides, 24 saponins, and 110 free phenolic compounds) were systematically and quantitatively analyzed [1]. Triterpene saponins were the most abundant, followed by glycosylated flavanones and chalcones; however, glycyrrhizic acid (referred to as glycyrrhizin in some studies) was the main constituent of all the examined samples, as expected [1, 35]. The content of glycyrrhetinic acid in *Gancao* extract was much lower than that of glycyrrhizic acid [1]. However, the majority of glycyrrhizic acid is metabolized to glycyrrhetinic acid by intestinal bacteria and then absorbed [36]. Liquiritin and isoliquiritin were the major flavonoids in 83 batches of GU from different regions and accounted for more than 90% of the 15 detected flavonoids [37]. Each species of *Gancao* contains a different distribution of components. For example, GG contains a relatively higher abundance of flavonoid and chalcone apiosides and is the only species to contain glabridin; conversely, licoricidin and glycycomararin are the most abundant in the GU extract, but only GI contains licochalcone A [1, 29].

The pharmacokinetic interactions caused by *Gancao* extract result from various constituents. For example, the inhibitory effects of the methanol extract of GU on CYP3A4 activity were attributable to various constituents, including (3R)-vestitol, 4-hydroxyguaiacol apioglucoside, liquiritigenin 7,4'-diglucoside, isoliquiritigenin, and liquiritin, which inhibit CYP3A4 activity between concentrations of several micromolar and hundreds of micromolar [28]. The
inhibition potencies of 40 major *Gancao* compounds for the activities of several human CYPs, i.e., CYPs 1A2, 2C9, 2C19, 2D6, and 3A4 in human liver microsomes (HLMs) were evaluated by using a liquid chromatography/tandem mass spectrometry (LC-MS/MS) cocktail assay [38]. The results showed that free flavonoids (for example, liquiritigenin, isoliquiritigenin) and arylcoumarins, generally exhibited potent inhibitory activities against the five CYP isozymes, especially 1A2 and 2C9 [38]. Hence, they were identified as key constituents responsible for the herb-drug interactions between CYPs and *Gancao*. In contrast, flavonoid glycosides, saponins, and phenolic acids showed weak regulatory activities [38]. However, they may be converted into the above-identified CYPs inhibitors such as free flavonoids *in vivo* [38], suggesting the complexity of the interaction potentials of the constituents in *Gancao* extracts. Below, we have discussed the detailed interactions and related mechanisms of some key constituents by component.

### 3.1 Large molecules: *Gancao* proteins

*Gancao* proteins self-assemble into near-spherical nanoparticles with an average diameter of 206.2 ± 2.0 nm and are stable at 25°C for 7 days [34]. They form nanoparticles that encapsulate and form stable complexes with aconitine, a toxic alkaloid in TCMs such as *Fuzi*, with a molar ratio of approximately 1:5.2 and an encapsulation rate of 28.2% [34]. Ionic interactions, hydrogen bonds, or hydrophobic forces were assumed to be involved in the formation of the complexes between protein and small molecules [39]. Complex formation with *Gancao* proteins reduced the acute toxicity of intraperitoneally injected aconitine in ICR mice [34]. All mice administered aconitine died at approximately 18 min after injection,
whereas the mice that received the same dosage of *Gancao* protein-encapsulated aconitine experienced only mild toxic reactions, which subsided within 3 h [34]. The formation of the complex was assumed to decrease the blood exposure level of aconitine [34]. However, the exact mechanisms and whether *Gancao* proteins attenuate the toxicity of oral aconitine or aconitine-containing TCMs remain to be studied.

### 3.2 Small molecules

#### 3.2.1 Saponins: glycyrrhizic acid

Glycyrrhizic acid influences the pharmacokinetics of co-administered drugs. For example, the AUC of methotrexate, an anticancer agent and immunosuppressant with a narrow therapeutic window, was increased by a single (270% increase) or repeated doses (increased by 480% after 7 doses) of 150 mg/kg oral glycyrrhizic acid [40]. Multiple action mechanisms of glycyrrhizic acid with the ability to mediate pharmacokinetic interactions are discussed below.

#### 3.2.1.1 Influences on drug solubility

Glycyrrhizic acid forms “guest-host” complexes, which comprise one or two glycyrrhizic acid molecules per guest molecule, at lower concentration (10^{-5}–10^{-3} M) but forms micelles at critical concentrations above 10^{-3} M [41].

The stoichiometry of supramolecular complexes and their association constants were estimated by observing the change in either the nuclear magnetic resonance (NMR) chemical shift of protons in the NMR spectra or the parameters of absorption spectrum [42, 43]. The formation of complexes dramatically increases the solubility of certain hydrophobic
compounds. For example, complexation with glycyrrhizic acid prevented the reversible aggregation and enhanced the stability and solubility of xanthophyll carotenoids including astaxanthin [44]. Glycyrrhizic acid forms supramolecular complexes with simvastatin [45] that increased its solubility by up to more than 100-fold [46].

Structurally, glycyrrhizic acid contains a hydrophobic portion (a triterpenoid aglycone) and hydrophilic portion (two glycosyl groups). Owing to its amphiphilic properties, in a solution of pH 5–6, glycyrrhizic acid can form rod-like micelles with an estimated radius of 1.5 nm and length of 21 nm [47]. The micelle formation is due to the hydrophobic interactions between the triterpene moieties of glycyrrhizic acid [42], which highlights the potential applications of glycyrrhizic acid as an emulsifier and solubilization agent, i.e., a plant-derived surfactant [47].

3.2.1.2 Influences on drug permeability

Glycyrrhizic acid not only increases drug solubility, but also enhances drug permeability through cell membranes [48]. Using NMR and molecular dynamics, it was revealed that in the membrane of liposomes, glycyrrhizic acid could penetrate the lipid bilayer, change the mobility of lipids, form pores, and carry a few water molecules inside the membrane [48]. In addition, glycyrrhizic acid increased the permeability by approximately 60% and decreased the elasticity modulus of red blood cell membranes by an order of magnitude, even at micromolar concentrations [49]. Glycyrrhizic acid substantially increased the membrane permeability of myeloblastic leukemia cells (K562) [50]. The maximum rate of diffusion of formate ions through K562 cell membranes was 5.5 times higher than those in the absence of
3.2.1.3 Influences on drug metabolizing enzymes

Glycyrrhizic acid inhibited CYP 2C9, 2C19, and 3A4 activity in HLMs, with IC$_{50}$ values of 32.85, 74.21, and 13.79 µM, respectively [51]. However, the inhibitory effect on the activity of CYPs 1A2, 2D6, and 2E1 in HLMs was relatively lower [51]. In rats, a single dose of glycyrrhizic acid (240 or 480 mg/kg, p.o.) did not affect the hepatic CYP superfamily, including CYPs 3A, 1A1, 1A2, 2B1, 2C11, and 2E1 [52].

In contrast, long-term treatment with glycyrrhizic acid induced drug metabolizing enzymes. For example, glycyrrhizic acid induced CYP3A4 by activating the pregnane X receptor (PXR) [53]. In healthy volunteers, treatment with 150 mg glycyrrhizic acid salt tablet twice daily for 14 days decreased mean midazolam AUC$_{0-\infty}$ (20%) and C$_{\text{max}}$ (12%), which were suggestive of a modest induction of CYP3A [54]. Given that T$_{1/2}$ was unaltered for midazolam, glycyrrhizic acid predominantly induced intestinal, rather than hepatic, CYP3A [54]. In addition, treatment with glycyrrhizic acid (150 mg, twice daily for 14 days) induced the CYP3A4-catalyzed sulfoxidation of omeprazole in healthy volunteers [55]. In contrast, daily doses of glycyrrhizic acid (240 or 480 mg/kg, p.o.) for 4 or 10 consecutive days induced several-fold increase in both the content and activities of hepatic CYPs, especially CYP3A, in mice [24]. In rats, four daily doses of glycyrrhizic acid (240 or 480 mg/kg, p.o.) also induced several-fold increase in CYPs, including CYP3A [52]. These results suggested the species-dependent influence of glycyrrhizic acid on the induction of CYP3A.

In addition, glycyrrhizic acid induced CYP 2Bl and lA2 in mice [24] and rats [52] and
activated glucuronidation in rats [25]. The administration of glycyrrhizic acid (23 mg/kg, p.o.) for 6 days caused a 96% increase in the specific activity of UGT1A [18]. Consequently, the concentration of UDP-glucuronic acid increased by 484% [25]. In brief, the influences of glycyrrhizic acid on drug solubility and permeability through cell membranes are suggestive of its potential positive influences on the intestinal absorption of drugs; however, the inductive effects on drug metabolizing enzymes may negatively impact systemic drug exposure, especially for treatments involving repeated doses.

3.2.2 Saponins: glycyrrhetinic acid

Although the content of glycyrrhetinic acid in Gancao extract is relatively low compared with that of glycyrrhizic acid [1], given that the majority of glycyrrhizic acid is metabolized into glycyrrhetinic acid by intestinal bacteria and then absorbed [36], glycyrrhetinic acid remains a key constituent in Gancao extract for the mediation of pharmacokinetic interactions.

3.2.2.1 Influences on drug distribution

Glycyrrhetinic acid exists in the form of two isomers: 18 α-glycyrrhetinic acid (the trans isomer) and 18 β-glycyrrhetinic acid (the cis-isomer) [56]. Verified by using electrospray ionization mass spectrometry, glycyrrhetinic acid stereoisomers self-associate owing to the presence of free carboxyl groups [56]. Similarly, glycyrrhetinic acid forms supramolecular complexes with other compounds via noncovalent interactions [56]. In contrast, glycyrrhetinic acid was reported to specifically bind receptors in the membranes of normal liver cells [57]. In addition, glycyrrhetinic acid binds its receptors in hepatocellular carcinoma cells [58]. The
beta-configuration hydrogen atom on the C18 position of glycyrrhetinic acid was most important for the binding [59]. In addition, glycyrrhetic acid inhibited efflux drug transporters such as MRP4 and breast cancer resistance protein (BCRP) [60]. Furthermore, glycyrrhetinic acid was identified as an efficient mitochondria-targeting ligand [61]. Therefore, glycyrrhetinic acid exhibited pronounced influences on the tissue, cellular, and subcellular distribution of TCMs when administered in combination.

For example, glycyrrhetic acid-modified drug delivery systems, including liposomes, micelles, and nanoparticles, have been developed to achieve hepatocellular carcinoma-targeted drug delivery [62]. Specifically, glycyrrhizic acid-functionalized graphene oxide acted as a nano-carrier to deliver doxorubicin into the mitochondria of HepG2 cells, which improved doxorubicin efficacy and decreased its toxicity [61]. Glycyrrhetinic acid-modified PEG-PCL copolymeric micelles caused the selective accumulation and enhanced the cytotoxicity of curcumin in HepG2 cells [63]. In addition, through MRP4 and BCRP inhibition, glycyrrhetic acid promoted the accumulation and subcellular (cytoplasmic and nuclear) distribution of entecavir [ETV, a superior nucleoside analog used to inhibit hepatitis B virus (HBV)] in hepatocytes, which ultimately augmented the antiviral efficiency of ETV [60].

3.2.2.2 Influences on drug metabolizing enzymes

The oral administration of glycyrrhetic acid at 50 mg/kg/day for 7 days increased the expression and activities of CYP1A1, CYP2B1, and CYP2C11 by several folds in mice [22]. However, the activities and mRNA expression of the CYP 2C and 3A families decreased
significantly in the livers of mice treated with 25–100 mg/kg glycyrrhetinic acid for 15 days [64]. In addition, pretreatment with 18 $\beta$-glycyrrhetinic acid (100 mg/kg, s.c., 3 days) inhibited CYP2E1 activity and expression in mice [65].

The short-term inhibitory effects of glycyrrhetinic acid on CYPs have been widely reported in *in vitro* studies. For example, glycyrrhetinic acid strongly inhibited CYP3A4 in HLMs, with a low IC$_{50}$ value of 1.53 $\mu$M [66]. With midazolam as the probe substrate, glycyrrhetinic acid greatly decreased CYP3A4 activity with IC$_{50}$ values of 8.195 $\mu$M in HLMs and 7.498 $\mu$M in a recombinant cDNA-expressed CYP3A4 enzyme system [64]. In HLMs, the inhibitory effect of glycyrrhetinic acid on CYP3A4 was identified as competitive, with an inhibition constant (K$_{i}$) value of 1.57 $\mu$M [64]. Glycyrrhetinic acid was identified as a mixed inhibitor of recombinant human CYP3A4 (rCYP3A4) with an IC$_{50}$ of 7.25 $\mu$M and a K$_{i}$ of 6.4 $\mu$M [67]. In contrast, glycyrrhetinic acid weakly inhibited CYP1A2 (IC$_{50} = 61.06$ $\mu$M [66]), CYP2C9 (IC$_{50} = 26.46$ $\mu$M [66] or 32.85 $\mu$M [51]), CYP2C19 (IC$_{50} = 74.21$ $\mu$M [51]), but did not inhibit the activity of CYP2A6, CYP2D6, or CYP2E1 (IC$_{50} > 100$ $\mu$M in HLMs) [64].

In addition, glycyrrhetinic acid strongly inhibited several UGTs. The inhibition of UGT1A3 and UGT2B7 by glycyrrhetinic acid was best fit to competitive and noncompetitive inhibition, respectively, with K$_{i}$ values of 0.2 and 1.7 $\mu$M [68]. Glycyrrhetinic acid also strongly inhibited UGT 2B15 [69], with a K$_{i}$ value of 0.35 $\mu$M [69].

### 3.2.2.3 Influences on drug transporters

Glycyrrhetinic acid has almost 10-fold stronger inhibitory activity on organic anion transport polypeptides 2B1 (OATP2B1) than glycyrrhizic acid [70]. The IC$_{50}$ values of glycyrrhetinic
acid and glycyrrhizic acid on OATP2B1-mediate estrone-3-sulfate (E3S) were 13.0, and 125.7 μM, respectively, which were comparable with its plasma concentrations in clinical trials [70]. At 100 μm, glycyrrhetinic acid inhibited more than 60% of the OATP-mediated uptake of statins including atorvastatin, fluvastatin, and rosuvastatin [70].

Regarding efflux drug transporters, in addition to inhibiting MRP4 and BCRP [60], glycyrrhetinic acid inhibited the function of P-gp and MRP1 in KBC2 cells and KB/MRP cells, respectively [71]. Consequently, KB-C2 and KB/MRP cells were sensitized to anticancer agents, including vinblastine and doxorubicin, by glycyrrhetinic acid [71].

In summary, glycyrrhetinic acid may exert profound effects on the intracorporal processes of oral drugs. Inhibition of intestinal drug metabolizing enzymes and intestinal efflux transporters suggested the enhancement of drug intestinal absorption by glycyrrhetinic acid, whereas the inhibition of uptake transporters such as OATP may perturb the intestinal absorption of the corresponding substrates. Overall, the potent inhibitory effects of glycyrrhetinic acid on drug metabolizing enzymes may increase the systemic exposure of co-administered TCM drugs. For drug distribution, the promoting effect of glycyrrhetinic acid is clear.

3.2.3 Flavonoids

Liquiritin and isoliquiritin, but not liquiritigenin and isoliquiritigenin, form complexes with compounds like aconitine [72]. In addition, liquiritin inhibits CYP3A4 activity [28], but weakly inhibits UGTs compared with liquiritigenin [73]. Furthermore, liquiritin and isoliquiritin inhibit the activity of P-gp [74].
Liquiritigenin and isoliquiritigenin significantly inhibit CYPs 1A2, 2C9, 2C19, 2D6, and 3A4, especially 1A2 and 2C9, in HLMs [38]. Furthermore, liquiritigenin is a UGT inhibitor, with \( K_i \) values for UGT1A1 and UGT1A9-mediated 4-MU glucuronidation reactions of 9.1 and 3.2 \( \mu \text{M} \), respectively [73]. Additionally, liquiritigenin and isoliquiritigenin inhibited monoamine oxidase (MAO) A and B in a dose-dependent manner, with IC\(_{50}\) values of 32 and 13.9 \( \mu \text{M} \), and 104.6 and 47.2 \( \mu \text{M} \), respectively [75].

In contrast, the repeated treatment with liquiritigenin (15 mg/kg per day, i.v., for 3 days) induced UGT1A in the liver of rats [76]. In addition, real-time reverse transcription-polymerase chain reaction (RT-PCR) analysis revealed that the treatment with liquiritigenin significantly increased the mRNA expression of transporters including MRP2, bile salt export pump (BSEP), OATP1A1, taurocholic acid co-transporter protein (NTCP), and MRP3, but not MRP1 in the liver of rats [76]. The transactivation of UGT1A1 and MRP2 \textit{in vitro} and \textit{in vivo} by isoliquiritigenin was Nrf2-dependent [77].

In short, liquiritin and isoliquiritin characteristically form complexes with certain compounds; they are weak inhibitors of drug metabolizing enzymes, but potent inhibitors of P-gp; in contrast, liquiritigenin and isoliquiritigenin exhibit significant inhibitory effects on CYPs, UGTs, and MAOs, but repeated treatment with liquiritigenin and isoliquiritigenin may induce some UGTs and transporters.

Glabridin is a specific constituent in GG [1]. It was found to result in mechanism-based inactivation (i.e., not reversible) of the activities of CYP3A4 (with a \( K_i \) value for inactivation of 7 \( \mu \text{M} \) and the time required for a 50% decrease of the activity of CYP3A4 as 5 min) and CYP 2B6 (63% loss in enzymatic activity after incubation with 30 \( \mu \text{M} \) glabridin for 30 min)
in the presence of nicotinamide adenine dinucleotide phosphate (NADPH) [78]. In addition, glabridin competitively inhibited (i.e., in a reversible manner), but did not inactivate CYP2C9, whereas CYP2D6 and CYP2E1 were virtually unaffected [78]. In addition to its action as a substrate of P-gp [79], glabridin inhibited the function of P-gp in KBC2 cells and increased the cellular accumulation of daunorubicin in a concentration-dependent manner [71]. Licochalcone A is a specific constituent in GI [1]. In HLMs, the Ki values of licochalcone A for CYPs 1A2, 2C9, 2C19, 2C8, and 3A4, are 1.02, 0.17, 3.89, 0.89, and 2.29 μM, respectively [80]. In addition, licochalcone A strongly inhibits UGT1A1, 1A3, 1A4, 1A6, 1A7, 1A9, and 2B7 [with both IC50 and Ki values below 5 μM], but moderately inhibited UGT1A8, 1A10, 2B4, 2B15, and 2B17 in HLMs [81]. Hence, licochalcone A exhibits a high herb-drug interaction potential owing to inhibition of CYPs and UGTs.

3.2.4 Others

Glycyrol has IC50 values of 1.3 and 16.1 μM in human recombinant cDNA-expressed CYP1A1 and CYP1A2, respectively [82]. In addition, it inhibited CYP2C9 with IC50 values of 0.67 μM in human recombinant cDNA-expressed CYP2C9 [82].

3.3 Brief summary

In general, most of the constituents discussed could inhibit drug metabolizing enzymes and some efflux drug transporters. Hence, a single dose of the constituents would increase the systemic exposure of drugs administered in combination. In contrast, repeated intake of the constituents would decrease the systemic exposure of the co-administered drugs owing to the
induction of the enzymes and efflux transporters.

In addition, some constituents have specific features that exert profound influences on the pharmacokinetics of the combined drugs. For example, glycyrrhizic acid improves drug solubility and permeability, and glycyrrhetinic acid is an effective promoter of drug distribution. *Gancao* proteins share common features with glycyrrhizic acid, glycyrrhetinic acid, liquiritin, and isoliquiritin, that is, the formation of complexes when co-administered with TCM drugs. However, the opposite results are seen: the formation of complexes with glycyrrhizic acid and glycyrrhetinic acid increases the solubility and promotes the distribution of the co-administered drugs; in contrast, the formation of complexes with *Gancao* proteins may cause precipitation and limit the absorption of the combined drugs.

4 Representative *Gancao*-TCM pharmacokinetic interactions

4.1 *Gancao-Aconiti Radix* (Chuanwu) and *Gancao-Aconiti Lateralis Radix Praeparata* (Fuzi)

*Chuanwu* and *Fuzi* are produced from the dried root or daughter root, respectively, of the plant aconitum (*Aconitum carmichaeli* Debx.), and exert various pharmacological effects but also lethal cardiotoxicity [83]. The toxicity of *Chuanwu* and *Fuzi* is mainly caused by the presence of diester-diterpene alkaloids (DDAs), such as aconitine, mesaconitine, and hypaconitine, whereas the monoester-diterpenoid alkaloids (MDAs) were less toxic [83]. *Gancao* was usually used in association with *Chuanwu* and *Fuzi* to reduce their potential toxicity [2]. From a pharmacokinetic perspective, *Gancao* was helpful for the reduction of the systemic exposure of toxic DDAs after the ingestion of *Chuanwu* or *Fuzi*. 
Compared with the aconitum decoction, the addition of *Gancao* decreased the content of aconitine in the decoction by 84% [34], which was in accordance with a previous report [72]. The formation of complexes with DDAs has been observed [34, 72, 84]. For example, liquiritin and isoliquiritin could form complexes with aconitine that have a 1:1 stoichiometry [72]. In addition, *Gancao* contained proteins stable to boiling (with molecular weight of approximately 31 kDa) that were able to encapsulate aconitine with a molar ratio of approximately 1:5.2 to form a stable complex [34]. The formation of complexes with proteins from *Gancao* finally reduced the acute toxicity of i.p.-injected aconitine in ICR mice [34]. The basic assumption was that the formation of complexes results in precipitation and is directly associated with the decreased content and, consequently, intake of DDAs [84].

In addition, the repeated administration of GU extract enhanced the metabolic rate of DDAs such as hypaconitine; the mechanism may be related to the induction of hepatic metabolizing enzymes [85].

Some constituents in *Gancao* extract promote the intestinal absorption of DDAs. For example, liquiritin and isoliquiritin promote the transportation of hypaconitine across the Caco-2 cell monolayer by reducing its efflux mediated by P-gp [86]. In addition, pretreatment as well as co-administration of liquiritin enhanced the intestinal absorption of aconitine and thereby increased systemic drug exposure by 1.5 or 2.0 times through the inhibition of intestinal P-gp activity [74].

In summary, sediment formation and the resulting decrease in the content and intake of DDAs contributed significantly to the detoxification effect of *Gancao* extract on *Chuanwu* and *Fuzi*. However, given the positive effects of the formation of complexes with glycyrrhizic acid on
drug solubility, the exact mechanism of sedimentation formation should be determined. In addition, the toxicity and the pharmacokinetics of the formed sediment should be evaluated.

4.2 Gancao-Tripterygium wilfordii (Leigongteng)

Leigongteng is a woody vine of the Celastraceae family [87]. It is widely used in clinics to treat various autoimmune and inflammation-related conditions [87]. However, according to a meta-analysis, it induces a wide range of adverse events, with an overall incidence of 26.7% in 23,256 Leigongteng users [88]. Gancao could effectively decrease the toxicity and increase the efficacy of Leigongteng for the treatment of patients with rheumatoid arthritis [89]. Leigongteng mainly contains effective, but also toxic constituents, such as triptolide [90] and celastrol [91]. Hence, the modification of the pharmacokinetics of the constituents could lead to beneficial effects on their toxicity.

A Gancao extract protected against triptolide-induced oxidative stress, partly via the activation of the Nrf2 pathway [92], which regulates the transactivation of drug metabolizing enzymes such as UGT1A1 and efflux drug transporters such as MRP2 [77]. Pretreatment with glycyrrhizic acid at 100 mg/kg/day for 7 days consecutively accelerated the metabolic elimination of triptolide from the body [93]. In female rats, the AUC, mean residence time (MRT), and T1/2 of triptolide in the glycyrrhizic acid group were approximately half that in the control group and the clearance rate (CL/F) was doubled; conversely, in male rats, the AUC was approximately one-quarter of that of the control group and CL/F was three times higher [93]. The detoxification effect on triptolide was attributed to the induction of CYP3A by glycyrrhizic acid, as evidenced by the reversing effect of ketoconazole, a CYP3A inhibitor.
In addition, pretreatment with glycyrrhetinic acid enhanced the P-gp-dependent elimination and reduced the accumulation of triptolide in HK-2 cells [94]. Isoliquiritigenin may reduce the hepatic oxidative stress and hepatic accumulation of triptolide, as well as its metabolites, through the enhanced expression of Nrf2 and efflux transporters, including P-gp, MRP2, and MRP4 [95]. Glycyrrhizinic acid significantly decreased the plasma concentration (from 64.36 to 38.42 ng/mL) and AUC(0-t) (from 705.39 to 403.43 g/L) of celastrol in rats [96]. Glycyrrhizinic acid could decrease the intestinal absorption of celastrol via induction of the activity of P-gp and acceleration of its hepatic metabolism [96].

In brief, the detoxification effects of *Gancao* and its constituents on *Leigongteng* and its toxic constituents are associated with the induction of drug metabolizing enzymes and efflux drug transporters.

### 4.3 *Gancao*-*Coptidis Rhizoma* (*Huanglian*)

*Huanglian* is produced from the medicinal plants of the family Ranunculaceae, such as *Coptis chinensis* Franch [97]. It has multiple pharmacological effects [97] as well as acute toxicity [98], which was associated with cholinesterase inhibition [99]. Isoquinoline alkaloids, in particular berberine, were identified as the major bioactive constituents of *Huanglian* [97, 98].

*Huanglian* is usually combined with *Gancao* [2]. In a single-dose pharmacokinetic study, *Gancao* dramatically decreased the intestinal absorption and, consequently, the exposure levels of berberine in mice [100].

Berberine is a substrate of P-gp [101] and is eliminated via metabolism [102, 103]. However, the effects of *Gancao* on the pharmacokinetics of berberine could not be attributed to
intestinal P-gp inhibition or the induction of drug metabolizing enzymes: the inhibition of P-gp would lead to an increase in the intestinal absorption of berberine, whereas enzyme induction occurs only after repeated administration. Indeed, Gancao extract promoted the intestinal absorption of berberine, jateorhizine, and palmatine from a Huanglian extract in a study using single pass intestinal perfusion model rat with jugular vein cannulation [104]. In addition, the inhibition of P-gp by glycyrrhizic acid improved the permeability of berberine in a Caco-2 cell monolayer and increased its AUC value by approximately 6-folds in rats [105]. Hence, other mechanisms are expected to be involved in the reduced intestinal absorption of berberine.

The results of Fourier transform infrared spectroscopy (FTIR) spectroscopic characterization and HPLC and time-off-flight mass spectrometry (TOF-MS) measurements suggested that some new chemical compounds, i.e., complexes, were formed during the co-extraction of Huanglian and Gancao [106]. The co-decoction of Huanglian and Gancao not only caused significant variations in the concentration of constituents, but also produced at least 16 complexes with structures that consisted of a saponin part from Gancao and an alkaloid part from Huanglian in a 1:1 stoichiometry [107]. Interestingly, all these saponins contained two glucuronic acid units (GluA-GluA), whereas the alkaloid part was predominantly berberine/epiberberine [107]. It was assumed that in the produced complex, the association of the hydrophilic carboxylic group in the saponins with the quaternary ammonium ion in the alkaloid caused their retention inside the complex, whereas the hydrophobic part was exposed to the aqueous phase and therefore induced the generation of precipitates [107]. In addition, the physical mixture of the extracts of Huanglian and Gancao produced similar complexes,
which suggested that co-decoction was not necessary to achieve the interactions between these two TCMs [107]. The formation of complexes with simvastatin increased the solubility of simvastatin by no more than 100-fold [46], whereas the formation of complexes with berberine ultimately resulted in precipitation. An exploration of the underlying mechanisms is therefore of interest.

In brief, the generation of complexes with *Gancao* constituents, including saponins, may lead to precipitates, the consequent reduction of the intake of isoquinoline alkaloids in *Huanglian*, and a reduction in the risk of acute toxicity. However, the intestinal absorption and systemic exposure of berberine in these precipitates warrant further study.

5 Conclusions

*Gancao* exerts synergistic effects when administered in combination with other TCMs and is used widely to increase the efficacy and decrease the adverse reactions of the combined TCMs. From a pharmacokinetic perspective, the detoxification effect of *Gancao* is attributable to the contained constituents such as macromolecules, i.e., proteins from *Gancao*, and small molecules such as glycyrrhizic acid, glycyrrhetinic acid, liquiritigenin, isoliquiritigenin, glabridin, and licochalcone A. The mechanisms are related to the formation of complexes, the influences on drug solubility, permeability, distribution, and metabolism; however, these occur in a TCM-dependent manner and an integrated explanation has not yet been proposed. The detoxification effect of a single dose of *Gancao* is mediated by constituents that form complexes with the toxic constituents in the co-administered TCMs and ultimately cause the formation of precipitates; hence, decreasing intestinal absorption is the
major mechanism. In contrast, repeated doses of Gancao may exert detoxification effects via the induction of drug metabolizing enzymes and efflux drug transporters.

6 Expert opinion

Studies on the pharmacokinetic mechanisms underlying the detoxification effect of Gancao are clinically important. For example, with the knowledge that the formation of precipitate is important for Gancao to detoxify the co-administered TCMs, the patients would like to keep the sediments when they are taking the herb extract. On the other hand, it should be noted that the detoxification effects of Gancao is conflicted by its potential toxicological effects, including hypertension, hypokalemia [108], and hypokalemic-induced secondary disorders [109]. Given that the adverse reactions are Gancao dose related, the daily dose of Gancao extract and its bioactive constituents should be restricted. For example, the daily intake of 0.015-0.229 mg glycyrrhizin/kg body weight/day had been suggested [110].

In future studies, the details of the components of Gancao extract that mediate pharmacokinetic interactions remained to be elucidated. In addition to the several constituents discussed above, Gancao extract contains more than 250 low-molecular-weight constituents. Indeed, some of the constituents, such as liquiritin apioside [1], are present at close to, or even higher than the amounts of the constituents discussed above. Their pharmacokinetic interaction potentials have not yet been revealed. Interestingly, some constituents in Gancao extract were shown to reduce the bioavailability of glycyrrhizic acid in rats and humans [111, 112]. The lipophilic constituents of Gancao extract (e.g. polyphenols) reduced the intestinal absorption of glycyrrhizic acid via a reduction in the gastric emptying rate and, consequently,
its bioavailability [113]. In contrast, the hydrophilic components (such as amino acids and polyamines) increased the bioavailability of glycyrrhizic acid and glycyrrhetic acid [113]. Compared to that of the pure constituents, the bioavailability of free flavonoids and coumarins in \textit{Gancao} water extract significantly improved; for example, 133- and 109-fold increases were observed for liquiritigenin and isoliquiritigenin, respectively [112]. It is reasonable to assume the potential and potent pharmacokinetic influences of these constituents on co-administered TCMs or drugs. With respect to feasibility, the pharmacokinetic interactions of several fractions of \textit{Gancao} with certain properties, rather than specific constituents, should be explored.

\textit{Gancao} not only decreases the exposure to toxic constituents but also increases the exposure to some of the effective constituents. For example, for the Peony-Glycyrrhiza decoction (also known as \textit{Shao-Yao-Gan-Cao-Tang}, \textit{Shaoyao-Gancao Decoction} in some studies literatures), which is composed of \textit{Shaoyao} (\textit{Paeoniae Radix}) and \textit{Gancao}, an overall study based on chromatographic fingerprinting experiments showed that the blood exposure of some \textit{Shaoyao} constituents was dramatically increased (by 1.3 to 11.3-fold) with the \textit{Shaoyao-Gancao} decoction [114]. Some constituents in \textit{Gancao}, including glycyrrhizic acid and 18 beta-glycyrrhetinic acid, increased the intestinal absorption of paeoniflorin, the major bioactive constituent in \textit{Shaoyao} extract, which was related to intervention with the efflux transport of paeoniflorin mediated by intestinal P-gp [115]. In addition, \textit{Gancao} increased the exposure to cinnamic acid in \textit{Cinnamomi Ramulus} (\textit{Guizhi}) [116], daphnetin in \textit{Daphnes Cortex} (\textit{Zhushima}) [117], and platycodin D and deapio-platycodin in \textit{Platycodonis Radix} (\textit{Jiegeng}) [118]. The material responsible and the mechanisms of the enhancive
pharmacokinetic effects of *Gancao* should be explored.

Furthermore, the species difference of the pharmacokinetic interactions of *Gancao* should be explored. For example, unlike in rats [21, 23], *Gancao* extract did not induce CYP3A4-catalyzed midazolam hydroxylation in humans [119]. This may have been a result of the different experimental designs as well as the intrinsic species difference with regard to drug metabolism and drug disposition [19]. Hence, the clinical basis of the pharmacokinetic interactions of *Gancao* should also be evaluated.

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