Front. Med. 2020, 14(1): 68–80
https://doi.org/10.1007/s11684-019-0690-z

RESEARCH ARTICLE

New incompatible pair of TCM: Epimedii Folium combined with Psoraleae Fructus induces idiosyncratic hepatotoxicity under immunological stress conditions

Yuan Gao[1,2,*], Zhilei Wang[2,3,*], Jinfa Tang[4], Xiaoyi Liu[2], Wei Shi[2,5], Nan Qin[2,5], Xiaoyan Wang[4], Yu Pang[6], Ruisheng Li[7], Yaming Zhang[2], Jiabo Wang[2], Ming Niu (✉)[2], Zhaofang Bai (✉)[2], Xiaohe Xiao (✉)[8]

[1]School of Chinese Materia Medica, Beijing University of Chinese Medicine, Beijing 100029, China; [2]China Military Institute of Chinese Materia, the Fifth Medical Centre, Chinese PLA General Hospital, Beijing 100039, China; [3]School of Pharmacy, Chengdu University of Traditional Chinese Medicine, Chengdu 611137, China; [4]The First Affiliated Hospital of Henan University of Chinese Medicine, Zhengzhou 450000, China; [5]School of Pharmacy, Jiangxi University of Traditional Chinese Medicine, Jiangxi 330004, China; [6]National Institutes for Food and Drug Control, Beijing 100050, China; [7]Research Center for Clinical and Translational Medicine, the Fifth Medical Centre, Chinese PLA General Hospital, Beijing 100039, China; [8]Integrative Medical Centre, the Fifth Medical Centre, Chinese PLA General Hospital, Beijing 100039, China

Abstract **Epimedii Folium (EF) combined with Psoraleae Fructus (PF) is a common modern preparation, but liver injury caused by Chinese patent medicine preparations containing EF and PF has been frequently reported in recent years. Zhuangguguanjiewan pills (ZGW), which contain EF and PF, could induce immune idiosyncratic liver injury according to clinical case reports and a nonhepatotoxic dose of lipopolysaccharide (LPS) model. This present study evaluated the liver injury induced by EF or PF alone or in combination and investigated the related mechanism by using the LPS model. Liver function indexes and pathological results showed that either EF or PF alone or in combination led to liver injury in normal rats; however, EF or PF alone could lead to liver injury in LPS-treated rats. Moreover, EF combined with PF could induce a greater degree of injury than that caused by EF or PF alone in LPS-treated rats. Furthermore, EF or PF alone or in combination enhanced the LPS-stimulated inflammatory cytokine production, implying that IL-1β, which is processed and released by activating the NLRP3 inflammasome, is a specific indicator of EF-induced immune idiosyncratic hepatotoxicity. Thus, EF may induce liver injury through enhancing the LPS-mediated proinflammatory cytokine production and activating the NLRP3 inflammasome. In addition, the metabolomics analysis results showed that PF affected more metabolites in glycerophospholipid and sphingolipid metabolic pathways compared with EF in LPS model, suggesting that PF increased the responsiveness of the liver to LPS or other inflammatory mediators via modulation of multiple metabolic pathways. Therefore, EF and PF combination indicates traditional Chinese medicine incompatibility, considering that it induces idiosyncratic hepatotoxicity under immunological stress conditions.**

Keywords Epimedii Folium; Psoraleae Fructus; idiosyncratic hepatotoxicity; traditional Chinese medicine incompatibility

Introduction

Traditional Chinese medicine compatibility (TCMC) is a basic form of clinical medication of traditional Chinese medicine (TCM) for the treatment and prevention of diseases. Proper TCMC cannot only improve clinical efficacy but also reduce toxicity caused by drug interactions. However, administering incompatible drugs may increase toxicity, create new toxicity, and even negatively affect treatment efficacy [1]. Traditional Chinese medicine incompatibility (TCMI), which primarily comprises "eighteen incompatible pairs" and "nineteen medicaments of mutual antagonism," makes great progresses and plays a

Received September 12, 2018; accepted December 27, 2018
Correspondence: Ming Niu, nmbright@163.com;
Zhaofang Bai, baizf2008@hotmail.com;
Xiaohe Xiao, pharmacy_302@126.com

*These authors contribute equally to this work.

crucial role in reducing the risk of clinical medication [2]. However, with the endless number of reports about liver injury related to traditional nontoxic medicines, new challenges emerged, and they should be viewed objectively and managed scientifically. Furthermore, TCMI that affects clinical medication safety should be further studied.

Epimedii Folium (EF) combined with Psoraleae Fructus (PF) is a common TCM preparation used to invigorate the liver and kidney; however, related preparations can lead to liver injury. The Chinese Food and Drug Administration has reported that liver injury can be caused by two Chinese patent medicines, namely, Zhuangguguanjiewan pills (ZGW) and Xianlinggubao capsule (XLG), which both contain EF and PF [3–9]. Increasing clinical reports have indicated potential hepatotoxicity in patients who use PF-containing drugs [10,11]. One clinical case demonstrated that a PF-induced liver injury can obtain a score of 3 by Roussel Uclaf Causality Assessment Method (RUCAM)-based causality, which may be related to drug metabolism *in vivo* and immune responses [12]. Moreover, with the updated RUCAM, a major step forward has been made to facilitate causality assessment in suspected herb-induced liver injury (HILI) cases; then, the updated RUCAM is improved by providing a better definition of the elements to consider and more accuracy in data and elements to assist the exclusion of alternative causes [13]. EF could induce liver injury according to clinical research, but no hepatotoxicity was observed in a long-term toxicity experiment [14]. Additionally, ZGW, which contains both EF and PF, has shown immune idiosyncratic liver injury on the basis of clinical case reports and a nonhepatotoxic dose of lipopolysaccharide (LPS)-mediated rat model of idiosyncratic drug-induced liver injury (IDILI). Nevertheless, the insight into whether EF or PF alone or in combination can induce idiosyncratic liver injury remains to be studied. In this study, we evaluated the liver injury induced by EF or PF alone or in combination and investigated the related mechanism by using the LPS model.

Materials and methods

Chemicals and reagents

Acetonitrile was purchased from Merck (Darmstadt, Germany). Methanol was purchased from Burdick and Jackson (Ulsan, Korea). Formic acid was obtained from Sigma-Aldrich Co. (St. Louis, USA). These three substances were of HPLC grade. Furthermore, double-distilled water was purified using the Millipore water purification system (Millipore, Bedford, MA, USA). LPS and pentobarbital sodium (20151217) were purchased from Sinopharm Chemical Reagent Co., Ltd. LPS was derived from *Escherichia coli* 055:B5 (Lot #057M4013V). Assay kits to detect plasma alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were purchased from Jiancheng Biological Technology, Co., Ltd. (Nanjing, China). All of the other reagents and solvents were of the highest commercially available grade.

Preparation and analysis of test materials

EF and PF were supplied by Sanjiu Medical and Pharmaceutical Co., Ltd. (Batch No. 1512007S; shelf time: 3 years). *Epimedium koreanum* Nakai and *Psoralea corylifolia* L. were authenticated by Dr. Xiaohe Xiao, director of Integrative Medical Centre, the Fifth Medical Centre, Chinese PLA General Hospital (Beijing, China). Voucher specimens were deposited in the Herbarium of Sanjiu Medical and Pharmaceutical Co., Ltd., China. In addition, EF and PF were crushed into fine powders, and the quality of these herbs for use in animal studies were tested and verified. Next, EF and PF powders were suspended in 0.5% sodium carboxymethyl cellulose (CMC-Na) for administration.

Animal handling and experimental design

Male Sprague–Dawley rats (180–220 g) were obtained from the Laboratory Animal Center of the Academy of Military Medical Sciences (License No. SCXK2012-004, Beijing, China). The rats were housed in the Laboratory Animal Center of the Fifth Medical Centre, Chinese PLA General Hospital (animal ethics committee approval No. IACUC-2017-003). The room temperature was regulated at 25 ± 2 °C with 50%–60% humidity. A 12 h light/dark cycle was maintained, and the animals were allowed to access food and water *ad libitum*. All of the animals were acclimatized for 1 week prior to the start of the experiments.

The assessment of idiosyncratic hepatotoxicity was based on our previously reported rat model, which was modified from the literature. On the basis of one of our published articles and the specification of ZGW, we adopted thrice the clinical dose of ZGW (3.78 g/kg) to perform our research [3]. Then, on the basis of the manufacturing process provided by the manufacturer, 0.22 g/kg of EF and PF were adopted to accomplish our research. The animals were randomly divided into eight separate groups, which were administered with deionized water (control group, Con); EF powder, 0.22 g/kg (EF); PF powder, 0.22 g/kg (PF); EF powder and PF powder, 0.22 g/kg (PE); LPS, 2.8 mg/kg (model group, Mod); EF powder (0.22 g/kg) and LPS (2.8 mg/kg) (EL); PF powder (0.22 g/kg) and LPS (2.8 mg/kg) (PL); or EF powder, PF powder (0.22 g/kg), and LPS (2.8 mg/kg) (PEL). The animals were injected with LPS or normal saline in the tail vein by using standard techniques, followed by the intragastric administration of the aforementioned herbs or

an equivalent volume of normal saline 2 h later. Then, 6 h after oral administration, the rats were anesthetized with pentobarbital sodium. Blood samples were collected from the inferior vena cava by using a syringe containing sodium heparin after a midline abdominal incision, and the livers were removed from the rats immediately after sacrifice. The isolated livers were utilized for histopathological examination, and the plasma samples were separated from the collected blood and analyzed for ALT and AST activities. The experimental protocol was approved by the Committee on the Ethics of Animal Experiments of the Fifth Medical Centre, Chinese PLA General Hospital.

Serum biochemistry, histopathological analysis, and immunohistochemistry

After centrifugation (3500 rpm, 10 min, 4 °C), plasma ALT and AST activities were determined according to the microplate assay kit instructions. The left lateral liver lobes obtained were fixed and preserved in 10% neutral-buffered formalin for at least 72 h before being processed for histologic analysis. Paraffin-embedded samples were sectioned at approximately 5 μm and stained with hematoxylin and eosin (HE). TdT-mediated dUTP Nick-End Labeling (TUNEL) staining was performed using the *In Situ* Cell Death Detection Kit, POD (Roche, Switzerland), following the manufacturer's instructions. The samples were visualized with a fluorescence microscope (Nikon, Japan) and then imaged.

Serum sample handling

In a polypropylene tube (1.5 mL), 200 μL thawed serum sample and 600 μL methanol were mixed and incubated for 20 min at 4 °C. After the solid debris was removed by centrifugation at 12 000 rpm for 10 min at 4 °C, the supernatants were moved into a polypropylene tube and filtered with a syringe filter (0.22 μm). Finally, the UHPLC–mass spectrometry (MS) system was injected with 4 μL supernatant.

Metabolomics analysis

Metabolic profiling analysis of the biofluids was performed using the Waters Xevo G2-XS QTOF/MS (Waters, Manchester, UK). An Acquity UPLC HSS T3 C18 column analytical column (temperature 30 °C) was injected with 4 μL aliquots of each sample. For positive electrospray ionization source (ESI) analysis, samples were isolated using a 30 min linear gradient of solvent A (water spiked with 0.1% formic acid) and solvent B (acetonitrile spiked with 0.1% formic acid) as mobile phases. The flow rate was fixed at 0.30 mL/min. Then, 10 μL of each sample was drawn as a quality control sample to ensure that the system was stable and the analyses were repeatable. Every 10th sample was injected with the control sample and subsequently analyzed. In analysis, the adopted mobile phases were (A) water and (B) acetonitrile. The same conditions were applied for other analyses. MS was performed with the Waters Xevo G2-XS QTOF/MS instrument (Waters, Manchester, UK) by using an ESI in both positive and negative modes. Progenesis QI (v. 2.0, Waters Technologies, UK) was used to preprocess all of the data. The online software program MetaboAnalyst 3.0 was used to estimate the missing value and to filter and normalize the data. For multivariate statistical analyses, including principal component analysis (PCA) and orthogonal partial least-squares discriminant analysis (OPLS-DA), the resultant data matrices were loaded into SIMCA 13.0 (Umetrics, Umeå, Sweden). All of the variables from the data matrix were mean-centered and scaled to the Pareto variance before PCA. The score plot of the PCA was used to demonstrate the natural interrelationship of the observations. Variables with a high VIP and |p(corr)| value (VIP $\geqslant$ 1.0 and |p(corr)| $\geqslant$ 0.5) in the OPLS-DA model and with significant differences between groups ($P < 0.05$ and folder change > 1.5) were selected as potential biomarkers. All of the selected variables were identified by Agilent MassHunter PCDL Manager software with the KEGG database.

Statistical analysis

The data were analyzed using SPSS (v. 17.0, Chicago, IL, USA). Unless otherwise indicated, the data were expressed as the mean $\pm$ SD. A one-way analysis of variance (ANOVA) was used to statistically analyze the results. The differences were considered significant when $P < 0.05$ and highly significant when $P < 0.01$.

Results

Development of liver injury in LPS-treated rats after EF, PF, or PE administration

The serum biochemistry results are presented in Fig. 1A and 1B. Plasma ALT and AST are well-recognized markers of various types of liver damage and were thereby used for the analysis. As shown in Fig. 1A and 1B, the ALT and AST levels of rats remained unchanged when exposed to low-dose LPS (model group) compared with those of the control group, suggesting that this dose of LPS alone was not associated with liver injury. In addition, no significant differences were found between the AST and ALT levels of rats treated with EF, PF, or PE alone compared with those of the control group. In contrast, the plasma ALT and AST levels of LPS-exposed rats were markedly increased when administered with EF, PF, or PE compared with those of both the control and model groups. Furthermore, the

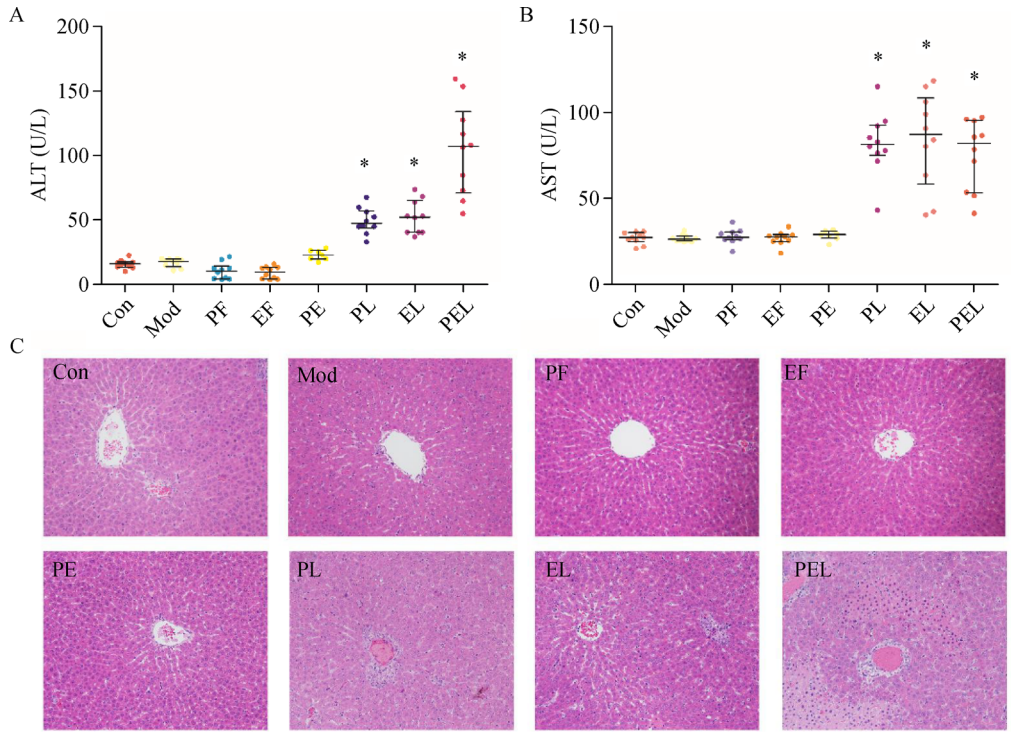

Fig. 1 PF, EF, and PE can induce liver injury in LPS-treated rats. (A) Serum ALT activity in rats co-treated with EF, PF, or PE (normal saline or LPS-treated rats). (B) Serum AST activity in rats co-treated with EF, PF, or PE (normal saline- or LPS-treated rats). * $P < 0.001$, compared with that in the model group. (C) Typical histopathological section photographs of rat liver specimens for HE analysis (magnification 200×).

transaminase activity in PEL-treated rats was the highest among all the groups, and the increase in plasma transaminase activity in EL-treated rats was slightly higher than that in PL-treated rats.

Liver tissues were examined by microscopy to obtain visual evidence of the degree of liver damage. As shown in Fig. 1C, the liver sections of the control group showed normal hepatocyte structures. The liver samples from EF-, PF-, and PE-treated rats were almost indistinguishable from those from the normal rats, although the EF-treated rats exhibited minimal steatosis. The samples from the model group displayed slight inflammatory cell infiltration in the portal area but no evident hepatocyte injury. Liver samples from rats co-treated with PF and LPS exhibited hepatocyte focal necrosis, loss of central vein intima, and inflammatory cell infiltration in portal areas. Notably, the liver damage in specimens co-treated with EF and LPS exhibited more severe hepatocyte focal necrosis and extensive inflammatory cell infiltration in portal areas. Moreover, hepatocyte focal necrosis was most severe in the group co-treated with PE and LPS, further showing the highest plasma ALT level among the groups. The drug-induced liver injury observed in the PEL group was more serious than that observed in the other groups, indicating that PF aggravated the EF hepatotoxicity.

The TUNEL assay did not reveal any apparent hepatocyte apoptosis in the control group and model group, with positive TUNEL signals occasionally scattered in the nuclei as small round or circular shapes (Fig. 2). Compared with the model group, the PL group ($P < 0.05$) exhibited both scattered and clustered positive signals, whereas the EL and PEL groups exhibited significant hepatocyte apoptosis ($P < 0.001$). Furthermore, hepatocyte apoptosis was significantly increased in the PEL group compared with that in the EL group, and a large number of positive signals (block distribution) was observed in the PEL group. Thus, compared with treatment with EF or PF alone, treatment with PE led to a significantly increased hepatocyte apoptosis.

EF or PF alone or PE modulated the inflammatory cytokine expression in an LPS model

ELISA was used to determine the TNF-α, IL-1β, IL-6, and IFN-γ levels in rat plasma (Fig. 3). Compared with those in the control group, the TNF-α, IL-1β, and IL-6 levels in the model group were significantly increased, thus demonstrating that low-dose LPS injected into the tail vein did not cause liver toxicity but instead stimulated the production of considerably numerous inflammatory cytokines. The PF,

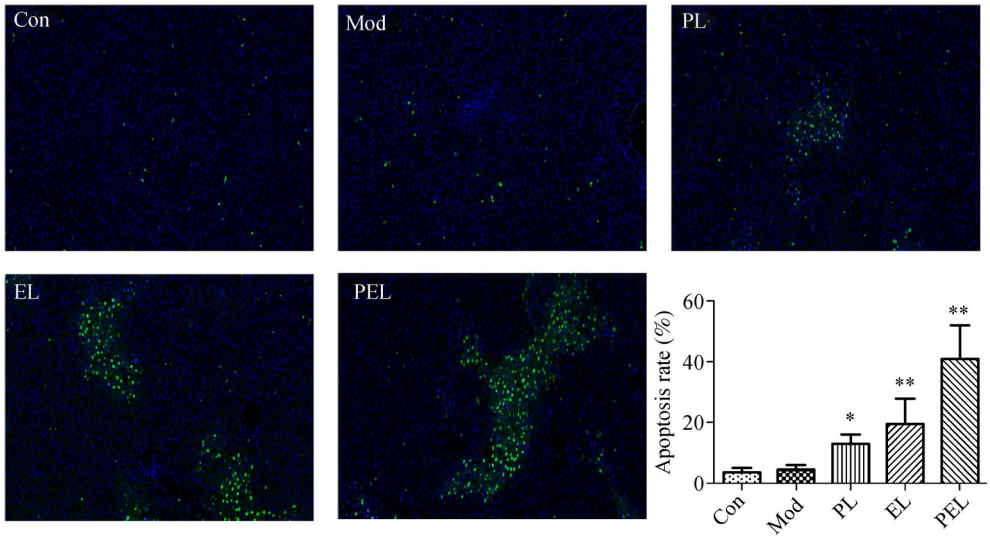

Fig. 2 Positive TUNEL staining (indicative of cell apoptosis) was observed in liver tissues. A representative photomicrograph at 200× magnification is shown. Liver sections were stained for TUNEL (green) and nuclei (blue).*$P < 0.05$, **$P < 0.001$, compared with those in the model group.

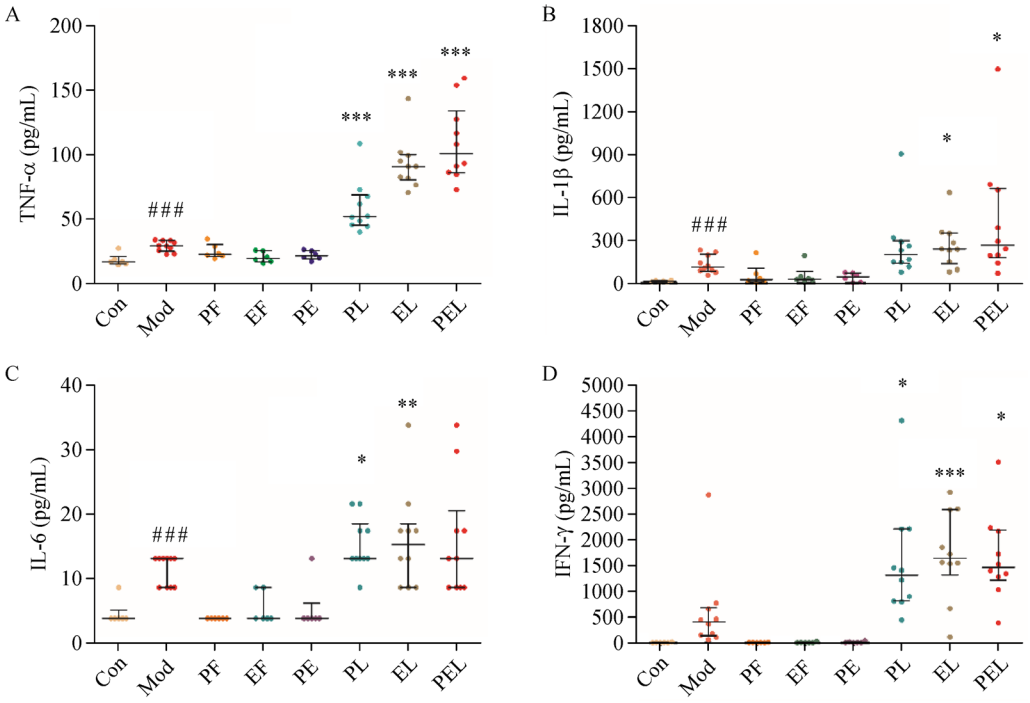

Fig. 3 Effect of PF, EF, or PE on inflammatory cytokines. (A) plasma concentrations of TNF-α; (B) plasma concentrations of IL-1β; (C) plasma concentrations of IL-6; (D) plasma concentrations of IFN-γ. Mod, PL, EL, and PEL groups ($n = 10$); Con, PF, EF, and PE groups ($n = 6$); *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, compared with those in the model group. $^{###}P < 0.001$, compared with that in the control group.

EF, and PE groups exhibited no significant difference compared with the control group. Compared with those in the model group, the TNF-α, IL-6, and IFN-γ levels were significantly increased in the PL group, whereas plasma TNF-α, IL-1β, IL-6, and IFN-γ levels were significantly increased in the EL group. However, the TNF-α, IL-1β, and IFN-γ levels increased significantly in the PEL group compared with those in the model group. Thus, EF, PF, or

PE enhanced the LPS-stimulated production of inflammatory cytokines, implying that IL-1β is a specific indicator of EF-induced immune idiosyncratic hepatotoxicity.

Metabolomics analysis of EF, PF, or PE in an LPS model

The UHPLC system provides a rapid, effective, and convenient method to analyze the variance in chemical constituents between different rat samples. The base peak intensity chromatograms (BPC) of samples from the control, model, EL, PL, and PEL groups in positive and negative ion modes are presented in Figs. S1 and S2, respectively. Visual inspection of these spectra revealed differences in BPC profiles among the control, model, EL, PL, and PEL groups, indicating that metabolite levels were altered by LPS co-administered with EF, PF, or PE.

As the PEL group exhibited the most severe liver damage, the control, model, EL, PL, and PEL groups were specifically selected for explicit classification. Initially, PCA was used as an unsupervised statistical method to study metabolic differences between the control, model, EL, PL, and PEL groups. The score plots for the PCA analysis derived from ESI^+ mode and ESI^- mode data are shown in Fig. S3A and S3B, respectively. As shown in Fig. S3, the QC samples clustered closely in both PCA score plots, demonstrating the stability of the LC/MS system throughout the analysis. Furthermore, apparent separation was observed among the control, model, EL, PL, and PEL groups in both PCA models, indicating considerable metabolite differences between the five groups.

OPLS-DA was used for the in-depth investigation of the differences between the control and model groups, the model and PL groups, the model and EL groups, and the model and PEL groups to identify potential biomarkers for discriminating idiosyncratic liver injury and drug types. Fig. S4A–S4J displays the result of an OPLS-DA model derived from ESI^+ data analysis. Moreover, data analysis of the ESI^- mode was performed, and the results are shown in Fig. S5. Finally, the metabolites from the ESI^+ and ESI^- mode analyses were combined and subsequently identified by their molecular formulas. All of the biomarkers were tentatively identified with the accurate mass–charge ratio by the online METLIN database. The metabolomics analysis results shown in Figs. 4 and 5 and Table 1 revealed that the levels of biomarkers such as phosphatidic acid (PA), phosphatidylethanolamine, phosphatidylinositol (PI), phosphatidylglycerol (PG), phosphatidylserine (PS), sphingomyelin, and N-acylsphingosine in the PEL, EL, and PL groups were significantly different from those in the control group. The specific trend is shown in Table 1. These biomarkers were mainly enriched in the glycerophospholipid metabolic pathway, the biomarker intensity in the EL group changed, whereas that in the PL group changed more evidently. The specificity of certain chemical compounds in the PEL group was significantly increased, indicating that the LPS treatment combined with EF and PF caused a disordered glycerophospholipid metabolism, which in turn caused liver damage. Additional analysis showed increased levels of PA, sphingomyelin, and N-acylsphingosine, as well as decreased levels of phosphatidylethanolamine, PG, and PS in the PL, EL, and PEL groups, especially in the PL group. Thus, liver damage was potentially caused by the disruption of PA and sphingomyelin metabolism.

Discussion

In recent years, the safety problems of traditional nontoxic Chinese medicine have frequently occurred, and some literature have reported many traditional nontoxic Chinese medicines, such as Dictamni Cortex, Menthae Haplocalycis Herba, PF, Bupleuri Radix, Toosendan Fructus, Puerariae Lobatae Radix, Polygoni Multiflori Radix (PM), and Kava, that can cause HILI [15–18]. HILI can be divided into intrinsic or idiosyncratic types. Intrinsic HILI is generally dose or time dependent and predictable with relatively short latency and slight individual differences. However, idiosyncratic HILI is unpredictable, affects only susceptible individuals, is less dose dependent, and has variable latency, presentation, and course [19]. Therefore, the insight into how to scientifically evaluate idiosyncratic HILI induced by traditional nontoxic Chinese medicines has become one of the most challenging problems in the field of TCM safety research [20,21].

RUCAM is the worldwide most commonly used causality assessment method (CAM) for HILI and provides a straightforward application in HILI with scored items. Especially, the updated RUCAM can identify HILI cases early in clinical development, enabling physicians and researchers to take measures to minimize the risk of hepatic reactions [22–24]. In recent years, we have established an integrated chain of evidence for the diagnosis of liver injury induced by Chinese herbal medicines; the integrated evidence-chain method (iEC) is then proposed [25]. On the basis of this method, we demonstrated that the objectivity of liver injury was caused by PM, and the idiosyncratic liver injury of PM may be immune mediated, as proven through an IDILI model [19]. We also have verified that ZGW, which contains both EF and PF, has a property of immune idiosyncratic hepatotoxicity according to iEC and a nonhepatotoxic dose of LPS model [3]. PF may be the main substance in ZGW that induced idiosyncratic liver injury [5]. Furthermore, Teschke used the structured causality assessment to indicate that Ayurvedic herb PF is potentially hepatotoxic [26]. In another study, oral PF administered at 0.4 g versus 1.6 g (high dose) daily for 12 weeks in rats caused liver injury in the form of steatosis

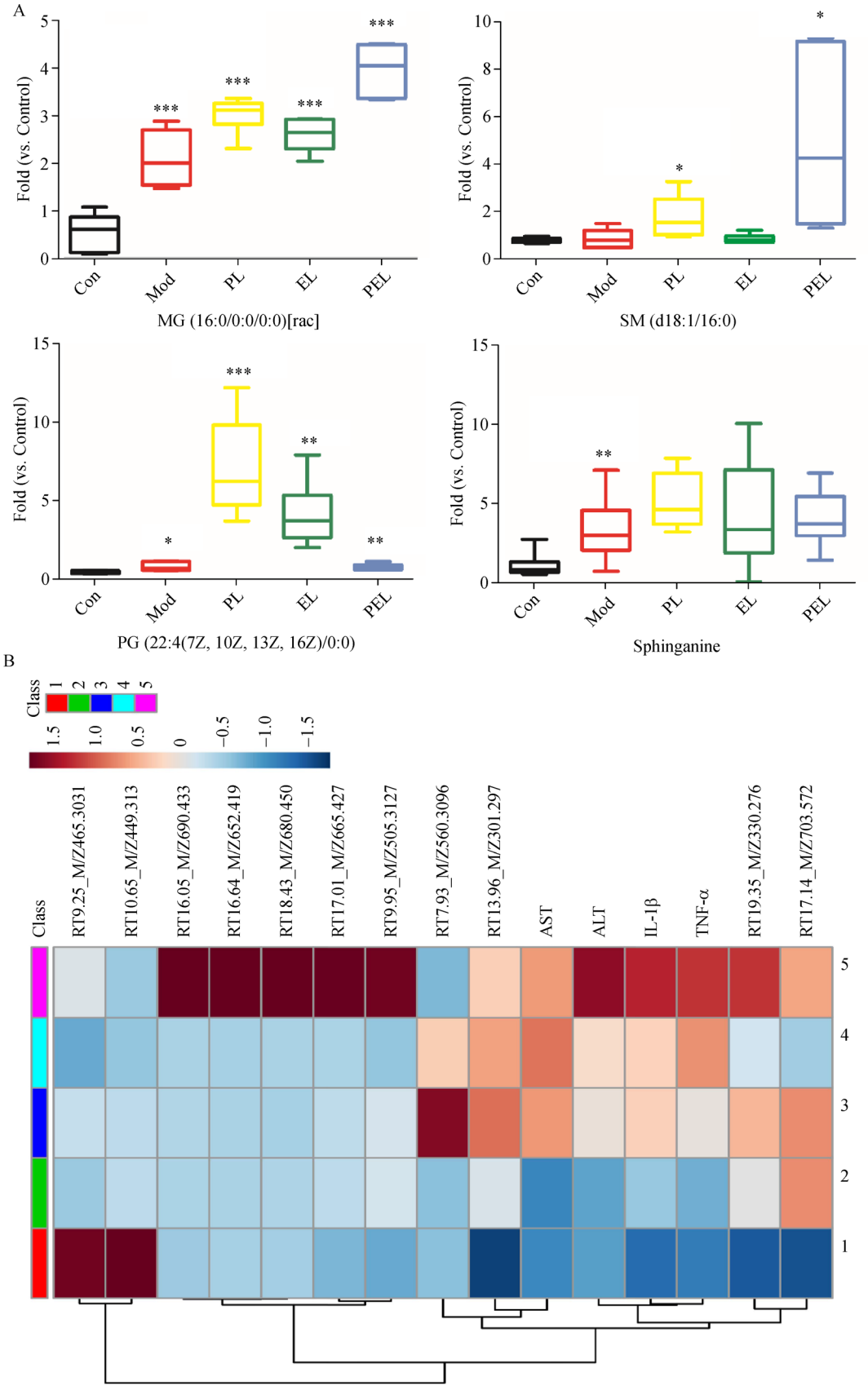

Fig. 4 Correlation analysis between metabolites and immune factors in different groups. (A) Variations in the trends of RT19.35_M/Z330.276, RT17.14_M/Z703.572, RT7.93_M/Z560.3096, and RT13.96_M/Z301.297 are shown. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, compared with those in the control group. (B) Class 1–5 represents the control, model, PL, EL, and PEL groups.

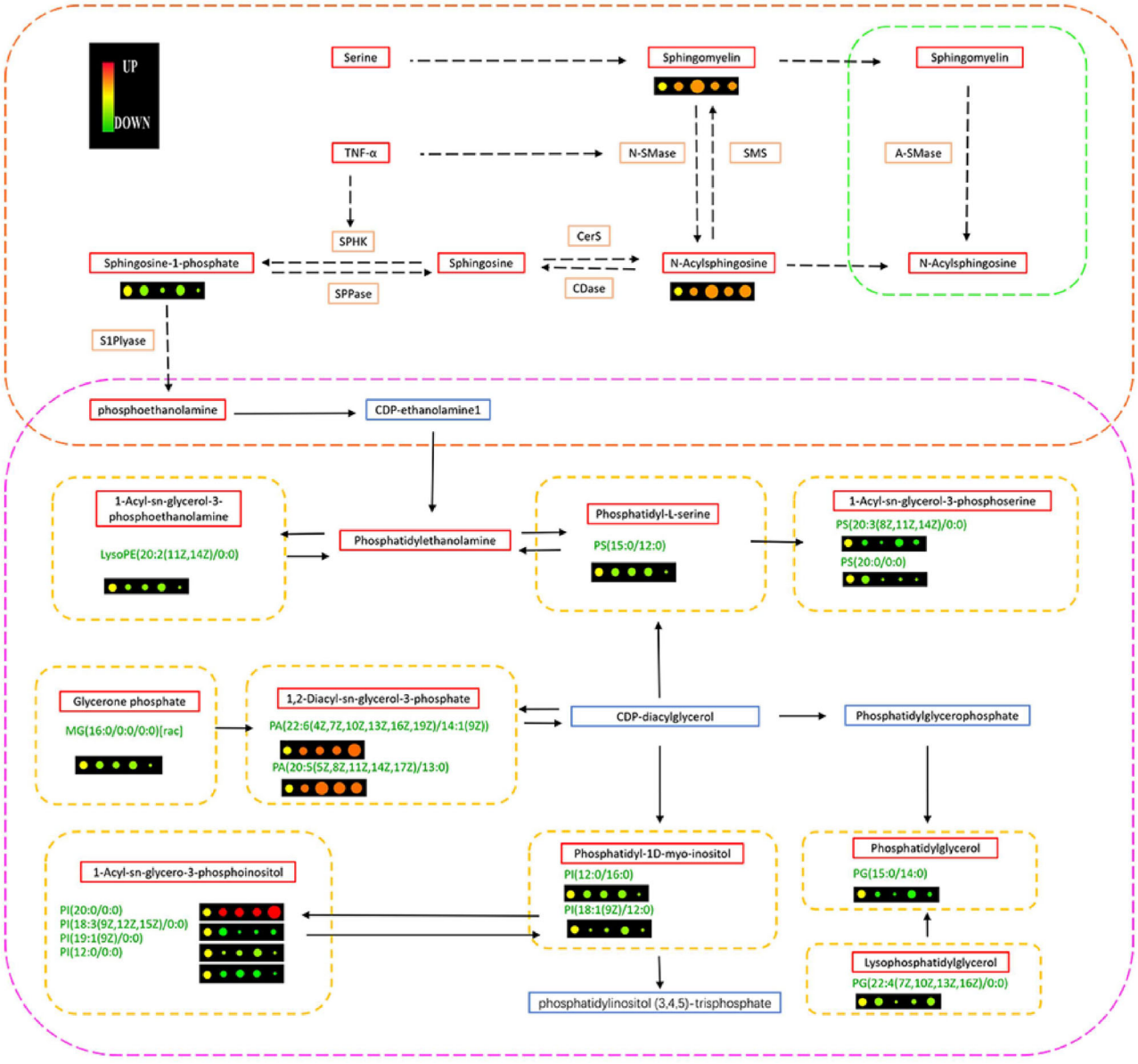

Fig. 5 Schematic diagram of the disturbed metabolic pathway. The size of the ball represents the relative degree of change between groups; the color of the ball represents the tendency of compounds to increase or decrease.

and hepatocyte necrosis [27]. Meanwhile, Bavachin, which is one of the flavonoids in PF, is a hepatotoxic substance [28]. Taken together, these results showed that PF can trigger a series of toxic events, including liver injury. However, the reason why minority individuals are more prone to the adverse reactions remains unanswered. In addition, EF in ZGW also has certain hepatotoxicity. Cheng verified that mice fed with EF for 3 days exhibited vomiting, poor appetite, and decreased activity, and hepatic steatosis was observed after 15 days of administration [29]. In chronic toxicity study, the rats were given 40, 80, and 160 times of human clinical dosage of total flavonoids of EF for 12 weeks, suggesting that the chronic toxicity of this drug was also weak [14]. To sum up, the insight into whether EF can induce liver injury remains controversial

As mentioned, ZGW, which contains EF and PF, can induce immune idiosyncratic hepatotoxicity. Therefore, we used an LPS model to clarify the reasonable or unreasonable compatibility of EF and PF. This study demonstrated that EF, PF, and PE combined with LPS not only increased plasma ALT and AST levels but also caused liver injury. ALT and AST are generally used as sensitive evaluation indexes of liver injury. In fact, liver injury often indicates

Table 1 Differentially identified metabolites for discrimination among the control, model, PL, EL, and PEL groups

Metabolite	Mass (Neutral)	Error (ppm)	Formulate	tR (min)	FOLD			
					Con vs. Mod	Con vs. PL	Con vs. EL	Con vs. PEL
DATA FROM THE ESI$^+$ MODE								
PA (22:6(4Z,7Z,10Z,13Z,16Z,19Z)/14:1(9Z))	690.4334	−10.63	C39H63O8P	16.05	1.5484	1.641	2.1565	22.931
PA (20:5(5Z,8Z,11Z,14Z,17Z)/13:0)	652.4192	−13.48	C36H61O8P	16.64	0.68069	6.817	4.5282	3.7127
PG (22:4(7Z,10Z,13Z,16Z)/0:0)	560.3096	3.24	C28H49O9P	7.93	0.96525	0.17944	0.33306	0.94477
PS (20:3(8Z,11Z,14Z)/0:0)	547.284	12.82	C26H46NO9P	9.40	5.49E-10	1.67E-11	1.00E-08	3.29E-10
MG (16:0/0:0/0:0)[rac]	330.276	3.05	C19H38O4	19.35	0.38613	0.35689	0.40864	0.23563
PI (12:0/18:1(9Z))	780.485	−7.84	C39H73O13P	13.72	4.1323	0.32628	0.75197	0.26141
PI (19:1(9Z)/0:0)	612.3304	−4.79	C28H53O12P	14.78	0.42428	0.49225	0.55941	0.41603
PI (12:0/0:0)	516.2375	−7.62	C21H41O12P	9.15	1.19E-11	3.37E-11	2.33E-11	1.01E-12
Sphingosine-1-phosphate	379.244	12.55	C18H38NO5P	17.04	0.15385	0.049752	0.15583	0.038203
Cer (t18:0/20:0)	611.5902	−8.07	C38H77NO4	13.05	1.1437	0.58018	1.2547	1.5889
SM (d18:1/16:0)	703.5726	4.01	C39H80N2O6P	17.14	1.0255	1.0348	1.0243	1.0245
DATA FROM THE ESI$^-$ MODE								
PS (20:0/0:0)	553.3361	3.37	C26H52NO9P	12.25	0.04056	0.008249	0.00953	0.008266
PI (16:0/12:0)	754.4657	−3.27	C37H71O13P	12.42	0.32803	0.25737	0.5367	0.003003
PI (20:0/0:0)	628.3617	−4.67	C29H57O12P	14.64	13.245	30.108	17.687	2227.6
PI (18:3(9Z,12Z,15Z)/0:0)	594.2807	−0.31	C27H47O12P	15.12	5.24E-12	6.89E-13	1.00E-12	1.72E-12
PS (15:0/12:0)	665.4275	−1.07	C33H64NO10P	17.01	0.39039	0.40176	0.45213	0.073747
PG (15:0/14:0)	680.4509	−13.51	C38H65O8P	18.43	8.61E-10	8.47E-11	2.41E-08	4.10E-10
LysoPE (20:2(11Z,14Z)/0:0)	505.3127	8.19	C25H48NO7P	9.95	0.092292	0.11025	0.26143	0.025222

degeneration or necrosis of hepatocytes, resulting in the leakage of cytosolic enzymes into the blood stream and the increase of serum enzymes. Moreover, ALT mainly exists in the cytoplasm of hepatocytes, but AST is mainly distributed in the mitochondria and cytoplasm of hepatocytes [30–32]. Thus, plasma ALT activity increased significantly in the EL, PL, and PEL groups, but no increase was observed in the remaining groups (Fig. 1). To further prove the result, we examined liver histology and TUNEL staining (Figs. 1 and 2) and found that TUNEL staining (marker for apoptosis) increased in rats treated with PL, EL, and PEL, indicating an increase in cell death caused by these combinations. Similar results were found in the liver histology evaluations. Hence, a minor inflammatory response triggered by a nonhepatotoxic dose of LPS decreased the hepatotoxicity threshold for EF and PF, leading to an increase in inflammatory cell infiltration and liver cell apoptosis. Notably, EF or PF did not cause liver injury in normal rats, whereas EF or PF could induce liver injury in LPS-treated rats. EF combined with PF can induce more severe liver injury in LPS-treated rats than the treatments administered separately. Therefore, EF combined with PF can cause serious adverse reactions during inflammation. Thus, this combination can be categorized as immune idiosyncratic hepatotoxicity.

Our inflammatory cytokine data revealed that TNF-α, IL-6, IL-1β, and IFN-γ positively correlated with EL-, PL-, and PEL-induced liver injury [33–35]. LPS interacted with cell surface receptors such as CD14 and TLR4, to induce NF-κB pathway activation and stimulate monocytes, macrophages, neutrophils, lymphocytes, and other cell types to release considerably numerous cellular factors and inflammatory mediators, such as IL-6 and TNF-α, generating a series of inflammatory reactions and liver injuries [36–39]. Our results revealed that TNF-α levels gradually increased, following the trend PL < EL < PEL (Fig. 3A). The most vital consequence of the idiosyncratic hepatotoxicity lethal stress is the promotion and amplification of inflammation through activating the transcription of cytokines and chemokines such as TNF-α, IL-6, and IL-1β in IDILI [40]. IDILI-associated drugs sensitize hepatocytes to cell death signaling from cytokines such as TNF-α and IFN-γ [41]. IL-1β, which is processed and released by NLRP3 inflammasome activation, is the most potent proinflammatory cytokine, and it is implicated as the effector molecule in many NLRP3-driven diseases, including IDILI [42–47]. Our study revealed that EF might significantly increase IL-1β secretion and activation compared with PF in LPS-treated rats. Thus, IL-1β is a marker for EF-related immune idiosyncratic hepatotoxicity. The results also showed that EF may induce immune idiosyncratic hepatotoxicity mainly by regulating LPS-mediated NLRP3 inflammasome activation. In summary, the activation of either receptor initiates signaling

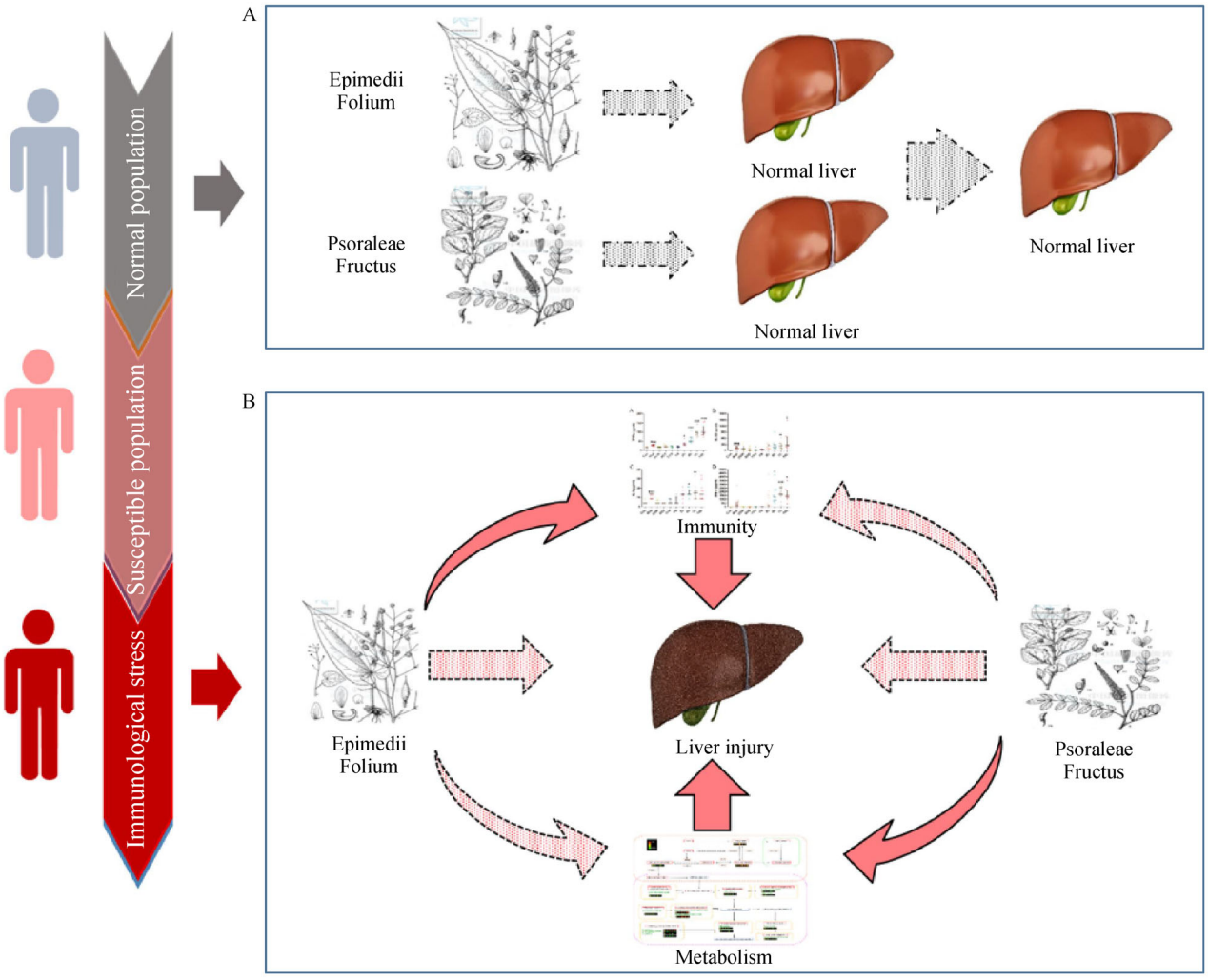

Fig. 6 Schematic diagram of liver injury induced by EF, PF, and PE under immunological stress conditions. (A) EF, PF or PE cannot induce liver injury under normal conditions. (B) EF, PF, or PE can lead to a serious liver injury under immunological stress conditions. The main mechanism of liver injury induced by EF, PF, and PE may be different: EF may regulate immune inflammation to induce liver injury; PF may regulate metabolism to induce liver injury; and PE may lead to a more serious liver injury by the comprehensive regulation of immune inflammation and metabolic dysfunction.

pathways that lead to NF-κB or NLRP3 inflammasome activation, resulting in proinflammatory cytokine production. Thus, an episode of inflammation during EF treatment predisposes animals to induce liver injury, suggesting that EF combined with LPS is an important factor for the occurrence of idiosyncratic drug-induced liver injury.

The metabolomics analysis showed that PF affects more metabolites in the glycerophospholipid and sphingolipid metabolic pathways compared with EF in an LPS model. Specifically, PA (20:5(5Z,8Z,11Z,14Z,17Z)/13:0); PG (22:4(7Z,10Z,13Z,16Z)/0:0); PS (20:3(8Z,11Z,14Z)/0:0); Sphingosine-1-phosphate; Cer (t18:0/20:0); PI (18:3 (9Z,12Z,15Z)/0:0); and PG (15:0/14:0) were greatly affected on the PL group. However, metabonomics analysis showed that the mechanism of liver injury induced by PF is mainly metabolic dysregulation, but EL and PEL groups also affect metabolism. Furthermore, EL, PL, and PEL groups might be regulated by TNF-α, with a gradually increasing trend (PL group < EL group < PEL group) observed for the sphingolipid pathway. TNF-α affects sphingolipid biosynthesis via sphingomyelin phosphodiesterase 2 in the liver [48]. Under normal circumstances, upregulated N-acylsphingosine levels increase sphingosine-1-phosphate levels [49,50]. However, the levels of sphingosine-1-phosphate and its metabolite phosphatidylethanolamine both decreased in our study, potentially causing inhibition and indicating that the PEL, EL, and PL groups inhibited the metabolic conversion of N-acylsphingosine to sphingosine-1-phosphate. Of the two key enzymes in this process, namely, ceramidase and

sphingosine kinase, sphingosine kinase is activated by TNF-α to regulate PA levels. However, both TNF-α and PA levels increased in our study; thus, we speculated that ceramidase but not sphingosine kinase was greatly affected. Ceramidase activity is closely related to hepatocyte apoptosis; although ceramidase mainly hydrolyzes N-acylsphingosine, ceramidase inhibition inevitably leads to the accumulation of upstream N-acylsphingosine and affects the metabolism of sphingomyelin [51,52]. Upstream sphingomyelin and PA metabolic disorders caused the abnormal metabolism of downstream glycerophospholipid metabolites such as phosphatidylethanolamine, PC, and PI. PC and phosphatidylethanolamine are sources of arachidonic acid (AA) and can be hydrolyzed by phospholipase to produce AA, contributing to inflammation by releasing factors such as prostaglandins and leukotrienes [53–58]. PIs are hydrolyzed by phosphatidylinositol 3-kinase (PI3K), which plays a key regulatory role in the occurrence and development of inflammation [59–61]. In fact, PF increases the responsiveness of the liver to LPS or other inflammatory mediators via the modulation of multiple metabolic pathways, thereby leading to hepatotoxicity. Therefore, PF may induce liver injury primarily by causing glycerophospholipid and sphingolipid metabolic dysfunctions under immunological stress conditions.

Overall, the combination of EF and PF can lead to a serious liver injury under immunological stress conditions. Furthermore, the main mechanism of liver injury induced by EF combined with PF may regulate immune inflammation and metabolic dysfunctions (Fig. 6), suggesting that immune and metabolic factors of the body should also be comprehensively considered when assessing the safety of EF combined with PF. Thus, EF combined with PF can be defined as a new incompatibility of TCM, thereby enriching our knowledge of the 18 incompatible pairs, one of the traditional theories of TCMI.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (Nos. 81874368 and 81630100), the Beijing Nova Program (No. Z181100006218001), the National Key Technology R&D Program (No. 2015ZX 09501-004-001-008), the National Key R&D Program of China (No. 2018YFC1707000), and the National TCM Industry Science and Technology Program (No. 201507004-4-2).

Compliance with ethics guidelines

Yuan Gao, Zhilei Wang, Jinfa Tang, Xiaoyi Liu, Wei Shi, Nan Qin, Xiaoyan Wang, Yu Pang, Ruisheng Li, Yaming Zhang, Jiabo Wang, Ming Niu, Zhaofang Bai, and Xiaohe Xiao declare that they have no conflicts of interest to disclose. The study was approved by the Experimental Animal Center of the Fifth Medical Centre, Chinese PLA General Hospital in Beijing, China. All animal experiments complied with the animal welfare and ethics guidelines. Male Sprague–Dawley rats (180–220 g) were obtained from the Laboratory Animal Center of the Academy of Military Medical Sciences (License No. SCXK2012-004). All procedures followed were in accordance with the ethical standards of the Ethics Committee in the Fifth Medical Centre, Chinese PLA General Hospital and the *Helsinki Declaration* of 1975.

Electronic Supplementary Material Supplementary material is available in the online version of this article at https://doi.org/10.1007/s11684-019-0690-z and is accessible for authorized users.

References

1. Xu Y, Ding J, An JN, Qu YK, Li X, Ma XP, Zhang YM, Dai GJ, Lin N. Effect of the interaction of Veratrum Nigrum with Panax Ginseng on estrogenic activity *in vivo* and *in vitro*. Sci Rep 2016; 6(1): 26924
2. Long W, Zhang XD, Wu HY, Jin J, Yu GY, He X, Wang H, Shen X, Zhou ZW, Liu PX, Fan SJ. Study on incompatibility of traditional chinese medicine: evidence from formula network, chemical space, and metabolism room. Evid Based Complement Alternat Med 2013; 2013: 352145
3. Tang JF, Wang XY, Wen Q, Tang S, Sang F, Li WX, Li YH, Zhang CN, Niu M, Bai ZF, Wang JB, Xiao XH. Idiosyncratic hepatotoxicity evaluation of Zhuangguguanjie wan mediated by immune stress. Acta Pharmaceutica Sinica (Yao Xue Xue Bao) 2017; 52: 1033–1040 (in Chinese)
4. Shaw PJ, Ganey PE, Roth RA. Idiosyncratic drug-induced liver injury and the role of inflammatory stress with an emphasis on an animal model of trovafloxacin hepatotoxicity. Toxicol Sci 2010; 118 (1): 7–18
5. Zhou K, Dai Z, Liu ZB, Tan Y, Hu LM, Zhang BL, Gao XM. Experimental study of screening the hepatotoxic medicinal materials in Zhuangguguanjie Pill. Chin J Pharmacovigilance (Zhongguo Yao Wu Jing Jie) 2009; 11: 641–648 (in Chinese)
6. Tang JF, Wang XY, Wen Q, Tang S, Sang F, Li WX, Li YH, Li CY, Zhang CE, Niu M, Bai ZF, Wang JB, Xiao XH. Cytokine analysis of Zhuangguguanjie wan-induced idiosyncratic liver injury based on mathematical modeling. Acta Pharmaceutica Sinica (Yao Xue Xue Bao) 2018; 53: 574–584 (in Chinese)
7. Tang JF, Wang XY, Li WX, Li YH, Wang JB, Bai ZF, Xiao XH, Yang W. Correlation analysis on idiosyncratic hepatotoxicity of Zhuangguguanjie Wan and 27 cytokines based on related visualization. Chin Tradit Herbal Drugs 2018; 49: 1624–1633
8. Cheng Y, Liu Y, Wang H, Li J, Ren J, Zhu L, Gong L. A 26-week repeated dose toxicity study of Xian-ling-gu-bao in Sprague-Dawley rats. J Ethnopharmacol 2013; 145(1): 85–93
9. Wu H, Zhong QX, Wang J, Wang M, Fang F, Xia Z, Zhong RL, Huang HC, Ke ZC, Wei YJ, Feng L, Shi ZQ, Sun E, Song J, Jia XB. Beneficial effects and toxicity studies of Xian-ling-gu-bao on bone metabolism in ovariectomized rats. Front Pharmacol 2017; 8:273–284
10. Cheung WI, Tse ML, Ngan T, Lin J, Lee WK, Poon WT, Mak TW, Leung VK, Chau TN. Liver injury associated with the use of Fructus Psoraleae (Bol-gol-zhee or Bu-gu-zhi) and its related proprietary medicine. Clin Toxicol (Phila) 2009; 47(7): 683–685

11. Nam SW, Baek JT, Lee DS, Kang SB, Ahn BM, Chung KW. A case of acute cholestatic hepatitis associated with the seeds of *Psoralea corylifolia* (Boh-Gol-Zhee). Clin Toxicol (Phila) 2005; 43(6): 589–591
12. Melchart D, Hager S, Albrecht S, Dai J, Weidenhammer W, Teschke R. Herbal traditional Chinese medicine and suspected liver injury: a prospective study. World J Hepatol 2017; 9(29): 1141–1157
13. Danan G, Teschke R. RUCAM in drug and herb induced liver injury: the update. Int J Mol Sci 2015; 17(1): 14–47
14. Li DM, Yin XF, Liu JH, Cai DW. Long term toxicity of total flavonoids from Epimedii Folium. Chin J of Exp Tradit Med Formulae (Zhongguo Shi Yan Fang Ji Xue Za Zhi) 2008; 14(7): 60–62 (in Chinese)
15. Teschke R, Larrey D, Melchart D, Danan G. Traditional Chinese medicine (TCM) and herbal hepatotoxicity: RUCAM and the role of novel diagnostic biomarkers such as microRNAs. Medicines (Basel) 2016; 3(3): 18
16. Jing J, Teschke R. Traditional Chinese medicine and herb-induced liver injury: comparison with drug-induced liver injury. J Clin Transl Hepatol 2018; 6(1): 57–68
17. Teschke R. Traditional Chinese medicine induced liver injury. J Clin Transl Hepatol 2014; 2(2): 80–94
18. Teschke R. Kava hepatotoxicity: pathogenetic aspects and prospective considerations. Liver Int 2010; 30(9): 1270–1279
19. Li C, Niu M, Bai Z, Zhang C, Zhao Y, Li R, Tu C, Li H, Jing J, Meng Y, Ma Z, Feng W, Tang J, Zhu Y, Li J, Shang X, Zou Z, Xiao X, Wang J. Screening for main components associated with the idiosyncratic hepatotoxicity of a tonic herb, *Polygonum multiflorum.* Front Med 2017; 11(2): 253–265
20. Wang JB. LI CY, Zhu Y, Song HB, Bai ZF, Xiao XH. Integrated evidence chain-based identification of Chinese herbal medicine-induced hepatotoxicity and rational usage: exemplification by *Polygonum multiflorum* (He shou wu). Chin Sci Bull (Ke Xue Tong Bao) 2016; 61(9): 971–980 (in Chinese)
21. Wang JB, Cui HR, Bai ZF, Xiao XH. Precision medicine-oriented safety assessment strategy for traditional Chinese medicines: disease-syndrome-based toxicology. Acta Pharmaceutica Sinica (Yao Xue Xue Bao) 2016; 51(11): 1681–1688 (in Chinese)
22. Danan G, Teschke R. Drug-induced liver injury: why is the Roussel Uclaf Causality Assessment Method (RUCAM) still used 25 years after its launch? Drug Saf 2018; 41(8): 735–743
23. Teschke R, Danan G. Drug induced liver injury with analysis of alternative causes as confounding variables. Br J Clin Pharmacol 2018; 84(7): 1467–1477
24. Teschke R, Schulze J, Eickhoff A, Danan G. Drug induced liver injury: can biomarkers assist RUCAM in causality assessment? Int J Mol Sci 2017; 18(4): 803
25. Wang J, Ma Z, Niu M, Zhu Y, Liang Q, Zhao Y, Song J, Bai Z, Zhang Y, Zhang P, Li N, Meng Y, Li Q, Qin L, Teng G, Cao J, Li B, Chen S, Li Y, Zou Z, Zhou H, Xiao X. Evidence chain-based causality identification in herb-induced liver injury: exemplification of a well-known liver-restorative herb *Polygonum multiflorum*. Front Med 2015; 9(4): 457–467
26. Teschke R, Bahre R. Severe hepatotoxicity by Indian Ayurvedic herbal products: a structured causality assessment. Ann Hepatol 2009; 8(3): 258–266
27. Deng PX, Xu M. Comparision on hepatotoxicity in rats by using Fructus Psoralea alone and in combination with other herbs. Guangxi J Tradit Chin Med (Guangxi Zhong Yi Yao) 2005; 28(2): 49–50 (in Chinese)
28. Yang Y, Tang X, Hao F, Ma Z, Wang Y, Wang L, Gao Y. Bavachin induces apoptosis through mitochondrial regulated ER stress pathway in HepG2 cells. Biol Pharm Bull 2018; 41(2): 198–207
29. Cheng JH, Cai HD. Adverse reactions to Zhuangguguanjie Wan and cause analysis. Adverse Drug Reactions J (Yao Wu Bu Liang Fan Ying Za Zhi) 2000; 1: 15–19 (in Chinese)
30. Qi SZ, Li N, Tuo ZD, Li JL, Xing SS, Li BB, Zhang L, Lee HS, Chen JG, Cui L. Effects of Morus root bark extract and active constituents on blood lipids in hyperlipidemia rats. J Ethnopharmacol 2016; 180: 54–59
31. Sarhadynejad Z, Sharififar F, Pardakhty A, Nematollahi MH, Sattaie-Mokhtari S, Mandegary A. Pharmacological safety evaluation of a traditional herbal medicine "Zereshk-e-Saghir" and assessment of its hepatoprotective effects on carbon tetrachloride induced hepatic damage in rats. J Ethnopharmacol 2016; 190: 387–395
32. Lou JW, Cao LL, Zhang Q, Zhang L, Ding AW. Comparative study on acute toxicity of different active ingredient fractions from Kansui stir-baked with vinegar on zebrafish embryos. China J Chin Materia Medica (Zhongguo Zhong Yao Za Zhi) 2017; 42(18): 3516–3522 (in Chinese)
33. Eguchi A, Wree A, Feldstein AE. Biomarkers of liver cell death. J Hepatol 2014; 60(5): 1063–1074
34. Kolarski V, Todorov A, Petrova D. Cytokines and the liver in health and disease. Vutr Boles 2000; 32(1): 19–24
35. Schmidt-Arras D, Rose-John S. IL-6 pathway in the liver: from physiopathology to therapy. J Hepatol 2016; 64(6): 1403–1415
36. Kwak A, Lee Y, Kim H, Kim S. Intracellular interleukin (IL)-1 family cytokine processing enzyme. Arch Pharm Res 2016; 39(11): 1556–1564
37. Waring JF, Liguori MJ, Luyendyk JP, Maddox JF, Ganey PE, Stachlewitz RF, North C, Blomme EA, Roth RA. Microarray analysis of lipopolysaccharide potentiation of trovafloxacin-induced liver injury in rats suggests a role for proinflammatory chemokines and neutrophils. J Pharmacol Exp Ther 2006; 316(3): 1080–1087
38. Luyendyk JP, Lehman-McKeeman LD, Nelson DM, Bhaskaran VM, Reilly TP, Car BD, Cantor GH, Deng X, Maddox JF, Ganey PE, Roth RA. Coagulation-dependent gene expression and liver injury in rats given lipopolysaccharide with ranitidine but not with famotidine. J Pharmacol Exp Ther 2006; 317(2): 635–643
39. Deng X, Stachlewitz RF, Liguori MJ, Blomme EA, Waring JF, Luyendyk JP, Maddox JF, Ganey PE, Roth RA. Modest inflammation enhances diclofenac hepatotoxicity in rats: role of neutrophils and bacterial translocation. J Pharmacol Exp Ther 2006; 319(3): 1191–1199
40. Lacour S, Gautier JC, Pallardy M, Roberts R. Cytokines as potential biomarkers of liver toxicity. Cancer Biomark 2005; 1(1): 29–39
41. Roth RA, Ganey PE. Animal models of idiosyncratic drug-induced liver injurycurrent status. Crit Rev Toxicol 2011; 41(9): 723–739
42. Jesus AA, Goldbach-Mansky R. IL-1 blockade in autoinflammatory syndromes. Annu Rev Med 2014; 65(1): 223–244
43. Dinarello CA, Simon A, van der Meer JWM. Treating inflammation by blocking interleukin-1 in a broad spectrum of diseases. Nat Rev Drug Discov 2012; 11(8): 633–652

44. Mangan MSJ, Olhava EJ, Roush WR, Seidel HM, Glick GD, Latz E. Targeting the NLRP3 inflammasome in inflammatory diseases. Nat Rev Drug Discov 2018; 17(8): 588–606
45. Guo H, Callaway JB, Ting JPY. Inflammasomes: mechanism of action, role in disease, and therapeutics. Nat Med 2015; 21(7): 677–687
46. Gross O, Thomas CJ, Guarda G, Tschopp J. The inflammasome: an integrated view. Immunol Rev 2011; 243(1): 136–151
47. Wree A, McGeough MD, Inzaugarat ME, Eguchi A, Schuster S, Johnson CD, Peña CA, Geisler LJ, Papouchado BG, Hoffman HM, Feldstein AE. NLRP3 inflammasome driven liver injury and fibrosis: roles of IL-17 and TNF in mice. Hepatology 2018; 67(2): 736–749
48. Brouwers JF. Liquid chromatographic-mass spectrometric analysis of phospholipids. Chromatography, ionization and quantification. Biochim Biophys Acta 2011; 1811(11): 763–775
49. Sims K, Haynes CA, Kelly S, Allegood JC, Wang E, Momin A, Leipelt M, Reichart D, Glass CK, Sullards MC, Merrill AH Jr. Kdo2-lipid A, a TLR4-specific agonist, induces *de novo* sphingolipid biosynthesis in RAW264.7 macrophages, which is essential for induction of autophagy. J Biol Chem 2010; 285(49): 38568–38579
50. Nixon GF. Sphingolipids in inflammation: pathological implications and potential therapeutic targets. Br J Pharmacol 2009; 158(4): 982–993
51. Le Stunff H, Milstien S, Spiegel S. Generation and metabolism of bioactive sphingosine-1-phosphate. J Cell Biochem 2004; 92(5): 882–899
52. Babenko NA, Shakhova EG. Effects of flavonoids on sphingolipid turnover in the toxin-damaged liver and liver cells. Lipids Health Dis 2008; 7(1): 1–11
53. Sacket SJ, Im DS. Activity change of sphingomyelin catabolic enzymes during dimethylnitrosamine-induced hepatic fibrosis in rats. Biomol Ther (Seoul) 2008; 16(16): 34–39
54. Rouzer CA, Ivanova PT, Byrne MO, Brown HA, Marnett LJ. Lipid profiling reveals glycerophospholipid remodeling in zymosan-stimulated macrophages. Biochemistry 2007; 46(20): 6026–6042
55. Fitzpatrick FA, Soberman R. Regulated formation of eicosanoids. J Clin Invest 2001; 107(11): 1347–1351
56. Pérez-Chacón G, Astudillo AM, Balgoma D, Balboa MA, Balsinde J. Control of free arachidonic acid levels by phospholipases A2 and lysophospholipid acyltransferases. Biochim Biophys Acta 2009; 1791(12): 1103–1113
57. Kawada N, Mizoguchi Y, Kobayashi K, Yamamoto S, Morisawa S. Arachidonic acid metabolites in carbon tetrachloride-induced liver injury. Gastroenterol Jpn 1990; 25(3): 363–368
58. Piao N, Ikejima K, Kon K, Aoyama T, Osada T, Takei Y, Sato N, Watanabe S. Synthetic triglyceride ocontaining an arachidonic acid branch (8A8) prevents lipopolysaccharide-induced liver injury. Life Sci 2009; 85(17-18): 617–624
59. Ghigo A, Damilano F, Braccini L, Hirsch E. PI3K inhibition in inflammation: toward tailored therapies for specific diseases. BioEssays 2010; 32(3): 185–196
60. Dhand R, Hiles I, Panayotou G, Roche S, Fry MJ, Gout I, Totty NF, Truong O, Vicendo P, Yonezawa K. PI 3-kinase is a dual specificity enzyme: autoregulation by an intrinsic protein-serine kinase activity. EMBO J 1994; 13(3): 522–533
61. Lehmann K, Müller JP, Schlott B, Skroblin P, Barz D, Norgauer J, Wetzker R. PI3Kγ controls oxidative bursts in neutrophils via interactions with PKCα and p47phox. Biochem J 2009; 419(3): 603–610