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Simultaneous determination of five bioactive components of *Gancao* in rat plasma by UHPLC-MS/MS and its application to comparative pharmacokinetic study of incompatible herb pair *Gansui-Gancao* and *Gansuibanxia Decoction*

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ABSTRACT

Incompatible herb pair Gansui-Gancao is recorded in "eighteen incompatible" medicaments in many monographs of TCM (Traditional Chinese Medicine) which means the two herbs can not be co-used in most cases. However, Gansuibanxia decoction composed of Gansui(Kansui), Banxia(Pinellia), Shaoyao(Peony) and Gancao(Liquorice) is a traditional Chinese formula which has been clinically employed for the treatment of cancerous ascites, pleural effusion, peritoneal effusion, etc. The purpose of the study was to investigate the pharmacokinetics of main bioactive components in Gancao to explore the reasons why Gansui-Gancao can be used in Gansuibanxia decoction. A simple, rapid and sensitive UHPLC-MS/MS method for simultaneous determination of liquiritigenin, isoliquiritigenin, liquiritin, glycyrrhetinic acid and glycyrrhizic acid of liquorice in rat plasma was developed and validated. After extraction from plasma, the analytes and internal standard were separated on a C18 column with the mobile phase consisting of 0.1% acetic acid containing 0.2 mM ammonium acetate in water and acetonitrile via gradient elution. The electrospary ionization source was adopted under the multiple reaction monitoring mode. The method was succesfully applied to a comparative pharmacokinetic study of main bioactive components of Gancao in rat plasma after oral administration of the extracts of Gancao (GC), Gansui-Gancao (GS-GC), Shaoyao-Gancao (SY-GC), Gansui-Shaoyao-Gancao (GS-SY-GC) and Gansuibanxia decoction (GSBXD), respectively. The pharmacokinetic parameters had significant differences ($P < 0.05$) in different groups which showed that Gansui decreased the bioavailability of Gancao, while Shaoyao increased the bioavailability of Gancao. Hence, these may be the pharmacokinetic mechanism of incompatible herb pair Gansui-Gancao and the reasons why the herb pair can be used in Gansuibanxia decoction.

1. Introduction

Incompatible herb pairs was first recorded in Shen Nong's herbal classic more than 2000 years ago. "Eighteen incompatible medicaments" as an important part of TCM theory warns that some herbs can't be co-used for their poor efficiency and even toxity. And Gansui (kansui radix, the root of *Euphorbia kansui* T. N. Liou) - Gancao (Glycyrrhizae radix et rhizoma, the root and rhizome of *Glycyrrhiza uralensis* Fisch.) is one of them. However, Gansuibanxia decoction composed of the two herbs as well as Banxia (Pinelliae rhizoma, the tuber of *Pinellia ternata* (Thunb.) Breit.) and Shaoyao (Paeoniae radix, the root of *Paeonia lactiflora* Pall.) was first recorded by Zhang Zhongjing in *Synopsis of Golden Chamber* in Han Dynasty. According to Zhang's book, the Gansuibanxia decoction has great curative effect of prolonged elema (Liuyin in Chinese). It was also used for the treatment of hydronephrosis, pleural effusion, cancerous ascites, pericardial effusion and chronic diarrhea [1–3]. Due to the incompatible herb pair in this decoction, much attention

Abbreviations: TCM, Traditional Chinese Medicine; QC, quality control; LLOQ, lower limit of quantification; IS, internal standard; RE, relative error; RSD, relative standard deviation; PPT, protein precipitation; LLE, liquid-liquid extraction; GC, Gancao; GS-GC, Gansui-Gancao; SY-GC, Shaoyao-Gancao; GS-SY-GC, Gansui-Shaoyao-Gancao; GSBXD, Gansuibanxia decoction.

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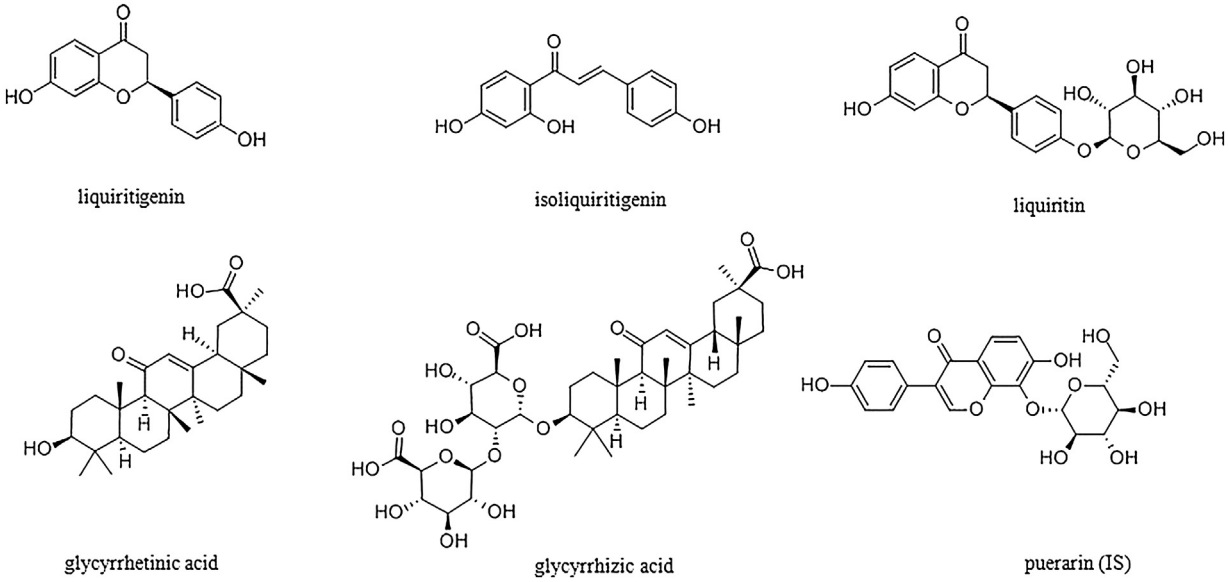

Fig. 1. Chemical strructures of analytes and IS.

has been paid when using Gansuibanxia decoction. However, there are plenty of cases showing great efficiency after treating with the decoction. Hence, the application of Gansuibanxia decoction should be reasonable.

Up to date, the mechanism of using incompatible herb pair Gansui-Gancao in Gansuibanxia decoction remains unclear. The toxicity may increase and the efficiency may decrease when using the eighteen incompatible medicaments, and the former has been widely accepted [4]. Based on this viewpoint, the majority of studies about Gansui-Gancao of Gansuibanxia decoction focused on the ratios, the times of administration, doses, the status of the herbs, etc. These studies concerned about the biomedical indicators and histopathology of experimental animals [5–7]. The results were differfent. In some studies, the results showed there were no significant toxicity when using Gansui and Gancao; on the contrary, Gancao can decrease the toxicity of Gansui [8], while in other studies, the results showed the combination increased the toxicity of Gansui [9]. To the best of our knowledge, there is little information avaliable in the literature about the pharmacokinetics of Gansui-Gancao and Gansuibanxia decoction. Although glycyrrhizinic acid, liquiritin, paeoniflorin and albiflorin had been determined in the rat plasma after oral administratration of GSBXD [10], the mechanism of Gansui-Gancao and Gansuibanxia decoction still has not been fully understood.

Gancao is described as National Venerable Master in Chinese medicine and usually combined with other herbs in many herbal formulas by harmonize the characteriestics of other herbs [11,12]. It has been reported that Gancao has anti-viral, anti-inflammatory, hepatoprotective, cytoprotective, spasmolytic, and anti-oxidative effects, etc [13,14]. Therefore, it is interesting to take consideration of the following questions: whether the pharmacokinetics of the bioactive components in liquorice would change due to co-using with other herbs in Gansuibanxia decoction? In the formula, Gansui-Gancao is incompatible herb pair, while Shaoyao-Gancao is considered as mutual-assistance herb pair in many Chinese formulas, therefore, whether Shaoyao may inhibit the incompatible herb pair in vivo? Based on the above thoughts, in this investigation, the Gansuibanxia decoction was decomposed into five different formulas, including Gancao(GC), Gansui-Gancao(GS-GC), Shaoyao-Gancao(SY-GC), Gansui-Shaoyao-Gancao(GS-SY-GC) and Gansuibanxia decoction(GSBXD). Five bioactive components in Gancao, including flavonoids (liquiritigenin, isoliquiritigenin and liquiritin) and triterpenes (glycyrrhetinic acid and glycyrrhizic acid) [15] were determined and analyzed the differences of absorption and elimination in rat plasma of different groups after oral administration of the decoctions. As reported in the literature, there are several studies about determination of bioactive components of Gancao in rat plasma, and most of them based on LC—MS/MS [16–19] and HPLC-UV [20,21]. However, the method in this paper can simultaneously determine five bioactive components of Gancao with better performance in time-consuming(6.5 min), injected volume(2 μL), LLOQ (liquiritigenin 0.26 ng/mL, isoliquiritigenin 0.25 ng/mL, liquiritin 2.32 ng/mL, glycyrrhetinic acid 0.44 ng/mL, glycyrrhizic acid 0.24 ng/mL) with single ionization mode, simultaneously.

In this study, a simple, rapid and sensitive UHPLC-MS/MS method was developed and validated for simultaneous deteremination of five bioactive componets of Gancao in rat plasma and it was successfully applied to the comparative pharmacokinetics of incompatible herb pair Gansui-Gancao and Gansuibanxia decoction. In the meanwhile, it can be the basis for the pharmacokinetic methodology of the herb pair and the decoction above. The current study elucidated the mechanism of incompatible herb pair Gansui-Gancao and provided information for the reasonability of the application of Gansuibanxia decoction in the pharmacokinetic perspective.

2. Materials and methods

2.1. Chemicals and reagents

Gansui, Gancao, Shaoyao and *Banxia* were purchased from GuoDa Pharmacy (Shenyang,China). Liquiritigenin, isoliquiritigenin, liquiritin, glycyrrhetinic acid, glycyrrhizic acid and puerarin (interal standard, IS) were purchased from Chengdu MUST Bio-tech Co. Ltd. (Sichuan,China). The purity of all the reference substances was higer than 98%. All of the chemical structures are shown in Fig.1. Acetonitrile and methanol (HPLC grade) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Ammonium acetate (HPLC grade) was purchased from Concord Tech. (Tianjin, China). Acetic acid (HPLC grade) was purchased from Tianjin Kermel Chemical Reagent factory (Tianjin, China). Deionized water was obtained from Hangzhou Wahaha Corporation (Hangzhou, China).