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Research Paper

Comparative pharmacokinetics of rhein and chrysophanol after oral administration of Quyu Qingre granules in normal and acute blood stasis rabbits

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ABSTRACT

Ethnopharmacological relevance: Quyu Qingre granules (QYQRGs) are useful traditional Chinese composite prescription in the treatment of blood stasis syndrome. Comparing differences of pharmacokinetic properties of compounds in QYQRG between normal and blood stasis syndrome rabbits can provide much helpful information. The primary objective of this study was to compare the pharmacokinetics of rhein and chrysophanol after orally administering 2.0 g/kg b.w. QYQRG in normal and acute blood stasis model rabbits.

Materials and methods: The blood samples were collected subsequently at 5, 10, 15, 20, 30, 45, 60, 75, 90, 120, 240, 360 and 480 min after orally administrating QYQRG. The concentrations of rhein and chrysophanol in rabbit plasma were determined by HPLC and main pharmacokinetic parameters were obtained.

Results: The pharmacokinetic parameters $AUC_{0-\infty}$, T_{lag}, C_{max} and K_{21} of both rhein and chrysophanol were markedly different in the acute blood stasis model rabbits. It was also found that parameters A, β, MRT and $T_{1/2\beta}$ of rhein and the parameters α and $T_{1/2\alpha}$ of chrysophanol all exhibited significant difference between the normal and acute blood stasis model rabbits.

Conclusions: The absorption time of rhein and chrysophanol was accelerated and the absorption amount of these two compounds was increased in rabbits with acute blood stasis, suggesting that rhein and chrysophanol would possibly be the two effective compounds in QYQRG.

1. Introduction

Blood stasis, indicating hemorheological abnormalities, is an essential factor in the formation of a hemostatic plug and a significant mechanism in developing hematologic disorders such as hemorrhage, congestion, thrombosis, local ischemia and tissue changes (Bensky et al., 1993; Chiu et al., 2002; Braun et al., 2012). It was reported that promoting blood circulation might dispel blood stasis and improve many relative hemorheological events (Li et al., 2009; Yao et al., 2009). The traditional Chinese medicine (TCM), most of which are formulations that have been applied in clinical practice for thousands of years, has been attracting considerable attention for its complementary therapeutic effects and a quite few advantages in treating blood stasis syndrome (Gu et al., 2008; Lu et al., 2008; Zhang et al., 2010; Liu et al., 2012).

Quyu Qingre granules (QYQRGs) are very useful traditional Chinese composite prescription in the treatment of blood stasis syndrome, containing *Rhizome Rhei*, *Semen Persicae*, *Fructus Cartaegi*, *Ramulus Cinnamomi* and *Radix Glycyrrhizae*. These granules are safe and effective in clinically application. *Rhizome Rhei* is a key ingredient herb and functional compounds found in this herb are quantitatively equivalent in the making of QYQRG. Rhein and chrysophanol (Fig. 1), both existing in the *Rhizome Rhei*, are two main representative components of QYQRG and also two index components of *Rhizome Rhei*, which possess a broad beneficial bioactivities of anti-tumor (Darzynkiewicz et al., 1989; Shi et al., 2008; Lee et al., 2011), anti-inflammatory (Legendre et al., 2007; Kim et al., 2010), anti-bacterium (Yu et al., 2008), anti-oxidant (Yen et al., 2000; Krenn et al., 2003; Cai et al., 2004), inhibiting human aortic smooth muscle cell proliferation (Heo et al., 2009) and are beneficial in heart diseases (Sharma and Moffatt, 2013). In our previous study, it was found that QYQRG could

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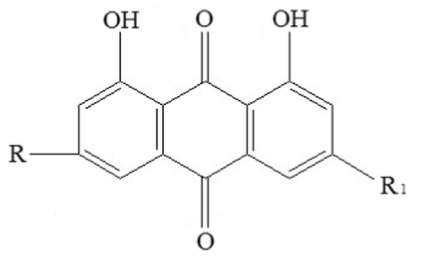

	R	R1
rhein	—COOH	—H
chrysophanol	—CH	—H

Fig. 1. The chemical structure of rhein and chrysophanol.

obviously reduce the values of whole blood viscosity, whole blood reductive viscosity, erythrocyte assembling index, erythrocyte electrophoresis time, casson viscosity and erythrocyte sedimentation rate equation *K* of blood stasis model rabbits, suggesting QYQRG could reduce erythrocyte aggregation and blood viscosity. QYQRG has been shown to effectively remove blood stasis and improve blood circulation, however, the therapeutic mechanism and the specific effective substances of this TCM are still indeterminate.

Pharmacokinetic studies play an important role in explaining and predicting efficacy of TCM and also can reveal the effective substances of TCM (Lu et al., 2007; Peng et al., 2009). Furthermore, pharmacokinetic characteristics of drugs usually vary with the condition of body (Ismail and El-Kattan, 2007; Bulitta et al., 2009) and much important information can be obtained through comparing the pharmacokinetics of drugs in the body with different status (Tian et al., 2010). Blood stasis syndrome may influence absorption, metabolism and elimination of drugs in blood. Therefore, it would be very helpful to gain the differences of pharmacokinetic properties of drugs in normal animals and animals with blood stasis syndrome. The primary objective of this study was to compare the pharmacokinetics of rhein and chrysophanol in normal and acute blood stasis model rabbits after oral administration of QYQRG. Differences in rhein and chrysophanol pharmacokinetics would allow further clarification on the mechanism of action of QYQRG and provide directions for further research on similar compound medicines.

2. Materials and methods

2.1. Material and reagents

All herbs used to prepare QYQRG were commercially available dry materials, which were purchased from Zhejiang herbal Pharmaceutical Co., and identified by associate professor Kongrong Chen, Zhejiang Chinese Medical University, China. To prepare the QYQRG, *Rhizome Rhei* (the rhizome of *Rheum palmatum* L., belonging to *Polygonaceae*, Voucher Ref. no. 2011-1222), *Semen Persicae* (the seed of *Prunus persica* (L.) Batsch, belonging to *Rosacea*, Voucher Ref. no. 2011-1222), *Fructus Cartaegi* (the fructification of *Cartaegus pinnatifida* Bge., belonging to *Rosacea*, Voucher Ref. no. 2011-1222), *Ramulus Cinnamomi* (the twig of *Cartaegus pinnatifida* Bge., belonging to *Lauraceae*, Voucher Ref. no. 2012-0110) and *Radix Glycyrrhizae* (the root of *Glycyrrhiza uralensis* Fisch., belonging to *Leguminosae*, Voucher Ref. no. 2011-1222) were combined in ratio of 4:4:3:2:2. *Rhizome Rhei* was extracted with 80% ethanol (1:30, w/v) for 1 h. After filtration, solution obtained was evaporated to extracta sicca (A) (relative density 1.30–1.35, 60 °C). Then the alcohol extract residue and the other herbs were mixed and the mixture was extracted with water 3 times, 1 h for each time. Alcohol was then added to the aqueous extract solution until the total content of alcohol was 70%. 12 h later, the filtrate (a) was obtained. After that, the residue was dealt again with the same alcohol precipitate processing and filtrate (b) was obtained after letting it sit for 4 h. The filtrate (a) and filtrate (b) were merged and evaporated to extracta sicca (B) (no alcohol taste, relative density 1.30–1.35, 60 °C). Extracta sicca (A) and extracta sicca (B) were combined and concentrated to give a total extracta sicca (relative density 1.40–1.43, 60 °C). Lactose and gummeline were added to the total extracta sicca (extracta sicca:lactose: gummeline = 15:12:13) for granulation with 12 mesh sieve. Finally, the obtained granules were dried at 60 °C.

QYQRG (Lot: 20120315) used in this study was prepared and provided by the Traditional Chinese Patent Medicine Preparation Centre, College of Pharmaceutical Science, Zhejiang Chinese Medical University, Hangzhou, China. Rhein and chrysophanol standards were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Methanol of chromatographic grade was purchased from SiYou chemical reagent Co. Ltd., Tianjing, China. All the other reagents were of analytical grade. Water was purified by redistilling and passing through a 0.22 μm membrane filter before use throughout the study.

2.2. Preparation of solution for intragastric administration

The contents of rhein and chrysophanol were determined to be 417.18 μg/g and 823.35 μg/g in QYQRG by HPLC, respectively. The HPLC method had been previously reported in China (Wu et al., 2012). The proposed dosage of QYQRG for human is 0.817 g/kg. As human to rabbit dosage conversion factor is 0.07 (Chen, 2011). So, the dosage of QYQRG for rabbit is 2.0 g/kg (0.817 g/kg × 70 kg × 0.07/2.0 kg = 2.0 g/kg). The QYQRG was dissolved in pure water at a concentration of 0.4 g/mL and was then ultrasonicated for 10 min. In this experiment, the dosage of QYQRG solution for oral administration is 5.00 mL/kg.

2.3. Animals

Rabbits weighing 2.0 ± 0.2 kg were used for this study. The rabbits were supplied by the Animal Experimental Center, Zhejiang Chinese Medical University, China. The rabbits were kept in an air-conditioned animal quarter at a temperature of 22 ± 2 °C and a relative humidity of 55 ± 5%. Water and food (laboratory rodent chow, Xi'an, China) were allowed ad libitum. The animals were acclimatized to the facilities for five days, and then were fasted with free access to water for 12 h prior to each experiment. Testing on rabbits followed nationally and institutionally accepted rules for use of animals.

2.4. in vivo study

Rabbits were randomly divided into the following two groups (n=6): normal control group administrated with QYQRG and acute blood stasis model group administrated with QYQRG. The acute blood stasis group was injected with 10% high molecular dextran injection (molecular weight, 200,000 Da; the dosage of injection was 10 mL/kg), twice daily for two days. An injection was administered once more in the morning of the third day. Each injection was administered within 3 min. Within 15 min from the last time of injection, an oral dose of 20.00 mL/kg of the prepared QYQRG solution was administered.

Compared with normal group, the values of whole blood viscosity, whole blood reductive viscosity, hematokrit, erythrocyte assembling index, erythrocyte electrophoresis time, casson viscosity, erythrocyte count, erythrocyte sedimentation rate equation and erythrocyte sedimentation rate equation *K* of blood stasis