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The rhubarb total free anthraquinone oral colon-specific drug delivery granules: Comparative pharmacokinetics study with rhubarb by UPLC-MS/MS after oral administration

Jinhua Chang[a,1], Lin Zhang[a,1], Xiaodong Gu[a], Liyan Liu[a], Zhongsi Li[a], Guangxin Miao[a], Xigang Liu[a], Pei Liu[a], Hefei Xue[a], Cuizhe Liu[a,*], Qiang Fu[b]

[a] Hebei Province Key Laboratory of Research and Development for Chinese Medicine, Chengde Medical College, Chengde, Hebei 067000, PR China
[b] Shenyang Pharmaceutical University, Shenyang, Liaoning 110016, PR China

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ABSTRACT

Objective: This study aimed to compare pharmacokinetics of rhubarb total free anthraquinones (AQs) oral colon-specific delivery granules (RTFA-OCDD-GN) and rhubarb after oral administration to evaluate the colonic-target releasing characteristic of RTFA-OCDD-GN and the impact of five AQs components on the contribution of the attenuation of nephrotoxicity.

Methods: The compare pharmacokinetics of RTFA-OCDD-GN and rhubarb was performed in rats. The plasma concentrations of aloe-emodin, rhein, emodin, chrysophanol and physcion were assayed by a validated UPLC-MS/MS method.

Results: The pharmacokinetics results showed that the $AUC_{0\text{-}72h}$ of aloe-emodin, emodin, chrysophanol and physcion was decreased by 3.09-fold ($p < 0.05$), 2.68-fold ($p < 0.05$), 2.97-fold ($p < 0.01$) and 2.84-fold ($p < 0.05$) respectively in group RTFA-OCDD-GN; whereas the $AUC_{0\text{-}72h}$ of rhein was increased slightly by 1.05-fold ($p > 0.05$) compared with group rhubarb samples. However the dose-normalized $AUC_{0\text{-}72h}$ of aloe-emodin, rhein, emodin and chrysophanol was decreased by 1.90-fold ($p < 0.05$), 1.82-fold, 1.33-fold and 2.27-fold ($p < 0.01$) respectively in RTFA-OCDD-GN group; whereas the dose-normalized $AUC_{0\text{-}72h}$ of physcion was increased by 2.33-fold ($p < 0.01$) compared with group rhubarb samples.

Conclusion: These differences on pharmacokinetic parameters indicated that RTFA-OCDD-GN delivered AQs to colon. The systemic exposure reduction of aloe-emodin, emodin and physcion in group RTFA-OCDD-GN could contribute to the attenuation of nephyrotoxicity caused by rhubarb.

1. Introduction

Rhei radix et rhizome (rhubarb), derived from the dried root and rhizomes of *Rheum palmatum* L., *Rheum tanguticum Maxim. ex Balf. or Rheum officially Baill.* (Polygonaceae family) [1], has extensive bioactivities, such as catharsis [2,3], anti-inflammation [4], antioxidant [5] and gastric protection [6]. It has been widely used in Chinese traditional medicine for thousands of years, especially as a laxative. The Chinese Pharmacopoeia recorded officially 104 types of traditional Chinese medicine (TCM) containing rhubarb. In these formulas rhubarb mainly plays the purgative effect. Anthraquinones (AQs) contained in rhubarb are considered as active components to play purgative effect [7], which mainly comprise of aloe-emodin, rhein, emodin, chrysophanol, physcion (free AQs) and their glycosides (conjugated AQs) (chemical structures of AQs and AQs glycosides are shown in Fig. 1).

The mechanism on purgative effect of AQs is to stimulate large intestinal paries and nervous plexus to promote intestinal peristalsis and reduce water absorption at colon [8]. However, most free AQs are absorbed and/or metabolized in stomach and small intestine after directly oral administration, not reaching the colon, further attenuating the purgative activity. Conjugated AQs can reach colon by the protection of β-glucoside bond to play purgative activity after being hydrolyzed to free AQs [9]. Thus the conjugated AQs were considered as the effective components playing purgative effect after the oral administration of rhubarb. However, conjugated AQs were destroyed easily in the decoction process [10]. Thus rhubarb is usually used as either all or part of crude powder form in TCM. However these TCM containing rhubarb exhibited inconsistent purgative effect due to the significant difference

* Corresponding author.
E-mail address: liucuizhexy@163.com (C. Liu).
[1] The first two authors contributed equally to this work.

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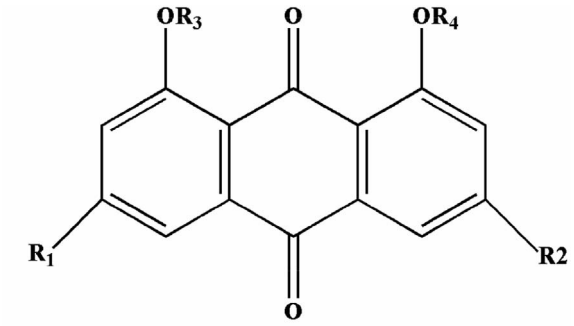

Combined AQs: R_3=H, R_4=Glc; R_3=Glc;R_4=H

Free AQs:	R_1	R_2
Chrysophanol:	H	CH_3
Aloe-emodin:	H	CH_2OH
Rhein:	H	COOH
Emodin:	OH	CH_3
Physcion	OCH_3	CH_3

Fig. 1. Chemical structures of AQs and AQs glycosides.

on growing region and culturing condition of rhubarb [11].

In recent years, many studies have demonstrated that AQs components such as emodin, rhein and physcion had potential renal and hepatic toxicity by the *in vivo* and *in vitro* tests [12–14]. Thus people have paid more and more attention to the safety of rhubarb and the preparations containing rhubarb. Interestingly, a recent study proved that processed rhubarb with a low amount of total conjugated AQs exhibited lower nephrotoxicity in comparison with crude rhubarb [15]. The hypothesis that rhubarb extract not containing conjugated AQs was used as the alternative of rhubarb contributing to the attenuation of nephrotoxicity was proposed. However, the decrease of the content of total conjugated AQs would lead to the attenuation of purgative effect. Thus it is an essential challenge how to improve the purgative effect and meanwhile reduce the toxicity after oral administration of rhubarb extract.

Oral colon-specific drug delivery systems (OCDDS) have been an important field of attention since decade years. OCDDS is being explored not only for local colonic pathologies, but also for systemic delivery of drugs like proteins and peptides [16]. The traditional strategies for OCDDS include time-dependent system and pH-dependent systems and microbially and/or enzymatically driven systems [17]. However these systems face some difficulties, such as the harsh gastrointestinal (GI) tract environment, the low concentration of the drug in the colon site [18]. To overcome these drawbacks, some nanomedicine technologies are used for the development of OCDDS, such as modified mesoporous silica nanoparticles [19,20], and nanosized drug depots by electrospinning [21–23] and eletrospraying [24] technology. In addition, various types of colon-specific drug delivery carriers such as guar gum [18], chitosan [25], pH-sensitive polymer [26] and Eudragit polymer combinations [27] have been developed. Moreover a novel concept of using di-dependent drug delivery system, i.e. microbially triggered system and pH-dependent system was proposed [28].

In our previous studies rhubarb total free AQs (RTFA) oral colon-specific drug delivery granules (RTFA-OCDD-GN) have been prepared successfully using the combination of microbially triggered system and pH-dependent system. The RTFA-OCDD-GN showed the same purgative effect and very low nephrotoxicity compared with rhubarb medical material (RMM) samples [29]. To explain the reason of toxicity-reducing, the comparative pharmacokinetics of RTFA-OCDD-GN and RMM samples were studied by an HPLC method [30]. The pharmacokinetics study demonstrated that the absorption of total AQs was decreased in comparison with RMM, which maybe further contributed to the attenuation of nephrotoxicity. However, it is not explained clearly that which AQs component is mostly related to the attenuation of nephrotoxicity due to the lack of the pharmacokinetic profile of physcion. Many studies on the pharmacokinetics of rhubarb have been reported [31–33], though there is still limited information on the pharmacokinetics due to the extensive two phase metabolic reaction and similar chemical structure of AQs components. Thereby a more sensitive UPLC-MS/MS method for the pharmacokinetic study of AQs components has been developed. And the aim of this paper is to compare the pharmacokinetics of RTFA-OCDD-GN and rhubarb after oral administration by the sensitive UPLC-MS/MS method to evaluate the impact of five AQs components on the contribution of the attenuation of nephrotoxicity.

2. Materials and methods

2.1. Plant materials, chemicals and reagents

The dried roots and rhizomes of raw rhubarb were obtained from Chinese Medicine Group Chengde Medicinal Materials Co., Ltd (Hebei, China) and authenticated to be Rheum officinale Baill. of Polygonaceae family by Professor Chunying Zhao, a botanist at Chengde Medical College (Hebei, China). Voucher specimens were deposited at the institution of Traditional Chinese Medicine, Chengde Medical University.

The RTFA extract was extracted using the method of Liu et al. [34], and contained 54.5% of the total free AQs.

The standard chemicals include aloe-emodin, rhein, emodin, chrysophanol, physcion and danthron (internal standard, IS) with the purity > 98% by HPLC were purchased from the Chinese National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Methacrylic acid copolymers (Eudragit S100), polyethylene glycol-6000 (PEG-6000), chitosan, hydroxypropyl-methyl cellulose (HPMC), sodium carboxymethyl cellulose (CMC-Na), sodium carboxymethyl starch (CMS-Na) and microcrystalline cellulose (MCC) were purchased from Changwei Medicine Corporation (Shanghai, China). Tween-80 and Sodium dodecyl sulfate (SDS) was obtained from Jiaxing Chemical Industry Co., Ltd. (Tianjin, China). Acetonitrile and formic acid of HPLC-grade was purchased from Fisher Scientific (Fair Lawn, NJ, USA). Deionized water was purified by a Milli-Q system (Millipore, Milford, MA, USA). Other chemicals and solvents were of analytical reagent grade and obtained from Tianjin Chemical Reagent Company (Tianjin, China).

2.2. Animals

Health Sprague-Dawley (SD) rats weighing 180–240 g were provided by Beijing Vital River Laboratory Animal Technology Co., Ltd. (License No. SCXK2012-0001). All animal experiments were performed in strict accordance with the Guidelines for the Care and Use of Laboratory Animals, as adopted and promulgated by the Ministry of Science and Technology of China. Before experiments, the rats were housed in a constant temperature (22 ± 2 °C) and humidity (40%–60%) environment with a 12 h light/dark cycles. A standard diet with water was applied during the period of acclimatization for one week.

2.3. RMM sample preparation

Fifty grams of rhubarb were powered and then extracted with eight-fold volumes of 40% ethanol (400 mL) for three times, and the filtrates were pooled and concentrated, then freeze-dried and stored at 4 °C until further analysis. The extract processes were performed below 40 °C as the chemical structure of conjugated AQs was destroyed easily. The yield of dried RMM samples was 11.1%. The contents of total AQs and free AQs in RMM samples were determined according to Chinese