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The pharmacokinetic study on the mechanism of toxicity attenuation of rhubarb total free anthraquinone oral colon-specific drug delivery system ☆

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ABSTRACT

Rhubarb is commonly used as laxatives in Asian countries, of which anthraquinones are the major active ingredients, but there are an increased number of concerns regarding the nephrotoxicity of anthraquinones. In this study, we compared the pharmacokinetic characteristics of rhubarb anthraquinones in rats after orally administered with rhubarb and rhubarb total free anthraquinone oral colon-specific drug delivery granules (RTFA-OCDD-GN), and then explained why these granules could reduce the nephrotoxicity of anthraquinones when they produced purgative efficacy. A sensitive and reliable high performance liquid chromatography (HPLC) method has been fully validated for simultaneous determination of the five active components of rhubarb, and successfully applied to investigate and compare the remarkable differences in pharmacokinetic study of rhubarb anthraquinones after orally administered with rhubarb and RTFA-OCDD-GN. The results showed that, compared with rhubarb group, the AUC, C_{max}, $t_{1/2z}$ and $V_{z/F}$ of aloe-emodin, rhein, emodin and chrysophanol in rats receiving the RTFA-OCDD-GN were significantly decreased, and the T_{max} of the four analytes was prolonged. Moreover, the T_{max} of rhein, the C_{max} of chrysophanol and emodin all have significant differences ($P < 0.05$). Simultaneously, anthraquinone prototype excretion rates in urine and feces of aloe-emodin, rhein, emodin, chrysophanol and physcion were all increased. These findings suggested that oral colon-specific drug delivery technology made anthraquinone aglycone to colon-specific release after oral administration. This allowed anthraquinones to not only play the corresponding purgative effect but also avoid intestinal absorption and promote excretion. And thereby greatly reduced the nephrotoxicity of rhubarb. The result is a new breakthrough in rhubarb toxicity attenuated research.

1. Introduction

Rhubarb, as a well-known traditional Chinese herbal medicine, is derived from the dried roots and rhizomes of *Rheum palmatum* L., *Rheum tanguticum* Maxim. ex Balf. or *Rheum officinale* Baill. (Polygonaceae family) [1], and has been widely used for febrifugal, cathartic and antidotal purposes for thousands of years [2]. In rhubarb, anthraquinones (AQs), including aloe-emodin, rhein, emodin, chrysophanol, physcion and their glycosides (the chemical structures of free anthraquinones shown in Fig. 1.), exhibit various biological and pharmacological activities, and are especially thought to be the major active components of rhubarb for their purgative efficacy [3]. The purgative mechanism of taking rhubarb orally is shown in Fig. 2. As shown, AQs exist both in combined (glycosides) and free (aglycones) forms of rhubarb. Only when combined AQs are taken orally can they play the purgative action, while free AQs are the final substance of combined AQs when they produce such purgative efficacy in vivo. But if free AQs are directly taken orally, most of them are destroyed or absorbed in the upper gastrointestinal tract before they reach the colon, giving rise to weakened purgative efficacy.

Combined AQs can change their structure easily and also lose the efficacy in the process of extraction and purification. Therefore, upto now, rhubarb in the preparations is all or partly used in original powder if only play its purgative efficacy. While the original powder is hard to be prepared in modern preparations. Moreover, the heterogeneity and complexity of rhubarb herbs, such as geographic origin, production batch and harvest season, may lead to the difference of constituent components and pharmacodynamic instability. In view of these problems, rhubarb total free AQs containing no less than 50% free AQs had been extracted and played purgative efficacy through OCDDS [4]. Anthraquinone

☆ The first and the second author made the same contribution for this manuscript.

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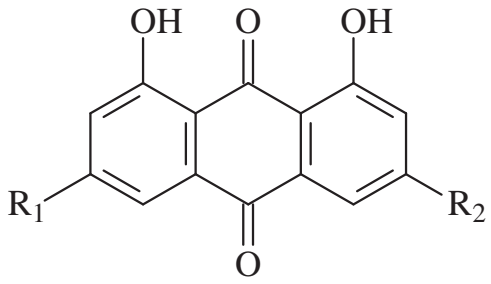

	R_1	R_2
Chrysophanol:	H	CH_3
Aloe-emodin:	H	CH_2OH
Rhein:	H	COOH
Emodin:	OH	CH_3
Physcion:	OCH_3	CH_3

Fig. 1. The chemical structures of free anthraquinones.

aglycone colon-targeted capsule was stable in the stomach and small intestine, and began to degrade in the colon. The capsule accelerated colonic transit and enhanced the role of water in the colon, and its purgative effect was dose-dependent [5].

Meanwhile, there are an increased number of concerns regarding the effectiveness and safety of rhubarb, especially after consecutive reports of adverse hepatotoxic effects and renal toxicity of AQs in in vitro and in vivo studies. The U.S. Department of Health and Human Services conducted a two-year National Toxicology Program study on emodin and demonstrated the potential hepatotoxicity and nephrotoxicity of emodin on mice and rats. The toxic effects included elevations of total bile acid levels (TBIL), alanine aminotransferase (ALT) levels and organ-weight-to-body-weight ratios (relative weights) of the kidney and liver, and also reduced the total protein and alkaline phosphatase levels [6]. Other researchers have reported that administration of rhubarb extracts caused elevations of serum ALT and TBIL in vivo, leading to hepatitis and nephritis [7–9]. Such concerns affect its application [10]. Therefore, according to the mechanism of purgative effect of AQs, if free AQs can all be released in the colon, it is possible to decrease the nephrotoxicity when they exert the purgative efficacy. In our previous study, we had prepared rhubarb total free AQ oral colon-specific drug delivery granules (RTFA-OCDD-GN) using pH-enzyme double controlled colon delivery technology, and made most of rhubarb total free AQs in granules that could be released in the colon. Through the test of time of the first black stool, the number and state of feces in 8 h, and the biochemical and histopathological examinations after continuous administration and a period of time of convalescence, we proved that RTFA-OCDD-GN could significantly reduce the nephrotoxicity of AQs when they produced purgative efficacy [11].

On this basis, in the present study, according to the rhubarb AQ content in plasma, urine and feces obtained after orally administered with rhubarb and RTFA-OCDD-GN to rats, we compared the similarities and differences in the pharmacokinetic study between the two groups to further explain how the granules reduced the nephrotoxicity of anthraquinones when they produced purgative efficacy. The results of the present investigations clearly indicated the mechanism of toxicity attenuation of RTFA-OCDD-GN. The relevant reports of this outcome are not found at home and abroad. The series of studies about rhubarb AQs also have a good model role in the modernization of traditional Chinese medicine preparations containing glycosides.

2. Materials and methods

2.1. Reagents, materials and animals

The standard chemicals which include aloe-emodin, rhein, emodin, chrysophanol, physcion and 1,8-dihydroxy anthraquinone (internal standard, IS) with a purity of over 98% by HPLC were purchased from the Chinese National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). HPLC-grade methanol was purchased from E. Merck (Mreda Company Inc, USA). Polyethylene glycol-6000 (PEG-6000) was purchased from Zhengxing Chemical Industry Research Institute (Suzhou, china), and Eudragit S100 was purchased from Changwei Medicine Corporation (Shanghai, China).

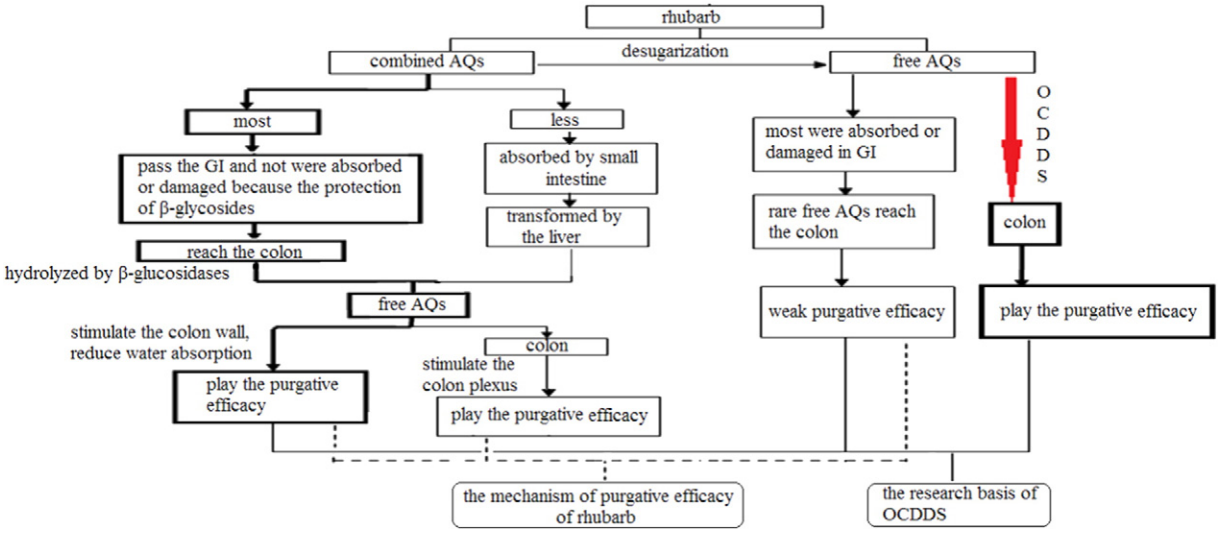

Fig. 2. The purgative mechanism of rhubarb and the research basis of oral colon-specific drug delivery system (OCDDS).