e-ISSN:2320-1215
p-ISSN:2322-0112

Research and Reviews: Journal of Pharmacy and Pharmaceutical Sciences

The Research on Effect-Enhancing and Toxicity-Reducing of Rhubarb Total Anthraquinones Caused by Oral Colon-Specific Drug Delivery System When Producing Purgative Efficacy

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Research Article

Received date: 26/03/2016
Accepted date: 11/04/2016
Published date: 17/04/2016

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Keywords: Purgative activities, Anthraquinones, Rhubarb, Oral colon-specific drug delivery system

Abbreviations: DAQs: Anthraquinones; CAQs: Combined AQs, FAQs: Free AQs; RMM: Rhubarb medical Material; OCDDS: Oral Colon-specific Drug Delivery System; RTA-OCDD-GN: Rhubarb Total AQs Oral Colon-Specific Drug Delivery Granules; RTA: Rhubarb Total AQs.

ABSTRACT

In the present study, we aimed to prepare rhubarb total anthraquinones oral colon-specific drug delivery granules (RTA-OCDD-GN) for delivering rhubarb free anthraquinones to colon. RTA-OCDD-GN was successfully prepared through a double-layer coating process using chitosan as an inner layer and Eudragit S100 as an outer enteric coating layer. The pharmacodynamics and toxicological results showed that RTA-OCDD-GN could produce considerable purgative efficacy with half dosage of rhubarb medical material (RMM), and significantly reduce the nephrotoxicity of anthraquinones compared with RMM. The comparative pharmacokinetics results *in vivo* gave a preliminary explanation to the mechanism of toxicity attenuation of RTA-OCDD-GN. Taken together, the oral colon-specific delivery system was a useful media to achieve the effect-enhancing and toxicity attenuation of orally administrated rhubarb total anthraquinones.

INTRODUCTION

Rhubarb, a well-known Chinese herbal medicine, has been widely used for thousands of years in China due to its purgative activities [1]. As one of main active constituents, anthraquinones (AQs) are usually considered as the chemical basis of purgative activity of rhubarb. AQs exist both in free and combined forms, and most of AQs is combined AQs (CAQs) in rhubarb medical material (RMM). Previous studies showed that free AQs (FAQs) are the ultimate substance playing such a purgative action [2,3]. The mechanism of purgative activity produced-FAQs is to stimulate large intestinal paries and nervous plexus to promote intestinal peristalsis and reduce water absorption at colon intestine [4,5]. But most of FAQs cannot be delivered to colon to produce purgative activity due to the absorption or destruction in upper gastrointestinal (GI) tract after directly oral administration. In contrast, CAQs, which bear β-glucosidase bonds, can avoid being hydrolyzed by α-glucosidase in the upper GI tract, reach colon and be hydrolyzed to FAQs by bacteria-producing β-glucosidase in colon to stimulate purgation. But CAQs are so easily damaged in the extract process

of rhubarb that lose the purgative efficacy, so the rhubarb in the preparations contained in Chinese Pharmacopoeia is all or partly used in original powder [2,6]. Recently, more and more studies have reported that AQs compounds could produce renal toxicity [7,8]. Colon-specific drug delivery system (OCDDS) can protect drug from absorption in the upper GI region and then promptly release into the proximal colon [9-11]. Therefore, we aimed to prepare rhubarb total AQs (RTA, containing only FAQs) oral colon-specific drug delivery granules (RTA-OCDD-GN) using pH-dependent and enzyme-triggered combination approach by a double-layer coating process. Moreover, *in vitro* drug release profiles of this newly developed drug delivery system were studied. Simultaneously, the further *in vivo* nephrotoxicity test compared with RMM and its relevant toxicity-reducing mechanism were investigated.

Preparation of RTA-OCDD-GN

RTA extract was prepared using the method of Liu et al [12]. The extract only contained FAQs, and the content of FAQs was determined by HPLC [13]. RTA-OCDD-GN was successfully prepared through a double-layer coating process using chitosan as an inner layer and Eudragit S100 as an outer enteric coating layer. The content of total FAQs in RTA-OCDD-GN was 8.49%.

***In vitro* release**

In vitro release study of double-layer coated granules was performed in triplicate using a Chinese Pharmacopoeia 2010 print part II method with some minor modifications [14]. Release test was carried out in three media with different pH values as follows: 0.1 M HCl aqueous solution (pH 1.2); phosphate buffer solution (PBS, pH 6.8); and PBS containing rat cercal contents (pH 7.4) [13,15,16]. The results of continuous release studies showed that the cumulative release rate of RTA-OCDD-GN was about 87% in the simulated colonic fluid containing rat cercal contents, while it was only about 6% in the simulated gastric and small-intestinal fluids. That means RTA-OCDD-GN could delivery basically all FAQs to the colon.

Purgative efficacy test *in vivo*

A total of 70 rats were randomly and evenly divided into seven groups. The groups were differentiated based on drug and dosage. We studied the purgation of RTA-OCDD-GN according to the first black stool time, the number and state of faeces. The results indicated that RMM and RTA-OCDD-GN could accelerate the intestinal enterocinesia and produce corresponding purgative activity. However, the amount of total AQs (only containing FAQs) in RTA-OCDD-GN was about half of that in the RMM. Therefore, purgative efficacy test confirmed that, in the same dosage, RTA-OCDD-GN containing the same content of total AQs compared with RMM could produce stronger purgative activity than RMM after directly oral administration (not published).

Nephrotoxicity test *in vivo*

210 rats were randomized into seven groups for 30 rats each according to the result of the purgative efficacy test. Through the observation of toxicity signs (including the body weight, general behavior, urine biochemistry and routine, and blood biochemistry after 20, 40 days of administration and 20 days of convalescence, respectively), and the histological sections to investigate the pathological changes of RTA-OCDD-GN and RMM.

After 40 days of RMM administration, the increase of organ coefficient in kidney, testicle and adrenal of the high-dosage RMM group was greater than that of the control group ($p<0.05$). Other tested organs did not show the obvious pathological changes compared with the control group. Histological examination showed that after 20 days of RMM administration at high-dosage, one (1/10) specimen showed the swelling/degeneration of RPCTECs, causing the lumen narrowing. After 40 days of RMM administration at high-dosage, all 10 specimens showed the swelling/degeneration of RPCTECs at different extents, causing the lumen narrowing (marked as "+"), as well as the epithelial cell shedding (marked as "++"). Four specimens of the middle-dosage RMM group exhibited these pathological changes mentioned above (marked as "+"). No pathological change was observed from the low-dosage RMM group. However, after the RTA-OCDD-GN administration, macroscopic pathological, organ coefficient and pathological changes were not observed from all groups (not published).

Mechanism of toxicity-reducing

We also through comparing the pharmacokinetic characteristics of rhubarb AQs in rats after orally administered RMM and RTA-OCDD-GN to explain the mechanism of reducing nephrotoxicity of RTA-OCDD-GN when producing purgative efficacy. A total of 18 male SD rats were randomly divided into three groups of 6 animals each. The medication groups were orally administered at a single intra gastric gavage dose of RMM and RTA-OCDD-GN, respectively. Under the above dosages, RMM and RTA-OCDD-GN could produce considerable purgative efficacy. The results shown that the AUC, $t_{1/2z}$, C_{max} and $V_{z/F}$ of four analytes (except physcion) in RTA-OCDD-GN group were significantly decreased, and the T_{max} was prolonged compared with RMM group. Simultaneously, AQs prototype excretion rates in urine and faece of five AQs in RTA-OCDD-GN group were all increased. The achieved comparative pharmacokinetics results suggested that OCDDS could not merely reduced the absorption of AQs, but more likely change their distribution and metabolism *in vivo*, so that the drug prototype absorbed into the bloodstream was rapidly excreted, and thereby reduced accumulation and nephrotoxicity [17].

CONCLUSION

RTA-OCDD-GN was successfully prepared through a double-layer coating process using chitosan as an inner layer and Eudragit

S100 as an outer layer. The cumulative release rate of RTA-OCDD-GN indicated that RTA-OCDD-GN could be specifically released in the colon. Moreover, the RTA-OCDD-GN could produce corresponding purgative activity with half dosage of total AQs compared with RMM group. In addition, the results of the comparison on renal toxicity test and pharmacokinetic study *in vivo* indicated that RTA-OCDD-GN could reduce nephrotoxicity through reducing drug accumulation and increased excretion. Therefore, the thorough investigation of toxicity attenuation of RTA-OCDD-GN will provide theoretical support for the further clinical application of rhubarb.

ACKNOWLEDGEMENTS

This work was financially supported by the National Natural Science Foundation of China (No. 81073146 and No.81341143); Key Discipline Construction Projects of Higher School; Hebei Province Natural Science Foundation of China (No. H2014406036); Science and Technology Research Key Project of Higher School in Hebei Province (No. ZH2012050); Science and Technology Research Youth Fund Project of Higher School in Hebei Province (No. QN2013020); A Hundred of Excellent Creative Talents Support Plan of Higher School in Hebei Province (II) (No. BR2-103).

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