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Original article

Pharmacokinetics and pharmacodynamics of rhubarb anthraquinones extract in normal and disease rats

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ABSTRACT

Background: Anthraquinones extract from *Rheum palmatum* L. (rhubarb) including rhein, emodin, aloe-emodin, chrysophanol, physcion and sennoside A, has been widely used in China to treat various diseases.
Objective: This study was designed to explore the pharmacokinetic and pharmacodynamic properties of rhubarb anthraquinones extract in diabetic nephropathy and acute liver injury rats.
Methods: The diabetic nephropathy and acute liver injury rats were induced by intraperitoneal injection with streptozotocin (STZ) and carbon tetrachloride (CCL_4), respectively. The rats were treated with different doses of rhubarb anthraquinones extract (37.5, 75 and 150 mg/kg) as administration groups. For pharmacokinetics, the drug concentrations of rhubarb anthraquinones consisting of rhein, emodin, aloe-emodin, chrysophanol, physcion and sennoside A were determined. For pharmacodynamics, the anti-diabetic nephropathy and hepatoprotective effects were assessed under different dosage regimens.
Results: The rhein, emodin, aloe-emodin, chrysophanol were considered as pharmacokinetic markers at three doses of rhubarb anthraquinones extract. In diabetic nephropathy rats, no obvious pharmacokinetic change of the four ingredients was observed compared with control rats. However, the plasma exposures of the four ingredients increased in acute liver injury rats compared with control rats. The serum creatinine (SCr), blood urea nitrogen (BUN) and urine protein (UP) values in diabetic nephropathy rats decreased compared with those in the model group, which suggested that rhubarb anthraquinones extract displayed certain therapeutic and preventive effects against the diabetic nephropathy. However, rhubarb anthraquinones extract cannot ameliorate the CCL_4-induced liver injury under the three different dosage regimens.
Conclusion: There was no significant pharmacokinetic difference after a single oral administration of rhubarb anthraquinones extract between control and diabetic nephropathy rats. However, apparent pharmacokinetic differences were observed between control and liver injury rats. Also, rhubarb anthraquinones extract had beneficial effects on diabetic nephropathy rats, while no marked effect on liver injury rats under the same dosage regimens.

1. Introduction

Traditional Chinese medicines (TCMs) are gaining growing attentions in both academic and industrial fields due to their unclear properties as well as long historical clinical practice in treating chronic or complex diseases. *Rheum palmatum* L. (Rhubarb), a well-known TCM, has been widely applied in China for several centuries to treat constipation, jaundice, gastrointestinal hemorrhage and ulcers [1]. With the advances in separation extraction method, various constituents have been isolated from rhubarb at present. Among them, rhubarb anthraquinones including rhein, emodin, aloe-emodin, chrysophanol, physcion and sennoside A are regarded as representative components in rhubarb (Fig. 1), which are often used as indexes in the quality control of rhubarb [2].

Multiple components detected simultaneously in TCM should be used to evaluate the integral pharmacokinetic profiles of TCM instead of the single component. Finding suitable pharmacokinetic markers as much as possible could attribute to study the pharmacokinetics of TCM. For rhubarb anthraquinones, there have been several studies investigating its pharmacokinetics in rats and

Abbreviations: rhubarb, *Rheum palmatum*; STZ, streptozotocin; CCL_4, carbon tetrachloride; SCr, serum creatinine; BUN, blood urea nitrogen; UP, urine protein; TCMs, Traditional Chinese medicines; BG, blood glucose; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; ESI, electrospray ionization.

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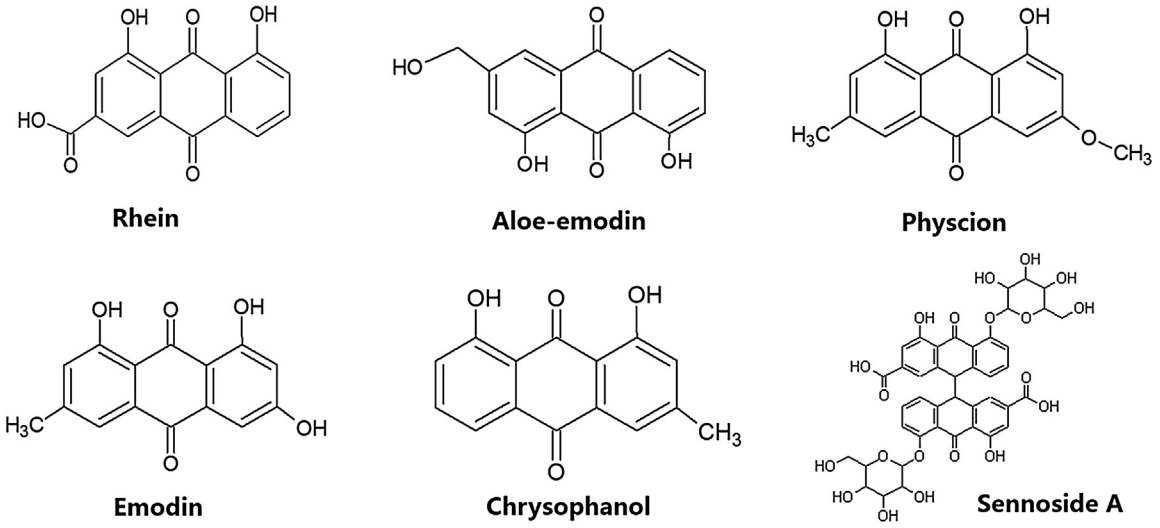

Fig. 1. Chemical structures of rhein, emodin, aloe-emodin, chrysophanol, physcion and sennoside A.

human. Zhu et al. compared the pharmacokinetic parameters of rhein after the administrations of rhubarb anthraquinones extract by retention enemas with those by conventional oral administrations to find a significant change in pharmacokinetic parameters of rhein in rats [3]. Additionally, the comparative pharmacokinetics of rhein in control and loperamide-induced constipation rats were investigated by Hou et al. The results demonstrated that the loperamide-induced constipation reduced the absorption of rhein, resulting in the decreased C_{max} and $AUC_{0-\infty}$ [4]. Pharmacokinetic characteristics of many drugs usually vary in different the body states, physiological functions and disease progressions. More important information could be obtained through comparing the drug pharmacokinetics in different conditions [5]. Diabetic nephropathy, known as diabetic kidney disease threatening human health for decades, is one of the major micro vascular complications of diabetes mellitus and the leading cause of end-stage renal disease [6]. It has been reported that about 20–30% patients of diabetes are prone to develop diabetic nephropathy after 5 or more years since the onset of diabetes mellitus [7]. The hypothesis we proposed was that diabetic nephropathy might influence renal excretion of drugs due to the damaged renal function. Therefore, it is essential to examine this hypothesis by investigating the pharmacokinetic profiles of rhubarb anthraquinones extract in diabetic nephropathy rats. Similarly, the pharmacokinetic changes often appeared in acute liver injury states because of alterations of metabolic enzymes and bile excretions in liver. Rhubarb anthraquinones were almost metabolized by CYP and UGT in liver [8,9], so the pharmacokinetic profiles were also needed to be evaluated in acute liver injury rats. Numerous studies reported that rhubarb anthraquinones exhibited beneficial pharmacological activities. It is included as an ingredient in many traditional Chinese medicine formulations for treatment of indications involving oxidative stress, inflammation (acute appendicitis, cholecystitis, and rheumatoid arthritis), hepatitis B, neuroectodermal tumor etc. [10–13]. However, systematic experimental results were lack to explain the role of organ protections of rhubarb anthraquinones in diabetic nephropathy and acute liver injury rats.

The objective of this study was to gain the systemic exposures of rhubarb anthraquinones extract in rats and compare the pharmacokinetic properties between the normal and disease rats. Simultaneously, we investigated the effects of rhubarb anthraquinones extract on diabetic nephropathy and acute liver injury rats. Thereby, the pharmacokinetic properties as well as pharmacological effects of rhubarb anthraquinones extract were evaluated in the present study to provide compelling evidence for the potential efficacy of rhubarb in clinical use.

2. Materials and methods

2.1. Chemicals and reagents

The decoction pieces of rhubarb were purchased from Bozhou traditional Chinese medicine market (Anhui Province, China). The herbal drugs were identified to be the rhizome of *Rheum palmatum* L. based on Chinese Pharmacopoeia by Prof. Yingjie Wei of Jiangsu Research Institute of Traditional Chinese Medicine. The representative specimen (No. 201506) was deposited in Jiangsu Research Institute of Traditional Chinese Medicine. Rhein, emodin, aloe-emodin, chrysophanol, physcion, sennoside A (purity 98%) and rhubarb anthraquinones extract were extracted and purchased from Nanjing Zelang Biological Technology Co. Ltd. (Nanjing, China). Estrone (purity 99%) was purchased from Beijing Bailingwei Technology Co. Ltd. (Beijing, China). Streptozotocin (STZ, purity $\geq$ 98%) was purchased from Sigma (St.Louis, USA). The assay kits for the measurements of blood glucose (BG), serum creatinine (SCr), blood urea nitrogen (BUN), urine protein (UP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) were bought from Nanjing Jiancheng Biological Engineering Institute (Nanjing, China). Acetonitrile, methanol and ethyl acetate were of HPLC grade and purchased from Fisher Scientific (Fair Lawn, NJ, USA). Deionized water was purified using a Milli-Q Ultrapure water system with the water outlet operating at 18.2 MΩ (Millipore, Bedford, USA). Other reagents were of HPLC grade or analytical grade.

2.2. Instrumentations

The HPLC system consisted of a Shimadzu DGU–$20A_3$ online degasser, two Shimadzu LC-20AD XR pumps with a high pressure mixer, a Shimadzu CTO-20A column oven and a Shimadzu SIL-20AC XR autosampler (Shimadzu, Japan). The chromatographic separation of the analyte was achieved by a Shim-pack VP-ODS column (5 μm, 150 mm × 2.0 mm, Shimadzu, Japan).

Mass spectrometric analysis and detection were acquired using an Applied Biosystems/MDS SCIEX 5500 Q-Trap triple quadrupole mass spectrometer (Foster City, CA, USA) equipped with an electrospray ionization (ESI) source, two Perkin-Elmer PE-200