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Pharmacokinetics and tissue distribution study of scoparone in rats by ultraperformance liquid-chromatography with tandem high-definition mass spectrometry

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ABSTRACT

Scoparone is an important constituent of Yinchenhao (*Artemisia annua* L.), a famous Chinese medicinal plant, and has several known bioactivities, and displayed bright prospects in prevention and therapy of jaundice and liver disorders. The aim of this study was to investigate the in vivo plasma pharmacokinetic and tissue distribution characteristics of scoparone after oral administration. The levels of scoparone in plasma, and tissues were measured by a rapid and sensitive UPLC–MS/MS method. The biosamples were prepared using methanolic precipitation and the separation of scoparone was achieved on a UPLC HSS T3 column by linear gradient elution using water (containing 0.1% formicacid) and acetonitrile (containing 0.1% formic acid) as the mobile phase at a flow rate of 0.5 mL/min The total run time was only 3.9 min. Our results successfully demonstrate that this method has excellent and satisfactory selectivity, sensitivity, linearity, precision, accuracy and recovery. The estimated pharmacokinetic parameters (i.e., C_{max}, *AUC* and *CL*), were C_{max} = 14.67 mg/L, *AUC* = 81.15 mg*h/L, *CL* = 1.23 L/h for scoparone. The pharmacokinetic study found that scoparone was distributed and eliminated rapidly in rats. Tissue distribution showed the highest level was observed in liver, followed by the kidney and spleen; the lower level appeared in the muscle, thyroid, and adrenal. It was not detected in the brain which indicated that scoparone does not cross the blood–brain barrier after oral administration. Our developed method was suitable for the study on pharmacokinetics and tissue distribution of scoparone after oral administration.

1. Introduction

Yinchenhao (*Artemisia annua* L.) is one of the most popular traditional Chinese medicinal plants for treatment of jaundice and liver disorders and has been used more than one thousand years. Biological and pharmacological studies have shown that it can be used clinically to treat cholestasis, hepatitis C, primary biliary cirrhosis, liver fibrosis, and cholestatic diseases [1,2]. Interestingly, as one of the main active constituents of Yinchenhao, scoparone (Fig. 1) has been proven effective in treating liver diseases, and shows hepato-protectivity and contributes directly to the therapeutic effect [3]. Additionally, scoparone was reported to be an effective drug for normalizing liver function primarily by enhancing the secretion of bile acids [4]. All these activities suggested that scoparone may be a good lead compound for further new drug studies. Therefore, these medicinal functions of the scoparone have attracted much attention and generated great interest from researchers for its further investigation and therapeutic application. However, information about scoparone's tissue distribution in vivo, which is very important for new drug discovery, has not been found in the published literatures up to now. It is well known that the pharmacokinetic study of a bioactive constituent can help us to understand its in vivo actions and explain a variety of

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events related to efficacy and toxicity [5]. Therefore, the importance of understanding the pharmacokinetic profiles of scoparone is evident, and a corresponding pharmacokinetic study is undoubtedly required. In order to explore the potential of scoparone as a liver protective agent, it is necessary to further study the pharmacokinetic and distribution characteristics of scoparone. Moreover, tissue distribution is also vital to investigate the major target sites and interpret the in vivo disposition. Considering the growing significance of a potential beneficial role of scoparone in human health, detailed in vivo pharmacokinetic and disposition studies of scoparone by proper administration route such as oral administration are required.

To our knowledge, analytical techniques applied to quantification of scoparone from Yinchenhao extracts included thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC) with UV detection and so on [6,7]. Single wavelength spectrophotometric detection, particularly in the end absorption region, lacks the specificity desirable for quantitation in physiological matrices. Due to the fact that the plasma sample at each time point cannot exceed 10% of the total plasma volume of an animal, if multiple time points need to be measured. On the other hand, the scoparone levels that can be reached in plasma are very low. Therefore, increasing evidence demonstrates that LC–MS is now the method of choice for such applications [8]. Recently, further improvements in chromatographic performance have been achieved by the routine availability of ultraperformance liquid chromatography (UPLC) relative to conventional HPLC method [9]. A number of analytical methods using mass spectrometry (MS) as the mode of detection have been reported for the determination of TCM in plasma and other tissues, and have been considered the methods of choice [10–13]. These methods are generally both reliable and sensitive. Therefore, a simple, specific and sensitive UPLC–MS/MS method assay for scoparone in rat plasma has been developed for the first time by our laboratory.

Up to now, there have been no reports of the pharmacokinetic properties of scoparone in the relevant literature although this information is important to our further exploration of the scoparone as a drug candidate. Accordingly, the present study describes a rapid and highly sensitive UPLC–MS/MS method to determine the concentration of scoparone in rat plasma and tissue samples. The conditions for analysis and sample preparation were optimized, and the method was validated in terms of selectivity, sensitivity, accuracy, precision and extraction recovery. The newly developed method was applied in pharmacokinetic and tissue distribution studies after oral administration of scoparone in rats.

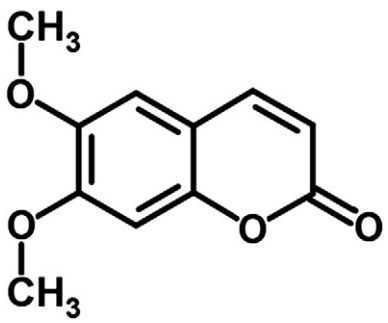

Fig. 1. Chemical structures of scoparone.

2. Materials and methods

2.1. Materials and reagents

Acetonitrile and methanol, HPLC grade, were purchased from Dikma Technology Corporation (Richmond Hill, Ontario, Canada). Deionized water was purified on a Milli-Q system (Millipore, Bedford, USA). Formic acid and phosphoric acid, analytical grade, were obtained from Beijing Reagent Company (Beijing, China). Glycerol was supplied from Chemicals Factory (Shanghai, P. R. China). Olive oil (Oliver grade) was supplied by Branch office of Shanghai of Olis olive oil Bloc (Catalonia, Spain). Scoparone (purify 99%) were purchased from Sichuan Provincial Institute for Food and Drug Control (Sichuan, P. R. China).

2.2. Animals, drug administration and sampling

Male Wistar rats in clean grade (weighting 250 ± 20 g) were supplied by the GLP Center of Heilongjiang University of Chinese Medicine (Harbin, China). Rats were individually housed in an Association for Assessment and Accreditation of Laboratory Animal Care-approved, temperature (24 ± 2 °C), humidity (60 ± 5%), and 12 h dark/light cycles controlled facility with ad libitum access to rat chow and tap water. The animals were housed under the above conditions for at least 1 week acclimation, and then fasted with free access to water for 12 h prior to each experiment. The experimental animal protocols described were approved by the Medicine Ethics Review Committee for animal experiments of Heilongjiang University of Chinese Medicine. Scoparone was dissolved in olive oil immediately before use, and as limited by their solubility. Thirty Wistar rats were randomly divided into six groups (n = 5 per group). Five groups were treated with scoparone solution by oral administration at a dose of 90 mg/kg, while the other one as the control group was treated with an equal volume of physiological saline. All rats were anesthetized with ether before blood and tissue sampling, and each rat was used only once for taking samples. After scoparone was orally administrated, blood (~1.0 mL), heart, spleen, liver, lung, kidney, brain, thyroid, adrenal, and muscle samples (~0.5 g) were collected at 10, 30 min and 1, 2, 4 h, respectively. Tissue samples were weighed rapidly and rinsed with physiological saline solution to remove the blood or content, blotted on filter paper, and then stored at − 80 °C until analysis.

2.3. UPLC–MS/MS analysis of scoparone

2.3.1. Chromatography

Chromatography was performed on a 2.1 mm i.d. × 100 mm ACQUITY 1.8 μm HSS T3 column (Waters Corp., Milford, USA) using an ACQUITY UPLC™ system (Waters Corp., Milford, USA). A "purge-wash-purge" cycle was employed on the autosampler, with 90% aqueous formic acid used for the wash solvent and 0.1% aqueous formic acid used as the purge solvent, this ensured that the carry-over between injections was minimized.