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UHPLC-Q-TOF-MS/MS based screening and identification of the metabolites *in vivo* after oral administration of betulin

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ABSTRACT

Betulin is an active natural pentacyclic triterpene ingredient with valuable anti-cancer and anti-HIV efficacies. In the present study, an efficient approach was developed to screening and identification of metabolites and to assess the metabolic profiles of betulin *in vivo* using UHPLC-Q-TOF-MS/MS system based on multiple mass defect filter data acquisition (MMDF) and multiple data processing techniques. Based on the proposed method, 56 phase I and 6 phase II metabolites were detected in rats after oral administration of betulin. The main biotransformation routes of betulin were identified as demethylation, dehydroxylation, deoxidization, dehydration. Conjugation with sulfate, taurine, cysteine and *N*-acetylcysteine groups produced 6 phase II metabolites. This study not only provided useful information for further study of the pharmacology and mechanism of betulin *in vivo*, but also provided essential data for further pharmaceutical studies of other pentacyclic triterpenes.

1. Introduction

Betulin, a pentacyclic triterpenes with a lupane skeleton, is extracted from birch tree bark and exhibits diverse pharmacological activities, such as anti-tumor, anti-viral, anti-bacterial and anti-inflammatory activities [1,2]. In addition, betulin also has the extensive biological activity of improving the immunity of the body, detoxifying and regulating blood lipids [3,4]. Currently, betulin has attracted increasing attention due to its significant anti-HIV activity [5] and possesses the advantages of the more targeted [6,7], low toxicity and low molecular weight [8], almost no adverse reactions [9], is a very promising leading compound [10]. In view of the growing role of betulin in human health, it is vital to study its pharmacokinetic features, efficacy and toxicity [11]. Therefore, an intensive investigation on absorption, distribution, excretion and metabolism of betulin *in vivo* was essential [12,13].

At present, betulin has a breakthrough in follow researches, such as pharmacology research, pharmacokinetic study, tissue distribution research and synthesis and application. Nevertheless, study on the metabolism of betulin have not been reported. Even though, metabolite identification is a part of drug discovery and development. Understanding drug metabolism can help to explain and predict a variety of events related to the efficacy and the toxicity of the parent drugs [14,15].

So far, with the development of various data acquisition and mining technologies [16–19], UHPLC-Q-TOF-MS/MS [20–23], especially for high-resolution mass spectrometry (HRMS) has exhibited excellent performances for metabolite detection because of its high-speed, high resolution and high detection sensitivity [24,25]. This technology was successfully developed and used for the detection and identification of metabolites in rat bile, plasma and urine samples. Comparing with the traditionally intensity-dependent data acquisition method, multiple mass defect filter method could give the information of low-level metabolites masked by background noise and endogenous components [26,27].

The purpose of this study was to set up a practical strategy for investigating the metabolic profile of betulin *in vivo*. First, we used a unique and novel MMDF and DBS dependent on line data acquisition method, was utilized to obtain the molecular ion peaks and MS2 spectrum of betulin metabolites. Then, many multiple data processing techniques using the MetabolitePilot™ software, such as extracted ion chromatogram (XIC), mass defect filter (MDF), product ion filter (PIF), neutral loss filter (NLF), were used to elucidate the structures of possible metabolites. Finally, UHPLC-Q-TOF-MS/MS with multiple data processing technologies was successfully developed and used for the detection and identification of betulin metabolites in rat bile, plasma and urine and we summarized the metabolic pathways of betulin *in vivo* for the first time. It is not only provided useful information for further

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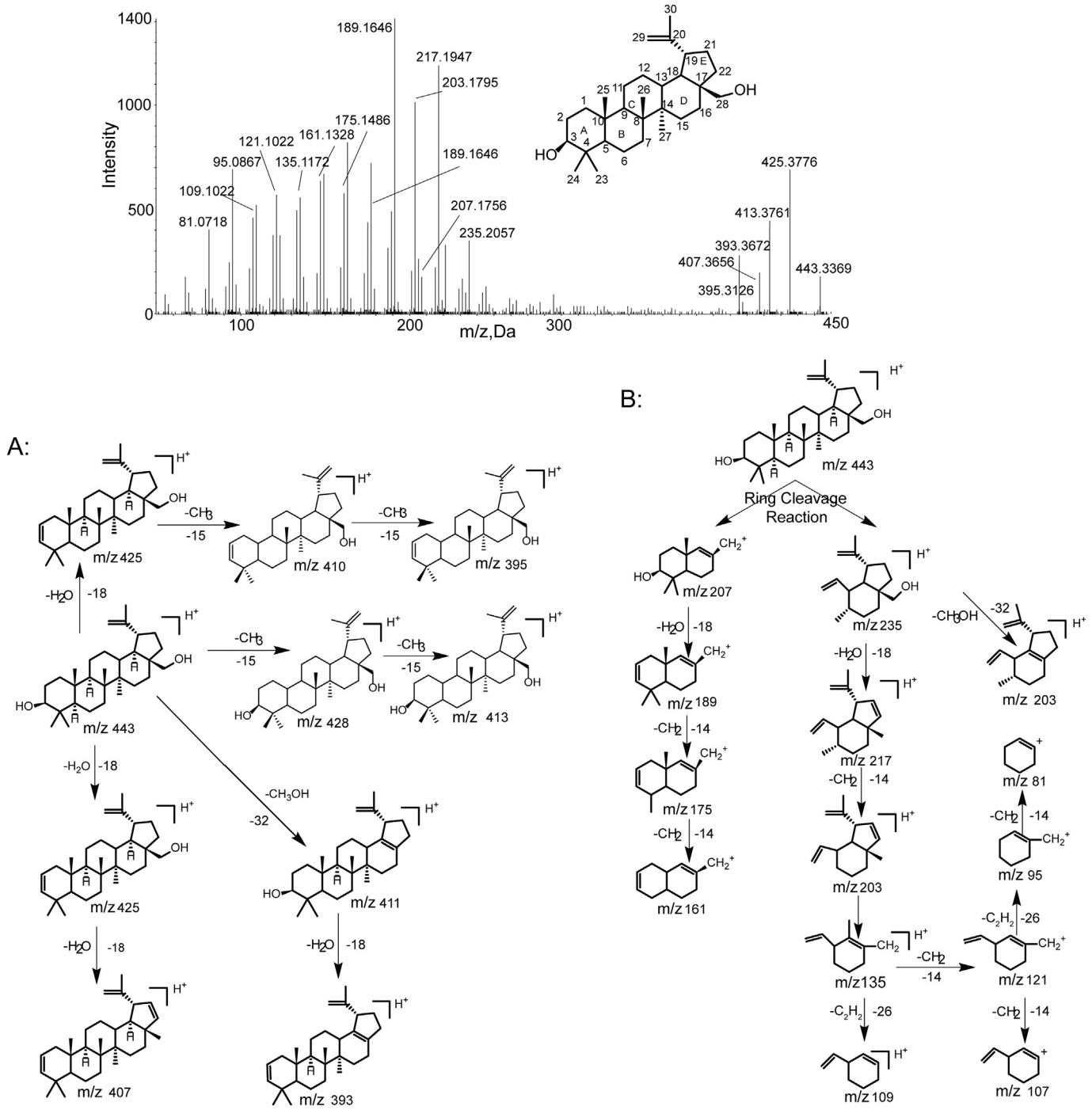

Fig. 1. The chemical structure and MS/MS spectrum of betulin and fragmentation pattern of M0.

study of the pharmacology and mechanism of betulin *in vivo*, but also provided essential data for the clinical application.

2. Experimental

2.1. Reagents and materials

Betulin (Fig. 1) (purity ≥ 98%, determined by the normalization method using HPLC) was purchased from Sichuan Weikeqi Bio- technology Co., Ltd. (Sichuan, People's Republic of China). HPLC-grade methanol and formic acid used for UPLC analysis were purchased from J. T. Baker (NJ, USA) and Fisher (NJ, USA), respectively. The ultrapure water was provided by Wahaha Corporation (Hangzhou, China) for preparing the mobile phase. Analytical grade sodium carboxymethyl cellulose (CMC-Na) and analytical grade ether, dichloromethane and n-butanol were purchased from Kermel Chemical Reagents Development Centre (Kermel, Tianjin, China). Other chemical were of analytical grade (Tianjin Chemical Corporation, People's Republic of China).

2.2. UHPLC-Q-TOF-MS/MS conditions

Sample analysis was conducted on an UHPLC system (Kyoto, Japan) coupled to a hybrid quadrupole time-of-flight tandem mass spectrometer (SCIEX, CA, USA) equipped with Duo-Spray™ ion sources and a Turbo ionspray interface (SCIEX Triple-TOF 5600 +), an auto-sampler and a column compartment. The chromatographic separation was

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