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Metabolomic study of raw and processed *Atractylodes macrocephala* Koidz by LC–MS

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ABSTRACT

Bai-Zhu, the dried rhizome of *Atractylodes macrocephala* Koidz (AMK), is widely used as a tonic herbal in eastern Asia. It is commonly used as prepared slices in clinic by stir-frying with wheat bran (processed AMK). In the theories of traditional Chinese medicine (TCM), Bai-Zhu possesses significantly different therapeutic effects before and after processing. However, the molecular mechanics of this processing is still unknown. In this paper, the strategy of metabolomics was employed to investigate the changes of chemical constituents in *Atractylodes macrocephala* Koidz after processing. Meanwhile, the cell activity test variation of processed and unprocessed medicine was used to interpret the processing mechanism of AMK. Using ultra-performance liquid chromatography quadrupole time of flight mass spectrometry (UPLC–QTOF/MS) with the method of multivariate statistic analyses including principal component analysis (PCA) and partial least square-discriminant analysis (PLS-DA), atractylenolide I, atractylenolide II, atractylenolide III, atractylenolide VI, 7-hydroxycoumarin, 8-β-methoxy atractylenolide I and Selina-4 (14), 7 (11)-dien-8-one were rapidly explored as the potential chemical markers of raw and processed AMK, respectively. Furthermore, it could be speculated that the processing mechanism of AMK was to increase the content of atractylenolide III which could strengthen the effect on gastrointestinal function.

1. Introduction

Processing of TCM is a pharmaceutical technique to fulfill the different requirements of therapy which is a significant trial discriminating western medicine from TCMs [1]. Proper processing may reduce toxicity or side effects, change the pharmacological properties, improve flavor or correct unpleasant taste and increase purity of TCMs [2–5]. In order to guarantee the safety in clinical trials, it is necessary to employ the correct processing technology in manufacturing clinical decoction pieces. What is more, the processing mechanism summarized from the processing procedure and clinical practice experience of TCM should be clarified reasonably. It could guide the production and application of processed products rationally.

Bai-Zhu, the rhizomes of *Atractylodes macrocephala* Koidz which has been widely used as a tonic in China and other Asian countries for thousands of years. In China, the processing methods for AMK have been practiced since the Tang dynasty [3] and are documented in the Chinese Pharmacopeia that stir-fry with wheat bran [6]. It was a typical example which has obvious different in clinical efficacy between raw and processed state. The raw of AMK was used for phlegm retention, edema and rheumatic pain, while the processed drug was usually used for the gastrointestinal upset, dyspepsia and anorexia, etc. [6]. Thus, it was necessary to discriminate raw and processed herbs in clinical practice. Generally, the main mechanisms underlying herb processing were found to be related to the changes in the composition and/or activity of the components in the herbs. In previous processing studies, the attention was mainly focused on the changes of terpenoids in AMK [7]. However, there are no holistic researches made in global metabolomic characters between raw and processed of AMK.

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Metabolomics is a branch of science concerned with the total metabolome of integrated biological systems and dynamic responses to alterations of endogenous and/or exogenous factors [8]. With the help of high sensitivity, high-throughput analytical tools and multivariate statistic analysis, not only could it be used to classify the samples of various origin, diverse status and different qualities in extensively complex herb or prescription [9–11], but it was also suitable to research the processing mechanism of TCM. In fact, several papers have illustrated that the metabolomics could be used to explain the processing mechanism of the TCMs such as Ginseng Radix et Rhizoma, Notoginseng Radix et Rhizoma, Rehmanniae Radix and Genkwa Flos, etc. [12–15]. In these researches, LC–MS or LC-NMR was used to scan the full metabolic profiling and multivariate statistical analysis was conducted to find potential chemical markers. Then, the processing mechanism was deduced from the report of potential chemical markers in the literature. However, there was no similarly research in AMK. Thus, this method would be carried out to study the processing mechanism of AMK.

In addition, there was a lack of sufficient activity reports which were relevant to the changes of efficacy after processing for the chemical markers in AMK. In order to explain the processing mechanism of AMK better, the rat intestinal eqithelial cell line (IEC-6) would be selected as a model to test the activity of potential chemical marker. IEC-6 was obtained from normal rat small intestinal crypt cells, isolated and established cell lines after continuous passage culture in vitro; it kept the characteristics of the intestinal epithelial stem cell undifferentiated and will differentiate into mature intestinal epithelial cell under certain condition [16]. It is widely used in the cellular, molecular and genetic mechanisms of intestinal mucosal repair [17]. Furthermore, intestinal mucosal repair mechanisms have relevance to the disease of disorder of gastrointestinal [17]. Thus, it was applied in this experiment to verify the activity of potential chemical markers and the result could be used to explain the processing mechanism of AMK.

In this research, the strategy of metabolomics was used to rapidly find potential chemical markers. That included two steps. Firstly, UPLC–QTOF/MS was used to scan the full metabolic profiling of raw and processed AMK. Secondly, a multivariate statistical analysis composed of PCA and PLS-DA of the spectra based on all chemical information was conducted to find potential chemical markers. Then, the mode of IEC-6 was applied to test the activity of potential chemical markers. On the basic of the above results, the mechanism of frying AMK with wheat bran was elucidated. This procedure of study would be a valuable strategy for uncovering the processing mechanisms of other herbs.

2. Materials and methods

2.1. Chemicals and herbal materials

Six batches of samples of AMK were collected from Yuqian County, Zhejiang Province, one of native cultivating regions in China and authenticated by the professor Yanjun Zai, Department of Pharmacognosy of Liaoning University of TCM, which was the rhizome of *A. macrocephala* Koidz. The raw AMK were obtained from sun-drying the slice of fresh rhizome of the *A macrocephala* Koidz. These samples were named BZP-01–BZP-06. Then, the processed AMK was produced by stir-baking raw AMK sliced (BZP-01–BZP-06) with honey wheat bran for 60 s, in the conditions of 200 °C, according to the processing method described in Chinese Pharmacopeia [6] and was named FBZ-001– FBZ-006. The detailed collecting time and locations of the raw and corresponding relations to the processed AMK were listed in Table 1. All of the raw and processed sliced voucher specimens were deposited in the Liaoning Province Engineering of proceeding Research Center, Liaoning University of Traditional Chinese Medicine.

Acetonitrile and methanol for HPLC analysis were supplied by Burdick & Jackson (Morris, NJ, USA). Formic acid (HPLC grade) was purchased from Sigma–Aldrich (St. Louis, MO, USA). Deionized water was purified by the Millipore-Q water purification system (Bedford, MA, USA). All other analytical grade chemicals were obtained from Kermel Chemical Reagent Co., Ltd. (Tianjin, China).

Atractylenolide I, II and III were purchased from Sichuan Wei Keqi biological Technology Co., Ltd. (Chengdu, China). Dulbecco's modification of Eagle's medium Dulbecco (DMEM) and fetal bovine serum (FBS) were purchased from Gibco/BRL (Grand Island, NY, USA). Cell Counting Kit-8 (CCK-8) was purchased from Sigma Chemical. Glucose Assay Kit was purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China).

2.2. Sample preparation

The standard solutions of atractylenolide I, atractylenolide II and atractylenolide III were prepared in methanol. The samples of raw and processed AMK were grounded into a powder, passed through a 0.45 mm sieve, and dried in an oven at 50 °C for 6 h in a vacuum desicator. An accurately weighed amount of the powdered (approximately 2.5 g) was introduced into a 100 mL amber vial, and 50 mL of methanol was added. The weight of the vial was recorded and the vial was sealed and sonicated at room temperature for 30 min. The original solvent weight was restored and the extract was filtered through a 0.22 μm membrane filter.

2.3. Ultra performance liquid chromatography tandem time of flight mass spectrometry

2.3.1. Liquid chromatography

Chromatographic analysis was performed in an Agilent 1290 Series UPLC system (Santa Clara, CA, USA) equipped with an online degasser, an autosampler, a binary pump system, and a thermostatically controlled column compartment, a diode array detector and an Agilent 6520 QTOF/MS.

Chromatographic separation was carried out at 50 °C on an Agilent Zorbax Eclipse Plus C18 (3.0 mm × 150 mm, 1.8 μm). The mobile phase consisted of acetonitrile (A) and water with 0.2% formic acid (B) using a gradient elution of 3–28% A (0–4 min), 28–60% A (4–6 min), 60–90% A (6–15 min), 90–100% A (15–20 min), 100–100% A (20–22 min) and 3 min post run time back to the initial mobile phase composition was used after each analysis. The flow rate was kept at 0.5 mL/min. The sample volume injected was set at 5 μL. Each injection, 1 μL standard calibration solution was injected which was used to calibrate the MS. And 5 μL methanol used as the blank was injected between selected analysis to validate inter-sample cross-talking effect.

2.3.2. Mass spectrometry

Mass spectrometry was performed by using an Agilent 6520 QTOF/MS equipped with an electrospray ionization (ESI) interface, and using the following optimum operating parameters: drying gas (N_2) flow rate, 10.0 L/min; drying gas temperature, 350 °C; nebulizer, 50 psi; voltage of capillary, 4000 V; skimmer, 65 V; OCT 1 RF Vpp, 750 V; fragmentor voltage, 200 V. Full-scan data acquisition in positive ion mode was performed from 100 to 1000 m/z with accurate mass of 5 ppm and acquisition rate of 2 spectra s^{-1}. Data were managed on the MassHunter WorkStation Data Acquisition software (Version B.02.01). The collision induced dissociation (CID) experiment was performed to get the fragments of putative chemical markers with argon as collision gas, while collision energy varied from 10 eV to 30 eV to get abundant fragment information. The mass was corrected with NaCsI before the study. Data was