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iTRAQ-based quantitative proteomic analysis reveals Bai-Hu-Tang enhances phagocytosis and cross-presentation against LPS fever in rabbit

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ABSTRACT

Ethnopharmacological relevance: Bai-Hu-Tang (BHT), a classical anti-febrile Chinese formula comprising of liquorice, anemarrhena rhizome, gypsum and rice, has been traditionally used to anti-febrile treatment and promote the production of body fluid to relieve thirst. In this paper, we aim to explore anti-febrile mechanism of BHT at protein level through analyzing alteration of differentially expressed proteins (DEPs) both lipopolysaccharide (LPS) fever syndrome and that was treated with BHT in rabbits.

Materials and methods: Febrile model was induced by LPS injection (*i.v.*) in rabbits, and BHT (750 mg dry extract/kg body weight) was gavaged to another group of LPS fever rabbits. After sacrifice of animals, total protein of liver tissue was isolated, and two-dimensional liquid chromatography (LC) - tandem mass spectrometry (MS) coupled with isobaric tags for relative and absolute quantification (iTRAQ) labeling analysis was employed to quantitatively identify differentially expressed proteins in two group animals, which were compared with control group. Then bioinformatic analysis of DEPs was conducted through hierarchical Clustering, Venn analysis, gene ontology (GO) annotation enrichment, and kyoto encyclopedia of genes and genomes (KEGG) pathways enrichment.

Result: The results demonstrated there were 63 and 109 DEPs in LPS fever group and BHT-treated group, respectively. Enrichment analysis of GO annotations indicated that BHT mainly regulated expression of some extracellular structural proteins for response to stimulus and stress. KEGG analysis showed that ribosome and phagosome were the most significant pathways. Thereinto, several proteins in phagosome pathway were significantly up-regulated by BHT, including F-actin, coronin, Rac, and major histocompatibility complex class I (MHC I), which work in phagocytosis and cross-presentation

Conclusion: BHT may contribute to pyrogen clearance by boosting antigenic phagocytosis, degradation, and cross presentation in the liver.

1. Introduction

Bai-Hu-Tang (BHT) is a classical traditional Chinese prescription that has important research value. It is firstly recorded in a medicine classics of Treatise on Cold Damage and Miscellaneous Diseases (in Chinese *Shanghan Zabing Lun*) compiled by Zhong-Jing Zhang about 2000 years ago in China. It is formulated with four herbs of liquorice, anemarrhena rhizome, gypsum and rice (Fig. 1), and is traditionally used to clear excessive heat and promote the secretion of saliva or body fluids (Charles, 2000; Zhu, 2007). In recent years, its new usage refers to many clinical diseases including diabetes mellitus (Chen et al., 2008), eczema, pruritus, some anxiety and emotional disorders (Fred and Bob, 2004). In Western countries, the efficacy of Chinese herbal medicine has been recognized (Tu, 2011), and traditional Chinese medicine (TCM) is becoming frequently used in Western countries (Cheung, 2011). BHT is widespreadly concerned, and also practiced in European countries (European Herbal and Traditional Medicine Practitioners Association, 2007).

It reported that BHT significantly decreased rectum temperature that was increased by lipopolysaccharide (LPS) in the rabbit (Zhang

Abbreviations: TCM, traditional Chinese medicine; BHT, Bai-Hu-Tang; LPS, lipopolysaccharide; iTRAQ, isobaric tags for relative and absolute quantification; LC-MS/MS, liquid chromatography - tandem mass spectrometry; DEPs, differently expressed proteins; GO, Gene Ontology; KEGG, kyoto encyclopedia of genes and genomes; MHC I, major histocompatibility complex class I; MHC II, major histocompatibility complex class II; NADPH, nicotinamide adenine dinucleotide phosphate; ROS, reactive oxygen species

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Fig. 1. Four herb voucher specimens are deposited in the institute herbarium. The specimen bottles were (A) Rice, (B) Liquorice, (C) Anemarrhena Rhizome, and (D) Gypsum. The pie charts were macro photography of herb specimens from the specimen bottles of A, B, C, and D.

et al., 2011; Wang et al., 2010), and BHT was a valuable antifebrile and anti-inflammatory natural drug in LPS fever symptom (Jia et al., 2013). Because LPS can be used to imitate bacterial infection and caused a typical fever in animal by intravenous or intraperitoneal injection (Shibata et al., 2005; Steiner et al., 2006; Ravanelli et al., 2007), researchers often used LPS to induce reproducible febrile responses in animals (Roth et al., 2002). Likewise, many TCM researchers believed that LPS fever rabbit shows a representative febrile syndrome of Chinese medicine pattern, and the febrile syndrome can be used to study antipyretic traditional herb medicine and ethnopharmacological research (Yu et al., 2010; Ai et al., 2011; Ni et al., 2012). Actually, according to the principle of defensive-qi-nutrient-blood syndrome differentiation (in Chinese *Wei-Qi-Ying-Xue Bianzheng*) in TCM, LPS fever is considered Qifen syndrome with the main symptom of unknown high fever (Yang, 2002; Ni et al., 2012). Based on the theory of prescription corresponding to syndrome (in Chinese *Fangzheng Xianguan*), BHT is the classical formula corresponding to Qifen syndrome, because it can significantly eliminate or improve febrile symptom (Li et al., 2007). However, the mechanism of BHT remains unclear.

As an essential part of TCM theory, TCM syndromes might be related to diverse and dynamic proteomics change, which is often along with alteration of different syndrome behaviors (Su et al., 2012). Proteomics can be used to elucidate the differential expressions of many proteins from body fluids, cells, tissues, and blood (Liu and Guo, 2011), and it allows the systematic quantitative and qualitative mapping of the whole proteome during disease (Hussain and Huygens, 2012). By analyzing these protein changes in tissue before and after TCM treatment, proteomics is powerful to study the mechanism of action of TCM remedies (Cho, 2007), and understanding of the characteristic changes in proteomics associated with a specific syndrome will facilitate syndrome identification and novel diagnostic approaches (Su et al., 2012), it also greatly promotes the quality evaluation and standardization, and the modernization and internationalization of TCM (Lu et al., 2010). Furthermore, many researchers have paid more attention to proteomics studies based on treatment-TCM syndrome animal models (Ji et al., 2015).

Against the above background, the study herein attempted to determine hepatic proteomic profiles using isobaric tags for relative and absolute quantification (iTRAQ) combined with two-dimensional liquid chromatography and tandem mass spectra analysis in LPS fever rabbits and that treated with BHT. Differently expressed proteins (DEPs) were analyzed using bioinformatics to explore the mechanism and effect of BHT, and it will support BHT traditional use at protein levels.

2. Materials and methods

2.1. Herbal materials and BHT extraction

The herbs of Liquorice (sliced root of Glycyrrhiza glabra *L.* in *Leguminosae*) and Anemarrhena Rhizome (sliced root of *Anemarrhena asphodeloides Bunge* in *Liliaceae*) and Gypsum (crystal of calcium sulfate) and Rice (nonglutinous rice, polished seed of *Oryza sativa* L. in *Gramineae*) were purchased from Antaitang Pharmaceutical Co., Ltd., China. All of the herbal pieces are in line with the standards of Chinese pharmacopoeia (National Pharmacopoeia Committee, 2015), which were identified by TCM expert Dr. Zuoting Yan. As showed in Fig. 1, the herb samples were deposited in a TCM Specimen Room with voucher numbers: NO. 100720 for Liquorice, NO. 101208 for Anemarrhena Rhizome, NO. 100906 for Gypsum, and NO. 101202 for Rice.

Liquorice 7.2 g, Anemarrhena Rhizome 21.8 g, Gypsum 60.2 g and Rice 10.8 g were extracted together by refluxing boiling water (1:10, w/v) for 2 h, and water (1:5, w/v) for another 1 h, respectively. The two-times decoction was blended together, then filtered with three-layer gauzes, and concentrated to 100 ml. The experimental decoction was autoclaved at 121 °C for 15 min, and kept in airtight containers at 4 °C until used. In order to calculate the concentration of the decoction, 10 ml of the experimental juice was completely dried at 65 °C for 24 h in an electric heating air-blowing drier, and yielded 1.08 g crude powder. Thus, the concentration of the experimental decoction was 108 mg/ml.

2.2. Animals and treatment

Adult New Zealand white rabbits weighting between 2.0 and 2.5 kg was used in the experiments, and housed individually in rabbit stocks under controlled room humidity of 50 ± 5% and a temperature of 25 ± 1 °C with a 12 h lights and 12 h darkness cycles. Animals were fed