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Investigation on the spectrum-effect relationships of Da-Huang-Fu-Zi-Tang in rats by UHPLC-ESI-Q-TOF-MS method

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Chemical compounds studied in this article:
Talatisamine
Hydroxy-chrysophanol
Aloe-emodin isomer
Benzoylmesaconine
Rhein isomer methylation
Mesaconine
Benzoylaconine
Hypaconine
Rhein isomer
Torachrysone

ABSTRACT

Ethnopharmacological relevance: Da-Huang-Fu-Zi-Tang (DHFZT) is a crucial TCM formula commonly used for the treatment of acute pancreatitis in Chinese clinical application. Our previous work found that DHFZT could act against pancreatic injury in rats with severe acute pancreatitis (SAP). The goal of this paper was to study the underlying correlations between the chemical spectra and the protective effect of DHFZT on pancreatic acinar cell to reveal the real bioactive compounds in DHFZT.

Materials and methods: The fingerprint chromatograms of rat serum after oral administration of DHFZT were established by UHPLC-ESI-Q-TOF-MS technique. At the same time, the model of anti-acute pancreatitis on cells was established by adding 10^{-7} mol/L cerulein to AR42J cell line, and the protective effects of the serum on pancreatic acinar cell from injury was evaluated by detecting the efficacy of amylase. Then, the spectrum–effect relationships between UHPLC fingerprints and anti-acute pancreatitis activities were evaluated using canonical correlation analysis (CCA) statistical method. The chromatogram separation was performed on a C_{18} reversed phase UHPLC column (2.1 mm × 100 mm, 3.5 μm, Agilent), the column temperature was set at 35 °C. The mobile phase consisted of 0.1% formic acid and acetonitrile with gradient elution. The serum samples were analyzed both in negative and positive ion mode. The mother and productive ions were scanned within the mass range of *m/z* 100–1200 and 50–1200, respectively. A thorough analysis of a great deal of information of the constituents in the rat serum was undertaken. The structure identification of the detected compounds was achieved by using high resolution MS values as well as the MS/MS fragments.

Results: Eighteen peaks in rat serum after oral administration of DHFZT were detected within only 30 min recorded chromatograms. The structure of the 18 compounds were then given out, of which 10 were the original form of compounds absorbed from DHFZT, 8 were the metabolites of the compounds existed in rat serum. According to the CCA results, talatisamine, rhein glucoside, rhein isomer methylation, hypaconine, hydroxyl-chrysophanol, emodin glucuronide conjugation, and chrysophanol glucuronide conjugation were finally found to be the main anti-acute pancreatitis components in DHFZT.

Conclusions: The model presented in this paper successfully discovered the spectrum–effect relationships of DHFZT, which showed a representative way to discover the primary active ingredients from the complicated herbal drugs.

Abbreviations: CCA, canonical correlation analysis; CE, collision energy; CE–MS, capillary electrophoresis–mass spectrometry; CNKI, Chinese National Knowledge Infrastructure; DBS, dynamic background subtraction; DP, declustering potential; GC–MS, gas chromatography–mass spectrometry; IDA, information-dependent acquisition; LC, liquid chromatography; NMR, nuclear magnetic resonance; SAP, severe acute pancreatitis; TCM, traditional Chinese medicine; TEM, turbo spray temperature; UHPLC-ESI-Q-TOF-MS, ultra-high performance liquid chromatography–electrospray ion source–quadrupole–time of flight–mass spectrometry; XICs, extracted ion chromatograms

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1. Introduction

Recently, Traditional Chinese Medicine (TCM) and their preparations become more and more popular in Asian and Western countries due to their stable therapeutic effects and weak toxicity in clinic (Chen et al., 2012; Qiu et al., 2011; Su et al., 2010). As a result, there is an increasing number of research institutions and universities studying optimal and bioactive chemical composition of TCM (Zhang et al., 2012; Sun et al., 2007). To develop rapid, reliable and sensitive analytical approaches for TCM quality control and efficacy assurance is becoming more and more significant and meaningful. Owing to the limitation of study ways on material basis research of TCM, it is of great necessary and significance to develop novel models to make clear what the effective compositions are during their clinical application.

The traditional chemical research methods for identification of constitutions of TCM are usually time consuming and expensive. No matter the conventional approaches such as LC, NMR or the applications of advanced techniques such as GC–MS and CE–MS, their application are greatly limited by the time-consuming periods and the lack of corresponding standards. Moreover, the effectiveness of a TCM could not be evaluated relying on only a few compounds identification. So, a combinative and powerful method which could offer higher quality structural information and comprehensive components inside is therefore required for the extensive characterization of TCM systems. UHPLC-ESI-Q-TOF-MS, as an important analytical method, has got quick development in recent years. It can be used to determine the contents, analyze the constituents of many compounds and get the fingerprints of the drug, food and other materials (Cabral et al., 2012; Gupta et al., 2013; Guo et al., 2013; Xiao et al., 2013; Yin et al., 2013). UHPLC-ESI-Q-TOF-MS is so powerful a tool for serum analysis also due to its high sensitivity, sound separation and identification ability to show chemical structures without standard reference (Wang et al., 2008; Xue et al., 2011; Zhou et al., 2013). Alongside this increased use of spectrum techniques, there have also been significant developments in combination with chemometrics (Massart et al., 1988). To combine CCA statistical method as one of the chemometrics with UHPLC-ESI-Q-TOF-MS method can better reveal the underlying bioactive compounds in TCM, which has been applied widely (Kong et al., 2009, 2008; Nie et al., 2011).

Da-Huang-Fu-Zi-Tang (DHFZT), a crucial TCM formula commonly used for the treatment of appendicitis, acute pancreatitis, biliary colic, chronic dysentery, acute ileus and adhesive ileus diseases (Liang et al., 2006; Wu et al., 2013a, 2013b), is composed of three herbs, *Rheum officinale Baill.* (Polygonaceae), *Aconitum carmichaelii Debx.* (Ranunculaceae) and *Asarum sieboldii Miq.* (Aristolochiaceae). It is first recorded in "Jin-Gui-Yao-Lue", a classical treatise on febrile and miscellaneous diseases written by a physician Zhong-Jing-Zhang (150–219 A.D.) in Eastern Han Dynasty. Though DHFZT was believed to be one of the main formulas to treat acute pancreatitis in TCM clinic, the exact mechanism of its action is still unknown until now, and the material basis of its anti-acute pancreatitis activities has not been reported yet, neither. The goal of this paper was to study the underlying correlations between the chemical spectra and the protective effect of DHFZT on pancreatic acinar cell to reveal the real bioactive compounds in DHFZT.

2. Materials and methods

2.1. Materials and reagents

Rheum officinale Baill., *Aconitum carmichaelii Debx.* and *Asarum sieboldii Miq.* were purchased from Nanjing Haichang Chinese medicine group corporation (Nanjing, China). Their species were identified by Prof. Jianwei Chen (College of Pharmacy, Nanjing University of Chinese Medicine). Acetonitrile and water were of LC–MS grade from Merk Company (Darmstadt, Germany), HPLC grade methanol was purchased from ANPEL Scientific Instrument Co., Ltd. (Shanghai, China), as well as the HPLC grade formic acid with a purity of 99% (Anaqua chemicals supply, USA). All other reagents were of analytical grade and obtained from Nanjing Chemical Reagent Company (Nanjing, China). Rat pancreatic acinar AR42J cells (ATCC CRL 1492) were obtained from the American Type Culture Collection. Cerulein and 0.25% trypsin–0.1% EDTA were purchased from Sigma Chemicals Co. (Spain). Fetal bovine serum was obtained from Invitrogen. Amylase assay kit was purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China).

2.2. Animals

Male Sprague-Dawley rats ($n=3$), weighed 200–220 g, were supplied by the Slaccas Experiment Animal Company (Shanghai, China). Temperature, humidity, and light conditions in the rats environment were kept constant, with food and water provided ad libitum. All rats were acclimated in the laboratory for at least one week prior to the experiment. Before testing, animals were fasted overnight with free access to water. All animal experiments were carried out according to the Guidelines for the Care and Use of Laboratory Animals, and were approved by the Animal Ethics Committee of Nanjing University of Chinese Medicine.

2.3. UHPLC-ESI-Q-TOF-MS

2.3.1. Sample collection and preparation

To prepare DHFZT, the prepared crude drugs of *Rheum officinale Baill.*, *Aconitum carmichaelii Debx.* and *Asarum sieboldii Miq.* were first mixed together in a ratio (3:4:1, w/w/w) and macerated in deionized water for 30 min, and then decocted twice with boiling water (1:8, w/v) each for 20 min, then the solution was filtered through a two-layer mesh, and was combined and concentrated to a density of 1 g/mL. A characteristic spectrum based on analyzing the features of the area and retention time of the main components in DHFZT was established to evaluate the quality of DHFZT. The fingerprint spectrum was shown in Fig. 1. A total of male Sprague-Dawley 3 rats were orally administered by gavage with a syringe: DHFZT (1.5 g/100 g body weight). Blood samples (about 0.75 mL) were collected from the orbital vein in tube without heparinized at pre-administration (0) and post-administration (0.5 h, 1 h, 2 h, 4 h, 5 h, 6 h, 7 h) and were immediately centrifuged at 4000 rpm for 5 min. The serum samples were collected and stored at −20 °C until analysis. 0.1 mL of serum was spiked in centrifuge tubes and then mixed with 500 μL of methanol by vortex mixing for 30 s. The aqueous and organic layers were separated by centrifugation at 12,000 rpm for 5 min and the organic layer was then transferred to another tube and evaporated to dryness under nitrogen at 40 °C. The residue was reconstituted with 100 μL of chromatographic methanol and vortexed for 30 s and centrifuged at 12,000 rpm for 3 min. A volume of 100 μL of the supernatant was injected for analysis.

2.3.2. Instruments and UHPLC-ESI-Q-TOF-MS conditions

2.3.2.1. Instruments. For analysis of multiple constituents in DHFZT, UHPLC system was couple with hybrid quadrupole time-of-flight tandem mass spectrometry LCMS–Q–TOF (LC/MS-Triple TOF™ 5600, AB SCIEX, Foster City, CA) equipped with an electrospray ionization (ESI) interface.

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