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Original article

Metabolomic profiles illuminate the efficacy of Chinese herbal Da-Cheng-Qi decoction on acute pancreatitis in rats

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ABSTRACT

Background and objectives: Chinese herbal drug Da-Cheng-Qi decoction (DCQD) has been widely used for decades to treat acute pancreatitis (AP). Previous trials are mostly designed to state the potential mechanisms of the therapeutic effects rather than to detect its whole effect on metabolism. This study aimed to investigate the efficacy of DCQD on metabolism in AP.
Methods: Twenty-two male adult Sprague–Dawley rats were randomized into three groups. AP was induced by retrograde ductal infusion of 3.5% sodium taurocholate solution in DCQD and AP group, while 0.9% saline solution was used in sham operation (SO) group. Blood samples were obtained 12 h after drug administration and a 600 MHz superconducting Nuclear Magnetic Resonance (NMR) spectrometer was used to detected plasma metabolites. Principal Components Analysis (PCA) and Partial Least Squares-Discriminant Analysis after Orthogonal Signal Correction (OSC-PLS-DA) were applied to analyze the Longitudinal Eddy-delay (LED) and Carr–Purcell–Meiboom–Gill (CPMG) spectra.
Results: Differences in concentrations of metabolites among the three groups were detected by OSC-PLS-DA of 1HNMR spectra (both LED and CPMG). Compared with SO group, DCQD group had higher levels of plasma glycerol, glutamic acid, low density lipoprotein (LDL), saturated fatty acid (FA) and lower levels of alanine and glutamine, while the metabolic changes were reversed in the AP group.
Conclusions: Our results demonstrated that DCQD was capable of altering the changed concentrations of metabolites in rats with AP and 1HNMR-based metabolomic approach provided a new methodological cue for systematically investigating the efficacies and mechanisms of DCQD in treating AP.

Introduction

Acute pancreatitis (AP), the inflammation of the pancreas caused by prematurely activated digestive enzymes, is an acute disaster of the abdomen with high morbidity and mortality without specific therapeutic measurements [1,2]. Da-Cheng-Qi decoction (DCQD), a well-known classical traditional Chinese medicinal purgative formula, has been widely adapted for the management of acute pancreatitis for decades [3–6]. Our previous studies have validated its excellence in promoting gastrointestinal peristalsis, protecting against cytokines and inflammatory mediators, preventing bacterial translocation and anti-endotoxin, improving microcirculation within the pancreas, lowering the content of P substance and modulating intracellular Ca^{2+}, Mg^{2+}, and −ATPase [7–9]. These studies demonstrated that patients with AP overall benefited from the comprehensive efficacy of DCQD. However, the above studies mostly focused solely on a single target or pathway but not on the whole effect of the herbal prescription. As shown above, DCQD has a multi-targeted role in AP, but its holistic efficacy evaluation and mechanistic understanding are yet lacking due to its complex components. Thus, its global effect on metabolomics was addressed in this study.

Metabolomics is a novel approach for the study of biological samples and it has been widely used in physiopathology, diagnostics, therapeutics, toxicology, pharmacology and discovery of biomarkers [10–12]. As a comprehensive and high throughput analysis methodology allowing the identification of global

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metabolic changes of the organism caused by endogenous or exogenous factors, metabolomics is in consensus with the "holistic conception" of traditional Chinese medicine in nature, which emphasis on integrity and dynamics. As analyzing the changes of metabolite profiles after treatment by Traditional Chinese Medicine (TCM) may help anatomize their underlying efficacies and mechanisms of action, metabolomics, consequently, is honored as the "spring" of the modernization of TCM and arouses a wide-spread application in TCM researches [13–17]. There are few reports about the metabolic profiles involved in the pathological process of AP and no studies concerning the impact of Chinese herbal DCQD on the global metabolic changes of AP using metabolomics approach so far, however.

In clinical practice, patients cannot receive Chinese herbal treatment solely due to its complicated pathophysiology and medical ethics. Because of this, Sprague–Dawley rats are commonly chosen as a suitable animal model system. In the present study, we performed 1H Nuclear Magnetic Resonance (NMR) based metabolomics analysis on the plasma samples obtained from three groups of experimental rats to determine if DCQD could rectify the altered metabolic profile in rats with AP.

Materials and methods

Design

Prospective, randomized controlled trial.

Settings

Ethnopharmacology laboratory at West China hospital.

Ethics statement

The protocol was approved by the Ethics Committee for Animal Experiments of Sichuan University. All rats were handled according to the University Guidelines and the Animal Care Committee Guidelines of West China Hospital. All surgeries were performed under chloral hydrate anesthesia, and all efforts were made to minimize suffering of rats.

Chemicals

Deuterium oxide (D_2O, 99.9%) was purchased from Cambridge Isotope Laboratories, Inc. Trimethylsilyl-propionate-2, 2, 3, 3-d_4 acid, sodium salt (TSP) was purchased from Merck, Germany. Sodium taurocholate was provided by Sigma Chemical (St. Louis, MO). Chloral hydrate was provided by Ke Long chemical reagent works, China.

DCQD preparation

Radix et Rhizoma Rhei Palmati, Mirabilite, Fructus Aurantii Immaturus, Cortex Magnolia Officinalis were provided by Cheng Du LvYuan Pharmaceuticals Co., Ltd, China.

The four herbal medicines were mixed together with an equal mass, then the mixture was diluted with normal saline at a concentration of 1 g/ml, the dose of DCQD selected for the study was 10 g/Kg BW, just the same as our previous studies [17].

Equipment

INOVA 600 MHz NMR spectrometer equipped with a triple-resonance probe and a z-axis pulsed field gradient was provided by Varian Unity (Varian, Inc.). Centrifuge was provided by Eppendorf MiniSpin Plus, Germany. The two-channel micro-injection pump was provided by Kd Scientific Company, USA.

Animals model of acute pancreatitis and intervention

Healthy male adult Sprague–Dawley rats (224 ± 21 g, 200–250 g b/w), were maintained in air-conditioned animal quarters at 22 ± 2 °C with a relative humidity of 65% ± 10%. They were acclimated for 1 week before the experiment with special feed and tap water. The animal experiments were carried out in the Laboratory of ethnopharmacology in West China hospital, Sichuan University.

Twenty-two 2-month-old (55–62 days old) rats obtained from the experimental animal center of Sichuan University were numbered and randomized into three groups. Randomization was generated according to random number table. Rats were randomly assigned to Sham operation (SO) group (n = 7), acute pancreatitis (AP) group (n = 7) or DCQD treated (DCQD) group (n = 8). After fasting for 12 h with free access to tap water prior to experiments, all animals were anaesthetized with 10% chloral hydrate intraperitoneal injection at a volume of 3 mL/kg in the morning. The hepatic duct was closed with a small bulldog clamp and the biliopancreatic duct was cannulated through the offside of the front opening of the duodenum papilla. AP was induced by retrograde pancreaticobiliary duct injection with 3.5% sodium taurocholate in a volume of 1 mL/kg using a micro-pump. Two hours after AP induction, rats in DCQD group were gavaged with DCQD in a concentration of 0.5 g/mL (crude drug), which was equal to 10 g/kg bw. In AP and SO groups, animals were gavaged with equal volume of 0.9% sterile saline instead. Blood samples were obtained from the angular vein 12 h later. All blood samples were anti-coagulated with heparin, and then centrifuged for 15 min at a speed of 3000 rpm. Plasma samples were collected and stored at −80 °C until use. The rats were euthanized after the experiment.

Sample preparation and NMR data acquisition

Plasma samples were dissolved prior to analysis. 100 μl Deuterium oxide (D2O) mixed with Trisodium Phosphate (TSP) at a concentration of 1 mg/ml, 150 μl blood plasma and 350 μl of D2O were added into numbered centrifuged tubes for NMR measurement. After centrifugation at 14,000 rpm for 10 min, 550 μl supernatant was transferred into 5 mm NMR tubes for use. All NMR spectra were recorded at 26 °C using an INOVA 600 MHz NMR spectrometer equipped with a triple-resonance probe and a z-axis pulsed field gradient. For each plasma sample, Carr–Purcell–Meiboom–Gill (CPMG) pulse sequence (CPMG) was performed for micro-molecules including sugar and amino acids and longitudinal eddy-delay (LED) for macro-molecules including lipid though it can also detect some small molecules. CPMG pulse sequence (−RD−90°−(r−180°−r)$_n$−ACQ) was performed during a relaxation delay of 2 s. Sixty-four free induction decays (FID) were collected into 64 k data points with a spectral width of 8000 Hz, an acquisition time of 4 s. LED pulse sequence (-RD-90SYMBOL 0 \f "Times New Roman" \s 10-G1-180SYMBOL 0 \f "Times New Roman" \s 10-G1-90SYMBOL 0 \f "Times New Roman" \s 10-T-90SYMBOL 0 \f "Times New Roman" \s 10-G1-180SYMBOL 0 \f "Times New Roman" \s 10-G1-90SYMBOL 0 \f "Times New Roman" \s 10-ACQ) was performed during a relaxation delay of 2 s. Sixty-four FID were collected into 64 k data points with a spectral width of 8000 Hz, an acquisition time of 4 s and a diffusion time of 100 min. FID was zero-filled and multiplied by an exponential line-broadening function of 1 Hz (for CPMG) and 3 Hz (for LED) prior to Fourier transformation.

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