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^{1}H NMR-based dynamic metabolomics delineates the therapeutic effects of *Baoyuan* decoction on isoproterenol-induced cardiac hypertrophy

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ABSTRACT

Cardiac hypertrophy (CH) is a major risk factor for many serious heart diseases. Sustained CH is catastrophic, resulting in cardiac dysfunction that eventually leads to heart failure (HF). *Baoyuan* decoction (BYD) is a famous traditional Chinese medicine (TCM) formula for supplementing and reinforcing Qi, clinically used for the treatment of cardiovascular diseases (CVDs). However, the therapeutic effects of BYD on CH remain unidentified. We herein investigated the effect of BYD on isoproterenol (ISO)-induced CH in rats and the underlying mechanisms by comprehensive pharmacodynamics and ^{1}H NMR-based dynamic metabolomics analysis of the plasma and urine samples. Results showed that BYD treatment markedly attenuated ISO-induced CH as evidenced by decreasing the left ventricular wall thickness, pathological cardiomyocyte hypertrophy, myocardial collagen fiber deposition and apoptosis, and plasma natriuretic peptide levels. Multivariate trajectory analysis revealed that the BYD treatment could restore the CH-disturbed plasma and urinary metabolite profiles towards the normal metabolic status featuring with a time-dependent tendency. Moreover, the key metabolic alterations in CH rats at different BYD-treated time stages involved energy metabolism, oxidative stress responses, amino acid metabolism, and gut microbiota metabolism. Of particularly, the significant roles of BYD for treating CH lie in the improvement of cardiac energy generation and antioxidant capacity. Our investigation provides a holistic view of BYD for therapeutic intervention of CH through monitoring of the dynamic metabolic changes and the results indicate that BYD may be applied as a potential agent for treating CH.

1. Introduction

Cardiac hypertrophy (CH) as one of the major causes of morbidity and mortality remains a major public health problem worldwide. CH is characterized by an abnormal thickening of the left ventricular wall and a decreased cavity of the ventricular chamber [1]. CH is a major risk factor for heart failure (HF). Prolonged hypertrophy results in the loss of compensation by cardiomyocytes and ultimate develops into HF [2]. Despite increasing understanding toward the pathophysical process of CH has been achieved, progress in its treatment remains sluggish in recent years [3]. Better understanding of CH can lead to a better diagnosis, and amelioration of pathological CH has been proven to be therapeutically beneficial for HF [4].

The β-adrenergic receptor-stimulated cardiac overload and myocyte loss may cause cardiac chamber remodeling and heart dysfunction, and thereby induces CH and HF, as demonstrated in humans and experimental animals [5]. It is well recognized that chronic stimulation with the non-selective agonist of β-adrenergic receptor, isoproterenol (ISO), could establish a well-standardized model of CH and HF [6]. Although ISO has been widely used to produce experimental model for the pharmacological evaluation of many cardioprotective agents, the precise molecular mechanisms of chronic ISO-induced CH have not been well clarified.

Traditional Chinese medicine (TCM) has been practiced for thousands of years in China and is getting increasingly popular worldwide for improving human health, especially for chronic disease therapy [7]. Nowadays, a variety of TCM combinations show their unique advantages in the clinical treatments of cardiovascular diseases (CVDs). *Baoyuan* decoction (BYD), a classic TCM formula (TCMF) with a long usage history for CVDs in China, consists of astragalus root, ginseng, processed licorice, and cinnamon. A

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lot of active ingredients (e.g., ginsenosides, astragalosides, licorice saponins, flavonoids, *etc.*) have been identified from BYD and exhibited cardioprotective effects against myocardial ischemia and heart failure post-acute myocardial infarction by attenuation of apoptosis, inflammation, oxidative stress, and myocardial fibrosis in our previous studies [8–10]. However, there is no evidence for the effect of BYD on CH, not to mention of its mechanism due to the multi-component mixtures' characteristic of BYD.

Metabolomics has experienced tremendous growth over the past decade. Metabolomics provides a strong driving force in the fields of early disease diagnosis, molecular basis of diseases, and mechanisms of drug efficacy [11]. Nuclear magnetic resonance (NMR)-based metabolomics is a reliable, reproducible, and non-invasive technique that provides important metabolic alterations and permits a dynamic monitoring of disease progression and drug interaction. Recent developments revealed that metabolomics has the potential to give a scientific explanation of molecular mechanisms of disease and TCM therapy. Metabolomics and TCM have similar properties in many respects, especially for the overall and systematic properties, thus metabolomics may enable an in-depth understanding for TCM therapy using the integrative approach [12]. In the present study, we proposed a dynamic metabolomic trajectory analysis based on proton NMR (^{1}H NMR) technique combined with pharmacodynamics approach to investigate the pathological processes of ISO-induced CH and to evaluate the potential protection effects and action mechanisms of BYD against CH.

2. Materials and methods

2.1. Animals and ethics statement

Twenty four Sprague-Dawley male rats (180–220 g) were obtained from the Department of Laboratory Animal Science, Peking University Health Science Center (Beijing, China). All animals were kept under standard conditions and fed with certified standard laboratory diet ad libitum, except prior to collection of the urine and plasma samples and the final necropsy. Tap water was provided ad libitum. The temperature and humidity were set at 21–23 °C and 40–60%, respectively. A 12 h light/dark cycle was used. All animal studies followed the relevant national legislation and local guidelines on the ethical use of animals, and were approved by the Institutional Animal Care and Use Committee of Peking University Health Science Center.

2.2. Chemicals and reagents

Dipotassium hydrogenphosphate (K_2HPO_4), sodium chloride (NaCl), sodium azide (NaN_3), and deuterium oxide (D_2O), 3-(trimethylsilyl) propionic-2,2,3,3-d_4 acid sodium salt (TSP-d_4), and potassium hydroxide (KOH) were purchased from Sigma-Aldrich (St. Louis., MO, USA). The antibodies against collagen I and collagen III were obtained from Abcam (Cambridge, USA). Terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) cell death detection kit was purchased from Roche (Basel, Switzerland); Enzyme-linked immunosorbent assay (ELISA) kits for A-type natriuretic peptide (ANP) and B-type natriuretic peptide (BNP) were obtained from Cusabio Biotech Co., Ltd. (Wuhan, China). Adenosine diphosphate (ADP) detection kit was purchased from Sigma-Aldrich (St. Louis., MO, USA). Detection kits for superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), malonaldehyde (MDA), and adenosine triphosphate (ATP) were obtained from Jiancheng Bioengineering Institute (Nanjing, China). All the other reagents used were of highest purity and commercially available.

2.3. Preparations of BYD

All crude materials were collected from a TCM market (Anguo, Hebei, China) and authenticated by Prof. Pengfei Tu to be the roots of *Astragalus membranaceus* (Fisch.) Bunge var. *mongolicus* (Bunge) Hsiao, *Panax ginseng* C. A. Mey., the prepared *Glycyrrhiza uralensis* Fisch., and the barks of *Cinnamomum cassia* Presl. All voucher specimens (GU-AG-20130312, PG-AG-20130312, AM-AG-20130312, and CC-AG-20130312) have been deposited at the Modern Research Center for Traditional Chinese Medicine, Peking University (Beijing, China). The decoction is the most commonly administrated form of TCMF, therefore, the crude astragalus roots (30 kg), ginseng (10 kg), processed liquorice (10 kg), and cinnamon (5 kg) in this formula were mixed and soaked with 550 L water for one night and subsequently refluxed for 2 h at 100 °C three times. The extraction solutions of BYD were combined and condensed in vacuo, and then 95% ethanol was added to make the concentration of ethanol in the solution to be 65%, stood for 2 h. The supernatant was subsequently freeze-dried to provide 8.5 kg BYD powders for experimental use. The qualitative and quantitative chemical profile of BYD has been described in a previous report [8].

2.4. Experimental procedure

After acclimation to the laboratory environment for seven days, the animals were randomly classified into three groups consisting of eight rats each. CH model was established by intraperitoneal injection of ISO as previously described [5]. Briefly, rats in the ISO and BYD groups were administered with ISO (15 mg/kg/day) for one week, while the rats in the normal control group simultaneously received an equal quantity of physiological saline. Then, the rats in the BYD group were administered with BYD at an optimized dose (1460 mg/kg) based on the previous studies [9,10], and the rats in the ISO and normal control groups were simultaneously given the same volume saline. The drugs and vehicle were orally administered once per day for three consecutive weeks.

Urine and blood samples were collected weekly. Before each sampling, the rats were fasten for 18 h, and then the urine samples (approximately 5 mL) were collected from metabolism cages and centrifuged at 3500 rpm and 4 °C for 5 min to remove the particle contaminants, and the urinary supernatants were then collected in the clean dry tubes. The blood samples (approximately 0.8 mL) were obtained from the ophthalmic vein and collected into the heparinized tubes and centrifuged at 4000 rpm and 4 °C for 10 min, and the obtained supernatants were collected in the clean dry tubes. All the samples were frozen at −80 °C until analysis.

2.5. Assessment of echocardiography and heart weight index

After the last collection of urine and plasma samples, all rats were deeply anaesthetized using sodium pentobarbital (50 mg/kg), and a Vevo 2100 ultrasound system (Visualsonics Inc., Toronto, Canada) with a 21 MHz probe was employed for echocardiography. The following parameters were measured from two-dimensional images and M-mode interrogation taken from the parasternal long-axis view at papillary muscle level (the sampling frequency in M-mode was 1000/s, and the scanning speed was 50–100 mm/s). For analysis of cardiac function and hypertrophy, the systolic and diastolic posterior wall dimensions (PWDs & PWDd) as well as systolic and diastolic left ventricular (LV) inner diameters (LVIDs & LVIDd) were measured. End systolic and diastolic volumes (ESV & EDV), fractional shortening (FS), and ejection fraction (EF) were calculated from LVIDs and LVIDd.

After the echocardiographic examination, all rats were subsequently sacrificed for collecting the blood from the abdominal aorta and harvesting the heart samples. The plasma samples were