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Deciphering biochemical basis of Qingkailing injection-induced anaphylaxis in a rat model by time-dependent metabolomic profiling based on metabolite polarity-oriented analysis

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ABSTRACT

Ethnopharmacological relevance: Qingkailing injection (QKLI) is prepared from eight traditional Chinese medicinal materials or their extracts, which is widely used in clinical practice to treat the upper respiratory inflammation, pneumonia, high fever and viral encephalitis, nonetheless, suffering from serious anaphylaxis.
Aim of study: This study aims to develop an integrative metabolomics approach for deciphering the biochemical basis of QKLI induced anaphylaxis (QKLI-IA).
Materials and methods: The accuracy of animal modeling, the coverage of detected metabolites and the timeliness of pathological reaction are three key factors for revealing the biochemical basis of disease with untargeted metabolomics. In this study, firstly, the allergic rats (responders) were first screened by passive cutaneous anaphylaxis experiment and then were utilized for modeling. To cover a wider range of metabolites, a large-scale untargeted metabolomics based on metabolites polarity-oriented analysis was performed using ultra performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry. Then, to evaluate the timeliness of QKLI-IA, a time-dependent metabolomic profiling including the early, mid and late anaphylaxis stages of QKLI-IA, was performed.
Results: Corresponding to early, mid and late anaphylaxis stages of QKLI-IA, 14, 9 and 4 potential biomarkers were identified, respectively. Metabolism pathway analysis revealed that QKLI-IA resulted in dynamic changes in serum amino acid, fatty acid, glycerolipid, and phospholipid metabolisms. Twenty-four metabolites were found with identical fluctuating trends across the three stages of QKLI-IA. The results indicate that the pathogenesis of QKLI-IA is closely related to arachidonic acid metabolism.
Conclusion: This research provides a methodology reference for revealing the biochemical basis of disease using metabolomic profiling and offers a new insight to understand the pathogenesis of QKLI-IA.

1. Introduction

Metabolomics was defined as a comprehensive analytical approach for the study of the complete set of endogenous metabolites (metabolome) present in a biological system (Arias et al., 2009). The major aim of metabolomics is to obtain an answer to, or insight into, a biological question. Therefore, metabolomics is suitable for revealing the biochemical changes of disease pathogenesis (Hu et al., 2012; Bujak et al., 2014). Anaphylaxis is an acute, systemic, metabolically abnormal reaction, and its developmental process is complicated (Brown et al., 2001; Singer, Zodda, 2015). Therefore, using metabolomics approach to explore biochemical changes is beneficial to help clarify the biochemical basis of anaphylaxis. The accuracy of animal modeling, the coverage of untargeted metabolomics data and the timeliness of pathological reaction are 3 key factors in metabolomics research aimed at elucidating the biochemical basis of disease pathogenesis, and each factor has a major influence on the research results.

In pharmacological or nutritional studies, 'intersubject variation' is often observed among laboratory animals, in which the induced effect is so variable that the animals can be classified as either 'responders' or 'nonresponders' (Li et al., 2007). Generally, the success rate of animal modeling is not 100% due to the individual differences in rats. The conventional animal modeling method for anaphylaxis uses behavioral indicators, such as scratching nose, piloerection and convulsions, to

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judge if anaphylaxis has indeed occurred, instead of inspecting the antibodies produced in the sensitization stage, thus ignoring the effects of individual differences in rats. This omission can result in an inaccurate assessment of which animals are allergic and can make subsequent toxicology research unclear. Therefore, establishing a new method to distinguish allergic animals was definitely the foundation of this anaphylaxis pathogenesis study. In this paper, the preparation method of antiserum was first investigated then the responders were screened based on a passive cutaneous anaphylaxis experiment, thus guaranteeing the accuracy of subsequent allergy models and leading to reliable research results.

As yet, knowledge about the underlying biochemical basis of anaphylaxis is largely unclear. Therefore, large-scale untargeted metabolomic profiling is necessary. The only reported anaphylaxis metabolomics study in mice models was induced with peanuts (Chalcraft et al., 2014). The study aimed at detecting basic and polar metabolites using a series of two columns: ZIC HILIC and RP-Amide. Consequently, most of the potential biomarkers were phosphatidylcholines and lyso-phosphatidylcholines rather than other kinds of metabolites such as amino acids, fatty acids, glycerolipids and phospholipids, which are involved with the anaphylactic reaction. In our study, each serum sample was pretreated with two different polarity solvents according to the polarity of metabolites and was determined under different experimental conditions. This was called the metabolites polarity-oriented analysis method. By using this method, a variety of allergy-related metabolites were able to be detected.

It is important to select appropriate time points when utilizing metabolomic tools to study the underlying biological processes involved with acute metabolic diseases such as anaphylaxis. According to our preliminary experiment, we found that Qingkailing injection induced anaphylaxis (QKLI-IA) can last for 120 min. Therefore, on the basis of this result, we carried out a dynamic study to reveal the trajectory of the metabolomic profiles corresponding to the stages of anaphylaxis.

QKLI is a traditional Chinese medicine which is widely used in clinical practice to treat the upper respiratory inflammation, pneumonia, high fever and viral encephalitis (Huang, 2012; Chen et al., 2002; Li et al., 2005; Fan, Liu, 2011); nonetheless, some people can suffer serious anaphylaxis from this substance. Therefore, deciphering the biochemical basis of QKLI-IA is important to its clinical application and secondary development. This study may also provide a reference approach for the investigating of adverse reactions of traditional Chinese medicine.

2. Materials and methods

2.1. Establishment of QKLI-IA animal models

2.1.1. Animals

Specific pathogen free grade male Sprague-Dawley rats (200 ± 20 g) were purchased from Beijing Weitonglihua Laboratory Animal Technology Co., Ltd. (Beijing, China). All rats were kept under a constant temperature (23 ± 2 °C) and humidity (60 ± 5%) with a 12 h/12 h light/dark cycle in controlled rooms. The rats were fed standard laboratory chow with water ad libitum and were acclimated to the room for 1 week. All animal experiments were approved by the Animal Ethics Committee of Beijing University of Chinese Medicine (Beijing, China), and all procedures were performed according to the Helsinki Declaration.

2.1.2. Preparation method of antiserum

Eighteen rats were randomly divided into six groups which were saline control group, QKLI control group, $Al(OH)_3$ gel control group, Freund's complete adjuvant (FCA) control group, QKLI + $Al(OH)_3$ gel group, QKLI + FCA group. The rats in the saline control group were subcutaneously injected with 4.2 mL/kg 0.9% saline five times at two-week intervals. The rats in the QKLI, $Al(OH)_3$ gel, FCA, QKLI + $Al(OH)_3$ gel and QKLI + FCA groups were injected with 4.2 mL/kg QKLI, 4.2 mL/kg $Al(OH)_3$ gel, 4.2 mL/kg FCA, 8.4 mL/kg QKLI + $Al(OH)_3$ gel and 8.4 mL/kg QKLI + FCA under the same conditions, respectively. Ten days after the first sensitization, whole blood was collected by orbital blood every three days until the 28th day. The whole blood was stand at room temperature for 30 min and then centrifuged (3000 rpm, 4 °C) for 15 min. The serum were collected and stored at −80 °C.

Another 18 rats were also randomly divided into six groups which were saline control group, QKLI control group, $Al(OH)_3$ gel control group, FCA control group, QKLI + $Al(OH)_3$ gel group, QKLI + FCA group. One day before the experiment, the back skin of rats were shaved, selected eight points on the shaved sides of rats, then 0.1 mL corresponding antiserum of each time point were injected intradermally to shaved sides of rats. Twenty-four hour after sensitization, the sera-transferred rats were challenged by intravenously injecting 8.4 mL/kg QKLI containing 1% Evan's Blue. 30 min after antigen challenge, rats were sacrificed. The skin at the reaction site was excised, if no blue spot excised the 3.0 × 1.5 cm^2 area of skin. The blue spot of the inner skin at the injection site was measured. Then the skin specimen was dissolved in 6 mL of acetone-saline (7:3) mixture solution. Twenty-four hour later, sample was centrifuged (2000 rpm, 4 °C) for 10 min. The absorbance of dye leakage extracted in the supernatant was measured at 610 nm with a tspectrophotometer. Blue spot with a diameter more than 5 mm was regarded as positive reaction.

2.1.3. Responders screening

Before the experiment, the back skin of rats was shaved, and 0.1 mL antiserum which contained QKLI antibody was injected intracutaneously. At 24 h after sensitization, sera-transferred rats were challenged by intravenously injecting 8.4 mL/kg QKLI containing 1% Evan's Blue. After thirty minutes the diameter of the blue spot on the skin was measured. Rats with a blue spot diameter of more than 3 mm were regarded as responders. Others were regarded as nonresponders.

2.1.4. Allergy model establishment and sample collection

In this study, twenty-eight qualified responders were selected and used to establish the allergy model and additional twenty-eight healthy rats were added as control group (CG). The rats of allergy group (AG) were sensitized to 1 mL antiserum, which contained QKLI antibody, by intraperitoneal injection. The CG rats were treated with an equal volume of 0.9% saline. Twenty-four hours later, all rats were challenged by intravenous injection with 8.4 mL/kg QKLI. At 0 min, 10 min, 30 min and 120 min after QKLI administration, seven rats were selected from each group randomly and anesthetized with 10% chloral hydrate, and the blood samples were obtained by abdominal aortic. Blood samples were stood at room temperature for 30 min and then centrifuged (3000 rpm, 4 °C) for 15 min, and then the serum was collected and stored at −80 °C.

2.2. Allergy model validations

Two indicatives of anaphylaxis, namely, histamine and β-hexosaminidase, were measured with enzyme-linked immunosorbent assay (ELISA) following the manufacturer's instructions of the ELISA kits. Results were expressed as the mean ± standard deviation (SD). The CG and AG groups were compared by *t*-test. $P < 0.05$ was considered statistically significant.

2.3. Metabolites polarity-oriented analysis method

To obtain comprehensive biomarkers associated with anaphylaxis, the polar and weak polar fractions of rat serum were processed and analyzed. Full details on serum sample preparation, ultra performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (UPLC Q-TOF/MS) analysis and validation are shown in