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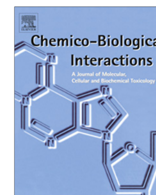

A urinary metabonomics study on biochemical changes in yeast-induced pyrexia rats: A new approach to elucidating the biochemical basis of the febrile response

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ABSTRACT

Fever is a prominent feature of many diseases, such as infection, inflammation and trauma. In the clinic, fever can be easily judged by measuring the body temperature; however, the pathogenesis of fever is still not fully understood. A febrile response is a systemic pathological process that can cause metabolic disorders. Metabonomics can provide powerful tools to reveal the pathological mechanisms for such a systemic disease. Thus, to reveal subtle metabolic changes under the condition of fever and to explore its mechanism, an ultra performance liquid chromatography coupled with a quadrupole time-of-flight mass spectrometry metabonomics approach was employed to investigate the urine biochemical characteristics of yeast-induced pyrexia rats. The acquired data were subjected to principal component analysis for differentiating the pyrexia rats from the control rats. Potential biomarkers were screened by using orthogonal partial least-squares-discriminant analysis and were identified by accurate mass, database, and MS/MS fragment information obtained from the MS^E technique. Sixteen metabolites in rat urine were identified as potential biomarkers. The relative intensities of the 15 potential biomarkers were calculated. The thermoregulatory circuitry of "endogenous pyrogen (EP) ↑-hypothalamus Na^+/Ca^{2+}-cAMP↑" was partially confirmed in this study. The results suggested that UPLC/MS-based metabolic profiling of rat urine identifies impaired tryptophan metabolism as the mechanism of yeast-induced fever. This research provided informative data that the impaired tryptophan metabolism might be one of the important reasons in elucidating the biochemical basis of the febrile response.

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1. Introduction

Fever was defined by the International Union of Physiological Sciences Commission as a state of elevated core temperature that is often, but not necessarily, part of the defensive responses of multicellular organisms (hosts) to the invasion of live (microorganisms) or inanimate matter recognised as pathogenic or alien [1]. Fever and the febrile response remain significant contributors to the pathogenesis and clinical presentation of many illnesses and diseases. It was reported that fever was accompanied by various sickness behaviours, changes in metabolic and physiological characteristics of body systems, and alterations in immune responses. Therefore, ascertaining fever mechanisms will help to explain the pathological features of diseases, to improve the accuracy of clinical diagnosis, and especially to elucidate the mechanism of clearing heat induced by some Chinese medicines. There are two basic pathways for thermoregulatory circuitry, namely the humoural and neural pathways [2–4]. The occurrence of fever is a complex process. Many factors are linked to fever responses, such as interleukin-1, tumor necrosis factors and interferon. The "set point" hypothesis is the dominant hypothesis which means that the thermal neurons in hypothalamus are activated to a higher thermal balance point causing fever. However, it is only a general hypothesis; many details are still unclear. This paper has aimed to study the urinary metabonomics characteristics of yeast-induced pyrexia rats and to reveal the impact of fever on the metabolism and its contribution to the thermoregulatory pathway.

Yeast is a type of fungus, in which the thallus, capsular polysaccharide and protein are the main components that induce pyrexia. Yeast can activate endogenous pyrogen (EP) cells to produce and release EP, which affect the thermoregulatory centre, causing a release of the heating medium, which in turn acts on the body

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temperature set-point. The yeast-induced pyrexia model has been widely used in pharmacological studies of antipyretic effects [5–8].

Metabonomics, defined as "the quantitative measurement of the dynamic multiparametric metabolic response of a living organism to pathophysiological stimulation or genetic modification", focuses on all small molecular metabolites that are produced by normal or abnormal cellular processes and is an important platform of systems biology [9–13]. To achieve the efficient analytical methods that can aid in the comprehensive diagnosis and/or prognosis of different diseases, metabonomic studies on biological fluids (serum, plasma, urine) and tissues have increased rapidly over the past decades [14,15]. The ability to measure global alterations in the metabolism in biological fluids and tissues is coincident with the integrity and systemic feature of fever. Among the data acquiring methods, such as nuclear magnetic resonance, high performance liquid chromatography (HPLC) coupled with mass spectrometry, and gas chromatography coupled with mass spectrometry, globality is very important, and it could help to reflect metabolic profiling of biological samples impersonally and integrally. Ultra performance liquid chromatography (UPLC) has a larger peak capacity and better chromatographic resolution than conventional HPLC because of its special column, which has a stationary phase of 1.8 μm particles. For the ionised component, quadrupole time-of-flight mass spectrometry (Q-TOF/MS) can provide its chromatographic peak area, an accurate value of m/z, and fragmentation information. UPLC Q-TOF/MS has been extensively used in metabonomic studies during the past several years [16–18]. MS^E is a new technique that is used in deducing the splitting disciplinary of MS [19]; it can provide parallel alternating scans for acquisition at a low collision energy to obtain precursor ion information or at a ramping of high collision energy to obtain a full-scan accurate mass of fragments, precursor ions, and neutral loss information [20,21].

Urine is a particularly attractive source for a biomarker study because it is easily accessible through non-invasive sampling [22]. It can reflect the metabolic disorder and provide insights into system-wide changes in response to physiological challenges or disease processes. To reveal the mechanism of the thermoregulatory circuitry, it is very important to identify and compare the contents of the potential biomarkers of pyrexia rats. In this article, pyrexia rat models were established by an injecting aqueous suspension of yeast in the back below the nape. Urine samples were collected from the model and control rats, respectively. The metabolite analysis was accomplished by using UPLC Q-TOF/MS. Orthogonal partial least-squares-discriminate analysis (OPLS-DA) was performed for investigating the metabolic changes of pyrexia rats. The potential biomarkers were identified, and their contents were compared between the model and the control groups accordingly. Meanwhile, the mechanism of the thermoregulatory circuitry of yeast-induced pyrexia was investigated.

2. Materials and methods

2.1. Chemicals and reagents

Yeast was purchased from Mauri Food Co., Ltd. (Hebei, China). Formic acid (No. 7000027413) was obtained from Sigma Chemical Co., Ltd (St. Louis, MO, USA). LC-grade methanol and acetonitrile was acquired from Baker Company (Baker Inc., USA). Ultra high purity water was prepared by Millipore-Q SAS 67120MOLSHEIM (France).

2.2. Animals and sample collection

All animal experiments were conducted in accordance with the Regulations of Experimental Animal Administration issued by the State Committee of Science and Technology of the People's Republic of China. Male Sprague–Dawley rats weighting 200 ± 20 g were obtained from Beijing Weitonglihua Laboratory Animal Technology Co., Ltd. (Beijing, China). All of the rats were maintained at a constant temperature (23 ± 2 °C) and humidity (60 ± 5%) in controlled rooms with a 12 h/12 h light/dark cycle. The rats were fed standard laboratory chow with water *ad libitum*. All of the animals were acclimated to the rooms for 7 days and then were transferred to individual metabolism cages and allowed to acclimatise for an additional 3 days. Every day, the rats' rectal temperatures were measured three times for the regular rhythm of body temperatures using a digital thermometer. The rats with a temperature difference that was greater than 0.5 °C were excluded. Sixteen qualified rats were selected and divided into two groups randomly, the control group (CG) and pyrexia group (PG). The pyrexia group was administered subcutaneously with a 20% aqueous suspension of yeast (15 mL/kg) in the back below the nape. The control group was similarly given an equal volume of 0.9% saline. The rectal temperature was measured, and the urine was collected at 0 h, 5 h, 9 h, 13 h, 25 h, 37 h, 49 h, 61 h, and 73 h after modeling. The urine volume at different time points, the pH and the osmolarity were recorded. The urine pH was measured by a pHS-3B pH meter (Shanghai, China). Urine osmolality was determined by freezing point depression (Shanghai, China). Urine was centrifuged at 14,000*g* for 10 min, and the supernatant was collected and stored at −20 °C.

2.3. Urine sample preparation

The urine samples were thawed at room temperature and were centrifuged. The supernatant was diluted at a ratio of 2:1 with distilled water and then was vortex mixed and passed through a 0.2-μm filter membrane before injection into the UPLC Q-TOF/MS system for analysis.

2.4. Chromatographic separation

Separation of the metabolites was performed on a Waters Acquity™ Ultra Performance LC system (Waters Corp., Milford, MA) that was equipped with an HSS T3 column (2.1 mm × 100 mm, 1.8 μm, Waters, UK). The gradient mobile phase was a mixture of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B). A solvent gradient system was used: 99% A for 0–0.5 min, 99–60% A for 0.5–10 min, 60–1% A for 10–11 min, 1% A for 11–12 min, and 1.0–99% A for 12.0–15.0 min. The flow rate was 0.40 mL/min. The analytical column and autosampler were maintained at temperatures of 45 °C and 4 °C, respectively. Then, 2 μL of sample solution was injected for each run.

2.5. Mass spectrometry

Mass spectrometry was performed on a Xevo™ G2 Q/TOF (Waters MS Technologies, Manchester, UK), a quadrupole and orthogonal acceleration time-of-flight tandem mass spectrometer. An electrospray ionisation source (ESI) interface was used and was set in positive ion mode. The profile data from m/z 50–1200 were recorded. The capillary and cone voltage were set at 3.0 kV and 45 V, respectively. The desolvation gas was set at 800 L/h at a temperature of 450 °C, the cone gas was set at 30 L/h, and the source temperature was set at 120 °C. All of the data were acquired using the lockspray to ensure accuracy and reproducibility. Leucine-enkephalin was used as the lockmass at a concentration of 1500 ng/mL and a flow rate of 5 μL/min. Data were collected in continuum mode, the lock spray frequency was set at 5 s, and the lock mass data were averaged over 10 scans for correction.

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