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A urinary metabonomics analysis of long-term effect of acetochlor exposure on rats by ultra-performance liquid chromatography/mass spectrometry

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ABSTRACT

The study was to assess the long-term toxic effects of acetochlor on rats. Two different doses (42.96 and 107.4 mg/kg body weight/day) of acetochlor were administered to Wistar rats through their food for over 24 weeks. Rat urine samples were collected at two time-points for the measurements of the metabonomics profiles with ultra-performance liquid chromatography–mass spectrometry (UPLC–MSMS). The results of clinical chemistry and histopathology suggested that long-term use of acetochlor in rats caused liver and kidney damage, and dysfunction of antioxidant system. The urinary metabonomics analysis indicated that the high and low-dose exposure of acetochlor could cause alterations of these metabonomics in urine in the rat. Significant changes of the levels of hippuric acid (0.403-fold decrease), citric acid (0.430-fold decrease), pantothenic acid (0.486-fold decrease), uracil (0.419-fold decrease), β-Alanine (0.325-fold decrease), nonanedioic acid (0.445-fold decrease), L-tyrosine (0.410-fold decrease), D-glucuronic acid (8.389-fold increase) and 2-ethyl-6-methyl-N-methyl-2-chloro-acetanilide in urine were observed. In addition, it may interfere with the fatty acid synthesis, the pyrimidine degradation and pantothenate biosynthesis. The level of 2-ethyl-6-methyl-N-methyl-2-chloro-acetanilide is detected in all treated groups which is not found in the control groups, indicating which can be used as an early, sensitive marker of acetochlor exposure in rat. This study illustrates the important utility of metabonomics approaches to understand the toxicity of long-term exposure of acetochlor.

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1. Introduction

Acetochlor (2-chloro-N-(ethoxymethyl)-N-(2-ethyl-6-methylphenyl) acetamide) belongs to the chloroacetanilide family [1]. It is a general pre-emergence herbicide which is commonly used in China and other countries around the world to inhibit early development of weeds by affecting cell integrity [2,3]. In China, more than 1.0×10^4 t of acetochlor have been used each year [3]. The studies from North Carolina in 2010 and Iowa in 2011 showed among a group of 33,484 people, 4026 applicators had used acetochlor and 3234 of them got cancers, with 304 acetochlor-exposed, indicating that there is increased incidence of cancer patients among acetochlor-exposed people [4]. Due to the wide use of chloroacetanilide, such as chlorinated herbicides, which has contaminated soils and surface water, there was growing concern for the environmental impact [5]. Acetochlor has been listed as a contaminant of drinking water by the US Environmental Protection Agency [6].

Previous research has shown that acetochlor may have genetic toxicity [7] and alter thyroid hormone-dependent gene expression [6]. Acetochlor has also been reported to interact with uterine estrogen receptors and affect brain development [8]. But, the effect of chronic acetochlor exposure at the level of organism metabolism has not been completely clarified. Therefore, a new approach to study the toxicity effects of long-term exposure of acetochlor is urgently needed.

Metabonomics, which involves 'dynamic multi-parametric metabolic response of living systems to pathophysiological stimuli or perturbations of whatever source' is an important part of systems biology [9]. An important part of metabonomics research is comparative analysis of the differences in levels of certain small molecule metabolites taken from healthy and diseased subjects, which helps people to better understand the metabolic pathways of disease processes, to discover biological markers and perform clinical auxiliary diagnostics [10]. Therefore, it have been widely employed in the evaluation of potential toxicity and

Abbreviations: UPLC, ultra performance liquid chromatography; ESI-Q-TOF-MSMS, electrospray ionization quadrupole time-of-flighttandem mass spectrometry; BPI, base peak intensity; PCA, principal component analysis; PLS-DA, partial least squares discriminant analysis; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALB, albumin; BUN, blood urea nitrogen; CR, creatinine; GHO, cholesterol; GLU, glucose; TP, total Protein; TG, triglyceride.

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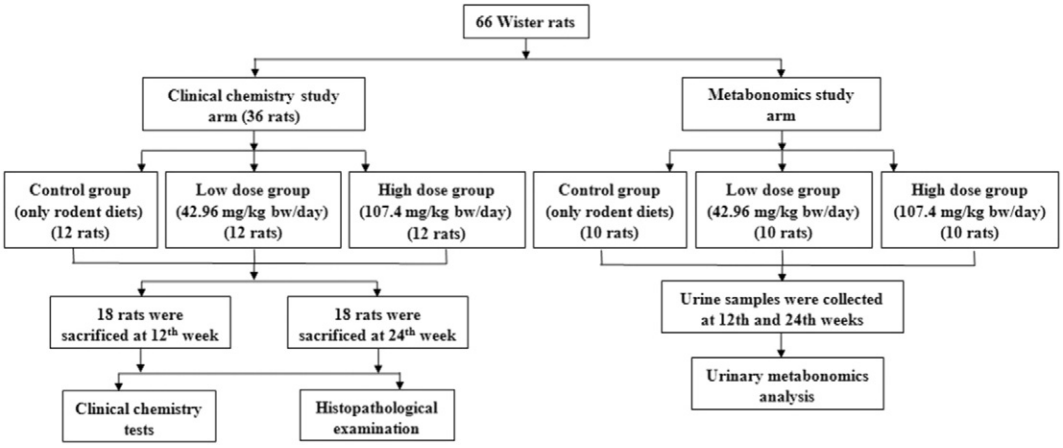

Fig. 1. Diagram of the experimental design.

monitoring toxicities [11]. Currently, liquid chromatography–mass spectrometry, gas chromatography–mass spectrometry (GC–LC), capillary electrophoresis–mass spectrometry (CE–LC) are among the most commonly used tools in chromatographic analysis of the metabonomics.

Urine is essentially a body's liquid waste repository. Any endogenous or exogenous metabolites that are not needed or are present in excess may be found in the urine [12]. Therefore, urine has been considered a potential source of biomarkers, especially in toxicology [13] and nutrition [14]. Compared to GC–LC and CE–LC, liquid chromatography–mass spectrometry was the better method for the urinary metabonomics analysis. In this study, we will analyze the potential toxicity of the herbicide acetochlor using urinary metabonomics based on UPLC/Q-TOF MSMS, then explore toxicity of the endogenous metabolites of acetochlor in the urine of rat, and further evaluate its safety.

2. Methods

2.1. Chemicals and reagents

Acetochlor (97% purity) was obtained from Anhui Huaxing Chemical Industry Co., Ltd. (China). HPLC grade (methanol and acetonitrile) were purchased from Dikma Science and Technology Co. Ltd. (Canada) and leucine enkephalin from Sigma–Aldrich (St. Louis, MO). Distilled water was obtained from a Milli-Q Ultrapurewatersystem (Millipore, Billerica, MA, USA). Kits for serum creatinine (CRE), blood urea nitrogen (BUN), aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin (ALB), total protein (TP), cholesterol (GHO), triglyceride (TG), glucose (GLU) were purchased from BioSino Bio-technology and Science Inc. (Beijing, China).

2.2. Animal treatment

In this study, a total of 66 Wistar rats weighing about 60–80 g with equal 1:1 sex ratio were supplied by Vital River Laboratory Animal Technology Co. Ltd. (Beijing, China). They were kept individually in metabolic cages at temperature (22 ± 2 °C) with 50–60% humidity and 12 h light–dark cycles. They were fed with the rodent diets and given drinking water ad libitum for at least 1 week, in order to familiarize them familiar with the environment. After acclimatization, according to match with the same sex and similar weight, 66 rats were randomly assigned into two study arms: clinical chemistry (36 rats) and metabonomics (30 rats). And then in the same way, 36 rats in clinical chemistry group were further randomly subdivided into three groups (low dose group [42.96 mg/kg bw/day, 1/50 of LD50], high dose group [107.4 mg/kg bw/day, 1/20 of LD50] and the control group [only rodent diets]). Acetochlor was administered to the rats consecutively for 24 weeks with rodent diets. The other 30 rats (the arm of metabonomics) were also randomly divided into three groups (high-dose group, low-dose group and control group) in the same way as the arm of clinical chemistry (10/group) (Fig. 1).

2.3. Sample collection and preparation

In the clinical chemistry group, at the end of weeks 12 and 24, eighteen rats were selected randomly, fasted for 12 h, anesthetized by intraperitoneal injection of sodium pentobarbital (40 mg/kg body weight), and then killed by bloodletting. Blood samples were taken from the abdominal aorta, and immediately separated by centrifugation at 3000 rpm (835 g) for 15 min. The serum samples were analyzed using an Autolab-PM4000 automated biochemical analyzer (AMS Co., Rome, Italy) to test for ALT, AST, BUN, CRE, TP, ALB, GHO, GLU and TG.

The liver and kidney of each rats were excised for histopathological studies immediately after blood collection. Liver and kidney samples were fixed in 10% formalin, processed into 4 mm paraffin sections, and stained with hematoxylin and eosin for histopathological assessment.

In the metabonomics group, the 24 h urine samples of 30 rats were collected at the end of 12th and 24th weeks. The urine samples were centrifuged at 10,000 rpm for 10 min. The supernatants were collected and stored at −80 °C. Before analysis, urine samples were allowed to thaw at room temperature, diluted 1:1 (v/v) with water, mixed by vortex for 1 min, and then separated by centrifugation at 12,000 rpm (13,362 *g*) for 10 min. The supernatants were then transferred to autosampler vials for urinary metabonomics analysis.

Table 1
The body weight of rats at each time point (g).

Doses (mg/kg bw)	4 week	8 week	12 week	16 week	20 week	24 week
0	177.4 ± 32.6	274.5 ± 52.6	350.2 ± 90.4	387.4 ± 102.1	408.9 ± 109.8	428.8 ± 111.5
42.96	177.5 ± 41.1	289 ± 67.5	348.6 ± 95.2	389.8 ± 117.2	409.9 ± 122	403.7 ± 117.1
107.4	189.2 ± 35.8	280.2 ± 72.1	353.5 ± 93	379.5 ± 95.2	394.3 ± 102.7	398 ± 112.7

Values expressed as mean ± SD (n = 10).

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