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Using combined bio-omics methods to evaluate the complicated toxic effects of mixed chemical wastewater and its treated effluent

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HIGHLIGHTS

- Mice exposed to mixed chemical wastewater and its treated effluent for 90 days.
- Hepatic transcriptomic alterations were analyzed by digital gene expression.
- Serum metabolomic alterations were analyzed by proton nuclear magnetic resonance.
- The water samples induced disruption of lipid metabolism and hepatotoxicity.
- Omics approaches are valuable to evaluate the complicated toxicity of wastewater.

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ABSTRACT

Mixed chemical wastewaters (MCWW) from industrial park contain complex mixtures of trace contaminants, which cannot be effectively removed by wastewater treatment plants (WWTP) and have become an unignored threat to ambient environment. However, limited information is available to evaluate the complicated toxic effects of MCWW and its effluent from wastewater treatment plant (WTPE) from the perspective of bio-omics. In this study, mice were exposed to the MCWW and WTPE for 90 days and distinct differences in the hepatic transcriptome and serum metabolome were analyzed by digital gene expression (DGE) and proton nuclear magnetic resonance (^{1}H-NMR) spectra, respectively. Our results indicated that disruption of lipid metabolism in liver and hepatotoxicity were induced by both MCWW and WTPE exposure. WTPE is still a health risk to the environment, which is in need of more attention. Furthermore, we demonstrated the potential ability of bio-omics approaches for evaluating toxic effects of MCWW and WTPE.

1. Introduction

The unceasing increasing demand for petrochemicals and other chemicals has been driving the rise and development of chemical industrial parks in many countries of the world [1–3]. The wastewater is usually collected for centralized treatment in the parks. The mixed chemical wastewaters (MCWW) from chemical industrial parks originates from various production lines and contains a rather complex composition of contaminants, most of which have poor biodegradability and high toxicities [4–6]. However, many trace contaminants in the MCWW cannot be effectively removed by conventional treatment process, although the conventional parameters of the effluents reach the local standards [7,8]. These contaminants are discharged into the aquatic environment via the effluents from wastewater treatment plants (WTPE) and may induce adverse effects to the ambient environment [9].

In order to protect the environmental health around chemical industry parks, it is urgent to obtain the accurate information about the complicated toxic effects of MCWW and its WTPE. However, there is still a lack in effective tools and approaches to evaluate the complicated toxic effects of MCWW and its WTPE. Many existing approaches, such as Ames bioassay [10], comet assay [11] and luminescent biosensor[12] are often used for routine monitoring of the toxicities induced by wastewater. However, these bioassays are all based on a given end-point or a mode of action, and can hardly identify novel biomarkers or key pathways for the toxicity of the mixed wastewater [13,14]. Therefore, more comprehensive and sensitive approaches are needed for the toxicological assessment of MCWW and its WTPE.

Growing evidence shows that "-omics" technology such as transcriptomics and metabolomics are reliable for toxicological assessment of various environmental contaminants. Santos et al.

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used transcriptomic and metabolomic methods to investigate the toxicity of copper on fish model in the aquatic environment [15]. These two methods were also used by Williams et al. to study the toxic effects of environmental dibenzanthracene on the stickleback [16]. More recently, transcriptomic and metabolomic approaches have also been used to evaluate toxicities on fish induced by wastewater [17,18]. However, little information is available about the toxic effects of MCWW and its WTPE on mammals based on omics investigation.

In this study, two omics approaches (trancriptomics and metabolmics) were used in profiling mode to evaluate the potential toxicity of MCWW and its WTPE on male mice and to identify novel potential biomarkers for the environment health risk assessment for chemical industrial parks.

2. Materials and methods

2.1. Water samples

Wastewater samples were collected from a wastewater treatment plant (WWTP) in a chemical industry park of Changzhou (east of China), which gathers hundreds of chemical plants. The WWTP uses conventional anoxic/oxic (A/O) biological treatment process and daily processes 10,000 m^3 of wastewater. The treatment steps include grit removal, regulating-pool, anoxic pond, aerobic tank and secondary clarifier. MCWW samples were collected prior to the regulation-pool and WTPE samples were collected from the outfall after the secondary clarifier. Immediately after the collection, the samples were transported to the laboratory and stored at −20 °C in the dark. The samples were collected in September 2012.

The conventional measurements of chemical oxygen demand (COD), ammonia nitrogen (NH_4^+–N), total nitrogen (TN) and total phosphorus (TP) were conducted using the standard methods recommended by the National Environmental Proctection Agency of China [19]. The target-analyses were applied for selected characteristic classes of organic contaminants such as 22 semivolatile organic compounds (SVOCs). These compounds are all included in the priority list of pollutants of US-EPA and are known to have potential adverse effects on the ecosystem and human health. Following previous studies [20], the SVOCs in the water samples were detected using DSQ II Single Quadruple GC/MS (ThermoQuest, San Jose, CA, USA) with selected ion monitoring (SIM) mode. Trace elements were detected by ICP-OES (ICP-J-A1100, Jarell-Ash Inc., USA). Recovery rates were above 90.3% for SVOCs and above 92.5% for trace elements. The detection limits for all contaminants ranged from 0.1 to 2 ng/L (a signal-to-noise ratio of 3).

2.2. Animal treatment

Male mice (*Mus musculus*, 20–25 g) were purchased from experimental animal center of the Academy of Military Medical Science of China and acclimated for two weeks. The mice were housed in an animal laboratory at 25 °C with 50% relative humidity and a 12/12 h light-dark cycle. A total of 30 healthy mice were selected and randomly assigned to three groups: the MCWW group (n = 10, fed with the MCWW samples), the WTPE group (n = 10, fed with the WTPE samples) and the control group (fed with distilled water). All mice were fed with standard mouse chow and received water (test water samples) *ad libitum* throughout the experiment, during which the food and water consumption was carefully recorded.

At the end of feeding trials (90 days), all mice were sacrificed under fasting for 12 h and anesthetized with diethyl ether before liver tissues were collected. Blood was collected and serum was separated by centrifugation at 3000 rpm for 15 min at 4 °C and stored at −80 °C. One lobe of the liver was fixed in 10% formalin solution for histopathological assessment.

2.3. Histopathological and biomedical measurements

Histopathological assessments were performed following the standard procedures [21]. In brief, the formalin-fixed livers were embedded in paraffin wax, sectioned, and stained using the hematoxylin and eosin (H&E) method, followed by microscopic assessments. These experiments were performed as double-blind trials.

A clinical-chemistry screen of the serum samples was performed using Olympus 2700 analyzer (Olympus Co., Japan), including total protein (TP), albumin (ALB), globulin (GLB), glutamate pyruvate transaminase (GPT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), creatinine (Cr), total cholesterol (T-CHO) and triglyceride (TG) [22].

2.4. Transcriptomic analysis

The hepatic trancriptomic profiles of mice were analyzed by using digital gene expression profiling (DGE). Equal amount of RNA (6 μg) extracted from three mouse livers in each group was mixed as one sample and then three biological replicates of each group was used for the DGE analysis. The transcriptome sequencing was performed using the Solexa Sequencing Chip (flowcell) on an Illumina Cluster Station and an Illumina HiSeq™ 2000 System. The filtering, tag expression, distribution and sequencing saturation were analyzed to assess the sequence quality. The number of unambiguous clean tags for each gene was calculated and was then normalized to the number of transcripts per million (TPM) clean tags [23,24].

The identification of differentially expressed genes (DEGs) between the control and the treatment groups (MCWW and WTPE) was accomplished according to the previous studies [25,26]. The false discovery rate (FDR) ≤0.001 and the absolute value of the log2 ratio (treatment group/control group) ≥1 were used as the threshold to identify the DEGs. Annotation, biological interpretation, and pathway enrichment analysis of DEGs were conducted on the basis of Molecule Annotation System (MAS) 3.0 (http://bioinfo.capitalbio.com/mas/).

Validation of the transcriptomic profiles was conducted using real-time quantitative polymerase chain reaction (RT-QPCR) analyses with five target genes, including Agpat2, Aldh9a1, Cyp2c29, Cyp2c54 and Serpina1e. Primer sequences, PCR product sizes, and annealing temperatures are summarized in Table S1. Four replicates of RT-QPCR were performed on each sample (see the Supplementary material for the full methodology).

2.5. Metabolomic analysis

Frozen serum samples were thawed at room temperature and shaken before use. A total of 300 μL of a phosphate sodium buffer (70 mM Na_2HPO_4; 0.025 (w/v) NaN_3; 20% (v/v) D_2O; 3 mM sodium trimethylsilyl [2,2,3,3-$^2H_4$] proionate (TSP); pH 7.4) was added to 300 μL of each serum sample. This mixture was homogenized and centrifuged at 10,000 rpm for 10 min and then 550 μL of the supernatants were transferred into a 5 mm NMR tube for analysis. The detailed detection was conducted according to the methods described previously [27].

Partial least-squares discriminant analysis (PLS-DA) with orthogonal signal correction (OSC) was used to explore the main effects in NMR data sets by using SIMCA-P software (Umetrics, Umea, Sweden). The coefficients of OPLS-DA were calculated to determine the dominant metabolites influencing the differentiation between control and treatment groups. Metabolites with coefficients ≥1.5 were regarded as significantly altered