Environmental Toxicology and Pharmacology 60 (2018) 157–164

Contents lists available at ScienceDirect

Environmental Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/etap

Proteome analysis of the potential serum biomarkers for chronic benzene poisoning

Zhimin Zhang, Peimao Li, Dafeng Lin, Dianpeng Wang, Yanfang Zhang*

Shenzhen Prevention and Treatment Center for Occupational Diseases, Shenzhen, 518001, Guangdong, China

ARTICLE INFO

Keywords:
Benzene
Chronic poisoning
Hematotoxicity
Serum proteins
Biomarkers

ABSTRACT

The aim of our study is to seek novel specific biomarkers which could provide clues to the mechanism of chronic benzene poisoning (CBP) and might also be used as specific markers for early detection and diagnosis. In this study, a comparative serological proteome analysis between normal controls and CBP patients at three different levels of poisoning were performed via a 2D-DIGE and MALDI-TOF-MS. As the result a total of 10 proteins were found significantly altered between the normal and the mild, moderate and severe poisoning. The identified differentially expressed proteins were classified according to their molecular functions, biological processes, and protein classes, and three important serum proteins among them, apolipoproteinA-1, alpha-1-antitrypsin and complement C3, were further confirmed by immune turbidimetric analysis for their significant up-regulation in the CBP patients. Our findings suggest that these differential proteins may help elucidate the mechanism of CBP and provide potential biomarkers for diagnosis.

1. Introduction

Benzene is one of the most common chemical pollutants that is widespread in industry (Santiago et al., 2014; Tchepel et al., 2014). It is extensively used as an industrial solvent in developing countries, representing a significant occupational hazard (Khalade et al., 2010). Occupational benzene exposures usually occur in the petrochemical industry, and manufacturing industry that require aromatic solvents or glues containing benzene such as shoe manufacturing, rubber production, and printing (Capleton and Levy, 2005; Wang et al., 2006; Williams et al., 2005). It is well known that chronic exposure to benzene leads to a range of hematotoxicity involving anemia, leucopenia, thrombocytopenia, pancytopenia, and even leukemia (Snyder, 2012; Williams, 2014). The International Agency for Research on Cancer (IARC) has classified benzene as a category 1 human carcinogens (Goldstein, 2010).

There are a variety of mechanistic studies in the literature which describe the effects of benzene, such as covalent binding (Longacre et al., 1981), immune suppression (Snyder, 2002), and chromosome aberrations induced by benzene (Zhang et al., 2002). In addition, a number of newly identified mechanisms in recent years that include DNA mutation (Billet et al., 2010), oxidative damage (Murugesan et al., 2013), and alterations in gene expression (McHale et al., 2011). Although many epidemiological investigations and experimental studies have been carried out, the mechanisms of benzene-induced toxicity is still a matter of debate and has not been clarified. There is still a lack of sensitive and specific indicators for its diagnosis at early stage. Thus, it is urgent to seek novel specific biomarkers which could provide clues to the mechanism of chronic benzene poisoning (CBP) and might also be used as specific markers for early detection and diagnosis.

Serum has been proved an ideal biological specimen and serum proteomics analysis has been developed for seeking insight into the pathophysiology and mechanisms of disease and helps identify potential biomarkers with diagnostic and prognostic significance (Ray et al., 2011). Two-dimensional difference gel electrophoresis (2D-DIGE) could resolve several thousand proteins based on the isoelectric points in the first dimension and the sizes in the second dimension (Lee et al., 2015; Westermeier and Gorg, 2011). Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) is also one of the most commonly used tools in proteomics research (Sandanayake et al., 2014), and has been reported sensitive and specific for clinical trials. In the present study, we compared the expression of various proteins by checking their contents in the sera of normal controls and chronic benzene poisoning patients at three different levels of poisoning

Abbreviations: CBP, chronic benzene poisoning; 2D-DIGE, two dimension difference gel electrophoresis; MALDI-TOF-MS, matrix-assisted laser desorption/ionization time of flight mass spectrometry; DTT, DL-dithiothreitol; Tris, tris(hydroxymethyl) aminomethane; CHCA, a-cyano-4-hydroxycinnamic acid; IEF, isoelectric focusing; CHAPS, 3-[3-cholamidopropyl]-dimethylammonio-1-propanesulfenate; IPG, immobilized pH gradient; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis

* Corresponding author at: Department of Clinical Laboratory of Shenzhen, Shenzhen Prevention and Treatment Center for Occupational Diseases, No.2019, Buxin Road, Luohu District, Shenzhen, 518001, China.

E-mail address: 13923431511@139.com (Y. Zhang).

https://doi.org/10.1016/j.etap.2018.04.017
Received 17 September 2017; Received in revised form 17 April 2018; Accepted 20 April 2018
Available online 27 April 2018
1382-6689/ © 2018 Elsevier B.V. All rights reserved.

by using 2D-DIGE coupled with matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) strategy. Differential proteins classification was performed by PANTHER (Protein Analysis through Evolutionary Relationships) (Mi et al., 2013a). The important serum proteins significantly altered among the Normal, Mild poisoning, Moderate poisoning and Severe poisoning were further validated by Immune turbidimetric analysis. This study not only provide scientific insight into the molecular mechanisms of chronic benzene poisoning but also reveal potential serum biomarkers that can be used for the diagnosis and monitoring the progression of the disease.

2. Materials and methods

2.1. Instruments and reagents

The major instruments employed for experiments in this study include UltrafleXtreme MALDI-TOF/TOF mass spectrometer from Bruker Daltonics (USA). The Ettan IPGphor 3 Integrated Isoelectric Focusing Unit Electrophoresis System and Ettan DALTsix Electrophoresis Unit from GE Healthcare (USA), and Beckman Coulter AU5800 automatic biochemical analyzer from Beckman Coulter (USA). The 2-D Quant Kit and the CyDye DIGE Fluor Labeling Kit were purchased from GE Healthcare (USA). The ProteoExtract Albumin/IgG removal kit was purchased from Merck KGaA (Germany). Research-grade acetonitrile (ACN), trifluoroacetic acid (TFA), Dimethyl formamide, ammonium bicarbonate, and DL-Dithiothreitol were purchased from Sigma-Aldrich (USA).

2.2. Subjects and serum collection

This study was conducted in accordance with the declaration of Helsink (1997). The protocol of experiments was approved by the Medical Ethics Committee of Shenzhen Prevention and Treatment Center for Occupational Diseases. Each subject gave written informed consent throughout the whole study period. All the serum from chronic benzene poisoning patients, as classified according to the diagnostic criteria for the "Diagnosis of occupational benzene poisoning (2013 Edition)" issued by National Health and Family Planning Commission of the People's Republic of China (http://www.nhfpc.gov.cn/ewebeditor/uploadfile/2014/10/20141030140929767.pdf), were collected from April 2014 to August 2015 at the Shenzhen Prevention and Treatment Center for Occupational Diseases. For 2D-DIGE analysis, four groups (normal, mild poisoning, moderate poisoning, and severe poisoning) were enrolled, and sex and age were matched among between groups (Table 1). In validation study, the same four groups were also enrolled to facilitate data analysis (Table 2). Normal subjects were selected to meet the following conditions: there was no benzene exposure before, without X-ray or other radiation exposure in the past 3 months, no contact history with other blood toxicity and genotoxic substances, without undergoing tumor and blood system diseases and other major disease history.

Venous blood sample of each subject was collected with BD vacutainer venous blood collection tubes (BD, USA) containing coagulant. Approximately 4 mL of blood was collected for each person. All blood samples were centrifuged at 4000 rpm for 10 min, and the supernatant liquid for each sample was divided and stored in aliquots at −80 °C for analysis. Each serum sample underwent no more than 2 freeze/thaw cycles prior to protein/peptide extraction and MS analysis.

2.3. Sample preparation for 2-D gel electrophoresis

For 2-D gel electrophoresis, the serum samples were processed by using the ProteoExtract Albumin/IgG removal kit (Merck, Germany) and following the standard protocol provided by the manufacturer. Briefly, 60 μL of each serum was diluted 10-fold with binding buffer, and then the diluted serum sample was added to the resin column and allowed to pass by gravity-flow. The resin column was washed twice with 600 μL of binding buffer. The flow-through fraction was concentrated using a 3 kDa cut off centrifugal filter device (Millipore, USA). The final serum protein concentration was determined with the 2-D Quant kit (GE Healthcare, USA) according to the manufacturer's instructions.

2.4. CyDye labeling

Protein extracts were minimally labeled (25 μg protein per 200 pmol dyes) with Cy2, Cy3 or Cy5 fluorescent dyes following the standard protocol provided by the manufacturer (GE Healthcare). An internal standard sample was first generated by pooling equal amounts (25 μg) of all of the samples together. The internal standard was labeled by Cy2 and used to minimize the gel-to-gel variation and assess the reproducibility. Then, each group was divided into two parts and one was labeled with Cy3 and another with Cy5, which were used to eliminate the effect of dyes in gel electrophoresis. The Cy3 and Cy5-labeled samples of different groups were mixed with 25 μg of Cy2-labeled internal standard and separated in a gel, and 12 gels were used for various labeled sample combinations (Table 3).

2.5. 2-D gel electrophoresis

Equal volume of 2 × lysis buffer (7 M urea, 2 M thiourea, 4% w/v CHAPS, 2% w/v DTT, 2% v/v IPG buffer pH 4-7) was added to each mixed labeled sample and incubated on ice for 10 min. Then, rehydration buffer (8 Murea, 2% w/v CHAPS, 0.28% w/v DTT, 0.5% v/v IPG buffer pH 4–17, 0.002% w/v bromophenol blue) was added to make the total volume of the sample up to 450 μL. The first dimension

Table 1
The clinical characteristics of the subjects for 2D-DIGE analysis.

Items	Normal	Mild poisoning	Moderate poisoning	Severe poisoning
number	6	6	6	6
Gender (Male/Female)	1/5	1/5	1/5	1/5
Age (Mean ± SD)	36.17 ± 6.46	37.83 ± 6.65	37.67 ± 8.66	40.33 ± 8.69

Table 2
The clinical characteristics of the subjects for validation analysis.

Items	Normal	Mild poisoning	Moderate poisoning	Severe poisoning
number	90	53	8	14
Gender (Male/Female)	64/26	14/39	1/7	4/10
Age (Mean ± SD)	29.85 ± 7.77	37.36 ± 8.41	41.25 ± 8.50	40.00 ± 5.88

Download English Version:

https://daneshyari.com/en/article/8545928

Download Persian Version:

https://daneshyari.com/article/8545928

Daneshyari.com