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Identification of serum biomarkers for occupational medicamentosa-like dermatitis induced by trichloroethylene using mass spectrometry

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ABSTRACT

Occupational medicamentosa-like dermatitis induced by trichloroethylene (OMLDT) is an autoimmune disease and it has become a serious occupational health hazard. In the present study, we collected fasting blood samples from patients with OMLDT (n = 18) and healthy volunteers (n = 33) to explore serum peptidome patterns. Peptides in sera were purified using weak cation exchange magnetic beads (MB-WCX), and analyzed by matrix-assisted laser desorption ionization time-of-flight-mass spectrometry (MALDI-TOF-MS) and ClinProTools bioinformatics software. The intensities of thirty protein/peptide peaks were significantly different between the healthy control and OMLDT patients. A pattern of three peaks (m/z 2106.3, 2134.5, and 3263.67) was selected for supervised neural network (SNN) model building to separate the OMLDT patients from the healthy controls with a sensitivity of 95.5% and a specificity of 73.8%. Furthermore, two peptide peaks of m/z 4091.61 and 4281.69 were identified as fragments of ATP-binding cassette transporter family A member 12 (ABCA12), and cationic trypsinogen (PRRS1), respectively. Our findings not only show that specific proteomic fingerprints in the sera of OMLDT patients can be served as a differentiated tool of OMLDT patients with high sensitivity and high specificity, but also reveal the novel correlation between OMLDT with ABC transports and PRRS1, which will be of potential value for clinical and mechanistic studies of OMLDT.

Introduction

Trichloroethylene (TCE) is a volatile chlorinated organic compound commonly used in industrial settings as a degreaser for metal parts and general-purpose solvent for lipophilic compounds (Ruder, 2006). Many studies have demonstrated that TCE exposure has severe adverse health effects, including neurotoxicity (Gash et al., 2008), immunotoxicity (Cai et al., 2007), hepatotoxicity (J. Liu et al., 2007; Ramdhan et al., 2008), renal toxicity and carcinogenesis (Tabrez and Ahmad, 2009). In 2011, the U.S. Environmental Protection Agency (EPA) formally characterized TCE as a human carcinogen in the Final Health Assessment for TCE (http:/www.epa.gov/IRIS). Due to increased concerns over its health effects, the use of TCE has been declined in the United States since 1970s (Bakke et al., 2007). However, the total volume used of TCE per year still remains high in China, and most of TCE is used in the industrialized regions, especially the Pearl River Delta Regions (Yu et al., 2012).

Occupational intoxication events from TCE sources have been frequently reported in the past decades (Jung et al., 2012; Kamijima et al., 2007, 2008; Watanabe, 2011; Xu et al., 2009). A close link between occupational exposure to TCE and severe skin disorders has been suggested (Kamijima et al., 2007). The number of patients suffering from TCE-related severe skin disorders has been increase in Asia, especially in the Philippines, Singapore and Taiwan (Kamijima et al., 2007). Since the first severe TCE-exposure exfoliative dermatitis patient, 394 cases have been reported until 2009 in Guangdong, China (Huang and Huang, 2010). Chronic TCE exposure can lead to severe skin lesions which include exfoliative dermatitis, Stevens–Johnson syndrome, erythema multiforme and toxic epidermal necrolysis (Huang et al., 2006). These skin disorders are grouped under "occupational medicamentasa-like dermatitis (OMLD) induced by TCE according to Chinese National Diagnostic Criteria" (Yu et al., 2012). Apart from that, occupational exposure to TCE is also known to cause harmful effects on the kidney, liver, heart, lung, brain, lymphatic and nervous system (Khan et al., 2009; Scott and Chiu, 2006; Xu et al., 2009).

More and more investigations have been conducted on OMLDT in recent years. However, the studies mainly include the analysis of clinical cases (Jung et al., 2012; Kamijima et al., 2007; Watanabe, 2011; Xu et al., 2009), retrospective epidemiological investigations (Kamijima

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et al., 2008), susceptible genetic polymorphisms (Dai et al., 2009; Watanabe et al., 2010), and immune injury mechanisms (Yang et al., 2011). Very few studies have focused on valid population studies. An increasing amount of evidence indicates that protein/polypeptide biomarkers in the serum are appropriate sources for successful OMLDT diagnosis (Hanash et al., 2008; Ma et al., 2009; Tanaka et al., 2006). Therefore, it is important to explore protein/polypeptide differences in the sera of OMLDT patients and healthy controls. In addition, these studies can provide additional insights into the molecular mechanism of OMLDT and reveal potential serum biomarkers.

Matrix-assisted laser desorption/ionization-time of flight-mass spectrometry (MALDI-TOF-MS) is a novel proteomics technique being widely applied to analyze various body fluids for the identification of biomarkers and for the disease diagnosis (Liang et al., 2010; Qiu et al., 2009). MALDI-TOF-MS has been applied for protein profiling and identification using two basic approaches: (I) the identification of specific proteins (Madonna et al., 2003) and (II) the analysis of complete proteomes. The latter can be based either on the detection of specific peaks (Yao et al., 2002) or on the analysis of complete spectral patterns (Bright et al., 2002). Affinity bead-based purification uses the different chemical chromatographic outer layers of magnetic beads to selectively purify certain subsets of peptides, which is regarded as sensitive, fast, and essential for clinical use (Baumann et al., 2005). In the present study, we performed a magnetic beads-based weak cation exchange chromatography (MB-WCX) for serum processing and MALDI-TOF-MS for peptide profiling. Based on alterations found within polypeptide fingerprinting of serum (PFS), we have inspected spectral overlays, compared intensities of relative peptide ions, and statistically analyzed hundreds of features obtained from OMLDT patients and healthy controls, in order to build a diagnostic model of OMLDT. A pattern of peaks was selected for model building to discriminate the OMLDT patients from the healthy controls with high sensitivity and specificity. By a liquid chromatography (LC) coupled with tandem mass spectrometry (MS/MS) approach, two differential peaks were identified as the fragment of two proteins, ATP-binding cassette transporter family A member 12 (ABCA12), and cationic trypsinogen (PRSS1) respectively (Fig. 1). These findings will be helpful for clinical diagnosis, and providing novel scientific clue for mechanisms studies of OMLDT.

Materials and methods

Reagents. Research-grade acetonitrile (ACN), trifluoroacetic acid (TFA), ammonium bicarbonate, ammonium acetate, acetone, ethanol, and methanol were purchased from Sigma-Aldrich (USA). The profiling kit 100 MB-WCX and the starter kit for MALDI-TOF-MS were purchased from Bruker Daltonics (USA). The 2D Quant Kit was purchased from GE Healthcare (USA). Sequencing Grade Modified Trypsin was purchased from Promega (USA).

Instruments and softwares. The UltrafleXtreme MALDI-TOF/TOF mass spectrometer, MTP 384 target plate polished steel, and MTP 384 target plate AnchorChip steel were acquired from Bruker Daltonics (USA). The Probot by LC Packings instrument and UltiMate 3000 Nano and Cap Systems were purchased from Dionex Corporation (USA). The following software programs were used: FlexControl Version 3.3, FlexAnalysis Version 3.3, ClinProTools Version 2.2, BioTools Version 3.2, WARP-LC Version 1.2 (Bruker Daltonik GmbH, Germany), Chromeleon 6.80 SR10 Build 2818, and μCarrier 2.0 Build 1621 (Dionex Company, USA).

Study subjects. This study was conducted in accordance with the principles of the Declaration of Helsinki (World Medical Association, 1997). All the subjects gave their written informed consent, and the Medical Ethics Committee of Shenzhen Center for Disease Control and Prevention approved the study protocols. The skin manifestations of the patients were classified according to the diagnostic criteria for occupational medicamentosa-like dermatitis induced by trichloroethylene (GBZ 185-2006) as defined by the Ministry of Health, the People's Republic of China. Between March 2010 and November 2011, 18 patients (13 males and 5 females; 24.06 ± 8.81 years of age (mean ± standard deviation)) were hospitalized for treatment. Of them, 11 males and 4 females whose serum was available (23.87 ± 8.54 years of age) were enrolled in the test set. Thirty-three normal subjects (21 males and 12 females;

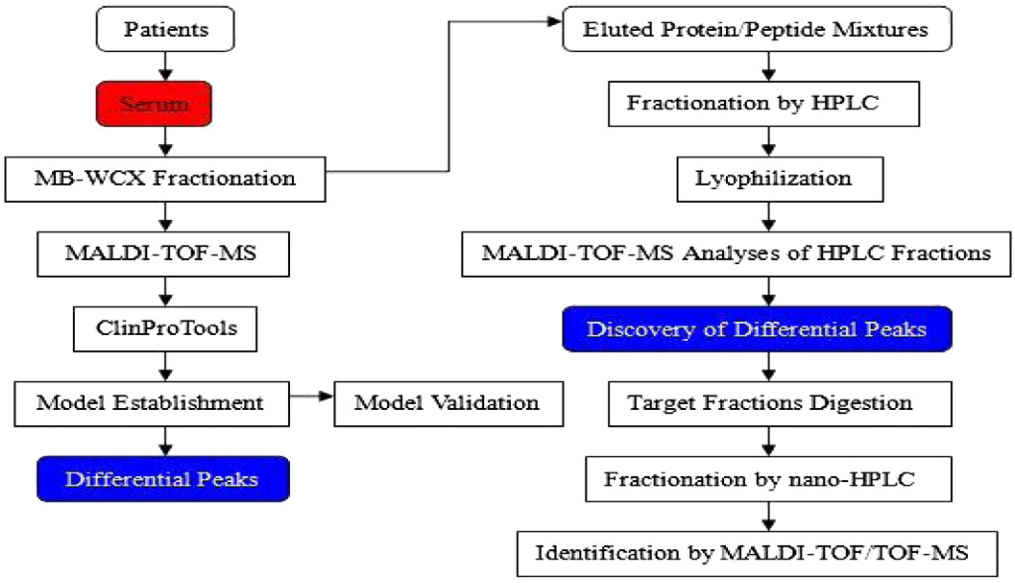

Fig. 1. Schematic diagram of the magnetic bead-based serum proteomic profiling method and protein identification. The collected sera from occupational medicamentosa-like dermatitis induced by trichloroethylene (OMLDT) patients and healthy controls were purified by a weak cation exchange magnetic beads (MB-WCX) chromatography. The serum proteomic profile was analyzed by matrix-assisted laser desorption/ionization-time of flight-mass spectrometry (MALDI-TOF-MS). After the statistical analysis by ClinProTools, 30 peptide peaks were significantly different between two groups. A diagnostic pattern of selected peptide peaks was established by ClinProTools to discriminate the OMLDT groups from healthy controls with high sensitivity and high specificity. In the protein identification assay, the eluted protein/peptide mixtures were fractionated by HPLC and the collected fractions were lyophilized for MALDI-TOF-MS. Two target fractions containing the m/z signal associated with the differential peaks were digested with trypsin. The enzymolysis mixture was separated by nano-liquid chromatography (nano-LC) and identified by tandem mass spectrometry (MALDI-TOF/TOF-MS).