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Molecular mechanisms involved in lead induced disruption of hepatic and pancreatic glucose metabolism

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ABSTRACT

Lead (Pb) is a toxic heavy metal known to be associated with pathology of various human chronic diseases. This study has focused on the effect of lead on glucose homeostasis with regard to metabolic function of pancreas and liver. Islets of Langerhans were isolated from the pancreas of rats and exposed to lead for 24 h, then insulin release along with markers of ER stress and oxidative stress were evaluated. In another part, lead was administered to rats for 32 days and after evaluating criteria of diabetes, the activity of gluconeogenesis and glycogenolysis enzymes, and markers of oxidative stress and inflammation were measured in the liver. Lead disrupted insulin secretory function of islets through activating GSK-3β and ER stress, and increased activity of gluconeogenic enzymes in the liver featured by glucose intolerance. Chronic exposure to lead can disrupt glucose homeostasis by affecting pancreas and liver mainly through induction of insulin resistance.

1. Introduction

The heavy metal lead (Pb) is an abundant environmental toxicant with a wide range and long history of use dating back to Roman times. Despite wide toxicological studies, there is still debate on the hazards of lead for the general population through long time exposure to low levels of this toxicant in drinking water, food, and air (Karrari et al., 2012; Mehrpour et al., 2012). During the last decade, the exposure levels below

Abbreviations: 8OHG, 8-hydroxy-2-deoxy guanosine; AUC, area under curve; BLL, blood lead level; CHOP, CCAAT/enhancer-binding protein (C/EBP) homologous protein; eIF2B, initiation factor 2B; ER, endoplasmic reticulum; FBS, fasting blood sugar; G6P, glucose 6-phaophatase; GLUT2, glucose transporte 2; GP, glycogen phosphorylase; GRP78, glucose regulated protein 78; GSIS, glucose stimulated insulin secretion; GSK-3β, glycogen synthase kinase-3 beta; GTT, glucose tolerance test; HOMA-IR, homeostatic model assessment-insulin resistance; HOMA-β, homeostatic model assessment-beta cells function; IR, insulin receptor; IRS, insulin receptor substrate; MTT, thiazolyl blue tetrazolium bromide; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; PEPCK, phosphoenolpyruvate carboxykinase; PI-3K, phophatidil inositil-3 kinase; ROS, reactive oxygen species; TBARS, thiobarbituric acid reactive substances; TNF-α, tumor necrosis factor-alpha.

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the limit of 25 μg/kg body weight/week established by WHO was considered to be safe, but now the experts believe that there is no known safe exposure limit for lead (Grandjean, 2010). The nervous system is the main target for lead toxicity; however, it has become clear that almost all organs and systems in the body can be affected by this agent. The mechanism of lead toxicity is inhibition of heme synthesis enzymes and thiol-containing antioxidants leading to the formation of reactive oxygen species and consequently oxidative stress (Nemsadze et al., 2009). In addition to confirmed neurological, hematological, immunological, gastrointestinal, renal, circulatory, carcinogenic, and reproductive pathologies (Gidlow, 2004), there are some reports on the association of exposure to lead with glucose dys-metabolism and diabetes complications (Bener et al., 2001). Two separated studies have reported higher levels of lead in biological samples of diabetic patients and type 1 diabetic mothers as well as their neonates (Afridi et al., 2008; Kolachi et al., 2011). Some evaluations have also detected a link between blood lead levels and progression of nephropathy in type 2 diabetic patients (Huang et al., 2013; Lin et al., 2006).

In this experimental setup consisting of both *in vitro* and *in vivo* models, we aimed to evaluate the effect of lead on glucose metabolism and involved cellular mechanisms with reference to the main insulin secreting and responding organs, pancreas and liver, respectively, in the rat.

2. Materials and methods

2.1. Chemicals

All chemicals were purchased from Sigma-Aldrich Chemie (GmbH, Munich, Germany) unless otherwise stated. Rat-specific TNF-α enzyme linked immunosorbent assay (ELISA) kits was purchased from Bender Med Systems (Vienna, Austria). ELISA kit for determination of DNA damage was obtained from Cayman Chemical Co. (Michigan, USA). ELISA kit for measuring activity of GSK-3β was purchased from Millipore Corporation. Tripure Isolation Reagent and Expand Reverse Transcriptase were prepared from Roche Applied Sciences. The primers were synthesized and delivered by Gen Fanavaran Ldt. SYBR® Premix Ex Taq from Takara Bio Inc. was also used for real time PCR reaction.

2.2. Animals

Adult male Wistar rats, weighting 200–250 g, were housed under standard laboratory conditions with free access to normal laboratory rat chow and water ad libitum. All ethical concerns about the use of animals were considered carefully and the protocol of the study was approved by Tehran University of Medical Sciences (TUMS) review board.

2.3. *In vivo model of subchronic toxicity toward liver*

2.3.1. *Treatment*

Animals were randomly divided into three groups comprising six animals, and all were treated for 32 days. Groups were as follows: group 1 or control received normal drinking water while groups 2 and 3 received lead acetate 0.05 and 0.2%, respectively, *via* drinking water.

2.3.2. *Oral glucose tolerance test (oGTT)*

Rats were fasted for 6 h before the test and 2 g/kg glucose solution was administered orally. Blood glucose was measured using a glucometer (Accu-Chek®) before and 30, 60, 120 and 180 min after glucose administration.

2.3.3. *Sampling*

At the end of the specified treatment (24 h after the last dose of lead acetate), the animals were anesthetized by intraperitoneal administration of pentobarbital sodium (55 mg/kg), and then the abdomen was rapidly incised to expose the liver which was rapidly removed and freeze-clamped at liquid nitrogen temperature. The frozen liver was stored at −80 °C and used for determination of metabolites' concentration and enzymatic activities. After blood sampling, serum was separated and stored at −80 °C for serological assays.

2.3.4. *Blood lead level (BLL) and insulin assay*

The amount of lead in serum samples was measured by graphite-furnace atomic absorption spectrophotometer. A rat insulin ELISA assay kit was also used to measure the concentration of insulin in the serum.

2.3.5. *Determination of enzymatic activity of PEPCK, G6Pase, and GP in the liver*

Liver PEPCK activity was measured in the liver homogenate in the reverse direction (carboxylation of phosphoenolpyruvate to form oxaloacetic acid in the presence of NADH). PEPCK activity was expressed in units per gram liver protein. One unit is defined as the amount of enzyme converting 1 mmol of substrate into products per minute under assay conditions (Saadat et al., 2004). G6Pase and GP activity was determined on the basis of release of inorganic phosphorus, as described previously. One unit of enzyme releases 1.0 mmol of inorganic phosphate per minute at pH 6.5 at 37 °C (Nordlie and Arion, 1966).

2.3.6. *Determination of cellular lipid, protein, and DNA oxidative and inflammatory markers in the liver*

As an indirect index of ROS production, the extent of lipid peroxidation was measured using the thiobarbituric acid that reacts with lipid peroxides to produce a complex called TBA reactive substance (TBARS) which is determined spectrophotometrically. Results are expressed as nmol/mg protein as $\varepsilon = 153\,M^{-1}\,cm^{-1}$ (Abdollahi et al., 2004). The oxidative damage to proteins was assessed spectrophotometrically by determination of carbonyl groups based on the reaction with dinitrophenylhydrazine. The carbonyl content in nmol/mg protein was calculated using a molar extinction coefficient of $22{,}000\,M^{-1}\,cm^{-1}$ at 370 nm after subtraction of the blank absorbance (Mostafalou et al., 2012a). The level of 8-deoxyguanosine was determined as an index of DNA oxidative damage using the ELISA kit according to the kit brochure. The TNF-α ELISA kit was also used to assay hepatic TNF-a.