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Assessment of benzene induced oxidative impairment in rat isolated pancreatic islets and effect on insulin secretion

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ABSTRACT

Benzene (C_6H_6) is an organic compound used in petrochemicals and numerous other industries. It is abundantly released to our environment as a chemical pollutant causing widespread human exposure. This study mainly focused on benzene induced toxicity on rat pancreatic islets with respect to oxidative damage, insulin secretion and glucokinase (GK) activity. Benzene was dissolved in corn oil and administered orally at doses 200, 400 and 800 mg/kg/day, for 4 weeks. In rats, benzene significantly raised the concentration of plasma insulin. Also the effect of benzene on the release of glucose-induced insulin was pronounced in isolated islets. Benzene caused oxidative DNA damage and lipid peroxidation, and also reduced the cell viability and total thiols groups, in the islets of exposed rats.

In conclusion, the current study revealed that pancreatic glucose metabolism is susceptible to benzene toxicity and the resultant oxidative stress could lead to functional abnormalities in the pancreas.

1. Introduction

Benzene (C_6H_6) is an organic compound, used as an industrial solvent and a component of petrochemicals. It is one of the environmental contaminants released from various sources affecting human life. Refined petroleum products generally contain benzene 2–3% by volume. But in certain regions of the world, the use of benzene in petrochemicals has reached to more than 5% by volume (Verma and Tombe, 2002; Karakitsios et al., 2007). Release of benzene in our environment takes place from industrial wastes, combustion of petrochemicals, and cigarette smoke. Its absorption takes place from all natural routes and rapidly metabolized in the liver and bone marrow. And the resultant toxic metabolites and free radicals cause various lethal effects on the body (Travis et al., 1990; Bahadar et al., 2014a).

Free radicals and reactive oxygen species (ROS) have been thought as important physiological mediators playing an

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important role in glucose homeostasis and insulin signaling. For instance, H_2O_2 has been reported to regulate glucose stimulated insulin release from pancreatic β-cells. And it also causes the inhibition of protein tyrosine phosphatase activity which further lead to an increase in the phosphorylation of insulin receptor (IR) and insulin receptor substrate (IRS) proteins (Pi et al., 2007; Mahadev et al., 2001). Although, under defined constraints these signaling pathways are thought to be controlled by ROS, but, excessive and sustained production of ROS may lead to oxidation of cell macromolecules and subsequent disturbance in the physiological functions and integrity of DNA, proteins and lipids.

Benzene after being metabolized in the liver and bone marrow produces toxic metabolites and free radicals, which are known to be responsible for oxidative stress (Uzma et al., 2010). Due to decreased level of antioxidant enzymes, pancreatic β-cells are readily prone to oxidative stress caused by increased levels of ROS (Modak et al., 2009). And the resultant oxidative impairment may lead to pancreatic β-cells dysfunction and disturbance in glucose homeostasis (Evans et al., 2003). Similarly, there are clinical reports of elevated level of urinary 8-deoxyguanosine (8-OHdG), plasma thiobarbituric acid substances (TBARS), insulin resistance, and loss of insulin sensitivity in type 2 diabetes (Dandona et al., 1996; Al-aubaidy and Jelinek, 2011). Moreover, in experimental investigations, it has been demonstrated that oxidative stress disturbs insulin signaling pathway along with a significant decrease in insulin sensitivity (Gardner et al., 2003; Dokken et al., 2008; Archuleta et al., 2009). The exact mechanism by which oxidative stress causes insulin resistance is still not clear, but it has been suggested that alterations in mitogen activated protein kinase (MAPK) and over expression of certain pro inflammatory cytokines may be responsible (Styskal et al., 2012).

The concern on the role of chemical pollutants in the incidence of metabolic diseases is growing. Recently, many environmental pollutants have been reported to cause disruption in pancreatic β-cells function. For example, cadmium (Cd), arsenic (As), pesticides and mercury (Hg) have been reported to cause oxidative stress in pancreatic β-cells and subsequent disturbance in insulin release and blood glucose (Hectors et al., 2011; Lu et al., 2011; Chang et al., 2013).

Benzene exposure has been documented to cause genotoxicity, reproductive toxicity, and immune toxicity (Bahadar et al., 2014a). This study aimed to examine benzene induced oxidative impairment, effect on glucokinase (GK) activity, and insulin secretion, in rat pancreatic islets.

2. Materials and methods

2.1. Chemicals

Benzene purity (>99%) lot #270709-1L, adenosine diphosphate (ADP) sodium salt, adenosine triphosphate (ATP), bovine serum albumin (BSA), collagenase, D-glucose, 2,7-dichlorodihydrofluorescein-diacetate (DCFH-DA), 5,5-dithiobis (2-nitrobenzoic acid (DTNB), ethylenediaminetetraacetic acid (EDTA), 3-(4,5-Dimethylthiazol-2-yl-)-2-5-Diphenyltetrazolium bromide (MTT), glucose-6-phosphate dehydrogenase (G6PD), glucose oxidase, glucose peroxidase, HEPES, K_2HPO_4, $MgCl_2$, nicotinamide adenine dinucleotide (NADH), O-dianizidine, phosphate buffer, phosphoenolpyruvate, rhodamine 123, tetrabutylammonium hydrogen sulfate, thiobarbituric acid (TBA), trichloroacetic acid (TCA) and Tris–HCl, were purchased from Sigma Aldrich (Dorset, England). Rat specific insulin kit was purchased from Mercodia Co, Sweden and rat specific 8-OHdG ELISA kit from Cusabio.

2.2. Animals and experimental design

Total 24 adult male Wistar rats (weight 230–240 g, age 2–3 months old) were acquired from the animal house of Pharmaceutical Sciences Research Center, Tehran University of Medical Sciences (TUMS), Iran. All animals were housed under standard laboratory conditions with free access to water ad libitum and balanced pellet food. Before the experiment, all animals were acclimatized for one week to laboratory conditions. The housing temperature was ($25 \pm 1\,^{\circ}C$) with 12 h dark/light cycle and 50% humidity. All ethical guidelines on the use of animals for investigation purposes were followed and the experimental protocols were approved by the TUMS ethics committee on medical research with code number 92-03-103-23969.

Rats were randomly divided into four groups ($n = 6$ in each group). All groups were separately housed in polypropylene cages. Groups were divided as;

Group 1: Served as a control and only received corn oil.
Group 2: Received 200 mg/kg benzene dissolved in corn oil.
Group 3: Received 400 mg/kg benzene dissolved in corn oil.
Group 4: Received 800 mg/kg benzene dissolved in corn oil.

Both vehicle and benzene were administered orally via gavage, at 5 mL/kg of body weight, for 4 weeks. The doses that had shown effects on oxidative stress markers in our pilot study were considered in the main study. Food and water intake were recorded throughout the experiment. Moreover, fresh benzene solution of the above concentration was prepared on a daily basis.

2.3. Selection of doses and route of administration

The doses were selected on the basis of benzene oral LD50 in rats and in light of previously published studies. In the present study, the highest dose is approximately 1/3 of LD50 (2990 mg/kg, Material Safety Data Sheet (MSDS), Sigma Aldrich) and other doses were calculated around 1/2, 1/4 of maximum dose in order to see any concentration-dependent toxicity on pancreatic islets. Previously, for assessing systemic toxicity in animals, oral route has been employed as the most preferable one for administering a precise dose of benzene.

2.4. Measurement of fasting blood sugar (FBS) and insulin

At the end of the specified treatment period, rats of all groups were kept fasted for 10 h. Blood samples were collected by heart puncture for measuring FBS and plasma insulin.