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The effect of occupational exposure on pro/antioxidant balance in the blood of non-smoking and smoking smelters with diabetes

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ABSTRACT

Arsenic, lead and cadmium, potent environmental toxicants have been reported to induce diabetes mellitus, but their potential biological mechanism(s) have not been much investigated. The present study was designed to correlate parameters of pro/antioxidant balance with occupational exposure on heavy metals and smoking in smelters with diabetes compared on control group.

The results showed a significant increase in the concentration of arsenic, cadmium and lead in the blood and urine of smelters, while smoking caused a further increase in the concentration of these metals. Increasing γ-glutamyltransferase activity and lead concentration due to occupational exposure in copper foundry, tobacco smoke and co-existing diabetes were observed. Also these factors have synergistic effects on metallothionein and glutathione concentrations as well as glutathione dependent enzymes activities.

Our data suggests that sub-chronic arsenic, lead and cadmium exposure induces diabetic condition which may be mediated due to increased oxidative stress in blood.

1. Background

Workers at copper foundry smelting are occupational exposure to high level of arsenic (As), lead (Pb) and cadmium (Cd) mainly in air atmosphere and are absorbed to human organism via respiratory system. Harmful effects of the exposure to these toxicants alone have been documented for many years (Chlebda et al., 2004). These metals do not play any physiological role, but can cause variable biochemical and functional changes in exposed person (Chlebda et al., 2004; Sabolić et al., 2010). It is know that exposure to As can induce various complications involving dermatological effects, cardiovascular effects, pulmonary disorders, reproductive and neurological effects (Mohammed et al., 2015). Cd toxicity is associated with pulmonary, renal, hepatic, skeletal, reproductive and cardiovascular dysfunctions and is classified as a group I human carcinogen by the International Agency for Research on Cancer (Zhai et al., 2015). Nervous system, haemopoietic system and kidney belong to crucial organs during exposure to Pb (Chlebda et al., 2004). The level of Cd exposure in copper smelting is lower, but it can accumulate and remain in the system for 15–30 years, which is responsible for the toxicity in various organs. Therefore not only high concentrations, but also long-term exposure to these elements, may induce different diseases such as diabetes (Wang et al., 2014). Also it was confirmed that the deficiency and overload of some essential trace elements such as zinc (Zn) and copper (Cu) may play a major role in the development of diabetes. As, Pb and Cd are able to induce, directly or indirectly, diabetes development. Concomitant influence of As, Pb and Cd as well as their interactions on diabetes development have not been fully examined. Mechanisms involved in As induced diabetes are mainly insulin resistance and pancreatic β-cell dysfunction. Arsenic is a well-known disruptor of endocrine system including pancreas, which can result diabetes development. Apoptotic death/damage of pancreatic β-cell due to increased levels of reactive oxygen species (ROS) and oxidative stress (OS) is also considered to be a common mecha-

Abbreviations: As, arsenic; CAT, catalase; Cd, cadmium; Cu, copper; Cu/Zn SOD, superoxide dismutase; FAAS, flame atomic absorption spectrometry; GFAAS, graphite furnace atomic absorption spectrometry; GGT, gamma-glutamyltransferase; GPx, peroxidase glutathione; GSH, glutathione; GST, glutathione transferase; H_2O_2, hydrogen peroxide; MT, metallothionein; OS, oxidative stress; Pb, lead; ROS, reactive oxygen species; $O_2^{-\bullet}$, superoxide anion; TBARS, thiobarbituric acid reactive substances; Zn, zinc.

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nism in increasing the risks of diabetes due to As exposure (Lu et al., 2011).

Mechanism of OS inducing by heavy metals is explained in two ways. One of them is increased ROS accumulation and as a result OS. Second is decrease of antioxidant defense system via their binding to sulfhydryl groups or displacement of essential elements in the reactive centers of many proteins/peptides such as metallothionein (MT) and glutathione (GSH) as well as enzymes eg. superoxide dismutase (Cu/Zn SOD), peroxidase glutathione (GPx), glutathione transferase (GST) (Bizon and Milnerowicz, 2014; Bizon et al., 2013; Śliwińska-Mossoń et al., 2012). Also, it was shown that gamma-glutamyltransferase (GGT) activity can be a useful marker of OS as well as higher GGT activity is associated with increased risk of type 2 diabetes (Bradley et al., 2013).

Exposure to tobacco smoke is an additional factor increases OS and heavy metals concentrations in human organism (Milnerowicz et al., 2015). Many epidemiological studies indicate that cigarette smoking is an independent risk factor for diabetes development (Śliwińska-Mossoń et al., 2015; Foy et al., 2005; Wannamethee et al., 2001).

In our earlier studies we have shown that intensity of tobacco smoke as well as length of work years in copper foundry industry on heavy metals concentrations disorder pro/antioxidant parameters in biological fluids (Bizon et al., 2013).

Connecting all these knowledge, in present study we have analyzed the effect of exposure to As, Pb and Cd as well as tobacco smoke on the functioning of pro-oxidant/antioxidant system in the groups of non-smoking and smoking smelters with diabetes in comparison to smelters without diabetes and control groups using following parameters:

1. As, Pb and Cd concentrations as a parameters of long-term occupational exposure and tobacco smoke.
2. Zn and Cu concentrations as essential elements.
3. Thiobarbituric acid reactive substances (TBARS) concentration and GGT activity as a markers of oxidative stress.
4. GSH and MT concentrations and Cu/Zn SOD, GPx, GST activities as a crucial antioxidants.

2. Materials and methods

2.1. Ethics

This work has been carried out in accordance with the Declaration of Helsinki (2000) of the World Medical Association. This study was approved in terms of ethics by the Commission of Bioethics at Wroclaw Medical University (No. KB: 469/2008).

2.2. Subjects

The study was conducted on 107 smelters (range aged: 24–60 years old) worked in copper foundry. The criteria for inclusion in the study of workers group was working in the copper foundry in section: metallurgical, metallic charge preparation, refiners or converter workers. Moreover the size and quality of the environmental exposure to lead and arsenic were the highest for smelters during at least last five years of work in comparison to other workers. All subjects were randomly selected and had not been taking any medications other than antidiabetes drugs for the past year (receiving only diabetes treatment). None of the subjects was receiving antioxidant supplementation.

The control group included 38 healthy men (range aged: 24–57 years old), occupationally not exposed to heavy metals, who were qualified by occupational physician as healthy, able to work, who had no previous history of occupational or environmental exposure to heavy metals, what confirms a concentrations of lead and arsenic in blood and urine. All persons inhabited the similar region, within the 50–80 km from copper foundry.

Our investigation was focused only on male subject. Table 1 presents data concerning age, body mass index, years of work in copper foundry, and cotinine concentration in serum of smelters and persons of control group divided into smokers and non-smokers. Data about smoking were obtained from direct personal interview and verified by the determination of serum cotinine concentration—the metabolite of nicotine. Among the study group of smelters, 14 non-smokers and 17 smokers were diagnosed with diabetes. The data on diabetes had been obtained from direct personal interview and were verified by determination of blood glucose and insulin concentration. Insulin value was interpreted together with the glucose concentration at the time of sampling. Glucose concentration within normal range confirmed the stable course of diabetes (Table 1).

2.3. Methods

Smoking and non-smoking smelters with diabetes have shorter time of employment in copper foundry than smoking and non-smoking smelters without diabetes. Mean age of smoking smelters with diabetes were significantly lower when compared to smoking and non-smoking smelters without diabetes. All groups were similar in case of BMI value (Table 1).

2.3.1. Blood and urine collections

Venous blood was collected in the morning, after 12-h fasting. Serum was obtained according to the standard procedure by taking venous blood for disposable trace element-free tubes (Cat. No: 03.1524.001, Sarstedt, Germany) with serum clotting activator, left at 25 °C to complete thrombosis, and centrifuged (1.200*g*/20 min). In order to obtain plasma, blood was collected into trace element-free tubes containing EDTA-K 2 (No. Cat: 04.1931.001, Sarstedt, Germany) and lithium heparin (Cat. No: 05.1106.001, Sarstedt, Germany), immediately gently mixed, and centrifuged (2.500*g*/15 min). The obtained serum, plasma, and whole blood were portioned and stored in sealed tubes (Cat. No: 0030102.002, Eppendorf, Germany). The pellet erythrocyte was washed in equal volume of ice-cold 0.9% NaCl. This process was repeated twice. The washed cells were lysed by addition of ice-cold double distilled water: in case of heparin 1:1.4 and in case of EDTA 1:4.

In order to obtain samples for analysis of GSH and GSSG, 1.050 mL of distilled water was added to 150 mL of whole blood, then mixed and incubated for 10 min. Next, 300 mL of 25% metaphosphoric acid was added (Cat. No: 253-433-4, Sigma-Aldrich, Germany), mixed and incubated for 10 min and centrifuged for 6 min at 13.000g.

The urine was collected in the morning, according to the standard procedure. Subjects were asked to wash their hands before supplying the urine, in order to reduce contamination. Urine samples were stored in special container containing liquid nitric acid.

The biological material was frozen at −80 °C until analysis.

2.3.2. Method for the determination of concentrations metals

All the elements examined: lead, cadmium, zinc, copper and arsenic were determined by atomic absorption spectrometry using SOLAAR M6, ThermoElemental Co.

Lead concentration in blood: graphite furnace atomic absorption spectrometry (GFAAS), absorbance measurement at 283.3 nm wave, with Zeeman background correction. The reference materials BCR-194, −195, −196 by IRMM, UE were used.