THE EFFECT OF ESTRADIOL ON THE OXIDATIVE DAMAGE AND TRACE ELEMENT LEVEL DETERMINED IN THE LIVER OF RATS TREATED WITH DIMETHYLARSINIC ACID

Eybl V., Kotyzová D., Koutenský J., Glattre E.
1Department of Pharmacology and Toxicology, Charles University in Prague, Faculty of Medicine in Plzeň, Czech Republic
2Cancer Registry of Norway, Oslo, Norway

SUMMARY
DMA – dimethylarsinic acid (cacodylic acid) – used as an herbicide, is the major metabolite formed after the exposure to inorganic arsenics in mammals. It is considered to have an important role in arsenic carcinogenesis through the induction of oxidative damage in various tissues. Estradiol, apart from its main hormonal effect, displays both prooxidative and antioxidative action depending on the condition of the treatment. The oxidative stress plays a crucial role in estrogen-induced carcinogenesis. In the experiments performed in female Wistar rats receiving drinking water ad libitum with 0.01% DMA for 10 weeks, one half of rats was treated with 17β-estradiol (0.1 mg/rat s.c., twice a week) starting the 3rd week. One more group received estradiol only and last group served as controls receiving drinking water without treatment. The DMA enhanced lipid peroxidation in the liver, estradiol treatment potentiated this effect of arsenic. The GSH level was enhanced in DMA+estradiol treated group. In estradiol-only treated group both the lipid peroxidation and GSH content were increased. The administration of estradiol caused an enhancement of several trace element concentrations in the liver, mainly that of iron and copper. The critical role of estrogen on the development of oxidative stress was thus proved.

Key words: dimethylarsinic acid, DMA, estrogens, estradiol, oxidative damage, trace elements

Address for correspondence: V. Eybl, Department of Pharmacology and Toxicology, Charles University in Prague, Faculty of Medicine in Plzeň, Karlovarska 48, 301 66 Plzeň, Czech Republic. E-mail: vladislav.eybl@lfp.cuni.cz

INTRODUCTION
Arsenic and its compounds represent one of the most dangerous environmental contaminants (1). The mode of action of arsenicals has been studied intensively in the last years (2).

The main metabolite of both trivalent and pentavalent arsenic compounds in human body is considered dimethylarsinic acid (DMA, cacodylic acid)(3). This compound, used also as herbicide, exerts expressive prooxidative effects and is considered to be a complete carcinogen (1). However, the contaminants are not isolated in their effects on living organism. Special positions among the contaminants have hormone steroids, mainly estrogen derivates. These compounds are denominated as endocrine-disrupting chemicals (4,5, 6,7). Among these estrogen chemicals belong nature hormones, their analogs and a group of phytoestrogens.

Estrogens are important not only from the point of view of their physiological and endocrinological role but they can also exhibit prooxidative or antioxidative effects, according to the conditions of action (8). They exert neuroprotective effects but on the other hand can cause liver damage. Long-term exposure to low level of estrogens is associated with carcinogenicity where oxidative stress may be the plausible mode of action (9).

In this paper we present the study on the effect of co-exposure of natural estrogen 17β-estradiol and dimethylarsinic acid on the liver, one of the target organs of their toxicity. The influence of these compounds on oxidative damage and trace elements status has been estimated.

METHODS
Female Wistar rats (Anlab Prague, CZ) weighing 260 ± 30g have been drinking distilled water containing dimethylarsinic acid (cacodylic acid, Sigma Chemical Co.) 214 mg/l (0.01% arsenic solution) ad libitum for 10 weeks. Control animals received distilled water only. Starting the third week of the experiment the half of DMA-treated and control rats was injected s.c. with 17β-estradiol (Agofollin, Biotika) 0.1 mg/rat twice a week, remaining animals received the equivalent amount of vehiculum (sunflower oil). The animals were kept under standard laboratory conditions with free access to standard pellet diet in a controlled temperature room (21-23 °C) and 12 h light/dark cycle. At the end of the experiment the rats were killed in ether anesthesia by decapitation. The liver and kidneys were quickly excised, washed in ice-cold saline, weighed and stored frozen at -70 °C until analysis. The level of reduced glutathione (GSH) was determined by Ellman’s reagent in fresh liver homogenates in the same day. Peroxidation of lipids (LP) was estimated in liver homogenate using thiobarbituric acid test and...
expressed as malondialdehyde (MDA) production. Tissue of liver was analyzed for arsenic and essential elements – zinc, copper, iron, calcium and magnesium – content using atomic absorption spectrometry (SpectrAA 220 FS instrument, Varian Australia Ltd.). Data are presented as means ± SD; the statistical significance of differences between the groups was determined using Student’s unpaired t-test. Numbers of animals (N) are stated in the Tables.

RESULTS

The results in Fig. 1 demonstrate the effect of estradiol administration on lipid peroxidation in the liver of DMA-exposed and control rats. Arsenic treatment alone as well as estradiol treatment increased lipid peroxidation (both by 15%) compared to control group. DMA and estradiol co-exposure produced a further increase in lipid peroxidation by 38% as compared to controls and by 20% as compared to DMA-treated group.

Figure 2 shows the level of glutathione in the liver of rats. DMA treatment alone had no effect on glutathione level. An elevated level of GSH was observed in estradiol treated group (by 21%) and also in DMA+estradiol co-treated group (by 27%).

Table 1 demonstrates the accumulation of arsenic in the liver of both DMA and DMA+estradiol treated rats. Estradiol co-treatment had no effect on total arsenic concentration in the liver.

The results presented in Table 2 show the effect of DMA and estradiol treatment on bioelements level in the liver of rats. The results indicate the significant increase in copper (by 52%), zinc (by 31%), iron (by 38%), magnesium (by 29%) and calcium (by 15%) when compared to control level. The administration of DMA alone or in combination with estradiol exerted no effect on hepatic bioelements concentration.

DISCUSSION

The 17β-estradiol at the dose used in our experiment exerted a prooxidative effect which was synergistic with the effect of DMA. Since it is known that both chemicals can induce carcinogenesis on the basis of induction of oxidative stress we consider this finding to be highly important. By this time no experimental data on this matter are available in the literature. These findings deserve further experimental studies. Since the hormone replacement therapy in postmenopausal women exerts a prooxidative effect (10), a further exposure to another prooxidative agent, e.g. DMA, might significantly increase the oxidative tissue damage. Relating to the global anthropogenic arsenic contamination and endemic occurrence of high level of arsenic in some parts of the world this problem is actual.

However, in our similar preliminary experiment in rats exposed to cadmium we were not able to demonstrate the synergistic effect of estradiol with this metal regarding to the oxidative damage.

In our experiments the 17β-estradiol caused a significant elevation of trace elements concentration in the liver. This finding is in agreement with the study of the impact of the hormonal replacement therapy on the trace mineral status in postmenopausal women (11). By now the problem was not studied experimentally.

Our results demonstrate the need to study the effects of coexposure to different contaminants on the organism.
ANTIOXIDANTS – CLINICAL ASPECTS

(REVIEW)

Hlúbík P., Střítecká H.
Purkyně Military Medical Academy, Department of Military Hygiene, Hradec Králové, Czech Republic

SUMMARY

Interest in nutrition and food consumption in advanced countries has become an important instrument of active care for maintaining the population’s health. Basic orientation in food composition and in topical knowledge about the effectiveness of individual nutrients in the human organism form an indispensable part of nutrition and health consciousness and it can significantly facilitate and improve activity in both professionals and non-professionals, or it can correct some subjective opinions and deep-rooted imaginations. At the end of the last century progress in molecular biological research led to better knowledge about previously unknown functions of nutrients in the metabolism and also knowledge about relationships among the individual nutrients. The interest of research institutes was concentrated on selected nutrient groups and also on vitamins. Attention was focused on verification of the role of vitamin E and β-carotene in strengthening the antioxidative capacity of the organism and immunity system as prevention against degenerative diseases, atherosclerosis and tumorous diseases. Attention was focused on verification of the role of vitamin E and β-carotene in strengthening the antioxidative capacity of the organism and immunity system as prevention against degenerative diseases, atherosclerosis and tumorous diseases. Attention was attracted to the fact that folacin acts preventively against defects of the neural tube and against hyperhomocysteinemia as an independent atherosclerosis risk factor. Also the data about the organism’s needs, for example vitamins, are mostly not quite complete, especially in the sphere of detailed knowledge of their preventive effect concerning the so-called civilization diseases, their use in the organism and the reaction of the organism to their insufficient or redundant supply.

Key words: vitamins, reactive oxygen species, civilization diseases, antioxidants

Address for correspondence: P. Hlúbík, Purkyně Military Medical Academy, Department of Military Hygiene, Třebešská 1575, 500 01 Hradec Králové, Czech Republic. E-mail: hlubik@pmfhk.cz

Many famous theoretical and clinical research institutes have intensively examined the negative role of oxidative stress and the protective effect of vitamins, possibly micronutrients with antioxidative effect. Researchers’ interest in the possible participation of antioxidative stress in the etiopathogenesis of civilization degenerative diseases is still increasing. Free radicals play an important role in the process of cellular senescence, in the pathogenesis of chronic diseases, in carcinogenesis, in the pathogenesis of glaucoma and in the acceleration of atherosclerotic changes of the vascular parries, which is mostly followed by the development of cardiovascular diseases. In the acceleration of the atherosclerosis processes the decisive role is probably played by the oxidation of low-density lipoproteins (LDL).

Free radicals are defined as chemical species that possess one or more unpaired electrons. The term “Reactive Oxygen Species” (ROS) collectively describes free radicals such as $O_2^-$, $OH$, and other non-radical oxygen derivatives such as hydrogen peroxide ($H_2O_2$). These reactive oxygen intermediates may participate in...