Selenium and Diazinon Neurotoxicity after an Intraperitoneal Administration in Rats

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Abstract

The purpose of this study was to evaluate the effect of selenium, diazinon and the mixture of diazinon with selenium on rat serum cholinesterase activity after an intraperitoneal administration. Forty rats were divided into three experimental and one control groups. Animals in the first group were dosed with selenium (2 mg/kg b.w.), in the second group with diazinon (20 mg/kg b.w.) and in third group with simultaneous administration of diazinon and selenium in the same doses as in the individual dosage intraperitoneally. Catalytic activity of cholinesterase was determined 36 hours after the compounds administration. Significat decreases ($P<0.001$) in cholinesterase catalytic activity were observed in the group with diazinon (to 1.81 μkat/L) and diazinon+selenium exposure (to 1.25 μkat/L) when compared to the control value 3.69 μkat/L. On the other hand, cholinesterase activity in the selenium group was not significantly affected. The results indicate that diazinon negatively affects the neuronal trasmission. Selenium was not able to prevent the neurotoxic effect of diazinon, moreover, the decrease in the cholinesterase activity was sharper in the diazinon+selenium group.

Keywords: acute, cholinesterase, diazinon, neurotoxicity, rat, selenium.

1. Introduction

Diazinon (O,O-diethyl O-2-isopropyl-6-methylpyrimidin-4-yl phosphorothioate) is a non-systemic insecticide used in agriculture to control soil and foliage insects and pests on a variety of fruit, vegetable, nut and field crops. Diazinon is also used on non-lactating cattle in an insecticidal ear tag. Prior to the cancellation of all residential uses by 2004, diazinon was used outdoors on lawns and gardens, indoors for fly control and in pet collars designed to control fleas and ticks. Diazinon was one of the most widely used insecticides for household and agricultural pest control. In 2000, the United States Environmental Protection Agency (U.S. EPA) announced an agreement with the registrants of diazinon to cancel all residential uses of diazinon. Indoor uses were cancelled in 2002 and outdoor uses in 2004, leaving only agricultural uses for diazinon [1]. Current agricultural uses of diazinon are limited to selected crops, and diazinon products (other than cattle ear tags) are regulated as restricted use pesticides [2]. Organophosphate insecticides are able to induce a number of distinct neurotoxicities [3]. Like other organophosphates, diazinon shows toxic action by inhibiting the activity of acetylcholine esterase (AChE) by phosphorylation of the serine hydroxyl group of the enzyme which results in accumulation of acetylcholine [4]. The neurotoxic effects of diazinon may be connected with the Toll-like receptor 4 (TLR4). Diazinon injection affects neurotrophin expression in the hippocampus in TLR4-defective mice but not in

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TLR4 intact mice. These results suggest that a defective TLR4 signaling pathway in the mouse hippocampus can be easily affected by diazinon administration [5]. Diazinon, as a thiophosphate, is metabolized by Cytochrome P450s into its oxon form that inhibits acetylcholinesterase (AChE). One model suggests that the result of AChE inhibition is an accumulation of the neurotransmitter acetylcholine (ACh) in the synapse which causes hyperstimulation of ACh receptors (AChR) [6, 7]. The oxon forms are, thus, responsible for most of the fatalities occurring following phosphorothionate OP insecticide intoxication [8]. These active metabolites (oxons) are hydrolyzed by paraoxonase (PON1) and low PON1 status may increase susceptibility to OP toxicity in humans. Low PON1 activity may also contribute to the developmental toxicity and neurotoxicity of OPs, as shown by animal and human studies [9]. Most frequently described symptoms of acute diazinon toxicity are headaches, nausea, vertigo, blurred vision, feeling of pressure in chest, respiration problems, muscular weakness or convulsions, diarrhea and vomiting. Typical symptoms of irritated nervous system are confuse, anguish, melancholy and insomnia. Symptoms of chronic poisoning are always connected with depression of cholinesterase activity [10, 11].

Selenium (Se) is toxic in large amounts, but trace amounts are necessary for cellular function in many organisms, including all animals and humans. Selenoproteins, in which selenium is present as selenocysteine, present an important role in many body functions, such as antioxidant defense and the formation of thyroid hormones. Some selenoprotein metabolites play a role in cancer prevention. In the immune system, selenium stimulates antibody formation and activity of helper T cells, cytotoxic T cells and Natural Killer (NK) cells [12]. Selenium is characterized by a narrow safety range between deficiency and toxic doses [13, 14]. It is hypothesized that heavy metal toxicity, along with the non-metals arsenic and selenium, all share in part at least one common interaction in biological systems that causes toxicity symptoms to occur, the generation of superoxide [15]. In rats, the median lethal dose (LD50) of intraperitoneal selenomethionine (SeMeth) was determined to be 4.25 mg Se/kg. The intravenous administration of selenium compounds in mice resulted predominantly in cardio-respiratory effects, hind limb paralysis and death. The LD50 was determined at 8.8 mg/kg for SeMeth and selenite was 4-fold more toxic than SeMeth [16]. The most common sign of human poisoning in China in 1983 was loss of hair and nails. In areas of higher incidence, lesions of the skin, nervous system, and possibly teeth may have been involved [17]. Chinese epidemiologic data suggest that 750–850 μg is the upper limit of a safe daily exposure [18]. The central nervous system and muscle function have been observed to be affected in both animals and humans after toxic Se exposures [19]. Isolated populations chronically exposed to Se in their environment were reported to have an increased risk of developing amyotrophic lateral sclerosis, a neuromuscular disease typified by progressive muscle weakness, paralysis, and death [20]. Estevez et al. [21] provide evidence that selenium induces neurodegeneration of cholinergic neurons through depletion of glutathione, a mechanism linked to the neuropathology of Alzheimer's disease, amyotrophic lateral sclerosis, and Parkinson's disease. In this connection, it is known that selenium may play different roles in the progression of Alzheimer's disease. Organoselenium compound supplemented in the diet normalized AChE activity [22].

The role of selenium in alleviation of the neurotoxic effects of many toxic compounds is confirmed [23]. Therefore, aim of this study was to find possible interactions between selenium and diazinon to induce or reduce the neurotoxic response after sole or simultaneous intake.

2. Materials and methods

Mature, 4 months old male rats of the Wistar strain (weighing approximately 410 g) were randomly divided into four groups of ten animals. The males were housed individually in plastic cages under constant temperature (20-22°C), humidity (55±10%), and 12/12 h cycle of light and darkness with access to food (feed mixture M3, Machal, Czech Republic) and drinking water *ad libitum*. All experiments were conducted in accordance with accepted standards of animal care in accredited laboratory (SK PC 50004, SUA Nitra, Slovakia). Rats in the group A were injected with a single dose (20 mg/kg body wt) of diazinon (Sigma-Aldrich, USA) intraperitoneally in

physiological solution, rats of the group B were injected with a single intraperitoneal dose (2 mg/kg body wt) of selenium in the form of sodium selenite (Reachem, Slovak Republic) in physiological solution, and rats in the C group were given a mixture of selenium (2 mg/kg body wt) and diazinon (20 mg/kg body wt) intraperitoneally in physiological solution. The fourth group served as a control and received only physiological solution. Animals were anaesthetized with ether and sacrificed 36 h following an experimental administration. Blood samples were taken from hearts to the sterile tubes and then centrifuged at 3500 rpm for 20 minutes to the blood serum. Catalytic activity of cholinesterase was determined using the Bio-La-Test® (Lachema, CZ). This assay is based on the method of Knedel and Böttger [24]. Reaction mixture consists of non-hemolytic blood serum and butyrilthiocholineiodide and dithio-bis-nitrobenzoic acid (warmed at 37°C). The catalytic concentration of the enzyme is determined from the increase of absorbance of the incubation mixture between 30 and 90 seconds after start of the reaction at wavelength 405 nm. Results of cholinesterase catalytic activity are presented in μkat/L. Comparisons between the groups were assessed by one-way analysis of variance (ANOVA) and post hoc Scheffe test using the Statgraphics Plus 5.1 software.

3. Results and discussion

Results of the cholinesterase (ChE) catalytic activity analysis are presented in Table 1. Significant changes in cholinesterase catalytic activity were observed in each group of experimental animals in comparison with control group except of selenium-exposed rats. The cholinesterase catalytic activity significantly ($P < 0.001$) decreased in diazinon-exposed group from 3.69 μkat/L to 1.81 μkat/L. Organophosphorus compounds irreversibly inhibit the enzyme acetylcholinesterase resulting in excessive accumulation of acetylcholine, leading to the paralysis of cholinergic transmission in the central nervous system, autonomic ganglia, parasympatic nerve endings, some sympathetic nerve endings and neuromuscular junction [3, 25, 26]. Diazinon is well known to exert its toxic effects by inhibiting cholinesterase activity in plasma, erythrocytes and brain [27, 28].

Selenium administration did not cause significant changes in the AChE activity in our experiment. Estevez et al. [21] revealed that selenium-induced oxidative stress leads to decreased cholinergic signaling and degeneration of cholinergic neurons required for movement. Several studies observed that the free radical production could be associated with the decrease in the activity of AChE in brain [29]. Other studies show possible involvement of excessive intake of selenium in the etiology of amyotrophic lateral sclerosis and other neurotoxic effects [19, 20, 30]. Aside from these toxic effects of selenium, there is increasing interest in the protective effect of selenium compounds against the neurotoxic effects of many toxic substances and neurologic disorders. Several organophosphates have been shown to interact as mixtures to produce synergistic AChE inhibition.

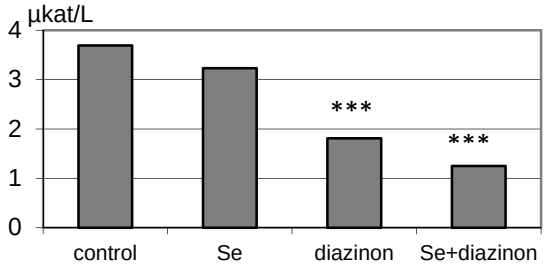
Table 1. Cholinesterase activity of experimental and control animals (μkat/L)

Group	n	Cholinesterase activity (x±s)	Variation coefficient (%)
Control	10	3.69±0.53	14.59
Selenium	10	3.23±0.60	18.69
Diazinon	10	1.81±0.83***	46.11
Selenium+Diazinon	10	1.25±0.31***	24.80

The values are expressed as means±standard deviation; ***P<0.001

Diazinon, malathion and ethropop inhibited the brain enzyme activity (90%) in salmon [31]. The interaction of various toxic compounds is important point in toxicity and risk assessment analysis. In our previous work, a decreased enzyme activity was more visible when cadmium and diazinon were injected simultaneously than in individual doses [32]. Some studies have demonstrated the neuroprotective action of selenium organic compounds [33- 35]. Selenium restored AChE activity and had antioxidant and antinitrosative effects in rats [22].

In the present work, the AChE activity decrease was more intense than in the diazinon exposed rats. We cannot confirm the protective effect of selenium on the ChE activity. The selenium and diazinon combination induced the stronger response in the exposed rats (Figure 1). The key factor seems to be a dose of selenium. The dose used in our experiment (2 mg/kg body wt) is higher than needed for protection of ChE activity and probably reached the level in which the oxidative stress can appear. However, this effect was not observed in the selenium-exposed group. The selenium dose alone was not able to cause any significant changes in the ChE activity. We can conclude that the significant changes recorded in selenium-diazinon group may be a result of synergistic effect of selenium and diazinon when selenium is administered in higher dose.



***P < 0.001

Figure 1. Cholinesterase activity in the control and experimental rats.

4. Conclusions

The results of this work have shown that acute intraperitoneal diazinon intoxication causes significant inhibition of the cholinesterase activity in the blood plasma. Selenium in the sole single dose had no effect but the inhibition of ChE increased when selenium and diazinon was administered simultaneously. We can conclude that intake of selenium in excessive doses with diazinon may elevate the neurotoxic effect of diazinon.

Acknowledgements

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