Diazinon and Cadmium Neurotoxicity in Rats after an Experimental Administration

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Abstract
The aim of this study was to describe the changes in cholinesterase activity in separate doses and after co-administration of cadmium and diazinon intraperitoneally and to assess toxicity and interactions of diazinon and cadmium on the nervous system in male rats. 40 male rats were randomly divided into three experimental and one control group (10 rats in each group). Blood analyzes were performed 36 hours after an intraperitoneal administration of observed compounds. The statistical evaluation of the results showed significantly ($P < 0.01$) reduced activity of cholinesterase in all experimental groups. The enzyme activity decreased from the control value 3.69 μkat/L to 1.81 μkat/L (diazinon group), 1.83 μkat/L (cadmium group) and 1.35 μkat/L (cadmium+diazinon group). These results indicate that both cadmium and diazinon are potent to manifest the neurotoxic effects. Moreover, a synergistic effect of the co-administered cadmium and diazinon in the nervous system has been observed.

Keywords: cadmium, cholinesterase, diazinon, neurotoxicity, rat

1. Introduction

Diazinon (O,O-diethyl O-2-isopropyl-6-methylpyrimidin-4-yl phosphorothioate) belongs to the group of organophosphate insecticides (OP). This large group of OP compounds includes such important insecticides as the phospho(ro)thio(n)ates parathion, methyl parathion, chlorpyrifos (CP) and diazinon (DZ), which are among the most extensively used OPs in industrialized and Third World countries [1]. Diazinon used to control cockroaches, fleas and ants. It is also used to control a wide variety of sucking and leaf eating insects. It is used on rice, fruit trees, sugarcane, corn, tobacco, potatoes and on horticultural plants. Diazinon has veterinary use against fleas and ticks [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]. Following their entry into the body, these substances undergo a biotransformation reaction in which the sulfur of their P=S group is replaced by oxygen. This results in the conversion of the parent phosphorothionates to their oxygen (or oxon) analogs. The desulfuration reaction results in a dramatic increase in the acute toxicity of the phosphorothionates as oxon forms exhibit a AChE-inhibiting potency that is two to three orders of magnitude higher than that of the parent phosphorothionates [5, 6]. The oxon forms are, thus, responsible for most of the fatalities occurring following phosphorothionate OP insecticide intoxication [7]. Accumulation of the neurotransmitter on the connections between

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nerves and muscles causes uncontrolled muscular contraction and algospasmus, between nerves and glands causes continual secretion of these glands, while acetylcholine accumulation between certain nerve cells in a brain causes sensory behavior disorders [2, 8, 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. [8, 10]

Cadmium (Cd) is a toxic metal which still attracts the attention of researchers and the public because its level in food products often exceeds the maximum allowable limits. The harmful effects of cadmium on living organisms are well described in many tissues and organs and its exposure has also been associated with nephrotoxicity, osteoporosis, neurotoxicity, carcinogenicity and genotoxicity, teratogenicity, and endocrine and reproductive effects [11- 14]. Since the blood-brain barrier keeps Cd outside the central nervous system, reported neurotoxic effects of Cd during development are likely to be secondary to an interference of Cd with Zn-metabolism and not a direct effect of Cd on brain cells [15]. *In vitro* effect of this metal on AChE and ectonucleotidase (NTPDase and ecto-5'-nucleotidase) activities in zebrafish brain has been previously tested. Cadmium treatments did not alter significantly the zebrafish brain AChE activity. However, cadmium inhibited ADP hydrolysis and ecto-5'-nucleotidase activity. Changes on NTPDase and ecto-5'-nucleotidase activities can be an important mechanism involved in neurotoxic effects promoted by cadmium [16]. In rats exposed to Cd (3mg/kg/day subcutaneously) for 3 weeks, a significant increase in the levels of lipid peroxidation and protein carbonyls along with significant decrease in the levels of reduced glutathione and total sulphydryl groups and the activities of AChE, superoxide dismutase, catalase, glutathione peroxidase, gluthione-S-transeferase, membrane bound enzymes were observed in brain tissue [17].

To understand the body burdens of environmental contaminants, knowledge on the health risks associated with current patterns of exposure is needed, including increased risk resulting from exposures to multiple chemical [18]. Therefore, aim of this study was to find possible interactions between cadmium and diazinon to induce the neurotoxic response after sole or simultaneous intake as they occur in the animal and human food.

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). 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 cadmium in the form of $CdCl_2$ (Reachem, Slovak Republic) in physiological solution, and rats in the C group were given a mixture of cadmium (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 [19]. 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

After the cadmium administration, one male died but no more deaths were observed in any of the experimental groups. Experimental animals did not show any visible behaviour change in comparison with the control group. Results of the cholinesterase catalytic activity analysis are presented in Table 1.

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
Cadmium	9	1.83±0.70**	38.18
Diazinon	10	1.81±0.83**	46.11
Cadmium+Diazinon	10	1.35±0.40**	30.29

The values are expressed as means±standard deviation; **P < 0.01

Changes in cholinesterase catalytic activity were observed in each group of experimental animals in comparison with control group. We observed statistically significant (P < 0.01) decrease in cholinesterase catalytic activity in cadmium exposed group from 3.69 μkat/L to 1.83 μkat/L. A similar decreased enzyme activity was detected in diazinon exposed group (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, 20, 21]. Diazinon is well known to exert its toxic effects by inhibiting cholinesterase activity in plasma, erythrocytes and brain [22-23]. An increasing number of publications have pointed out that the effects of toxic substances depend not only on their administration levels but also on the interaction with other substances. In the experiment realized by Pari and Murugavel [17], the activity of AChE in brain and plasma was significantly (P < 0.05) decreased in Cd intoxicated rats which correspond with our results. However, the administration of diallyl tetrasulfide (40 mg/kg body weight) significantly (P < 0.05) reversed the activities of AChE to near normal in Cd exposed rats. The impairment of the catecholaminergic and serotoninergic transmission has been found in Cd treated animals [24]. The study of brain enzyme activities, such as AChE is essential in detecting the neurotoxic effects of certain heavy metals [17]. Since brain AChE activity is an important regulator of the behavioral processes, the decreased level of AChE in plasma and brain might be one of the indicators for Cd induced hurdle in brain although it is not clear to what extent it contributes directly [17]. Several studies observed that the free radical production could be associated with the decrease in the activity of AChE in brain [25]. According to recent findings, diazinon toxicity is particularly related not only with acetylcholinesterase inhibition, but also with oxidative stress [2]. Similarly, cadmium can penetrate the blood brain barrier and accumulate into the brain, which is easily susceptible to Cd-induced lipid peroxidation and oxidative stress has been implicated in the pathogenesis of the cadmium-induced pathotoxicity [26, 27]. The effect of certain substance in the mixture with another one may manifest antagonistic, additive or synergistic effect. In our experiment, the simultaneous administration of cadmium and diazinon caused increased inhibition of cholinesterase activity when compared with the control. The activity of enzyme significantly (P < 0.01) decreased from the control 3.69 μkat/L to 1.35 μkat/L. Decreased enzyme activity observed in the cadmium-exposed and diazinon-exposed groups was even more visible when cadmium and diazinon were injected simultaneously. This reaction revealed the

synergistic effect of both substances on the nervous system. Figure 1 shows the comparison of the cholinesterase activity in control and experimental groups and clearly presents the inhibiting activity of cadmium and diazinon no matter if administered solely or simultaneously.

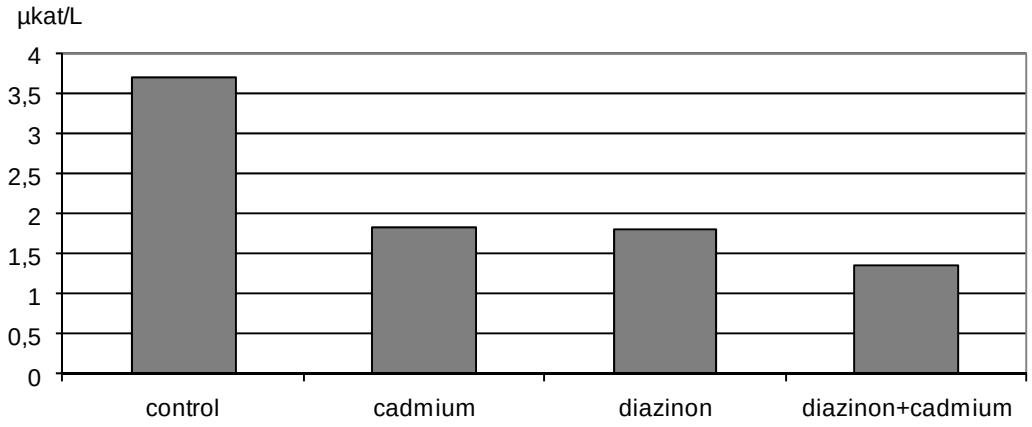

Figure 1. Cholinesterase activity in the control and experimental rats

Dietary exposure analysis of selected metals and pesticides revealed interesting correlations between the contaminants in food [28]. Pairwise correlation coefficients between contaminants in the respective pesticide groups ranged from 0.3 to 0.72, indicating that an individual highly exposed to one of these compounds is likely to also be highly exposed to others. Moreover, correlation coefficients found between cadmium and diazinon in men (0.70) and women (0.69) indicate that there is very high probability to be exposed to both compounds.

4. Conclusions

The results of this work have shown that acute intraperitoneal cadmium and diazinon intoxication causes significant inhibition of the cholinesterase activity in the blood plasma. This effect may be enhanced when both compounds enter the body. We can conclude that simultaneous intake cadmium and diazinon exhibits the synergistic effect in decreasing the activity of cholinesterase in blood plasma.

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