IN VITRO INHIBITION OF ACETYLCHOLINESTERASE ACTIVITY IN HUMAN RED BLOOD CELLS BY CADMIUM AND LEAD

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Abstract-The effects of cadmium and lead on human erythrocyte acetylcholinesterase activity were studied. Blood used in this study was obtained from 24 healthy individuals, then after hemolysation, treated with 3 various concentrations of cadmium and lead. A strong inhibition of acetylcholinesterase was noted in treated samples by cadmium and lead. The remaining activity was found to be 65% with the highest concentration of cadmium (10^{-5} M, $p<0.01$), 82% with the middle (10^{-6} M, $p<0.01$) and 90% with the lowest one (10^{-7} M, $p<0.01$). In the case of lead, the remaining activity was found to be 81% with the highest concentration (50 μ g/dl, $p<0.01$), 87% with the middle (40 μ g/dl, $p<0.01$) and 94% with the lowest one (30 μ g/dl, $p<0.05$). Cadmium showed a nearly linear correlation between doses used and decrease in activity ($r^2 = 0.83$), lead showed a better correlation ($r^2 = 0.92$). The direct effect of metal ions on AChE, i.e. a decrease in quantity of the enzyme, may be a proposed mechanism for this depression.
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Key words: Acetylcholinesterase, human, erythrocyte, cadmium, lead

INTRODUCTION

Cadmium (Cd) is considered to have no biological function and is highly toxic (1). After absorption, cadmium is transported by blood cells and albumin (2). Lead (Pb) is biologically nonessential and if present in excessive levels in the body, it can cause clinical disorders both in human and animals (3). Once lead is absorbed, about 99% of that in blood stream binds to hemoglobin in erythrocytes (2). Pathogenesis associated with experimental lead poisoning have been described in fishes. Blood seems to be an important target of the deleterious effects of lead. Dawson (4) noted an anemia response in the lead exposed catfish, Ameiurs nebutosus. As in the higher vertebrates, basophilic stippling in the erythrocytes was observed following Pb poisoning in the rainbow trout, salmogairdeneri (5). There are many reports of the effects of cadmium and lead on several enzymes both in the intact animal and in vitro conditions. Cadmium activates, as well as inhibits acid phosphatase (9). It has been reported that Cadmium displays zinc in the alkaline phosphatase (ALP) (7). Cadmium inhibits lactate dehydrogenase (LDH) and glutamate oxaloacetate transaminase (GOT) (8). However, in vitro stimulation of LDH activty was reported (1,9). Seventy - four chemicals of different classes on the acetylcholinesterase (AChE) activity in the muscle of the fathead Minnows, pimephales promelas, were studied and it was found that transition metal cations, in general, are very toxic and inhibitory to this enzyme. They proposed the following order of effect: $Cu^{2+} > Au^{2+} > Pd^{2+} > Cd^{2+} > Pb^{2+} > Ag^{2+} > Hg^{2+} > Sn^{2+}$ (10). However, either depression or stimulation of AChE activity was reported in vivo(1). A decrease in glutathion, (GSH) level and GSH transferase in the red swamp crayfish by cadmium was reported(11). It has been demonstarted that oxidative enzymes, succinic dehydrogenase and NADPH-cytochrome P450 reductase are active in gills and can increase AChE activity(13). Also, there are some reports about significant decrease(14) or an increase then decrease in AChE activity(15). Numerous epidemiological studies, mortality studies and experiments in animals have also indicated the nephrotoxic effect of lead (16 - 18). Inhibition of 6 - aminolevulinic acid dehydratase (6 - ALAD), which catalyzes the formation of porphobilinogen from 6-amino levulinic acid in the hemoglobin biosynthesis, is perhaps the most conspicuous and specific effects of Pb observed both in fish as well as in mammals (19-21). Enzyme inhibition, or activiation by sublethal concentrations of chemicals including those having ecological significance has been described in a variety of organisms(22). Although the interaction between the

enzyme and its inhibitor/ activator may be highly specific, e.g. Pb-induced inhibition of 6-ALAD (23), estimation of erythrocyte cholinesterase (EC 3.1.1.8) activity provides additional information about many problems, because plasma cholinesterase (EC 3.1.1.7) can also be depressed by inherited traits or by other causes, notably liver disease (24, 25). In this respect our main purpose was to examine the effect of Cd^{2+} and Pb^{2+} on AChE actitivity in human red blood cells.

MATERIALS AND METHODS

Materials

All compounds used in this study were prepared from Merck chemical Co. (Germany). Water was distilled and deionized. Five - thio - nitrobenzoate was used to evaluate the micellar spectral shift.

Reagents

All reagents needed for measurement of AChE in this study were prepared as described previously (26, 27).

Samples

Blood was obtained from 24 healthy individuals who had no known exposure to cholinesterase inhibitors. Blood was centrifuged at 6000 rpm to separate erythrocytes from plasma. One - hundred microliters of the bottom layer was taken and added to 6 ml of distilled water. This procedure caused lysis of erythrocytes and release of acetylcholinesterase. Hemolysates were frozen until assayed.

Enzyme assays

Activity of AChE in erythrocytes was assayed according to the method of Ellman and coworkers (24) as modified by George and Abernethy (26). To 3 glass test tubes containing 3 ml of hemolyzed blood, was added 60, 80 and 100 μl from 0.794 mg/l lead nitrate solution for preparation of 30 , 40 and 50 μg lead per dl of blood , respectively.

These solutions were incubated for 30 min in a water bath set at 37°c and then were used for enzyme assay directly. Similarly, 3 various concentrations of cadmium (as cadmium sulfate) were prepared and used for enzyme assay.

Data analysis

Student t-test (paired two - tail) was applied to data of the control and treatment groups to determine the significance of any differences in AChE activity.

RESULTS

A strong inhibition of AChE activity was noted in vitro after exposure of erythrocytes to cadmium (Fig. 1) and lead (Fig. 2). The results in Fig. 1 and 2 reveal that cadmium and lead treatments decrease the AChE activity in the all concentrations used. There were significant differences ($P<0.01$) between cadmium treatments and the control. The remaining activity was found to be 65% with the highest concentration, 82% with the middle and 90% with the lowest cadmium concentration. In the case of lead, there were also significant differences ($P<0.01$) between two higher concentrations and the control.

The lower one also showed a slightly significant difference ($P < 0.05$). The remaining activity was found to be 81% , 87% and 94% respectively. Fig. 3 and 4 illustrate the dose response curve of cadmium and lead, respectively. Cadmium shows a good correlation coefficient ($r^2=0.82$) that improved a nearly linear correlation between decrease in activity according to cadmium concentrations. Lead showed the better correlation ($r^2 = 0.92$).

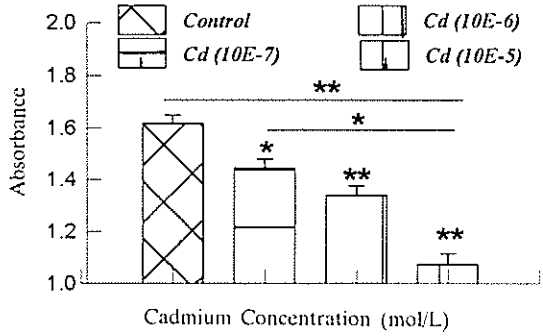

Fig. 1. Effects of various doses of cadmium on human erythrocyte acetylcholinesterase activity revealed by decreased absorbance at 440 nm. Data are mean ± SE, n = 24. *P<0.05; **P<0.01

In Vitro Inhibition of Acetylcholinesterase Activity

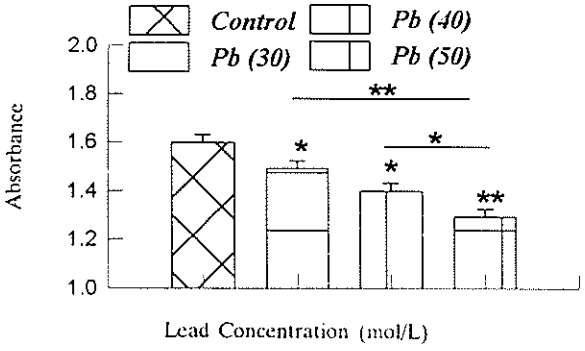

Fig. 2. Effects of various doses of lead on human erythrocyte acetylcholinesterase acivity revealed by decreased absorbance at 440 nm. Data are mean ± SE, n = 24.
* P<0.05; **P<0.01

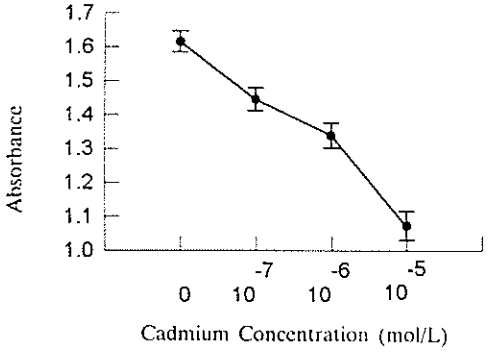

Fig. 3. Dose - response relationship between inhibtion of acetylcholinesterase activity revealed by decreased absorbance and increased concentration of cadmium.
Data are mean ± SE, n = 24. r^2= 0.83, P<0.01.

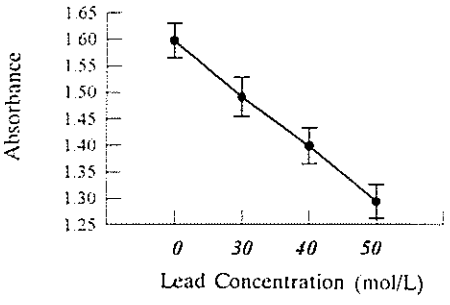

Fig. 4. Dose - response relationship between inhibtion of acetylcholinesterase activity revealed by decreased absorbance and increased concentration of lead.
Data are mean ± SE, n = 24; r^2= 0.92; **P<0.01.

DISCUSSION

Cadmium is an essential trace metal which progressively accumulates in the body. Cadmium appears in the workplaces in solder, a neutron absorbent in the nuclear industry, alkaline storage batteries, as amalgam in dentistry, a stabilizer for polyvinyl chloride etc. Cadmium exposure produces renal dysfunction, emphysema and osteomalacia (2). On the other hand, lead had been smelt, ingested as a homeopathic medicine, applied as a cosmetic, painted on buildings, and as a component of motor vehicles fuel. Lead serves no known useful purpose in the body. Recent works on the clinical effects of lead has focused on the subtle neuropsychiatric, reproductive, renal effects and endogenous enzyme interaction of chronic lead exposure (2,4). These two elements produce strong inhibition of a large number of enzymes that have functional sulfydryl group (6). AChE is one such enzyme. The depression of erythrocytes AChE activity might be caused by direct effects of metal ions, i.e. a decrease in quantity of the enzyme or may be due to interaction of metals and sulfydryl groups of the enzyme. In many cases of poisoning with organophosphorus agents and plasma anti - cholinesterases, precise determination of AChE is necessary. Chemical methods for cholinesterase assay fall into two groups; those that employ substrates that confer intrinsic action upon the method and those that do not. The former group includes those methods that rely on the hydrolysis of acetylthiocholine and butyrylthiocholine. Methods that do not use thiocholinesters as substrates lack specificity so that inhibitors must be used. Thus it is better to use the former mentioned mothods to determine the exact activity of ChE such as the method studied here.

Respectively it may be possible that in vitro exposure of blood to cadmium and lead as environmental pollutants during analysis. Based on data obtained here such exposure may result in false enzyme activity. On the other hand, cadmium and lead accumulate in the body and may be found in considerable concentrations in human blood. These elements in blood could interact with AChE or PChE and cause decreased activity of the enzymes. Thus it can cause false decrease in the measured activity of the enzyme and could lead to

incorrect diagnosis, treatment and prognosis prediction in anticholinesterase poisoning or other related cases. With regard to the great toxicological significance of precise determination of AChE actvity and the fact that environmental exposure to lead and cadmium still exists, it is important to take into account the results of this study to find the exact mechanism of AChE inhibition by lead and cadmium.

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