IN VITRO INHIBITION OF HUMAN ERYTHROCYTE ACETYLCHOLINESTERASE ACTIVITY BY ZINC AND MERCURY

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Abstract - The effects of zinc and mercury on human erythrocyte acetylcholinesterase activity were studied. Blood used in this study was obtained from 24 apparently healthy individuals and after hemolysation, was treated with 3 different concentrations of zinc and mercury. Significant suppressions in acetylcholinestrase activity were recorded in treated samples by zinc and mercury. When compared to controls the remaining activity was found to be 53% with the highest concentration of zinc (2.1 mg/dl, $p< 0.01$), 72% with the middle (1.4 mg/dl, $p<0.01$) and 85% with the lowest one (0.7 mg/dl, $p<0.01$). In the case of mercury, the remaining activity was found to be 55% with the highest concentration (8.4 ng/g, $p < 0.01$), 72% with the middle (5.6 ng/g, $p < 0.01$) and 79% with the lowest one (2.8 ng/g, $p < 0.01$). Mercury showed a good correlation between doses used and decreases in activity ($r=0.98$). Zinc also showed a linear correlation ($r= 0.99$). The direct interaction of metal ions with acetylcholinestrase is proposed as a mechanism for depressed enzyme activity. It is concluded that zinc and mercury contamination during acetylcholinesterase measurement can be a source of error that must be taken into account.
Acta Medica Iranica 38 (3): 156-159; 2000

Key Words: Acetylcholinesterase, mercury, zinc, human erythrocyte

INTRODUCTION

Recent years have witnessed significant attention being paid to the problems of environmental contamination by a wide variety of chemical pollutants including the heavy metals. Mercury and zinc, divalent group II B metals are the most prevalent commodities in the effluents and discharges entering the freshwater systems from industries involved in the production of caustic soda, paints, pesticides, batteries, petrochemicals, fertilizers, and alloys. Mercury, a non-essential metal, is a powerful biocide, and zinc, though essential in minute quantities, is also known for its high toxicity to freshwater fauna, especially fish which hold a strategic position in the transfer route to higher trophic levels (1). Mercury, one of the most toxic heavy metals, is a major pollutant in marine environment, and the human risks due to consumption of seafood contaminated with mercury have been reported (2). It has been proved that zinc causes gill damage and inhibition of normal growth besides causing severe damage to various tissues (1). The effects of heavy metals such as mercury on the activity of enzymes have been described in fishes (1,3,4,5). Mercury and zinc are listed along with cadmium as inhibitors of lactic dehydrogenase and glutamate oxaloacetate transaminase (4,6). The effects of 141 chemicals upon the activity of eight enzymes including mercury and zinc as inhibitors of lactate dehydrogenase have been reviewed with mercury being the strongest of all the chemicals tested (7). Evidence for a zinc-binding site in human serum butyrylcholinesterase has also been found (8). In vivo mercury exposure causes decreases in the acetylcholinestrase activities in the hepatopancrease of the shrimp Callianassa tyrrhena (3,9). A significant suppression in AChE activity was recorded in all the organs from both mercury and zinc intoxicated fish (1). Cholinesterase (ChE) activity was determined in adult heads of F1A thalassinus fed on a diet containing different concentrations of mercury, and it revealed that mercury treatments decreased the ChE activity in the three concentrations used (10). However, estimation of erythrocytes AChE activity provides additional information about many problems, because plasma cholinesterase can also be depressed by inherited traits or by other causes, notably liver disease (11,12). This enzyme has the potential for serving as a biochemical indicator of toxic stress (3). On the other hand it may be possible to in vitro exposure of blood to zinc and mercury as environmental pollutants during analysis. Hence, the present study was taken up to determine the effects of zinc and mercury on human erythrocyte AChE activity.

MATERIALS AND METHODS

Acetylthiocholine iodide (ATChI), quinidine sulfate and 5,5'-dithio-bis-2-nitrobenzoic acid (DTNB) were purchased from Sigma chemical company (UK). hyamine 1622 (benzethonium chloride) was supplied by BDH chemicals Ltd. (UK), and buffer salts by Fisons scientific apparatus Ltd. (UK). Water was distilled and deionized. The 5- thionitrobenzoate used to evaluate

the micellar spectral shift was prepared by the reaction between DTNB and thiocholine generated by erythrocyte AChE from acetylthiocholine iodide. Zinc sulfate and mercuric chloride (Merck, Germany) were used to prepare the stock solutions.

DTNB reagent was prepared in 1 L of 33 mmol/L (5.74 g/L) K_2HPO_4 and 100 ml of 0.10 mol/L KH_2PO_4 (1.36 g/100 ml). Enough amounts of the latter were added to bring the pH of the former to 7.6. Then 0.27 mmol (107 mg) of DTNB and 20 mol (16 mg) of quinidine sulfate were prepared in 1 L of the buffer and stored in a dark bottle, at 4C.

ATChI substrate (0.2 mol/L): 530 mg of ATChI was dissolved in 10.0 ml of water, and freezed for subsequent use. Immediately before use, the substrate was thawed and diluted with 1.0 ml of water, and mixed thoroughly.

Stopping reagent: This was 43 mmol/L (20 g/L) of hyamine 1622 in water, which was stored at 4C in dark bottle.

Samples: Bloods used in this study were obtained from 24 healthy individuals who had no known exposure to ChE inhibitors. Blood was centrifuged at 6000 rpm to separate erythrocytes from plasma. One hundred μl of the bottom layer was taken and added to 6 ml of distilled water. This procedure causes release of erythrocytes AChE. Hemolysates were frozen until assayed. Activity of AChE in erythrocytes was assayed according to the method of George and Abernethy (13), which is a modified procedure of Ellman (14). To 3 glass test tubes containing 3 ml of hemolyzed blood, zinc stock solution was added (0.092 g/L) to prepare a solution of 0.7, 1.40 and 2.1 mg/dl of zinc per erythrocyte. Mercury treatment was also done by adding certain concentrations of mercury stock solution (0.25×10^{-6} g/L) to prepare 2.8, 5.6 and 8.4 ng/g mercury per erythrocyte. These solutions were incubated for 30 min in a water bath set at 37C and then were used for enzyme assay, directly.

Student t-test (paired, two-tail) was applied to data of the control and treatment groups to determine the significance of differences in AChE activity. Z-test was performed to analyze significance of the relationships between decreased enzyme activity and metal doses.

RESULTS

A significant suppression in AChE activity was recorded in vitro after exposure of human erythrocytes to either metal, but these changes followed two different trends, depending upon the metal used. Effects of different concentrations of zinc and mercury on erythrocyte ChE activity have been presented in figures 1 and 2 respectively. As shown in these figures, both zinc and mercury treatments significantly ($P<0.01$) decreased the AChE activity in all concentrations used. In comparison with controls the remaining activity was found to be 53% with the highest, 72% with the middle and 85% with the lowest zinc concentrations. In the case of mercury, the remaining activity was found to be 55%, 72%, and 79% with the highest, middle and lowest concentrations respectively. Dose response correlation coefficients for zinc and mercury were $r=0.99$ and $r = 0.98$ respectively.

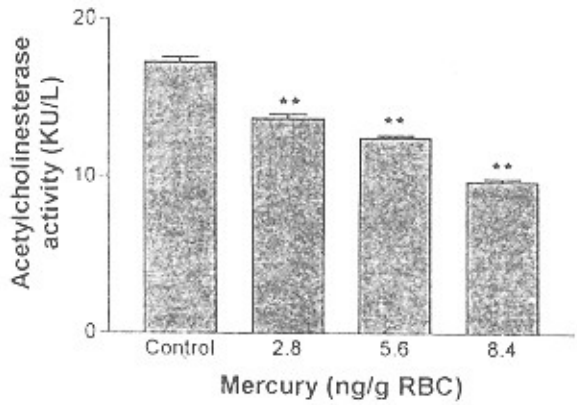

Fig. 1. Effects of different concentrations of mercury on human RBC acetylcholinesterase activity

Data are mean ± SEM of 24 samples.
** Difference between treated and control groups is significant at $P<0.01$.
$r = .98$

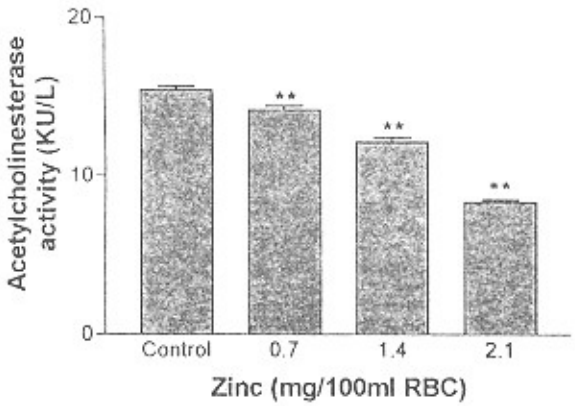

Fig. 2. Effects of different concentrations of zinc on human RBC acetylcholinesterase activity
Data are mean ± SEM of 24 samples.
** Difference between treated and control groups is significant at $P<0.01$.
$r = .99$

DISCUSSION

The activity of AChE, an enzyme that modulates the amount of the neurotransmitter substance acetylcholine at the nerve cell junction (which also occurs even in non-nervous tissues in addition to nervous tissues), is reported to vary in different organs in response to environmental stress, including heavy metal stress (1). As determined in the present study, the human erythrocytes exposed to three different concentrations of mercury and zinc relevant to human exposure exhibited remarkable variations with a decrease in AChE activity. Since mercury is a strong sulfhydryl inhibitor, it is conceivable that cellular integrity is seriously impaired in the presence of toxic mercury ions resulting in reduced enzyme turnover. Therefore in vivo, decreased AChE activity could be attributed to the structural damage to the cellular machinery concerned with enzyme production (15). On the other hand, the decrease in AChE activity could be due to the binding of zinc and mercury ions to lipid rich structural components of mitochondria and subsequently affecting the activities of enzymes like AChE which are associated directly with lipid-rich fraction, especially where integrity of the structural components is necessary for maximum catalytic activity. Furthermore the decrease in AChE activity could be due to the decreased synthesis of the enzyme by the inhibitory nature of toxicants and also due to asphyxiation (1). The decrease in AChE leads to ionic fluxes, differential membrane permeability and disturbed metabolic and nervous activity. In vivo suppression in the activity of AChE is organ-dependent and is attributed to the differential sensitivity of the AChE present in the organs. Added to it, the magnitude of metal accumulation in the target tissues and biological half-life of the metal are considered of prime importance in the modulation or modification of the AChE. The measurement of brain AChE activity in aquatic animals is considered to reveal the extent of pollution of aquatic environment, which is directly proportional to the concentrations of the pollutants. The studies of Gill et al. (4) revealed that death occurs in fish when AChE activity falls below a critical level and inhibition of the brain AChE to the level of 70 to 80% is critical to fishes. Next to the brain, the greater suppression in the activity of neuromuscular transmission and ionic fluxes is associated with its tension under imposed toxic metal stress. Studies on freshwater fish indicated that mercury inhibited AChE more potently than zinc. Also mercury was reported to affect more severely than zinc the sensory and motor organs in the central nervous system leading to accumulation of acetylcholine and inhibition of AChE activity in the nervous and muscular tissues (1). Our results show no significant difference between rate of AChE inhibition by zinc and mercury. As shown in figures 1 and 2, both zinc and mercury show approximately similar trends of AChE inhibition. This inharmonious finding between the present study and that of Suresh et al. (1) can be related to different species used for each study. Ultimately in vitro depression of erythrocyte AChE activity can be caused by direct effect of metal ions, i.e. a decrease in quantity of the enzyme, or may be due to the interaction of metals and sulfhydryl groups of the enzyme. The present paper represents the first record of the effect of zinc and mercury on AChE activity in human erythrocyte and reveals that both mercury, a nonessential metal, and zinc, an essential metal, which belong to group IIB in the periodic table suppress AChE activity in human red blood cells. The absence of detectable levels of mercury in the environment or human food cycle cannot be taken as a lack of toxicity. Residues may disappear from water, food or other possible sources of exposure but AChE depression at slightly low levels may continue for several days or weeks due to accumulation properties of zinc and mercury. The in vivo effects of zinc and mercury on human AChE activity remains to be cleared by further studies. The direct interaction of metal ions with AChE is proposed as a mechanism for depressed enzyme activity. In the end it is proposed that zinc and mercury contamination during acetylcholinesterase measurement can be a source of error that must be taken into account.

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