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(Research Article)

Determination of Activity of Lead, Alcohol and Vitamin-E on Catalase Activity in Brain Tissue of Rats

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ABSTRACT

Evaluation of catalase activity of lead, alcohol and vitamin E tested in brain tissue of rats. In four weeks of treatment, in lead treated rats, it was recorded 150 µmol/min/gram tissue. In alcohol (183.3), in lead with alcohol it was recorded 187.5µmol/min/gram tissue. In vitamin E treated rats, it was recorded 127.5 and in combination of alcohol, lead and vitamin E, it was recorded 215.0µmol/min/gram tissue. In eight weeks of treatment, control recorded 167.5 µmol/min/gram tissue and in combination of lead and alcohol, it was recorded 105.02µmol/min/gram tissue. In vitamin E treated rats, the catalase activity was 195.0 and in combination of lead, alcohol and vitamin E, it was recorded 170.0µmol/min/gram of brain tissue.

Key Words: Alcohol, Lead, Vitamin E, Catalase, Brain tissue.

INTRODUCTION

Catalase, an enzyme present in the peroxisome has shown to oxidize alcohol *in vitro* in the presence of an hydrogen peroxide-generating system [1].Previous studies have indicated that acetaldehyde may be produced by catalase in brain[2]. Homogenates of immature rat brains are able to generate acetaldehyde via catalase-mediated reaction and further indicated that, through the action of catalase, acetaldehyde is also produced in brain during ethanol intoxication. Regional differences were also noted as a function of time, indicating that processes other than catalase also contribute to acetaldehyde formation.Apparently, brain aldehyde dehydrogenase also plays a role in regulating the levels of acetaldehyde[3]. Acetaldehyde production has also recently been demonstrated in cultured atsrocytes. The primary mechanisms for the actions of ethanol on the central nervous system and brain tissue are largely unknown, there are several studies, which have postulated a role for acetaldehyde. The first metabolite of ethanol, in creating neurotoxicity[4].

MATERIALS AND METHODS

Test Animal

Male Sprague Dawley rats weighing around 150 grams at the age of three months old were used in this study. The animals were housed in polypropylene cages under hygienic conditions and feedings were done using rat pellet diet (Hindustan Lever Limited) and water *ad libitum*. Permission was taken from ethical committee to conduct experiment with its reference number CPCSEA/CH/org/2000/241.

Treatment of Rats with Lead, Alcohol and Vitamin-E

The test animals were divided into eight groups and each group consists of six animals. Group I acts as control receiving water. Group II were treated with lead acetate at 160mg/lt concentration dissolved in water. Group III animals were treated with 10% alcohol. Group IV animals were treated with 160 mg/lt concentration of lead acetate and 10%

alcohol. Group V animals served as control treated with Vitamin-E/kg diet. Group VI animals were treated with lead acetate at 160mg/lt concentration dissolved in water and Vitamin-E/Kg diet. Group VII animals were treated with 10% alcohol and Vitamin-E/kg diet.Group VIII animals were treated with 160 mg/lt concentration of lead acetate , 10% alcohol and Vitamin-E/kg diet [5,6].

Chemicals Used

Phosphate buffer 0.01M, pH 7.0 (86.55mg of disodium hydrogen phosphate and 61mg of sodium dihydrogen phosphate were dissolved in 100 ml of water). Dichromate-acetic reagent 5% (5 gm of potassium dichromate was dissolved in water and mixed with acetic acid (1:3 v/v in distilled water). The solution was further diluted to 1:5 with distilled water). Hydrogen peroxide 0.2M (One ml of H_2O_2 was mixed with 44 ml of water).

Catalase Activity Assay

Catalase activity assay was conducted by the method of Sinha, 1972**.** 0.5 ml of tissue homogenate was taken and 1.0 ml of buffer and 0.5 ml of hydrogen peroxide were added and the time was noted. The reaction was arrested by the addition of 2.0 ml of dichromate acetic acid reagent. Standard hydrogen peroxide in the range of 4 to 20 µmoles were taken and treated similarly. Both the tubes were heated in a boiling water bath for 10 minutes. The green color developed was read at 570 nm. Catalase activity is expressed as µmoles of hydrogen peroxide consumed/min/gm of wet brain.

RESULTS

The data on catalase activity in rats treated with lead, rats treated with alcohol and rats coexposed to lead and alcohol for four weeks are given in Table-1. The catalase activity ranged from 100 to 130 µmol/min/gram tissue (117.50) in the cerebral cortex of control rats. The lead treatment was characterized by a significant increase (28%) in catalase activity (150.0). In alcohol treated rats, the increase in catalase activity levels (183.33) were higher (49%) compared to lead treated rats. The catalase activity levels were significantly increased in rats coexposed to alcohol and lead, and the values ranged from 160 to 220 µmol/min/gram tissue (187.5). The increase in lipid peroxidation was 60% in rats coexposed to alcohol and lead. In vitamin-E treated rats, the catalase activity was 127.5µmol/min/gram tissue and in lead with vitamin-E treated rats, it was recorded 152.5 µmol/min/gram tissue. In alcohol and vitamin-E treated rats, 176.7 µmol/min/gram tissue was recorded and in combination of alcohol, lead and vitamin-E, the catalase activity was 215.0 µmol/min/gram tissue of brain tissue.

The data on catalase activity in rats treated with lead, rats treated with alcohol and rats coexposed to lead and alcohol for eight weeks are given in Table-2. The catalase activity ranged from 140 to 220 µmol/min/gram tissue (167.5) in the cerebral cortex of control rats. After eight weeks of treatment, catalase activity was decreased in all the treatment groups. The lead treatment was characterized by a significant increase in catalase activity. In lead treated rats, the catalse activity ranged from 110 to 160 µmol/min/gram tissue (132.50). The percent decrease in catalase activity was 20% in lead treated rats. In alcohol treated rats, decrease in catalase activity (25.00) was further decreased (26%) compared to lead treated rats. The catalase activity were significantly decreased (41%) in rats coexposed to alcohol and lead, and the values (105.02) ranged from 50 to 170µmol/min/gram tissue. Rats treated with vitamin-E was 195.0µmol/min/gram tissue. In lead with vitamin-E, the catalase activity was 182.5µmol/min/gram tissue. In alcohol with vitamin-E it was recorded 172.5 and in alcohol, lead and vitamin-E treated rats, the catalase activity was 170.0µmol/min/gram tissue.

DISCUSSION

Increased oxidative stress in the presence of increased glutathione levels suggest that mechanisms other than glutathione depletion are responsible for oxidative stress during adaptive response to alcohol such as ongoing metabolism of alcohol and alterations in other antioxidant defense systems. In a recent study, brain catalase levels have been found to be decreased after lead exposure, where as ethanol treatment was accompanied by increased catalase activity. When the rats were treated with both lead and ethanol, the alcohol induced increase in catalase was suppressed by lead treatment [8]. In contrast, our results are consistent with few studies that either show increased activity of catalase and glutathione after treatment with lead alone [9] .or alcohol alone [10] or decreased glutathione and catalase activity after long standing exposure in studies involving either lead alone [11] or alcohol alone [12,13].

It has been proposed that the putative role of the enzyme catalase in mediating some psychopharmacological effects of alcohol may be through its ability to produce acetaldehyde in the central nervous system[14]. In several studies, brain homogenates of rodents incubated in the presence of alcohol have been shown to oxidize alcohol to acetaldehyde[15]. Similar results have been obtained in neural tissue cultures[2]. Moreover, other authors

have demonstrated that prior i.p. administration of alcohol to rats protects brain catalase activity from inhibition by 3-amino-1,2,4,-triazole (AT)[16], cyanamide and 4-hydroxipyrazole[12].This prevention of the inhibitory effect in vivo by these compounds has been taken as indirect supportive evidence for the oxidation of alcohol in the central nervous system via catalase [12,16]. Thus, the ongoing oxidative stress at four weeks of treatment after alcohol exposure and coexposure to alcohol and lead may be partly accounted by alcohol metabolism mediated by catalase even when glutathione levels are increased.

CONCLUSION

The increased catalase activity at four weeks concomitant with ongoing oxidative stress suggests an initial adaptation to the chemical insult caused by lead or alcohol. The unaltered vitamin-E content, increased glutathione content, increased catalase activity, but decreased vitamin-C content at four weeks in the presence of oxidative stress suggests depletion of vitamin-C as an important determinant of neurotoxicity caused by lead and alcohol.

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Table 1: Catalase activity in rats treated for four weeks with lead, alcohol and lead + alcohol with and without vitamin-E treatment

Group	Catalase Activity (μmol/min/gm)
Control	117.50 ± 0.0
Lead	150.0 ± 0.1 [a]
Alcohol	183.33 ± 0.0[ab]
Lead + Alcohol	187.5 ± 25.0[ab]
Control + Vitamin E	127.5 ± 0.2 (8.5%↑)**
Lead + Vitamin E	152.5 ± 0.0 [a] (1.67%↑)
Alcohol + Vitamin E	176.7 ± 0.0[a] (3.62%↓)
Alcohol + Lead + Vitamin E	215.0 ± 0.0 [ab] (14.67%↑)

* Values were expressed as μmol/min/gm.
a = significant at p<0.05 vs. control, b = significant at p<0.05 vs. lead
c = significant at p<0.05 vs. alcohol.
**The values in the parenthesis indicate percent change from the corresponding group without vitamin-E.

Table 2: Catalase activity in rats treated for eight weeks with lead, alcohol and lead + alcohol with and without vitamin-E treatment

Group	Catalase Activity (μmol/min/gm)
Control	167.5 ±0.0
Lead	132.50 ±0.1
Alcohol	125.00 ±0.2
Lead + Alcohol	105.02 ± 0.0
Control + Vitamin E	195.0 ± 0.0 (16.41%↑)**
Lead + Vitamin E	182.5 ± 0.0 (37.74%↑)
Alcohol + Vitamin E	172.5 ±0.0 (38.00%↑)
Alcohol + Lead + Vitamin E	170.0 ± 0.0 (61.87%↑)

* Values were expressed as μmol/min/gm.
a = significant at p<0.05 vs. control, b = significant at p<0.05 vs. Lead
c = significant at p<0.05 vs. alcohol
** The values in the parenthesis indicate percent change from the corresponding group without vitamin-E.

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