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Effect of Lead, Alcohol and Vitamin E on Protein carbonyl content in rats

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ABSTRACT

Protein carbonyl content assay was conducted among eight groups of animals treated with lead, alcohol and vitamin E in individuals and in combination at two, four and eight weeks of duration. In two weeks of treatment, lead treated rats recorded 2.45 nmol/grams. In alcohol treated rats, it was recorded 2.60 nmol/gram of protein carbonyl content in liver tissue. Compared to control, it was recorded 2.01 nmol/gram. In vitamin E treated with lead and alcohol, protein carbonyl content was recorded 3.02 nmol/gram of tissue. In four weeks of duration, lead treated rats recorded 2.64 nmol/gram of tissue and in alcohol treated rats, protein carbonyl content was 2.87 nmol/gram and in lead with alcohol and vitamin E treated rats, protein carbonyl content was 3.29 nmol/gram. In eight weeks of treatment, maximum protein carbonyl content was recorded in lead with alcohol treated rats compared to control.

Keywords: Lead, Alcohol, Vitamin E, Protein carbonyl content, liver tissue.

INTRODUCTION

Lead is considered as one of the most hazards and cumulative environmental pollutants that affect all biological systems through exposure from air, water and food sources. (Patra and Swarup, 2000). Lead exposure induces clinicopathological changes through toxicity occurred to kidney and endocrine system (Jadhav et al., 2007). Lead is a toxic heavy metal which is known to induce a broad range of physiological, biochemical and behavioral dysfunctions in humans. Because of its pervasiveness in the environment, lead poisoning still remains an important health problem (Hande et al., 1999). Recent studies suggest that oxidative stress is a potential contributor to lead's toxicity and that lead directly or indirectly can change the prooxidant/ antioxidant balance in biological tissues (Quinlan et al., 1998). Lead exposure mainly occurs through the respiratory and gastrointestinal systems. Absorbed lead (whether inhaled or ingested) is stored in soft tissues. Autopsy studies of lead exposed humans indicate that liver tissue is the largest repository (33%) of lead from among the soft tissues followed by kidney cortex and medulla. As environmental exposures to lead have increased, the toxic effects of lead on various organ systems in the body have been recognized (Lyn, 2006). Liver injury from alcohol has many components, including intrahepatic events [such as hepatocyte alcohol metabolism, generation of reactive oxygen species (ROS) that result in cellular oxidative stress, loss of protective enzymes and transporters] and extrahepatic stimuli, such as gut-derived endotoxin, induced by ethanol exposure. These factors can act separately or in concert to trigger common pathways involving an inflammatory cytokine cascade (Patricia, 2010). In the present study, the effect of lead acetate, alcohol and lead acetate combined with alcohol in the presence and absence of vitamin E on Protein carbonyl content in liver tissues were tested *in vitro*.

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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/lit concentration dissolved in water. Group III animals were treated with 10% alcohol. Group IV animals were treated with 160 mg/lit 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/lit 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/lit concentration of lead acetate, 10% alcohol and Vitamin E/kg diet (AL-Jobory, 2006; Alkatan M, 2006).

Chemicals Used

2 M HCl (16.6 ml concentrated HCl make up to 100ml with distilled water), 10mM 2,4 – dinitrophenylhydrazine in 2M HCl, 19.81mg of DNPH dissolved in 100ml 2M HCl, 20% trichloroacetic acid (20gm trichloro acetic acid make up to 100ml with distilled water), Absolute ethanol, Ethyl acetate, 6 M Guanidine hydrochloride in 20 mM potassium phosphate solution (57.09gm of Guanidine hydrochloride make up to 100ml with potassium phosphate solution, pH is adjusted to 2.3 by adding trifluoro acetic acid), Potassium phosphate 20mM (27.38gm of potassium dihydrogen phosphate dissolved in 100ml distilled water).

Protein Carbonyl Assay

Liver protein carbonyl content was determined by colorimetric method (Levine et al., 1990). The liver homogenate (0.5ml) was pipetted into 1.5ml centrifuge tube and 0.5 ml of 10mM 2,4-dinitrophenylhydrazine in 2 M HCl was added and allowed to stand at room temperature for 1 hour, with vortexing every 10-15 minutes. Then, 0.5ml of 20% Trichloroacetic acid was added followed by centrifugation. The supernatant was discarded and the pellets was washed 3 times with 1 ml ethanol – ethyl acetate (1:1) to remove free reagent. The obtained precipitated protein was redissolved in 0.6 ml guanidine solution. Carbonyl content was calculated from maximum absorbance (390nm) using molar absorption coefficient of $21 \text{ mM}^{-1} \text{ cm}^{-1}$.

RESULT

Protein carbonyl content at Two weeks

Among the eight groups of animals treated, in lead treated animals, the protein carbonyl content was 2.45nmol/gram and in

alcohol treated tissue, it was recorded 2.60 nmol/gram of liver tissue. In lead combined with alcohol, the protein carbonyl content was 3.56 nmol/gram. In vitamin E treated tissue, the protein carbonyl content 1.90 nmol/gram. In lead with vitamin E treated rats, the protein carbonyl content was 2.19 nmol/gram. In alcohol with vitamin E treated animals, the protein carbonyl content was 2.37 nmol/gram and in lead with alcohol and vitamin E, the protein carbonyl content was 3.02 nmol/gram (Table 1).

Table 1: Protein carbonyl content in rats treated for two weeks with lead, alcohol and alcohol and lead with and without vitamin E.

Group	Protein carbonyl content
	Mean \pm S.D.
Control	2.01 ^b \pm 0.1
Lead	2.45 ^c \pm 0.0
Alcohol	2.60 ^f \pm 0.0
Lead + Alcohol	3.56 ^h \pm 0.1
Control + Vitamin E	1.90 ^a \pm 0.2
Lead + Vitamin E	2.19 ^e \pm 0.1
Alcohol + Vitamin E	2.37 ^d \pm 0.1
Lead + Alcohol + Vitamin E	3.02 ^g \pm 0.1

Values are the mean of three replicates. \pm standard error. The means followed by the same letter (S) are not significantly different at $P < 0.05$ when subjected to Tukey's HSD.

Table 2: Protein carbonyl content in rats treated for four weeks with lead, alcohol and alcohol and lead with and without vitamin E.

Group	Protein carbonyl content
	Mean \pm S.D.
Control	2.08 ^b \pm 0.1
Lead	2.64 ^e \pm 0.0
Alcohol	2.87 ^f \pm 0.0
Lead + Alcohol	3.72 ^h \pm 0.1
Control + Vitamin E	2.04 ^a \pm 0.0
Lead + Vitamin E	2.35 ^c \pm 0.0
Alcohol + Vitamin E	2.59 ^d \pm 0.1
Lead + Alcohol + Vitamin E	3.29 ^g \pm 0.0

Values are the mean of three replicates. \pm standard error. The means followed by the same letter (S) are not significantly different at $P < 0.05$ when subjected to Tukey's HSD.

Protein carbonyl content at Four weeks

The protein carbonyl content values ranged from 1.91 to 2.43 nmol/gm tissue in the liver of control rats. The lead treatment was characterized by a significant increase in protein carbonyl content (2.64 nmol/gm). In lead treated rats, the protein carbonyl content ranged from 2.35 to 2.89nmol/gm of liver tissue and the percent increase in protein carbonyl content was 27%. In alcohol treated rats, the increase in protein carbonyl content was higher (2.87nmol/gm of liver tissue). when compared to lead treated rats and the percent increase in protein carbonyl content was 39%. The protein carbonyl content was significantly increased (78%) in rats coexposed to alcohol and lead, and the values ranged from 3.07 to 4.47nmol/gm of liver tissue. Thus, the magnitude of deleterious effects of oxidative stress on protein oxidation was more significant in combined treatment group when compared to lead alone treated rats or alcohol alone treated rats (Table 2).

Protein carbonyl content at Eight weeks

The protein carbonyl content ranged from 1.80 to 2.75nmol/gm tissue in the liver of control rats. The lead treatment

was characterized by increase in protein carbonyl content (2.94 nmol/gm]. In lead treated rats, the protein carbonyl content ranged from 2.37 to 3.43nmol/gm of liver tissue. In alcohol treated rats, the increase in protein carbonyl content was higher when compared to lead treated rats. The percent increase in protein carbonyl content was 62% in alcohol treated rats compared to 35% in lead treated groups. The protein carbonyl content was significantly increased in rats coexposed to alcohol and lead, and the values ranged from 3.75 to 4.65nmol/gm of liver tissue. The percent increase in protein carbonyl content was 92% in rats coexposed to alcohol and lead (Table 3).

DISCUSSION

Protein oxidation is predominant during lead and alcohol exposure. Reactive oxygen species can attack amino acid residues (particularly histidine, arginine and lysine) to produce carbonyl (> C=O) functions that can be measured after reaction with 2,4-dinitrophenylhydrazine (Reznick and Packer, 1994). Thus the 'carbonyl assay' has become the most widely used protocol to measure protein oxidation. The enzymes that have been shown to be inactivated *in vitro* by free radicals generated by metal catalysed oxidation reactions include key metabolic enzymes such as glutamine Synthase, creatine kinase, pyruvate kinase, enolase, glyceraldehydes-3-phosphate-dehydrogenase and lactate dehydrogenase (Fucci et al., 1983). The formation of protein carbonyl derivatives is associated with pathological conditions both in humans and in animal model systems as well as with aging (Stadtman, 1992). The pathological conditions reported include inflammatory diseases such as rheumatoid arthritis, atherosclerosis, neurological disorders, cataractogenesis, and ischemia – reperfusion tissue damage (Oliver et al., 1990). Recent studies have reported increased carbonyl content after ethanol treatment (Rouach et al., 1997) or lead exposure, but there are no reports on the effects of coexposure of lead and ethanol on protein carbonyl content.

In the present study, protein carbonyl content is significantly increased after lead, alcohol and lead combined with alcohol treatment. The magnitude of accumulation of protein carbonyls in rats coexposed to lead and alcohol is more pronounced compared to lead alone or alcohol treated rats. The increased protein oxidation occurs by both lipid peroxidation dependent and lipid peroxidation independent manner. Recently significant increase in protein carbonyl content was observed even within an hour of exposure to CCl₄ vapors, when no change in MDA level was observed. This suggests that the accumulation of oxidized proteins in the liver may be an early indication of CCl₄ induced liver damage and that protein oxidation may be independent of lipid peroxidation (Sundari et al., 1998). Similarly, diquat mediated protein oxidation also occurs by lipid independent mechanism (Blakeman et al., 1998).

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