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Effect of Co-Exposure to Ethanol and Cadmium in Rats

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Metabolism and toxicity of heavy metals may be influenced by certain factors such as protein malnutrition, essential element deficiency or alcoholism. Ethanol has been found to enhance the absorption of lead in body and alcoholics have been reported to be more susceptible to lead intoxication (Goyer and Mahaffey 1972). However, subsequent experiments with rats fed isocaloric diets and controlled nutritional content suggested that clinically suspected synergism between ethanol consumption and lead intoxication observed among industry workers was more likely due to nutritional factors than mutual enhancement of closely related cellular effects of the two toxins (Mahaffey et al. 1974). Recently, it has been observed in this laboratory that the animals co-exposed to ethanol and lead are more vulnerable to systemic toxicity of lead including neurotoxic effects (Flora and Tandon 1987). We have observed that low dietary protein disturbs cadmium induced alterations in carbohydrate metabolism, affects hepatic and renal process of cadmium detoxification and enhances susceptibility to cadmium intoxication in rats (Tewari et al. 1986).

As alcoholism may be common among industry workers and a significant section of population, who may be exposed to cadmium, it was considered of interest to investigate the influence of ethanol-cadmium co-exposure on cadmium sensitive hepatic, renal and serum enzymes, tissue accumulation of cadmium, essential trace element status and cadmium induced hepatic metallothionein synthesis in rats.

MATERIALS AND METHODS

Male albino rats weighing 110 ± 10 g of Industrial Toxicology Research Centre's colony maintained on commercial rodent pellet diet (Hindustan Lever Ltd., India) and drinking water *ad libitum* were acclimatized in experimental room until they weighed 150 ± 10 g. They were randomly divided into four groups of 10 each and treated for eight weeks as follows -

Group I - No treatment (Normal control)
Group II - Ethanol, 1 g/kg, by gastric gavage daily for first week
5 g/kg, by gastric gavage daily for second week
10g/kg, by gastric gavage daily for rest of the weeks
Group III - Cadmium, 40 ppm in drinking water was $CdCl_2$
Group IV - Ethanol, as in group II+Cadmium, as in group III.

The average water consumption in each group was 16 ml/rat/day. The animals were anesthetized mildly under ether; liver, kidney and spleen removed and washed free of extraneous material. The blood was drawn from the heart and serum separated.

A portion of the liver and a kidney were homogenized in ice-cold 0.25 M sucrose to obtain 10% (w/v) homogenate. Standard procedures were employed to assay the hepatic and renal glucose 6-phosphatase (EC 3.1.3.9) (Swanson 1955), fructose 1,6-diphosphatase (EC 3.1.3.11) (McGilvery 1955), serum glutamic oxalacetic transaminase (GOT; EC 2.6.1.1), glutamate pyruvic transaminase (GPT; EC 2.6.1.2) (Reitman and Frankel 1957) and hepatic and renal glutathione levels (Jollow et al. 1974). Inorganic phosphorous and protein contents of the homogenates were determined by the methods of Fiske and SubbaRow (1957) and Lowry et al. (1951) respectively. Total hepatic metallothionein (MT) was estimated according to the procedure of Eaton and Toal (1982).

The measured quantities of liver, kidney and spleen were acid disgested (HNO_3:H_2SO_4:$HClO_4$::6:1:1) and the carbon free residues dissolved in 5 ml of 5% HNO_3. The digested samples and MT fractions were read at 228.8, 213.9 and 324.7 nm for Cd, Zn and Cu contents respectively on a flame atomic absorption spectrometer using air-acetylene fuel (Perkin-Elmer 5000). The suitable standards were prepared identically for comparison.

RESULTS

The administration of Cd caused significant retardation in weight gain as compared to normal control or ethanol exposed rats. The group simultaneously exposed to ethanol and Cd showed further loss of weight.

1. The administration of Cd alone significantly increased the activities of glucose 6-phosphatase and fructose 1,6-diphosphatase in both liver and kidney, while the administration of ethanol alone increased their activities in only kidney. The co-exposure to ethanol did not modify the effect of Cd, except enhancing the activity of renal glucose 6-phosphatase further (Table 1).

Table 1. Effect of ethanol, cadmium or their combination on the activities of hepatic and renal gluconeogenic enzymes in rats.

	Glucose-6-phosphatase (n mole Pi liberated/min/mg protein)		Fructose-1,6-diphosphatase (n mole Pi liberated/min/mg protein)	
	Liver	Kidney	Liver	Kidney
Normal control	33.99±1.13	40.19±2.14	43.31±2.50	59.04±3.66
Ethanol	36.69±1.19	56.38±5.67^{b}	48.02±1.97	76.15±4.96^{b}
Cadmium	63.82±3.09^{a}	69.69±2.10^{a}	67.74±4.23^{a}	106.73±4.00^{a}
Ethanol + Cadmium	60.06±4.34^{a}	92.51±5.38^{a,*}	68.17±2.34^{a}	94.65±4.84^{a}

Each figure is mean ± SE of 6 animals,

$^{a}p<0.001$, $^{b}p<0.05$ versus Normal control, $^{*}p<0.01$ versus cadmium treated group as evaluated by the Student's 't' test.

2. The activity of serum GPT increased upon exposure to Cd or ethanol alone; serum GOT did not alter significantly. However, the combined exposure to Cd and ethanol significantly raised both serum GOT and GPT levels. The treatment with ethanol or Cd alone increased hepatic and renal GSH content; their combination, however, did not influence the individual effects (Table 2).

Table 2. Effect of ethanol, cadmium or their combination on serum transaminases and hepatic and renal glutathione content in rats.

	SGOT (n mole hydrazone formed/min/mg protein)	SGPT (n mole hydrazone formed/min/mg protein)	Glutathione (μ mole/g) Liver	Glutathione (μ mole/g) Kidney
Normal control	38.75±2.80	151.70±1.85	5.48±0.17	3.00±0.09
Ethanol	43.81±1.04	167.07±4.77^{c}	6.38±0.35	3.85±0.26^{c}
Cadmium	40.33±1.54	170.41±5.12^{b}	8.84±1.20^{c}	3.46±0.14^{c}
Ethanol + Cadmium	72.86±2.18^{a,*}	207.65±4.78^{a,*}	7.58±0.66^{c}	3.84±0.13^{c}

Each figure is mean ± SE of 6 animals,

$^{a}p<0.001$, $^{b}p<0.01$, $^{c}p<0.05$ versus Normal control; $^{*}p<0.05$ versus Cadmium treated group as evaluated by the Student's 't' test.

3. The accumulation of Cd in liver, kidney and spleen enhanced significantly upon co-exposure to ethanol and Cd. The hepatic Zn decreased while the renal Zn increased following exposure

Table 3. Effect of ethanol, cadmium or their combination on tissue concentration of cadmium and essential trace elements in rats

	Cd (μg/g)			Zn (μg/g)			Cu (μg/g)		
	Liver	Kidney	Spleen	Liver	Kidney	Spleen	Liver	Kidney	Spleen
Normal control	1.3 ± 0.31	0.5 ± 0.08	1.2 ± 0.24	41.6 ± 2.47	28.8 ± 1.54	31.0 ± 4.80	6.1 ± 1.21	9.5 ± 0.67	3.0 ± 0.55
Ethanol	1.3 ± 0.11	0.4 ± 0.10	1.4 ± 0.13	29.0 ± 1.02^{a}	36.4 ± 2.51^{c}	32.9 ± 2.21	6.2 ± 1.42	7.5 ± 1.27	5.6 ± 0.46^{b}
Cadmium	22.0 ± 2.21^{a}	12.2 ± 1.55^{a}	2.7 ± 0.33^{b}	51.9 ± 5.07	38.8 ± 3.86^{c}	34.7 ± 2.97	5.1 ± 0.58	9.6 ± 1.32	5.0 ± 1.80
Ethanol + Cadmium	$44.4 \pm 3.62^{a,*}$	18.0 ± 2.23^{a}	$8.6 \pm 1.45^{a,**}$	$78.4 \pm 8.01^{b,***}$	$63.2 \pm 7.74^{b,***}$	46.5 ± 4.41^{c}	$10.5 \pm 2.26^{***}$	11.4 ± 3.22	9.0 ± 2.82

Each figure is mean $\pm$ S.E. of 6 animals,
$^{a}p<0.001$, $^{b}p<0.01$, $^{c}p<0.05$ versus Normal control; $^{*}p<0.001$, $^{**}p<0.01$, $^{***}p<0.05$ versus Cadmium treated group as evaluated by the Student's 't' test.

to ethanol alone. The uptake of Zn in both the organs increased upon administration of Cd, which was more marked in animals co-exposed to Cd and ethanol. The Zn content of spleen increased only upon combined treatment. The hepatic and renal levels of Cu remained uninfluenced by exposure to Cd or ethanol alone, while the spleen level of Cu increased upon treatment with ethanol. The combined exposure, however, increased the hepatic content of Cu significantly (Table 3).

4. The hepatic MT content increased upon administration of Cd, which was significantly more marked in animals co-exposed to Cd and ethanol. The concentration of Cd and Zn in hepatic MT fraction was also more marked in animals exposed to the combination of Cd and ethanol compared to those treated with Cd alone. The increased Cu level in hepatic MT fraction due to Cd remained unaffected upon co-exposure to ethanol (Table 4).

Table 4. Effect of ethanol, cadmium or their combination on total hepatic metallothionein and its cadmium, zinc and copper contents in rats.

	Total hepatic MT (μg/g)	Hepatic MT metals (μg/g)		
		Cd	Zn	Cu
Normal control	1.88±0.18	0.21±0.02	1.71±0.41	1.18±0.16
Ethanol	1.77±0.20	0.27±0.03	1.69±0.14	1.01±0.13
Cadmium	76.09±8.58[a]	8.55±0.96[a]	5.85±0.80[a]	3.34±0.50[b]
Ethanol + Cadmium	120.53±12.65[a,**]	18.79±0.93[a,*]	13.46±0.77[a,*]	2.65±0.26[a]

Each figure is mean ± SE of 6 animals,
[a]$p<0.001$, [b]$p<0.01$ versus Normal control; [*]$p<0.001$, [**]$p<0.05$ versus Cadmium treated group as evaluated by the Student's 't' test.

DISCUSSION

Cadmium induced hepatotoxicity and nephrotoxicity may be modified by extraneous factors such as alcoholism either by increasing the permeability of the membranes resulting into enhanced accumulation of Cd in tissues (Gulati et al. 1982, Flora and Tandon 1987) or by affecting hepatic and renal metabolism (Axelrod 1974, Lieber 1985). The observed Cd induced increase in the activities of hepatic and renal glucose 6-phosphatase and fructose 1,6-diphosphatase is due to the enhanced gluconeogenesis (Chapatwala et al. 1980, 1982, Tewari et al. 1986). The marked elevation in these rate limiting key gluconeogenic enzymes indicates enhanced synthesis of glucose from non-carbohydrate sources, that is mobilization and utilisation of fat deposits in liver and kidney which is reflected by significant loss in weight gain among Cd exposed animals (Weber and Singhal 1964). The administration of ethanol alone also increased the activities of these gluconeogenic enzymes particularly in kidney apparently to meet body's need for extra energy (Shaw and Lieber 1979) which is otherwise depleted due to diuretic effect of ethanol or alterations in ATP related systems under the influence of ethanol or its metabolites. However, ethanol did not modify the Cd induced increase in hepatic or renal gluconeogenesis which indicates that there is no synergistic effect on related enzymes. On the other hand, Cd elevated serum GOT and GPT increased further upon co-exposure to ethanol which shows that ethanol augments Cd hepatotoxicity.

Ethanol feeding has been shown to increase the rates of GSH turnover as well as steady state GSH levels, important for protection of cells in rats (Morton and Mitchell 1985), which support the present observation of the increased hepatic and renal GSH levels after ethanol administration. Likewise, the Cd induced increase in hepatic and renal GSH may be emphasized as a protective mechanism against Cd toxicity (Dudley and Klaassen 1984) wherein Cd might be binding with GSH in order to overcome the toxicity (Hsu 1981). The role of GSH in metabolising or disposing of various toxic substances such as peroxides and heavy metals as conjugates has been well recognized (Javitt 1961). However, co-exposure of ethanol had no influence on induction of GSH by Cd and apparently did not affect Cd induced self protective mechanism through conjugation with GSH.

The influence of ethanol on the accumulation of Cd and Cd-induced alterations in essential trace element levels in tissues may be an important measure of Cd intoxication during alcoholism. The co-exposure to ethanol significantly enhanced the uptake of Cd and Cd-induced increase of Zn in liver, kidney and spleen, the hepatic content of MT and Cd and Zn bound to hepatic MT fraction. The induction of tissue MT synthesis is a known protective response of cells against toxic metals. Hopf et al. (1986) reported that long term ingestion of ethanol reduced total amount of MT in liver but not in kidney and decreased hepatic Zn and Cu contents. In contrast, an increase of hepatic MT content after short-term treatment with a high dose of ethanol has been observed by Waalkes et al. (1984) and Bracken and Klaassen(1987). However, no change in either hepatic MT or Zn and Cu contents was observed in animals exposed to ethanol alone in the present study which may be due to the adaptation to the increasing dose of ethanol. While more accumulation of Cd and Zn in liver,kidney and spleen of animals co-exposed to Cd and ethanol as compared to those exposed to Cd alone shows their increased vulnerability towards Cd due to ethanol, the increased content of hepatic MT accompanied by significantly higher concentration of Cd and Zn in MT fraction due to increased body burden of Cd suggests the steped-up protective mechanism under the influence of ethanol.

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