Role of Zinc on Ethanol Metabolism in Intestine of Alcohol Intoxicated Rats

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***Abstract*----**There has been escalation in alcohol abuse with an enormous increase in alcohol-related gastrointestinal tract disorders. The study was conducted to evaluate the role of zinc on intestine of rats under ethanol toxicity. Rats were segregated into four groups: Control, Ethanol treated, Zinc treated and Combined zinc and ethanol treated. 1ml of 30% ethanol was given to rats orally everyday. Zinc (as zinc sulphate; 227mg/L) was mixed in drinking water of animals. All treatments were given for 2, 4 and 8 weeks and activities of drug metabolising enzymes i.e Cytochrome P450, Cytochrome b_5, NADPH and NADH cytochrome-C- reductase and Glutathione-s- transferase were estimated. Significant elevation was observed in the activities of all enzymes in intestine in response to toxicity induced by ethanol after all intervals. Zinc supplementation resulted in normalization of these values. This may be due to its antioxidant potential which provides protection against the toxic effects of ethanol.

***Keywords*----**Drug metabolism, Ethanol, Intestine, Zinc

I. Introduction

ETHANOL is widely consumed and is associated with increasing global health burden. This fact has stimulated the study of ethanol metabolism in man and in animals. Ethanol may exert its effects either directly or through profound derangements in [i]metabolic, hormonal and nutritional mechanisms [1]. Alcohol consumption can interfere with the function of all parts of the GI tract [2]. The mammalian small intestine serves principally as the site for absorption of nutrients, water, and both beneficial and potentially harmful xenobiotics [3]. Both Phase I and Phase II metabolic enzymes are expressed [4] in the intestine. The administration of ethanol to experimental animals and man results in the induction of the biotransformation of drugs, xenochemicals and the oxidation of ethanol itself, suggesting that ethanol induces a specific form of Cytochrome P450. The direct contact of alcohol with the GIT mucosa may elicit several metabolic changes among which is the induction of ethanol-inducible cytochrome P450 (CYP2E1) with generation of toxic acetaldehyde and reactive oxygen species [5]. However till date information is lacking regarding various other components of mixed function oxidases which also play a significant role in this complete process of ethanol toxicity.

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Zinc is an essential micronutrient and plays an important role in maintaining intestinal epithelial integrity [6]. It plays a key role in body's defenses against free radicals and reactive oxygen molecules as constituents of antioxidant enzymes [7]. Alcoholics frequently show zinc deficiency [8] that produces several pathological disorders both in laboratory animals and humans. It is well known that ethanol consumption damages the GI tract and influences the absorption of various metals. Zinc sulphate has been reported to protect the gastric mucosa subjected to various injurious against including ethanol [9]. The main purpose of this study was to assess the mechanistic understanding of ethanol toxicity with respect to specific enzymes in intestine involved in bioactivation and detoxification of ethanol.

II. Materials And Methods

Male Wistar rats (100-120g weight range) were obtained from Central Animal House, Panjab University, Chandigarh and were segregated into four main groups. **Group I: Normal Control** in which animals were fed pelleted diet and water ad-libitum. **Group II: Ethanol treated**. Rats in this group were fed 1 ml of 30% ethanol daily. **Group III: Zinc treated.** Rats in this group were supplemented with zinc in the form of zinc sulphate ($ZnSO_4.7H_2O$) at a dose of 227mg/L added to the drinking water of the animals [7]. **Group IV: Zinc and Ethanol treated. R**ats in this group were fed ethanol as was given to Group II rats and zinc as in Group III animals. There were 8-10 rats in each group and all treatments were given for different time intervals of 2 weeks, 4 weeks and 8 weeks.

After different treatment intervals, overnight fasted animals were sacrificed; the intestines were removed and flushed with ice cold saline. Then they were inverted and mucosal cells were scrapped off. 25% homogenates were prepared with cold 50mM Tris-HCl buffer (pH 7.4) containing 150mM KCl and 0.25M sucrose. These homogenates were subjected to cold centrifugation at 10,000g for 30 minutes and the supernatants (Post mitochondrial supernatant; PMS) were used for various biochemical estimations.

Cytochrome P450 was measured by carbon monoxide differences spectrophotometry of dithionite reduced samples by the method of Omura and Sato [10]. The assay of NADPH- cytochrome C reductase was carried out in PMS fraction by the method of Yasukochi and Masters [12]. Cytochrome b_5 activity was measured by the method of Omura and Sato [11]. NADH- cytochrome C reductase was assayed by the method of Yasukochi and Masters [12]. The assay of glutathione-s- transferase (GST) was done by the method of Habig et al. [13] using 1-chloro-2,4-dinitrobenzene (CDNB) as a substrate.

III. Results And Discussion

Cytochrome P450 is a superfamily of enzymes that catalyze the biotransformation of numerous foreign and endogenous compounds [14], [15]. Intake of alcohol has been shown to induce the activity of cytochrome P450 after all the treatment intervals, in comparison with the normal controls (Table I). This might be due to increased production of oxy radicals that promote lipid peroxidation [16] and also due to increased acetaldehyde generation which in turn impairs defense system against oxidatve stress [16], [17].

NADPH- cytochrome C reductase, a flavoprotein, has been thought to be involved in the oxidation of various drugs, steroids and other chemicals [18]. It is an essential component of the P-450 mono-oxygenase system [19, 20]. It is essential for the catalytic activity of cytochrome P450 [21]. The present study indicated an elevation in the enzyme activity after ethanol feeding. A reactive product of the interaction between ethanol and the cytochrome P-450 system may be responsible for the toxic effects of this compound (Table II). The induction of the enzyme following prolonged ethanol consumption conceivably could lead to increased metabolism of ethanol to acetaldehyde, a major metabolite presumed to be responsible for many of the deleterious effects of long-term ethanol intake.

NADH cytochrome-C-reductase has also been proposed to participate in mono-oxygenaase activity through electron transport to cytochrome b_5 and subsequently to cytochrome P450. During the present study, no significant alteration in cytochrome b_5 was noticed after ethanol feeding in any treatment interval, thus indicating that this is not playing a role in detoxification of ethanol (Table III). NADH cytochrome-C-reductase activity was found to be enhanced significantly after ethanol intoxication after all the treatment durations as reported by other workers also [21] (Table IV). This enzyme is present in outer mitochondrial menmbrane that can interact with NADH and NADPH which are impermeable to mitochondrial membrane. When this enzyme binds with these species, they initiate a series of events in the mitochondria which are responsible for initiating lipid peroxidation. Since oxidation of ethanol by alcohol dehydrogenase increases the NADH/NAD+ ratio, increased availability of NADH via metabolism of ethanol and induction of enzyme systems after chronic ethanol treatment may play important roles in the development of a state of oxidative stress in intestine by ethanol.

Glutathione-S-transferases (GSTs), a key Phase II biotransformation enzyme, are a large family of multifunctional proteins that are essential for disposal of exogenous toxic compounds and the adaptive, antioxidant response to reactive oxygen species (ROS) [22, 23]. In addition, the GST is an essential part of the cellular antioxidant defense [24]. The increase in the GST activity in the intestine as a response to the ethanol consumption suggests its activation due to oxidative stress [25] (Table V).

Zinc treated rats also did not show any significant change in all these enzyme activities in the present investigation. Regarding the animals which were administered zinc alongwith ethanol, it was noticed that the activities of the enzymes reverted to within normal limits. It has been reported by other researchers also that zinc supplementation improves barrier dysfunction in many pathological conditions in small intestine [26]. It is well known that zinc is a potent inducer of metallothionein (MT) production in multiple organ systems. Thus, it has been proposed that zinc inhibition of tissue damage is mediated by increased MT synthesis [27]. In earlier studies also, zinc was found to interact with cytochrome P450 thus decreasing its ability to bind various drugs [6]. Therefore, it may be inferred that zinc inhibits drug metabolism either by altering the oxidation- reduction potential of the flavo-protein. Previous studies in our laboratory have also shown the protective effects of zinc in animals with respect to normalization of the activities of these enzymes [28]. Future studies will examine the mechanisms of action of zinc in the cytoprotection against alcohol toxicity.

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TABLE I
EFFECT OF ZINC ON CYTOCHROME P450 LEVELS IN INTESTINE OF ETHANOL TREATED WISTAR MALE RATS
(Values are expressed as nmol mg^{-1}protein)

	Group	Time Periods	
	2 weeks	4 weeks	8 weeks
I Control	0.10±0.03	0.11±0.02	0.12±0.02
II Ethanol	0.13±0.03	0.14±0.04	0.14±0.02
III Zinc	0.09±0.03	0.09±0.02	0.12±0.03
IV Zinc+ Ethanol	0.07±0.01[y]	0.07±0.01[y]	0.06±0.01[b,q,z]

nmol=nanomole; mg=milligram
Values are expressed as Mean±S.D.
[b]p<0.01 by Newman-Keul's test when the values of Group II, III, and IV are compared with those of group I.
[q]p<0.01 by Newman-Keul's test when the values of Group IV are compared with those of group III.
[y]p<0.01; [z]p<0.001 by Newman-Keul's test when the values of Group IV are compared with those of group II.

TABLE II
EFFECT OF ZINC ON NADPH- CYTOCHROME- C-REDUCTASE ACTIVITY IN INTESTINE OF ETHANOL TREATED WISTAR MALE RATS
(Values are expressed as nmol cytochrome – c reduced $min^{-1}mg^{-1}$protein)

Group	Time Periods		
	2 weeks	4 weeks	8 weeks
I Control	61.23±6.16	64.88±3.13	67.16±5.98
II Ethanol	97.63±14.87[c]	107.55±11.74[c]	124.25±8.89[c]
III Zinc	65.45±3.56	72.07±6.49	75.89±3.85
IV Zinc+ Ethanol	88.18±9.82[c,r]	91.22±6.83[c,r,z]	95.36±6.40[c,r,z]

nmol=nanomole; min=minute; mg=milligram
Values are expressed as Mean±S.D.
[c]p<0.001 by Newman-Keul's test when the values of Group II, III, and IV are compared with those of group I.
[r]p<0.001 by Newman-Keul's test when the values of Group IV are compared with those of group III.
[z]p<0.001 by Newman-Keul's test when the values of Group IV are compared with those of group II.

TABLE III
EFFECT OF ZINC ON CYTOCHROME B5 ACTIVITY IN INTESTINE OF ETHANOL TREATED WISTAR MALE RATS
(Values are expressed as nmol b5 mg^{-1}protein)

Group	Time Periods		
	2 weeks	4 weeks	8 weeks
I Control	0.08±0.03	0.09±0.01	0.12±0.02
II Ethanol	0.09±0.02	0.12±0.04	0.16±0.04
III Zinc	0.09±0.02	0.11±0.01	0.15±0.01
IV Zinc+ Ethanol	0.07±0.01	0.10±0.02	0.14±0.02

mg=milligram
Values are expressed as Mean±S.D.

TABLE IV
EFFECT OF ZINC ON NADH- CYTOCHROME- C-REDUCTASE ACTIVITY IN INTESTINE OF ETHANOL TREATED WISTAR MALE RATS

Group	Time Periods		
	2 weeks	4 weeks	8 weeks
I Control	47.58±4.82	55.27±4.94	69.79±12.34
II Ethanol	68.85±9.11[c]	80.59±6.06[c]	188.89±16.27[c]
III Zinc	49.75±4.11	60.84±4.71	90.11±8.71
IV Zinc+ Ethanol	57.11±4.47[a,p,y]	68.84±5.45[c,q,z]	137.31±23.78[c,r,z]

(Values are expressed as nmol cytochrome c reduced $min^{-1}mg^{-1}$protein)
nmol=nanomole; min=minute; mg=milligram
Values are expressed as Mean±S.D.
[a]p<0.05; [c]p<0.001 by Newman-Keul's test when the values of Group II, III, and IV are compared with those of group I.
[p]p<0.05 ; [q]p<0.01 ; [r]p<0.001 by Newman-Keul's test when the values of Group IV are compared with those of group III.
[y]p<0.01 ; [z]p<0.001 by Newman-Keul's test when the values of Group IV are compared with those of group II.

TABLE V
EFFECT OF ZINC ON GLUTATHIONE-S-TRANSFERASE (GST) ACTIVITY IN INTESTINE OF ETHANOL TREATED WISTAR MALE RATS
(Values are expressed as nmol conjugate formed min-1mg-1protein)

Group	Time Periods		
	2weeks	4 weeks	8 weeks
I Control	0.07±0.01	0.05±0.0794	0.05±0.006
II Ethanol	0.11±0.03[b]	0.11±0.03[b]	0.11±0.03[b]
III Zinc	0.08±0.02	0.06±0.006	0.06±0.01
IVZinc+ Ethanol	0.09±0.01[a]	0.10±0.01[a,q]	0.09±0.02[a,p]

nmol=nanomole; min=minute; mg=milligram
Values are expressed as Mean±S.D.
[a]p<0.05; [b]p<0.01 by Newman-Keul's test when the values of Group II, III, and IV are compared with those of group I.
[p]p<0.05 ; [q]p<0.01 by Newman-Keul's test when the values of Group IV are compared with those of group III.