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FREE RADICAL CHANGES IN METHANOL TOXICITY

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Abstract : Role of free radicals in methanol toxicity was evaluated in methanol treated albino rats. Methanol intoxication increased lipid peroxidation and depleted the free radical scavenging enzyme systems. The free radical quenching effect of vitamin E protected the animals from methanol induced free radical damage.

Key words : free radicals, methanol, folate deficiency, vitamin E

INTRODUCTION

Methyl alcohol, the simplest of all alcohols is toxic to humans. It is being widely used in the industry as a solvent. Handling of products which contain methyl alcohol exposes the population to its toxic vapours. Recently there has been a proposal to use methyl alcohol as an alternate fuel in automobiles due to the rapid dwindling of petroleum resources. If this proposal is implemented it would mean an enormous exposure of public to methanol vapours in comparison to the limited contact present today, thereby increasing the frequency of toxic exposures. In addition, in developing countries like India, methyl alcohol is a common adulterant in country liquor which increases the chance of accidental poisoning enormously.

Toxic effects of methanol in monkeys and human are (1), central nervous system depression of short duration, characterised by asymptomatic latent period of 12–24 hours followed by a severe metabolic acidosis, ocular toxicity, coma and death. Most of the reports point out acidosis as the cause for this toxicity. Metabolic acidosis is due to accumulation of formic acid, which is a product of oxidation of methanol by 3 enzyme systems namely, the alcohol dehydrogenase, catalase and microsomal oxidising systems. Among these, the microsomal oxidising system is known to produce free radicals during the oxidation process. It is also well documented that these generated free radicals cause extensive damage to cellular membranes by depleting endogenous antioxidant defences, thereby leading to cellular dysfunction and

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death. Ethanol, which is also an aliphatic alcohol has been proved to induce a part of its toxicity through free radical mechanism (2–4). However, the role of free radicals in methanol toxicity has not been extensively studied. In a recent report, Skrzydlewska and Farbiszewski (5) have reported that methanol administration increases lipid peroxidation products and decreased the liver glutathione peroxidase and glutathione reductase activities. Hence the present study is aimed at elucidating the possible role of free radicals in methanol toxicity and the beneficial effect of a free radical quencher – vitamin E in modulating the toxic effects of methyl alcohol in albino rats.

METHODS

Wistar strain adult albino rats of either sex weighing 150–200 g were used for this study. They were maintained under standard laboratory condition and fed as libitum with commercial rodent chow (M/s Hindustan Liver Ltd. Bombay, India) and water.

Methanol was administered intraperitoneally (to avoid uncertainties of gastrointestinal absorption) at half the LD 50 dose determined in this laboratory (4 mg/kg). Vitamin E was administered at a dose of 400 mg/kg body weight/day intragastrically. The rats were divided into 7 groups of 15 animals each according to the treatment regime as follows. Group I were the control animals which were administered saline intraperitoneally. Group II animals were administered methanol. In order to study the efficacy of free radical quenching vitamin, vitamin E was administered to group III and group IV animals. Group III animals were administered saline and group IV animals were administered methanol on the 8th day following 7 days of vitamin E treatment.

Human beings have a very low hepatic folate content (6). In methanol metabolism conversion of formate to carbon dioxide is folate dependent. Hence in the deficiency of folic acid, methanol metablism could take the alternate pathway (microsomal pathway) (7). To simulate this, rats were made folate deficient by feeding them on a special dietary regime for 45 days (8). Folate deficiency was further confirmed by estimating urinary excretion of formamino glutamic acid (FIGLU) on the 45th day (9). Rats on a folate deficient diet excreted an average of 64 mg FIGLU/kg body weight/day (Range 25–125) while animals on the control diet excreted an average of 0.29 mg/kg body weight/day (Range 0.15-0.55). These folate deficient animals showed a significant increase in FIGLU excretion when compared to the control animals ($P<0.05$). The folate deficient animals were further divided into 3 groups. Group V animals were the folate deficient control animals administered saline. Group VI animals were treated with methanol on the 45th day. Group VII animals were maintained on folate deficient diet for 45 days with vitamin E treatment on the last 7 days. On the 8th day of vitamin E supplementation these rats were treated with methanol intraperitoneally. Two hours after the administration of methanol/saline, animals of all the seven groups were sacrificed by cervical dislocation and the livers were excised immediately.

Biochemical assays

The livers were washed, weighed and homogenised in ice cold 0.1M tris HCI buffer of pH 7.4, using motor-driven teflon-glass tissue homogeniser. The ability of SOD to inhibit the auto oxidation of epinephrine at pH 10.2 was used as the basis for the assay of SOD (10). The production of hydroxy radicals was assayed by measuring the generation of formaldehyde from dimethylsulphoxide by the liver microsomes (11). The reaction of lipid peroxides with TBA was adopted as a sensitive method for lipid peroxidation in animal tissues (12). Microsomal protein was estimated by the method of Lowry et al (13).

Statistical analysis

Statistical significance between the groups were evaluated using one way analysis of variance (Anova) and on significant F test ratio tests, Tukey's multiple comparison were carried out.

RESULTS

Administration of methanol to rats increases ($P<0.05$) the hydroxyl radicals (Table I). In folate deficient rats also hydroxyl radical significantly increase ($P<0.05$). Vitamin E supplementation prior to methanol administration prevents this change. Folate deficient rats administered vitamin E prior to methanol administration showed a significant decrease in hydroxyl radical compared to folate deficient animals administered methanol only. The activity of superoxide dismutase in liver is presented in Table I. The engyme activity was found to be decreased significantly ($P<0.05$) in group II (methanol treated) and group VI (folate deficient rats treated with methanol) animals. Vitamin E supplemented groups (group III, IV and VII) showed a restoration of the enzyme activity to near normal level.

MDA in methanol treated animals increased significantly ($P<0.05$) when

TABLE I: Effect of methanol on liver biochemical parameters.

Group (n = 7 in each group)	*SOD (U/mg pr)*	*OH (HCHO/mg pr/min)*	*LPO (nM MDA/mg pr)*
Group I (Control)	7.113±0.011	0.287±0.0004	1.198±0.0052
Group II (Methanol)	5.615±0.029*	2.006±0.0004*	1.740±0.017*
Group III (Vit E)	7.094±0.003	0.289±0.0005	1.415±0.001*
Group IV (Vit E + Meoh)	6.741±0.006	1.457±0.027*@	1.274±0.002
Group V (Folate deficient)	7.130±0.010	0.343±0.0019	1.239±0.006
Group VI (Fol def + Meoh)	3.446±0.017*#	3.203±0.030*#	1.888±0.001*
Group VII (Fol.def + Vit E + Meoh)	4.931±0.0002*@	1.407±0.0013*@	1.647±0.001*
Anova	F = 52.265	F = 46.926	F = 119.151

Values given are mean ± SE, df = 6,98
*–compared with control
#–Methanol vs Folate def. + Methanol
@–Methanol vs vit E + Methanol & fol def. Methanol vs fol def. + vit E + Methanol
Significant at $P<0.05$

compared to controls, more so in folate deficient animals administered methanol. Vitamin E supplementation prevented the MDA elevation as compared to group II and VI.

DISCUSSION

Methanol is known to be oxidised via three main oxidative pathways among which the alcohol dehydrogenase (folate dependent) and catalase peroxidative system have been extensively studied. Goodman and Tephly (14), have also reported the role of hepatic microbodies in the peroxidation of methanol. Oxidation of methanol in the liver by the microsomal oxidising system enhances the production of free radicals in the liver, as also evidenced by the increase of hydroxyl ions in this study.

Exposure of tissue to free radicals in a variety of experimental systems have documented the ability of free radicals to produce damage. It was well documented that the detection and measurement of lipid peroxidation is the evidence most frequently cited to support the involvement of free radicals in toxicology and human diseases (15). The consequence of lipid peroxidation in cellular membranes is damage to poly unsaturated fatty acids which tends to reduce membrane fluidity which is essential for proper functioning. The increase in MDA level observed in this study, which is an index of lipid peroxidation, indicates cell membrane damage after methanol administration. Antioxidant and antioxidant enzymes have evolved to limit the rate of production of free radical damage throughout the cells and tissue. The first line of defence is the preventive antioxidants which suppress the formation of the free radicals. Superoxide dismutase which is a key antioxidant enzyme in tissue, catalyses the disproportionation of superoxide to hydrogen peroxide. The second line of defence is the antioxidant vitamin E. Methanol administration to rats induces free radical generation and hence the first line of defence comes to rescue as shown by the significant fall in SOD in methanol treated animals (group II) and folate deficient animals treated with methanol (group VI). An increase in MDA in these animals shows that the generated free radicals might have caused damage to the cellular membranes resulting in lipid peroxidation. In E supplemented animals, adverse biochemical changes brought about by methanol administration reverse to normal owing to the fact that vitamin E is potent free radical quencher which has the ability to preserve membrane fluidity and stability.

Rats with folate deficiency forms the ideal model which will mimic the effects of methanol poisoning in man (6). Hence metabolism of methanol in folate deficient animals will have to take an alternate pathway which may be either the microsomal or the catalase peroxidative pathway both of which generates free radicals during the oxidation process. The significant difference in levels of the hydroxyl radicals in normal and folate deficient animals treated with methanol, is a clear evidence for the above hypothesis. In rats maintained on folate deficient diet for 45 days and then treated with methanol, free radical level significantly decreased in

animals pre-treated with vitamin E showing the beneficial effect of this vitamin in methanol toxicity.

Thus from this study, we conclude that methanol administration in rats increases the free radical generation both in normal and more so in folate deficient rats and vitamin E supplementation has proved to be beneficial in suppressing the circulating levels of free radicals and the free radical induced damage.

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