3-Hydroxy-3-Methylglutaric Acid and Triton-Induced Hyperlipidemia in Rats

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Summary. 3-Hydroxy-3-methylglutaric acid (HMG) significantly decreased cholesterol, triglyceride and phospholipid levels in whole serum, serum β-lipoproteins and liver of Triton-induced hyperlipidemic rats. Therapeutically 50 mg HMG/kg is equivalent to 200 mg nicotinic acid/kg in lowering all these lipid parameters. HMG may exert its hypolipidemic effect through inhibition of lipoprotein synthesis.

Triton WR-1339 is known physically to alter very low density lipoprotein (VLDL), rendering them refractive to the action of lipolytic enzymes of blood and tissue. This prevents or delays their removal from blood and secondarily stimulates the synthesis of lipids enhancing the hyperlipidimia[2–9]. SCHURR et al.[10] have described a procedure for induction of hyperlipidemia in rats with Triton WR-1339 and used this method for screening new lipid-lowering agents having a wide variety of activities. Considering the potential hypolipidemic properties of 3-hydroxy-3-methylglutaric acid (HMG) in animals[11–17] and man[17,18], we describe here the effect of HMG in counteracting the Triton-induced hyperlipidemia in rats. A well-known hypolipidemic drug, nicotinic acid[19], was included in this study for comparative purposes.

Materials and methods. 24 adult male albino rats (stock colony of Indian Veterinary Research Institute, Izzatnagar, India) of average body weight 200 g (range 180–220 g) were fasted for 24 h and i.p. injected 250 mg Triton WR 1339/kg body weight dissolved in 0.15 *M* NaCl at the concentration of 50 mg/ml. The animals were randomly divided into 4 groups of 6 animals each. 2 groups received i.p. HMC (Schwarz/Mann, USA) at the concentration of 25 and 50 mg/kg, and a 3rd group received i.p. 100 mg nicotinic acid (Nutritional Biochemicals, USA)/kg body weight in 1 ml saline. Each rat of the treated groups received two doses of compound, first immediately after Triton injection, and subsequently 20 h later. The total average dose for 200 g rat for each group was, therefore, 50 mg HMG/kg, 100 mg HMG/kg and 200 mg nicotinic acid/kg in 2 ml saline. The animals receiving 1 ml saline i.p. each time served as control. Fasting was continued during the post Triton period. Blood was withdrawn by cardiac puncture 43 h after Triton administration under ether anaesthesia. Serum β-lipoproteins were separated by dextran sulfate precipitation method[20]. Liver lipids were extracted as described earlier[15]. Cholesterol[21], triglyceride[22] and phospholipid[23] levels were analyzed in whole serum, serum β-lipoproteins and liver. Statistical significance was calculated by Student's *t*-test.

Results and discussion. As evident from the Table, HMG at both concentrations significantly decreased cholesterol, triglyceride and phospholipid levels in whole serum, serum β-lipoproteins and liver of rats. The extent of decrease in all these lipid parameters was more marked at 100 mg HMG/kg. At a dose level of 50 mg HMG/kg, the liver phospholipids were not significantly lowered. Nicotinic acid lowered all lipid parameters except liver phospholipid in whole serum, serum β-lipoproteins and liver. A comparative study of nicotinic acid showed that therapeutically 50 mg HMG/kg is equivalent to 200 mg nicotinic acid/kg in lowering lipid values in blood and liver. This is in agreement with an earlier report where 50 mg HMG/kg has been found is equivalent to 200 mg nicotinic acid/kg in offering almost total protection against the lipemic effect of alcohol[17]. The ratio between treated and control values of serum cholesterol and triglyceride of HMG-treated rats was less than that reported for well-known drugs like clofibrate, D-thyroxin and nicotinic acid in Triton test[10]. This could mean that HMG is superior to these compounds in counteracting Triton-induced hyperlipidemia.

Among several possibilities of mechanism of action of HMG reported earlier, it has been emphasized that hypo-

Comparison of hypolipidemic activities of HMG and nicotinic acid in Triton-test (Mean ± SE)

Lipids	Control group	Treated groups		
		50 mg HMG/kg	100 mg HMG/kg	200 mg nicotinic acid/kg
Whole serum (mg/100 ml)				
Cholesterol	222 ± 20	140 ± 12[b] (37)[a]	101 ± 5[b] (54)	135 ± 10[b] (39)
Phospholipid	275 ± 30	210 ± 21[c] (24)	165 ± 8[b] (40)	198 ± 16[d] (28)
Triglyceride	390 ± 39	239 ± 15[c] (39)	182 ± 7[b] (53)	220 ± 18[b] (43)
Serum β-lipoproteins (mg/100 ml)				
Cholesterol	142 ± 24	94 ± 14[b] (34)	71 ± 6[b] (49)	85 ± 5[b] (40)
Phospholipid	147 ± 10	114 ± 8[d] (22)	86 ± 4[b] (41)	98 ± 10[c] (33)
Triglyceride	348 ± 30	190 ± 20[b] (45)	167 ± 9[b] (52)	181 ± 16[b] (47)
Liver (mg/g)				
Cholesterol	3.5 ± 0.1	2.7 ± 0.2[b] (23)	2.2 ± 0.2[b] (37)	2.5 ± 0.2[c] (30)
Phospholipid	30.2 ± 1.9	28.1 ± 1.8 (7)	24.2 ± 1.9[c] (22)	26.4 ± 2.4 (12)
Triglyceride	16.8 ± 1.2	11.8 ± 1.0[d] (30)	9.6 ± 2.5[d] (42)	10.9 ± 1.2[d] (35)

[a] Values in parentheses indicate the percent reduction with respect to control group. Significantly different from control; [b] $p < 0.001$; [c] $p < 0.01$; [d] $p < 0.02$.

lipidemic response of HMG could involve a shift in lipoprotein spectrum[14,15]. Recently we have shown that HMG has no effect on orotic acid fatty liver in rats[24]. Since Triton is known physically to alter VLDL, and HMG is capable of counteracting Triton-induced hyperlipidemia, the possibility of HMG exerting its hypolipidemic effect through inhibition of lipoproteins synthesis appears more plausible. Furthermore, the method used detects compounds which inhibit lipid biosynthesis or its catabolism[10], the hypolipidemic activity of HMG may be mediated through its effect on lipid metabolism. FOGELMAN et al.[25] have very recently shown that normally 12% of mevalonic acid is catabolized through a shunt pathway in mammalian system involving *trans*-3-methylglutaconyl CoA. A derailment in the operation of this pathway might explain the hypercholesterolemic condition in rats and man. In view of earlier reports on hypolipidemic activity of HMG[11–18] in animals and man, it is tempting to suggest, therefore, that HMG may in some way correct the derailed pathway.

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Resistance of Cold-Exposed Rats to Aflatoxin

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Summary. Male rats kept at a temperature of 4°–5 °C were refractory to a lethal dose of aflatoxin compared to animals at 20°–21°C which exhibited a high mortality and marked liver damage. It is suggested that this decreased susceptibility is mediated through a stimulated microsomal drug-metabolizing system in cold environment.

The harmful effects of aflatoxin have been found to be modified by a variety of conditions including protein deficiency[1], vitamin A-deficiency[2], hypophysectomy[3] and pregnancy[4]. A critical review of these conditions had led to the suggestion that the increased susceptibility of rats in these conditions is due to a defective drug-metabolizing system in the liver[5]. The influence of environmental factors such as cold exposure, which modify the hepatic microsomal drug-metabolizing system have been studied in other hepatotoxins[6]. However, no such studies appear to have been investigated in aflatoxicosis.

Materials and methods. 12 male albino rats weighing approximately 120 g were divided into 2 groups. One group was kept at room temperature (20–21 °C) while the other group was placed in an adequately ventilated cold room having a temperature of 4–5 °C. All the animals received the colony stock diet and water ad libitum. At the end of 72 h, all the animals received i.p. a pure preparation of aflatoxin containing predominantly B_1 in a dose of 8 mg/kg body weight. The experiment was terminated by sacrificing all animals in both groups which were alive 72 h after administration of the toxin. The livers of the dead as well as the sacrificed animals were fixed in formalin and processed for microscopic examination in the usual manner.

Results and discussion. Between 40 and 48 h following the administration of the toxin, 5 of the 6 animals at room temperature died. Livers of these animals exhibited extensive necrosis of liver cells in the periportal and midzones. The rats exposed to low temperature, however, were apparently unaffected. Livers of these animals showed no parenchymal damage. There was, however, bile-duct and ductular proliferation with prominent portal tracts.

On the basis of the mortality and the histology of livers, it is clear that the susceptibility of rats to aflatoxin is decreased by exposure to cold. Exposure to lower temperature has been found to result in an increase in the microsomal drug-metabolizing enzymes[7]. Earlier, we had

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