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Preventive effects of Liv.52 on the activities of Cytochrome P-450 and Nicotinamide Adenine Dinucleotide Phosphate Hydrogen (NADPH) – Dependent Lipid Peroxidation in the Liver of Carbon Tetrachloride-Intoxicated rats

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ABSTRACT

This experimental, controlled study with rats exposed to carbon tetrachloride intoxication and given Liv.52 has suggested that when Liv.52 is given along with CCl_4 there may be decreased formation of CCl_4 radical derivatives. It is concluded that Liv.52 is hepatoprotective because it prevents lipid peroxidation, or possibly because it prevents lipid peroxidation, or possibly because it prevents irreversible binding of CCl_4 to important cellular proteins for its metabolism.

INTRODUCTION

In humans, impaired drug metabolism is often associated with hepatic disorders, especially cirrhosis, which may ultimately lead to complications of drug therapy[1]. Earlier studies have shown that levels of hepatic cytochrome P-450, the principal component of the mixed function oxidase system (MFOS) which is active in drug metabolism, are lowered in experimental animals rendered hepatotoxic by chronic or single exposure to carbon tetrachloride[2,3]. Also, it is well established that lipid peroxidative degradation of biomembranes occurs in carbon tetrachloride (CCl_4)-induced liver injury and is one of the principal causes of hepatotoxicity[4]. This is supported by evidence that some antiperoxidative agents such as cystamine[5], silamyrin[6] and malotilate[7] prevent CCl_4-mediated hepatic injury.

Hepatoprotection has also been reported by many investigators using a novel multi-herbal hepatoprotective agent, Liv.52 in hepatic disorders induced by CCl_4[8-10], alcohol[11] and radiation[12], as well as in severe cases of infective hepatitis, chronic active hepatitis and cirrhosis of the liver[13]. Even though it is a composite extract of various plants, Liv.52 has been found to be effective in normalising the activities of liver microsomal enzymes (viz. aniline hydrooxylase and aminopyrine demethylase) as well as in restoring disturbed levels of DNA and RNA in CCl_4-treated animals[8].

The present study was carried out to investigate whether Liv.52 can ameliorate CCl_4-induced changes in hepatic microsomal cytochrome (cyt) P-450 content and NADPH-dependent lipid peroxidation.

MATERIALS AND METHODS

Liv.52 syrup was obtained from The Himalaya Drug Company, NADPH, BSA, sodium dithionite and NADH were procured from other sources. All other chemicals used were of analytical grade.

Mature male albino rats (Swiss Porton strain) weighing between 150-180 g were procured from the Central Animal House, Punjab University, Chandigarh. The animals were fed pelleted standard laboratory feed (Hindustan Lever) and water *ad libitum*.

In all, 18 rats were segregated into three groups of six rats each. Group 1 served as control (untreated) and were injected subcutaneously with 0.2 ml of groundnut oil alone twice a week. In Groups 2 and 3, animals were injected subcutaneously (s.c.) with 0.2 ml of CCl_4 mixed with 0.2 ml of groundnut oil (1:1) twice a week. In addition, the animals in Group 3 were also given orally 0.5 ml of Liv.52 every day[10]. All treatments continued for a total duration of six weeks.

The animals were killed by exsanguination under light ether anaesthesia at the end of the study. After opening the peritoneal cavity, the livers were perfused *in situ* with cold 50 mM Tris-HCl buffer (*p*H 7.4) containing 0.15 M KCl and 2 mM EDTA. The tissues were excised, weighed and homogenised in 50 mM Tris-HCl buffer (*p*H 7.4) containing 150 mM KCl and 0.25 M sucrose. Finally, the homogenate was diluted to a concentration of 1 g wet wt/4.0 ml (w/v) with the same buffer. Tissue homogenate was subjected to cold centrifugation at 10,000 g for 30 min. The 10,000 g supernatant was used for biochemical assays.

The concentrations of total cyt P-450 in microsomal preparations was determined by the method of Omura and Sato[14] from CO difference spectrum of dithionite reduced samples, using an extinction coefficient of 91 mM^{-1} cm^{-1}. NADPH-dependent lipid peroxidation was assayed by the method of Pederson *et al.*[15]. Protein concentrations of microsomal suspension were determined by the method of Lowry *et al.*[16].

RESULTS AND DISCUSSION

The results of these investigations, shown in Table 1, demonstrate that CCl_4 toxicity to the animals resulted in decreased activity of cyt P-450 (p<0.001; 31.72%) and greatly increased the extent of NADPH-dependent lipid peroxidation (p<0.001; 259%) in the liver. However, treatment with Liv.52 ameliorated this observed decrease of cyt P-450 activity, reducing it to almost normal limits, although there was a persistent marginal increase of 27% as regards lipid peroxidation.

Table 1: Effect of Liv.52 on the activities of cytochrome P-450 and NADPH-dependent lipid peroxidation in rats intoxicated with CCl_4
(mean ± SD; n=6 animals in each group)

Group	Cyt P-450 (nmoles mg^{-1} protein)	NADPH-dependent lipid peroxidation (nmoles mg^{-1} protein/15 min)
(Control)	0.769 ± 0.10	0.203 ± 0.040
(CCl_4)	0.525 ± 0.07**$^{\#}$	0.729 ± 1.03**$^{\#}$
(CCl_4 + Liv.52)	0.720 ± 0.08	0.258 ± 0.046*

As compared with Group 1, *p<0.05; **p<0.001.
As compared with Group 3, $^{\#}p$<0.001.

It is generally accepted that CCl_4-induced liver injury is initiated by the formation of a reactive metabolite, trichloromethyl radical (CCl_3), by the microsomal mixed function oxidase system[17,18]. Formation of this reactive metabolite is catalyzed by microsomal enzymes. They are thus among the most vulnerable to the toxic effects of CCl_4 and ultimately levels of cyt P-450 are suppressed[18,19]. Further, there are reports that this activated CCl_3 radical binds covalently to macromolecules and induces peroxidative degradation of membrane lipids of the endoplasmic reticulum rich in polyunsaturated fatty acids[4].

This leads to disintegration of mitochondrial, lysosomal and cellular membranes and finally to cell necrosis[20].

Thus, it is likely that the potent antiperoxidative agents protect the liver by preventing CCl_3-induced peroxidative disintegration of membranes. In our study, Liv.52 treatment of CCl_4-intoxicated rats resulted in complete normalisation of cyt P-450 activity and also altered NADPH-dependent lipid peroxidation significantly as compared to hepatotoxic rats.

The protective effects of Liv.52 in reducing lipid peroxidation in hepatotoxic conditions have been shown earlier[21] and are attributed to the action of Liv.52 in reducing tocopherol levels. Although there is insufficient information to establish the mechanism of action of Liv.52 protection, this could be due to its antiperoxidative activity, which is dependent either on decreased production of CCl_4 radical derivatives, or due to its antioxidant action.

The results of our study suggest that the action may be due to the decreased formation of CCl_4 radical derivatives, as Liv.52 was given along with CCl_4. We conclude that Liv.52 is hepatoprotective because it prevents lipid peroxidation, or possibly because it prevents irreversible binding of CCl_4 to important cellular proteins for its metabolism.

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