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Effect of Liv.52 on Carbon Tetrachloride-induced Changes in Hepatic Microsomal Drug-Metabolizing Enzymes of the Rat

(Liver cirrhosis, aryl hydrocarbon hydroxylase; aniline hydroxylase; *p*-aminopyrine *N*-demethylase)

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SUMMARY

The effects of the hepatotonic Liv.52 on carbon tetrachloride (CCl_4)-induced changes in the activities of the microsomal enzymes, aniline hydroxylase, p-aminopyrine N-demethylase activities, although the decrease in AHH activity could not be prevented. Kinetic studies showed that the effect of Liv.52 was not due to an alteration in the Km values of the enzymes. The possible mechanism by which Liv.52 moderated the CCl_4-induced changes in the microsomal drug-metabolizing enzymes is discussed.

INTRODUCTION

Liv.52 is an indigenous herbal compound used in several hospitals in India in the treatment of various types of liver dysfunction. Experimental evidence of the efficacy of this drug in cirrhosis of the liver[1,2], and in hepatitis[3,4] has been recorded. Apart from its therapeutic effects, it has been reported to protect against hepatotoxins, e.g.CCl_4[5,6] and alcohol[7] as assessed by alterations in liver and serum lipids and transaminase levels.

The earliest alterations in liver cell structure and function following CCl_4 poisoning involve the endoplasmic reticulum[8]. The mixed-function oxidase enzymes involved in the metabolism of drugs and other foreign compounds are an integral part of the endoplasmic reticulum[9] and CCl_4 is known significantly to affect the activities of these enzymes[10]. Investigations have therefore been carried out to establish if Liv.52 can also offer protection against CCl_4-induced changes in the activities of liver microsomal drug-metabolizing enzymes.

MATERIALS AND METHODS

Liv.52 tablets were from The Himalaya Drug Co. (Bombay, India). All other chemicals were from Sigma Chemical Co. (London, U.K.).

Treatment of animals:

Male rats (Wistar Strain, 150-200 g) were divided into 6 groups of 10. Group 1 served as controls and were dosed orally 1 ml/day distilled water. Group 2 was similarly dosed for 10 days with 1 ml/day of a suspension of Liv.52 in distilled water. Group 3 was given a single i.p. dose of CCl_4 (0.2 ml/100 g body weight) and killed 24 hours later. Group 4 received Liv.52 for 10 days, then dosed with CCl_4 (0.2ml/100 g body weight) and killed after 24 hours. Group 5 was treated with a single dose of CCl_4 and after 24 hours dosed with Liv.52 daily for a further 7 days. Group 6 was administered a single i.p. dose of 0.2 ml/100 g CCl_4 and killed after 7 days. Livers of animals from all groups were excised and portions were fixed in buffered formalin for histological assessment of

hepatic damage. The remaining portions of liver were used to prepare microsomes by the method of Kamath *et al*.[11] for enzyme determinations.

Groups 5 and 6 were killed after 7 days because liver damage, induced by a single dose of CCl_4 is completely repaired in 5-7 days[10].

Composition of Liv.52

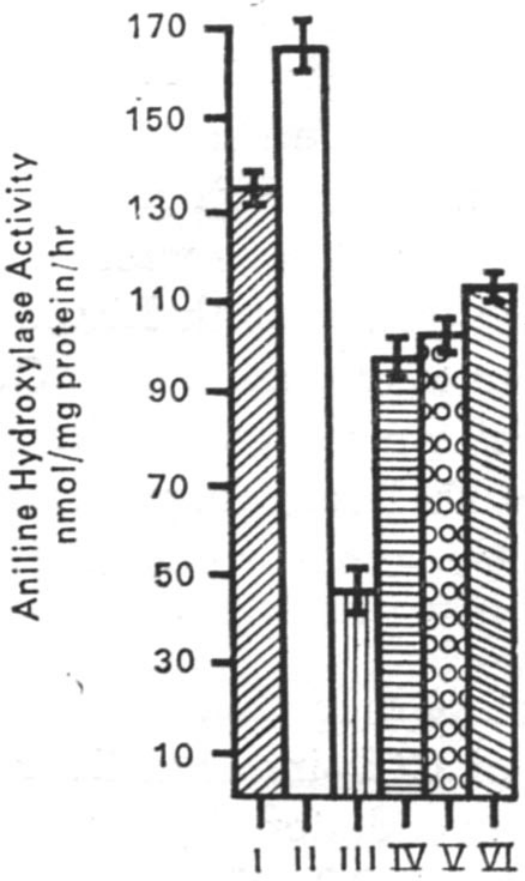
Each ml of Liv.52 contained:

Capparis spinosa	34 mg
Cichorium intybus	34 mg
Solanum nigrum	16 mg
Cassia occidentalis	8 mg
Terminalia arjuna	16 mg
Achillea millefolium	8 mg
Tamarix gallica	8 mg

Enzyme assays:

Aniline hydroxylase activity was determined by measuring the amount of p-aminophenol formed from aniline hydrochloride by the method of Schenkman *et al.*[12] *p*-Aminopyrine *N*-demethylase activity was assayed by the method of LaDu *et al*.[13] Estimation of the amount of formaldehyde formed during *N*-demethylation of aminopyrine was carried out by the method of Nash[14]. AHH activity was assayed by measuring the fluorescence due to the hydroxylated metabolites formed during the oxidation of 3,4-benzo (a) pyrene by the method of Nebert[15]. Microsomal protein was estimated by the method of Lowry *et al.*[16]

Fig. 1: Effect of CCl_4 and Liv.52 on microsomal aniline hydroxylase activity. Group I, control: Group 2, Liv.52-treated (1 ml/day) for 10 days; Group 3, 24 hours after CCl4 administration (0.2 ml/100 g); Group 4, 24 hours after CCl_4 administration to some Group 2 animals; Group 5, 7 days after CCl_4 treatment; Group 6, single dose CCl_4 + Liv.52 for 7 days. Results are expressed as mean values of 10 rats ± standard errors. The significance of the differences between the groups was verified by Student's *t*-test; $p<0.001$ was obtained in all cases.



RESULTS AND DISCUSSION

The effects of CCl_4 and Liv.52 on the activities of the hepatic microsomal enzymes, aniline hydroxylase, *p*-aminopyrine *N*-demethylase and AHH are shown in Figs.1, 2 and 3 respectively.

In agreement with results obtained earlier[10] the present study revealed a significant reduction in the microsomal enzymes, aniline hydroxylase and *p*-aminopyrine *N*-demethylase, and also of 3,4-benzo (a) pyrene hydroxylase, within 24 hours of exposure of rats to a single sub-lethal dose (0.2 ml/100 g) CCl_4. Results obtained in this study suggest that pre-treatment with the hepatotonic Liv.52 for 10 days prior to the challenge with CCl_4 could moderate to a considerable extent, the reduction in aniline hydroxylase (Fig.1) and aminopyrine *N*-demethylase (Fig.2) activities resulting from the administration of CCl_4 alone. The percentage reduction in aminopyrine *N*-demethylase and aniline hydroxylase activities by CCl_4 in the control rats was 25.6% and 66.1% respectively, while the

percentage reduction was 19.5% and 41.22% respectively, in Liv.52 treated rats. The apparent absence of any significant effect by Liv.52 with regard to the p-aminopyrine *N*-demethylase activity (see Fig.2) might have been influenced by the inductive effect of Liv.52 alone. The CCl_4-mediated decrease in 3,4 benzo (a) pyrene hydroxylase activity could not be prevented by 24 hours after exposure to CCl_4 also resulted in a faster recovery of enzyme activity (Figs.1 and 2).

The effects of Liv.52 on the kinetic properties of the microsomal enzymes, *p*-aminopyrine *N*-demethyolase and aniline hydroxylase, are seen in Fig.4. The fact that Km values for these enzymes in control and Liv.52-treated animals are the same, suggests that the Liv.52 mediated improvement in hydroxylase and demethylase activities was not due to an alteration in the affinity of the enzymes for their respective substrates.

Fig. 2: Effect of CCl_4 and Liv.52 on microsomal *p*-aminopyrine *N*-demethylase activity Group I, control; Group 2, Liv.52-treated (1 ml/day) for 10 days; Group 3, 24 hours after CCl_4 (0.2 ml/100 g) administration; Group 4, 24 hours after CCl_4 administration to some Group 2 animals; Group 5, 7 days after CCl4 treatment; Group 6, single dose CCl_4 + Liv.52 for 7 days. Results are expressed as mean values of 10 rats ± standard errors. The significance of the differences between the groups was verified by Student's *t*-test; $p<0.001$ was obtained in all cases.

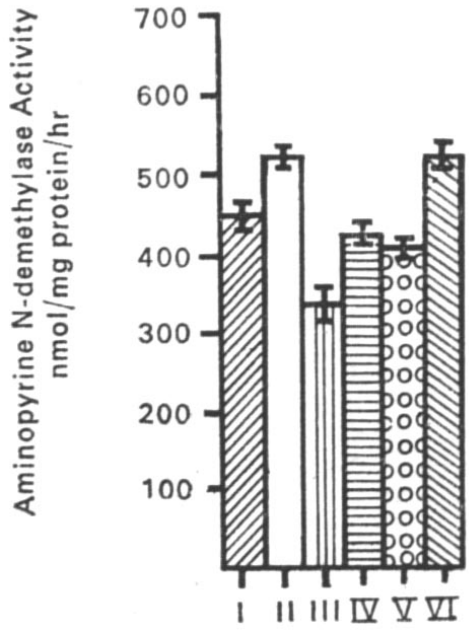
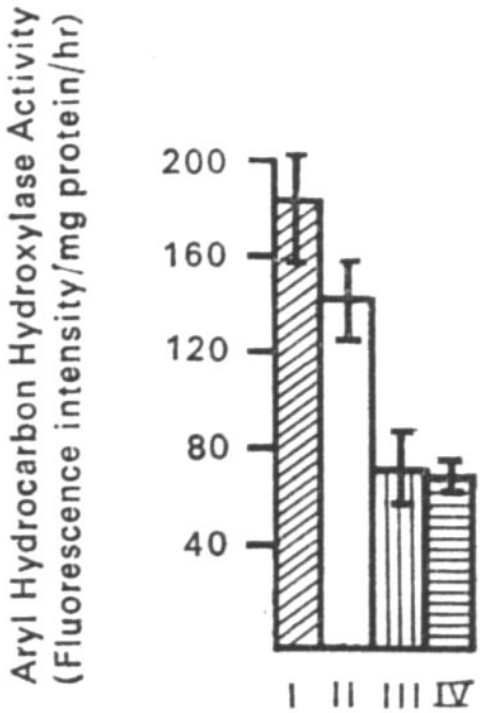


Fig. 3: Effect of CCl_4 and Liv.52 on microsomal AHH activity. Group I, control; Group 2, Liv.52-treated (1 ml/day) for 10 days; Group 3, 4 hours after CCl_4 administration (0.2 ml/100 g); Group 4, 24 hours after CCl_4 administration to some Group 2 animals. Results are expressed as mean of 10 animals ± standard errors. The significance of the differences between the groups was verified by Student's *t*-test; $p<0.001$ was obtained in all cases.

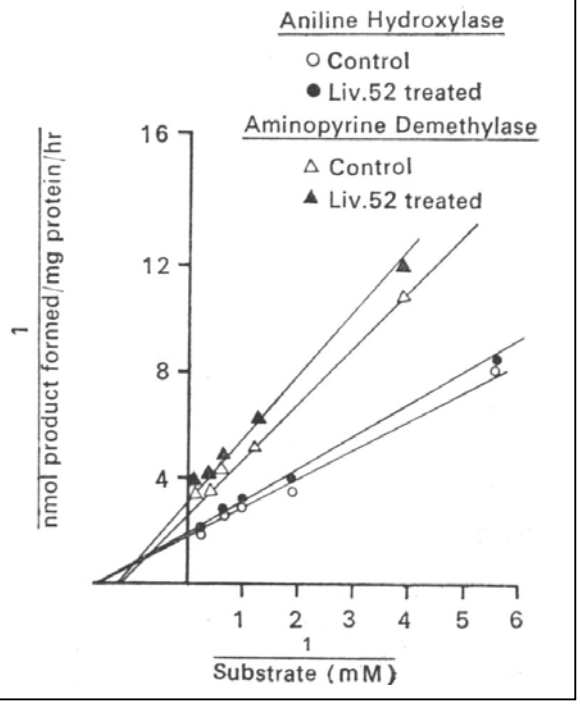


Histopathological examination showed that livers of rats challenged with CCl_4 alone showed centrilobular necrosis with mononuclear infiltration in the portal area, fatty deposition and loss of cell boundaries. In animals pre-treated with Liv.52 and subsequently given CCl_4, there was no noticeable hepatocellular necrosis or mononuclear infiltration and the reticulum framework was well retained. Livers of rats treated with Liv.52 for 7 days, subsequent to treatment with CCl_4 also showed a well preserved architecture in comparison with livers from animals killed 7 days after exposure to a single dose of CCl_4.

It has been established that one of the principal causes of CCl_4-induced liver injury is lipid peroxidation by the free radical derivates of CCl_4[17]. Hence it is not surprising that many compounds cited in the literature as being protective agents against CCl_4-induced liver injury, exert their action by impairment of CCl_4- mediated lipid peroxidation, through (a) a decreased production of CCl_4 free radical derivatives[18,19], or (b) due to the antioxidant activity of the protective agent itself[20].

Liv.52 can also exert protection against CCl_4-induced changes in microsomal functions by alterations in membrane lipid composition and lipid peroxidation, as is suggested by the observations of Saxena *et al*.[21] However, in the present investigation Liv.52 even when given subsequent to CCl_4 administration can produce a more rapid recovery of microsomal enzyme activities when compared with those exposed to CCl_4 only, indicating that the protective action of Liv.52 may not simply be due to antioxidant to that involved in the protective action of cysteine[22]. It is thought that the protective action of cysteine against CCl_4–induced necrosis is not due to interference with the initial stages of either activation or lipid peroxidation, but to the possible prevention by cysteine of the irreversible binding of CCl_4 to some important cellular protein. In this respect, Liv.52 may also condition the hepatic ells to cause accelerated regeneration by virtue of which the decrease in activity of membrane-bound enzymes would be prevented.

Fig. 4: Lineweaver–Burk plots of microsomal aniline hydroxylase and *p*-aminopyrine *N*-demethylase activities in control rats and rats pre-treated with Liv.52 suspension (1 ml/day) for 10 days. The enzyme activities were measured as nmol *p*-aminophenol or formal-dehyde, respectively, formed/mg protein/hr.



The reason why Liv.52 does not offer protection against CCl_4-induced decrease in AHH activity is not clear. It is possible that apart from its general effects on the components of the endoplasmic reticulum, CCl_4 may also have a selective effect on the AHH molecule, which cannot be reversed by pre-treatment with Liv.52.

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