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Hepatoprotection by malotilate against carbon tetrachloride-alcohol-induced liver fibrosis

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Abstract

Subchronic treatment of male rats with carbon tetrachloride (CCl_4, twice weekly 0.2 ml/kg p.o.) and feeding a 5% alcohol solution instead of drinking water led to a nearly complete liver cirrhosis in all animals within 4 weeks. This was also documented by a three fold increase in hepatic total hydroxyproline content. Steatosis was quantified by enhanced liver triglyceride concentrations and acute necroses by increments of serum enzyme activities (GPT, SDH). Daily oral treatment with malotilate (100 mg/kg) totally prevented the development of liver cirrhosis, hepatic hydroxyproline accumulation and increases in serum enzyme activities induced by CCl_4-alcohol. In cianidanol-treated rats (100 mg/kg p.o.) only portoseptal fibrosis was seen, however hydroxyproline and triglyceride accumulation as well as enhanced serum enzyme activities were not suppressed. D-penicillamine (300 mg/kg p.o.) and colchicine (50 μg/kg i.p.) failed to protect rats against CCl_4-alcohol induced fibrosis, necrosis and steatosis in this model.

Introduction

Experimental liver cirrhosis by treatment with CCl_4 has been accomplished by different techniques and in several animal species [for review see 1]. In our laboratory complete cirrhosis is produced by low oral dosage of CCl_4 (0.2 ml/kg twice weekly) and feeding a 5% alcohol solution within 4 weeks [2, 3]. In this model male rats proved to be more sensitive than females [4].

Malotilate (diisopropyl-1, 3-dithio-2-ylidene-malonate) has been shown to prevent acute experimental liver damage induced by hepatoxic agents like allyl alcohol, bromobenzene, carbon tetrachloride, chloroform, dimethyl-nitrosamine and thioacetamide [5]. This hepatoprotective activity was not related to an inhibition of the microsomal mixed-function oxidase system which activates many hepatotoxic agents to reactive intermediates [6–8]. In our model we compared the hepatoprotective efficacy of malotilate with that of D-penicillamine, colchicine and cianidanol which had been previously shown to exert antifibrotic activities in different models of hepatic fibrosis [9–11].

Methods

Male Wistar rats (Breeder: Winkelmann, Borchen) with an initial body weight of 200–220 g were used throughout and fed with Altromin® pellets. Ethanol was given as a 5% solution (v/v) instead of drinking water. Carbon tetrachloride was diluted with olive oil (0.2 ml/kg ≙ ml/kg instilled volume) and given by gavage twice weekly. Malotilate (100 mg/kg), D-pencillamine (300 mg/kg) and cianidanol (100 mg/kg) were suspended in a 1% tylose (carboxymethyl-cellulose) solution and given orally every day. Colchicine was dissolved in saline and injected i.p. daily at a dose of 50 μg/kg. Malotilate and cianidanol were a kind gift o Zyma, Munich. Munich. D-pencillamine was purchased from Sigma, Munich and colchicin (DAB7) from Serva, Heidelberg. All compounds were of the highest purity grade available and were stable in aqueous solutions. To avoid artefacts, however, solutions were prepared freshly on every day of application.

Blood samples were obtained by cutting the tip of the tail or, at the end of the observation period, by decapitation. Serum enzyume activities of alanine aminotransferase (GPT) and sorbitol dehydrogenase (SDH) were always estimated 3 days after the last CCl_4 treatment using commercial reagent kits of Boehringer, Mannheim. Hepatic triglyceride content was determined by means of a commercial reagent kit of Boehringer, Mannheim, and total hepatic hydroxyproline concentrations were measured according to Prockop and Udenfriend [12]. For morphological examinations the livers were fixed in formalin, and stained either with haematoxylin-eosin or with Gomori's silver impregnation.

Results

As shown in Figure 1 combined treatment with CCl_4/alcohol led to significant increases of serum enzyme activities of GPT and SDH indicating liver parenchymal damage. These were totally suppressed in the malotilate-treated rats during

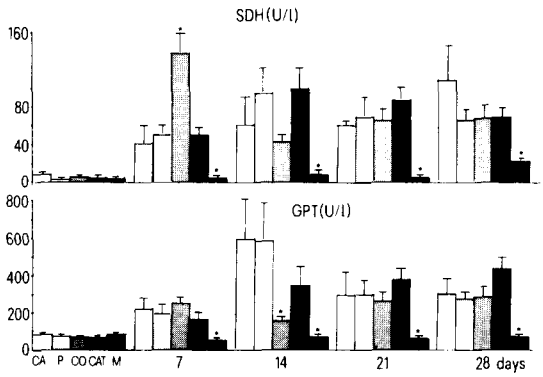

Figure 1
Serum enzyme activities in rats treated with CCl_4 (0.2 ml/kg p.o. twice weekly) and a 5% ethanol solution. Values represent means and standard errors out of 8 rats each. CA = CCl_4-alcohol; P = D-penicillamine, 300 mg/kg p.o.; C = colchicine, 50 µg/kg i.p.; CAT = cianidanol, 100 mg/kg p.o.; M = malotilate, 100 mg/kg p.o. Asterisks indicate $p < 0.05$ (Dunnett's t-Test) as compared to the CA-group.

the whole observation period. D-penicillamine, colchicine and cianidanol were not able to decrease the CCl_4-alcohol-induced increments of serum enzyme activities; significantly higher SDH-activities after 7 days and lower GPT-activities after 14 days in the colchicine group should not be over-emphasized.

Histomorphological examination of the livers at the end of the 4-weeks treatment period revealed the development of complete cirrhosis with broad fibrotic septa, pseudolobuli, ductular proliferations and increased mitotic activities, as well as fatty infiltration and acute necroses (Fig. 2a, b; Table 1). Fibrosis is further evidenced by a fourfold increase in hepatic hydroxyproline content (Fig. 3), steatosis by an enhanced triglyceride concentrations as compared to untreated controls. Malotilate totally protected against CCl_4-alcohol-induced liver fibrosis (Fig. 2c) and hydroxyproline accumulation, but did not influence steatosis (Fig. 3). Treatment with D-pencillamine and colchicine did not exert any antifibrotic or antisteatic activities in our model (Table 2, Fig. 3). Cianidanol, however, partially prevented complete cirrhosis in 6 out of 8 rats, only portoseptal fibrosis, acute necroses and fatty degeneration were observed (Fig. 2d). The minor fibrosis in cianidanol-treated rats was not correlated with a diminished hydroxyproline content (Fig. 3) and triglyceride content was even increased in this group.

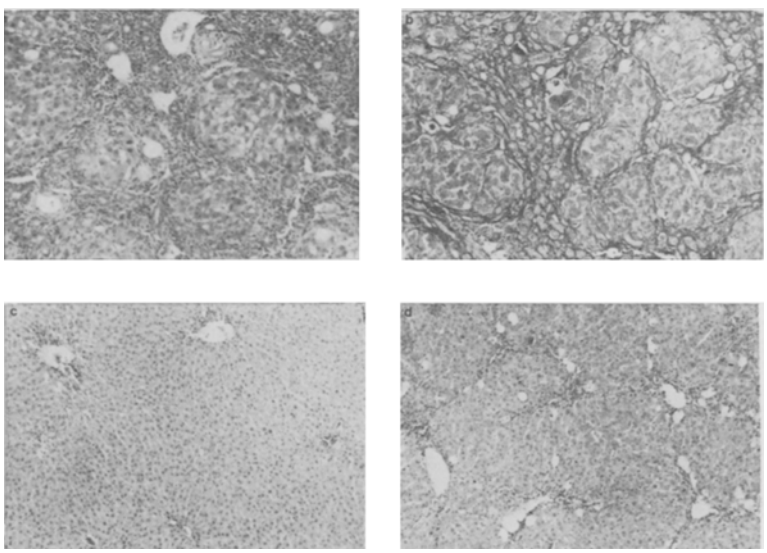

Figure 2
Histomorphological alterations in the livers
a (CCl_4-alcohol-group, HE × 125): broad fibrotic septa, pseudolobuli, ductular proliferations.
b (CCl_4-alcohol-group, Gomori's silver impr. × 125): same alterations as shown in a.
c (Malotilate-group, HE × 80): few singular necrosis, no fibrosis.
d (Cianidanol-group, HE × 80): frequent necroses, beginning fibrosis, collaptic zones.

Table 1
Histomorphological examinations of the livers

Group	Necroses	Extent of Fibrosis	Steatosis	Special observations
1 CCl_4-Alcohol	Frequent singular necroses	Complete cirrhosis	< 10%	Mitotic activity Ductular proliferations Hydropic swelling
2 + D-penicillamine	Singular necroses	Complete cirrhosis	< 10%	Mitotic activity Cell ballooning Ductular proliferations
3 + Colchicine	Frequent singular necroses	Complete cirrhosis	< 10%	Mitotic activity Cell ballooning Ductular proliferations
4 + Cianidanol	Frequent singular necroses	Portoseptal fibrosis Collaptic zones	10–20%	Mitotic activity Cell ballooning
5 + Malotilate	Singular necroses[a]	No fibrosis	< 10%	Cell ballooning[a]

[a]only 2 out of 8 rats

Discussion

In our model of carbon tetrachloride-alcohol induced liver fibrosis a nearly complete cirrhotic transformation of the liver parenchyma developed within 4 weeks as documented by broad fibrotic septa and pseudolobuli. In contrast to liver fibrosis induced by CCl_4 alone [1], the marked ductular proliferations seen in our model seem to mimick primary biliary cirrhosis in man. As compared to other experimental models of liver fibrosis including CCl_4 alone, the combined treatment with alcohol is of advantage with regard to the dose of CCl_4, which given alone is subtoxic, and the short duration of the experiment. The promoting effect of ethanol in this model seems to reflect the clinical situation of a toxic-nutritive liver injury, although it is still unclear in which way alcohol aggravates CCl_4-induced liver fibrosis; some possible factors are discussed by Strubelt [13].

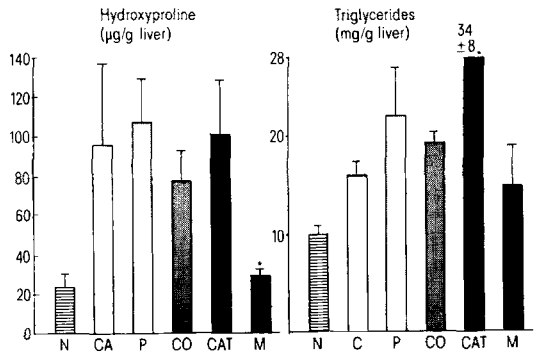

Figure 3
Hepatic hydroxyproline and triglyceride contents in CCl_4-alcohol-treated rats. Values represent means and standard errors out of 8 rats each. Asterisks indicate $p < 0.05$ as compared to the CA-group (Dunnett's t-Test). N = untreated controls; CA = CCl_4-alcohol; P = D-penicillamine; C = colchicine; CAT = cianidanol; M = malotilate.

Malotilate was shown to provide total hepato-protection against CCl_4-alcohol induced liver necroses and the development of cirrhosis. An interaction of malotilate with the bioactivation of CCl_4 can be excluded as malotilate did not suppress the activities of the microsomal monooxygenase system [6–8]. Whether this effect of malotilate allows the assumption of an antifibrotic action remains unclear, as the antinecrotic potency of this compound cannot be separated from its effect on the connective tissue accumulation. Thus, further studies require a better differentiation between the antihepatotoxic and antifibrotic activities of this drug.

With cianidanol, a different picture was seen; i.e., this compound did not antagonize the acute necrotization but partially reduced the development of complete cirrhosis. As shown previously [2] cianidanol acts in a dose-dependent way; the dose used in the present study was found to be the most effective. The discrepancy between its efficacy to reduce complete cirrhotic transformation as documented by the histomorphological examination and its inability to reduce the accumulation of hydroxyproline shows, that total hydroxyproline content is not necessarily a reliable parameter of hepatic collagen accumulation. Cianidanol, a drug with radical scavenging and membrane-stabilizing properties, has also been shown to inhibit prolyl hydroxylase activity [14, 15]. Thus it might interact with collagen proliferation on various levels.

D-penicillamine, a drug with beneficial clinical effects on primary biliary cirrhosis, is proposed to exert its antifibrotic activity by inhibiting

polyfunctional cross-linking. In our experiments, however, D-penicillamine was not able to influence the CCl_4-alcohol-induced liver necrosis and fibrosis, a fact which is in accordance with the observations made using the CCl_4-, the thioacetamide- and the cholin-deficiency-models [16]. The same is true for colchicine, which also failed to counteract the CCl_4-alcohol-induced liver lesions. This drug has been tested in the treatment of alcoholic liver cirrhosis [10].

In conclusion, malotilate showed the highest efficacy in inhibiting cirrhotic transformation of rat liver in a model of CCl_4-alcohol-induced fibrosis as compared to other antifibrotic drugs. Its exact mechanism of action, and its clincial applicability remain to be established.

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