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Brief Note
Influence of Ethanol Pretreatment on Serum Complement Levels in Rats Treated with Carbon Tetrachloride

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ABSTRACT. Pretreating male Wistar rats with a single oral dose (6 ml/kg) of ethanol clearly potentiated the CCl_4-induced hepatotoxicity (0.2 ml/kg ip), as shown by the elevated SGOT and SGPT levels, or histopathological observation of the liver sections.

Serum hemolytic complement (CH50) and C3 levels were slightly reduced by the administration of CCl_4, but significantly reduced when rats were treated with ethanol 18 hours prior to CCl_4 administration.

Key words : ethanol — carbon tetrachloride — serum complement

Carbon tetrachloride (CCl_4) is known to cause significant liver damage[1] and to lower serum complement levels.[2] It is also well documented that CCl_4-induced hepatotoxicity is potentiated by pretreatment with ethanol.[3] This article describes the influence of a pretreatment with a single oral dose of ethanol on serum complement levels in rats treated with CCl_4.

MATERIALS AND METHODS

Male Wistar rats were maintained *ad libitum* on commercial rat chow (Oriental MF pellet) and tap water. At the age of 11 weeks, the rats were randomly assigned to 4 groups of 8 rats each. One group of rats received 6 ml/kg, approximately 100 mMol, of ethanol as a 50 percent aqueous solution by oral gavage. Eighteen hours later, rats received intraperitoneally 0.2 ml/kg of CCl_4 dissolved in 0.8 ml olive oil. Orally administered distilled water (12 ml/kg) was used as a control for ethanol pretreatment, and intraperitoneally injected olive oil (1 ml/kg) was used as a control for CCl_4.

Twenty-four hours later, animals were sacrificed under ether anesthesia, and blood was drawn from the aorta with a plastic syringe. Blood samples were allowed to clot at 37°C for 2 hours, then serum was separated by centrifugation. The livers were immediately removed, cleaned, weighed and small portions of the livers were fixed in Bouin's solution, embedded in paraffin, sectioned and stained with hematoxylin-eosin for histologic examination.

The whole serum complement titer in terms of 50% hemolysis (CH50) was measured by a modification of the method of Mayer.[4] Serum C3 determination was performed by a single radial immunodiffusion technique (SRID).[5]

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Total protein concentration in the serum was determined by a modified biuret method (Wako). Serum glutamic oxalacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) were assayed by a commercial kit based on the method of Reitman and Frankel[6] (Transaminase B-Test, Wako).

RESULTS AND DISCUSSION

Serum hemolytic complement, C3, SGOT and SGPT levels are summarized in Table 1. SGOT and SGPT levels were used to evaluate CCl_4-induced liver damage.

TABLE 1 Influence of ethanol pretreatment on serum complement and transaminases levels in CCl_4 treated rats

Treatment	No. of rats	CH 50	C 3 %	Total protein g/dl	SGOT KU[b]	SGPT KU	Body weight g	Liver g/100gb.w.
water + olive oil	8	53.5±2.9[a]	100±2.9	5.9±0.05	108±8	24±2	276±3	3.3±0.05
ethanol+olive oil	8	68.6±3.7**	87.2±2.3**	5.4±0.11**	100±5	22±1	276±3	3.3±0.04
water + CCl_4	8	48.8±2.4	70.0±3.2**	5.9±0.15	453±78**	248±66**	289±2	3.7±0.05*
ethanol+CCl_4	8	39.7±4.0*	55.2±4.2**	5.2±0.13**	793±118**	668±222*	289±3	3.7±0.04*

a) Mean ± S.E. b) Karmen Unit
Statistically significant difference from the control value : *$p<0.05$ **$p<0.01$

The administration of ethanol alone did not alter SGOT and SGPT levels, while pretreating rats with ethanol clearly potentiated the CCl_4-induced hepatotoxicity, as shown by the elevated SGOT and SGPT levels.

The potentiation was also evident histologically, as shown in Figure 1 a, b. The findings of the present study are similar to those described in the literature.[7]

Livers from water-pretreated rats sacrificed 24 hr after an ip injection of 0.2 ml/kg of CCl_4 had small, focal areas of centrilobular necrosis with infiltration of monocytes and neutrophils and vacuolar degeneration of hepatocytes surrounding the necrotic cells (Fig. 1 a). The same type of lesions were present in the livers of rats pretreated with ethanol, but liver injury was more severe and widespread (Fig. 1 b). Liver sections appeared normal in the olive oil treated rats pretreated with either water or ethanol.

Serum hemolytic complement and C3 levels were slightly reduced by the administration of 0.2 ml/kg of CCl_4, but significantly reduced when rats were treated with ethanol before CCl_4 administration. Little is known about either the mechanism of the reduction of serum hemolytic complement activity or C3 levels after CCl_4 treatment or that of the ethanol potentiation of these changes.

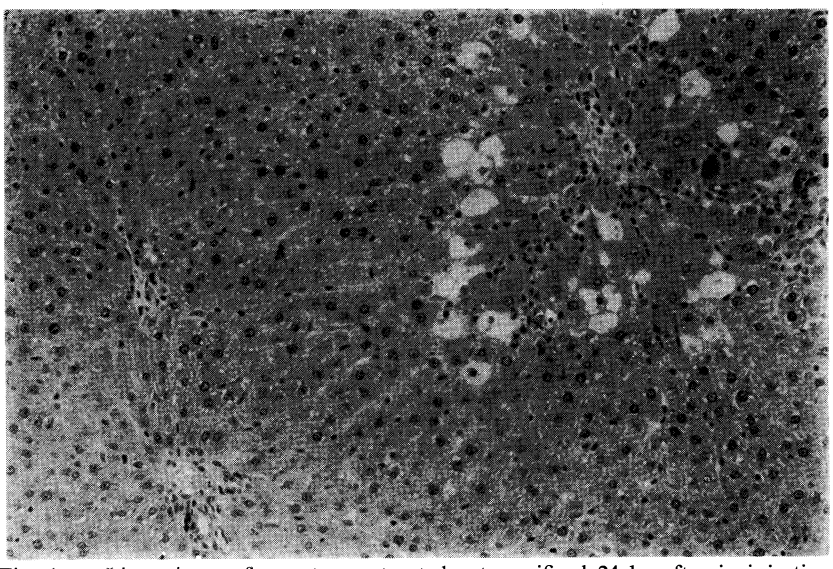

Fig. 1 a. Liver tissue of a water-pretreated rat sacrificed 24 hr after ip injection of 0.2 ml/kg of CCl_4. (HE, ×130)

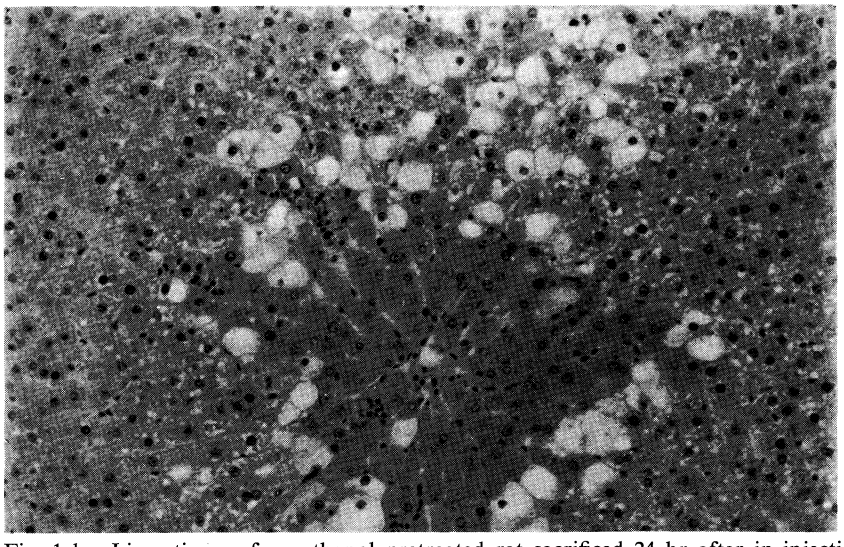

Fig. 1 b. Liver tissue of an ethanol-pretreated rat sacrificed 24 hr after ip injection of 0.2 ml/kg of CCl_4. (HE, ×130)

It is thought, however, that ethanol, by inducing or activating the microsomal drug metabolizing system, causes an acceleration of the biotransformation of CCl_4 to highly active metabolites which in turn may cause damage to the

hepatic ribosomes,[8] interfering with protein synthesis.[9] Since the liver is known to be one of the major sites of complement protein synthesis, including C3[10], hemolytic complement and C3 levels would be expected to decrease.

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