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EFFECT OF 3-AMINO-1,2,4-TRIAZOLE TREATMENT ON CATALASE ACTIVITY AND TRIGLYCERIDE LEVEL IN FATTY LIVER OF THE RAT

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Abstract—The effect of 3-amino-1,2,4-triazole (AT) pretreatment on the triglyceride (TG) level in CCl_4 (2.5 ml/kg, s.c.)-, ethanol (6 g/kg, p.o.)- or ethionine (1 g/kg, i.p.)- induced fatty liver in rats was studied, and the relationship between liver catalase activity and TG metabolism was investigated. The intoxication by each hepatotoxin increased the liver TG level in the rats. However, when the rats were pretreated with AT (1 g/kg, i.p.), the liver TG level decreased about 50 per cent, and the liver catalase activity was inhibited about 85 per cent. When AT and CCl_4 were injected simultaneously, they repressed about 50 per cent of the increase of the TG level caused by the intoxication of CCl_4. When AT alone was injected before the intoxication of ethanol, the liver catalase activity decreased by 50 per cent of that of the ethanol group and the increase in liver TG level was lowered by 40 per cent. On the other hand, when AT was injected after ethanol, the increase in liver TG level was depressed, although a decrease in catalase activity was not found. Although the serum TG level was decreased by CCl_4 treatment and increased by the ethanol treatment, AT did not show any effect on these changes. These results suggest that AT acts independently on the TG level and catalase activity in the liver.

Catalase is an enzyme that exists mainly in peroxisomes, [1, 2], but the essential physiological functions of peroxisomes and liver catalase still remain unclear. In recent years, several reports have proposed that peroxisomes and liver catalase participate in lipid metabolism [3–5]. 3-Amino-1,2,4-triazole (AT) is a strong inhibitor of liver catalase [6]. On the other hand, it has been reported that AT markedly decreased the triglyceride (TG) level among several lipids in the liver [7].

For the purpose of investigating the association between TG metabolism and liver catalase activity, we have inquired, in this paper, into the effect of AT on catalase activity and the TG level in the hepatotoxin (CCl_4, ethanol and ethionine)-induced fatty liver.

MATERIALS AND METHODS

Animals. For most experiments, with the exception of ethionine-induced fatty liver when female rats were used, male Wistar rats weighing about 150 g were used. All animals were fasted for 24 hr previous to the experiment and hepatotoxins were administered 12 hr before the rats were killed. The liver was perfused with 20 ml of ice-cold saline, and a 30% homogenate in saline was prepared.

Drugs. 3-Amino-1,2,4-triazole (AT) was purchased from Tokyo Kasei Kogyo Co. AT was repeatedly injected intraperitoneally into the rats at a dose of 1 g/kg body weight with 10% AT in saline at intervals of 12 hr starting from 72 hr before the experiment, and otherwise was injected once as stated in Tables 2 and 3. CCl_4 was injected subcutaneously into the rats at a dose of 2.5 ml/kg of body weight 12 hr before the experiment. Ethanol was orally administered to the rats at a dose of 6 g/kg of body weight 12 hr before the experiment. Ethionine was injected intraperitoneally into female rats at a dose of 0.5 g/kg of body weight 12–10 hr before the experiment. Isocaloric glucose was administered to the control group instead of the ethanol group, and a corresponding volume of saline was administered to the control rats.

Assay methods. Liver catalase activity and triglyceride content were measured according to the method described in a previous paper [7]. One unit of enzyme is defined as the amount with a k value of 1 where k has a constant of decrease in extinction at 240 nm in 1 sec at 25°.

RESULTS

The effect of AT pretreatment on the hepatotoxin-induced fatty liver was investigated (Table 1). In the experiment of ethanol-induced fatty liver (Expt. 2), control rats were given isocaloric glucose instead of ethanol. Female rats were used in the experiment of ethionine-induced fatty liver (Expt. 3). Liver catalase activity was lower and the liver TG level was higher in female rats than in male rats. In the AT-injected group (AT group), the liver catalase activity decreased to about 15 per cent of the control values, and the TG level in male rats showed a decrease of 32 per cent of the control value. The TG level in female rats was decreased by 50 per cent of the control value by AT injection. When injected with AT, serum TG level showed a decrease in Expt. 1, but did not show a statistically significant change in the other experiments when compared with the control values. The reason for this is not yet clear.

Table 1. Effect of aminotriazole (AT) pretreatment on the hepatotoxin-induced fatty liver*

Expt. No.	Group	No. of rats	Liver catalase activity† (units/g liver)	P	Liver triglyceride† (mg/g liver)	P	Serum triglyceride† (mg/dl)	P
1	Control	7	56.2 ± 6.2		5.7 ± 1.7		50.8 ± 12.9	
	AT	7	9.1 ± 1.6	< 0.01‡	3.9 ± 0.4	< 0.05‡	37.0 ± 8.0	< 0.05‡
	CCl_4	5	44.3 ± 4.9	< 0.01‡	20.7 ± 2.9	< 0.01‡	26.1 ± 5.3	< 0.05‡
	AT–CCl_4	7	5.5 ± 1.2	< 0.01§	10.0 ± 2.5	< 0.01§	20.1 ± 5.1	NS§,¶
2	Control	7	36.2 ± 3.7		5.1 ± 0.7		46.3 ± 7.6	
	AT	5	6.9 ± 3.7	< 0.01‡	3.4 ± 0.5	< 0.01‡	52.8 ± 3.8	NS‡,‖
	Ethanol	5	38.1 ± 5.9	NS‡,‖	34.8 ± 9.6	< 0.01‡	83.5 ± 9.0	< 0.01‡
	AT–ethanol	5	13.7 ± 2.1	< 0.01§	14.8 ± 6.5	< 0.01§	79.7 ± 20.0	NS§,¶
3	Control	6	22.3 ± 3.4		17.1 ± 1.9		87.2 ± 28.1	
	AT	7	3.2 ± 1.0	< 0.01‡	8.4 ± 2.0	< 0.01‡	89.3 ± 22.1	NS‡,¶
	Ethionine	7	20.3 ± 2.0	NS‡,¶	84.7 ± 7.6	< 0.01‡	63.3 ± 9.5	NS‡,‖
	AT–ethionine	7	1.7 ± 0.6	< 0.01§	42.8 ± 14.6	< 0.01§	74.4 ± 16.5	NS§,¶

* Male rats were used, with the exception of Expt. 3, where female rats were used. AT (1 g/kg, i.p.) was repeatedly injected into the rats at intervals of 12 hr starting from 72 hr before the sacrifice. CCl_4 (2.5 ml/kg, s.c.) was injected into the rats at 12 hr before the sacrifice. Ethanol (6 g/kg, p.o.) was administered to the rats at 12 hr before the sacrifice. Ethionine (0.5 g/kg, i.p.) was injected into the rats at 12 10 hr before the sacrifice. The control instead of the ethanol group was administered with isocaloric glucose, and other control groups were administered with a corresponding volume of saline.

† Results are expressed as the means ± S. D.

‡ Statistical comparisons by Student's *t*-test between the control and treated groups.

§ Statistical comparisons by Student's *t*-test between the hepatotoxin and AT-hepatotoxin group.

¶ P values larger than 0.05 were considered not significant (NS).

In the CCl_4-injected rats (CCl_4 group), the liver catalase activity decreased about 20 per cent, liver TG level increased 3.7-fold, and the serum TG level decreased 50 per cent when compared with control values. On the other hand, AT pretreatment (AT–CCl_4 group) repressed the increase in the liver TG level which was caused by CCl_4 injection. No effect of AT on serum TG was found.

The liver catalase activity was not affected by the administration of ethanol (ethanol group). However, the TG level in the liver and the serum increased by 6.9- and 1.8-fold of the control values. On the other hand, when pretreated with AT prior to the administration of ethanol (AT–ethanol group), liver catalase activity decreased by 37 per cent of the ethanol group, and the increase in liver TG level caused by ethanol was depressed markedly. The catalase activity in the AT–ethanol group remained higher than that of the AT group. This is thought to be caused by the protective effect of ethanol on the inhibiting action of AT upon catalase [8, 9]. AT did not affect the increase of serum TG level caused by the administration of ethanol.

In the ethionine-induced rats (ethionine group), the liver catalase activity showed no difference when compared to that of the control. The liver TG level, however, increased 5-fold over the control value. The AT pretreatment, which was done prior to the injection of ethionine (AT–ethionine group), induced an increase in liver TG level by 49 per cent, though serum TG level was not affected by AT.

The effect of simultaneous AT injection on the CCl_4-induced fatty liver is shown in Table 2. The relationship between each group corresponded to the results shown in Expt. 1 of Table 1. The increase in the liver TG level which was caused by CCl_4 was decreased by AT. However, the serum TG level in each group did not show any significant change.

The effect of AT injection on the catalase activity and the TG level in the ethanol-induced fatty liver was studied, using different injection times (Table 3). Both the catalase activity and the TG level were de-

Table 2. Effect of simultaneous aminotriazole (AT) injection on the carbon tetrachloride-induced fatty liver*

Group	No. of rats	Liver catalase activity† (units/g liver)	P	Liver triglyceride† (mg/g liver)	P	Serum triglyceride† (mg/dl)	P
Control	4	47.3 ± 4.5		8.2 ± 2.2		75.6 ± 21.9	
AT	4	5.6 ± 1.5	< 0.01‡	4.5 ± 0.6	< 0.01‡	80.9 ± 21.8	NS‡ §
CCl_4	4	37.1 ± 3.7	< 0.01‡	37.4 ± 8.2	< 0.01‡	67.9 ± 11.2	NS‡,§
AT–CCl_4	4	3.4 ± 0.7	< 0.01‖	14.3 ± 5.4	< 0.01‖	52.7 ± 11.6	NS§,‖

* Male rats were divided into four groups. All animals were fasted 24 hr previous to the sacrifice. One group was treated with AT (1 g/kg, i.p.) 12 hr before the sacrifice and the second group was treated with CCl_4 (2.5 ml/kg, s.c.) 12 hr before the sacrifice. The third group was treated with AT along with CCl_4. The control was treated with saline of equal volume to AT and CCl_4.

† Results are expressed as the means ± S. D.

‡ Statistical comparisons by Student's *t*-test between the control and treated groups.

§ P values larger than 0.05 were considered not significant (NS).

‖ Statistical comparisons by Student's *t*-test between the CCl_4 and AT–CCl_4 group.

Table 3. Effect of time of injection of aminotriazole (AT) on catalase activity and triglyceride in ethanol-induced fatty liver*

Group	No. of rats	Liver catalase activity† (units/g liver)	P	Liver triglyceride† (mg/g liver)	P	Serum triglyceride† (mg/dl)	P
Control	6	39.6 ± 5.3		5.4 ± 1.5		37.9 ± 5.2	
AT (12.0)	6	6.9 ± 1.7	< 0.01‡	2.4 ± 0.5	< 0.01‡	29.9 ± 3.7	< 0.02‡
Ethanol	4	41.9 ± 3.5	NS‡,§	34.7 ± 6.6	< 0.01‡	52.2 ± 17.2	< 0.01‡
AT (12.5)–ethanol	5	20.1 ± 4.9	< 0.01‖	13.8 ± 7.3	< 0.01‖	55.3 ± 37.5	NS§,‖
Ethanol–AT(11.5)	6	44.3 ± 2.5	NS§,‖ < 0.01¶	14.8 ± 5.5	< 0.01‖ NS§,¶	47.9 ± 23.1	NS§,‖ NS§,¶

* Male rats were divided into five groups. One group was treated with AT (1 g/kg, i.p.) 12 hr before sacrifice and the second group was treated with ethanol (6 g/kg, p.o.) 12 hr before sacrifice. The third group was treated with AT 30 min previous to the administration of ethanol which was given 12 hr before sacrifice. The fourth group was treated with ethanol 12 before sacrifice and AT was injected 30 min after the administration of ethanol. The numbers in brackets indicate the administration time of AT in hours before sacrifice. All animals were fasted 24 hr previous to sacrifice. The control was treated with saline of a volume equal to that of AT and a dose of glucose isocaloric to ethanol.

† Results are expressed as the means ± S. D.

‡ Statistical comparisons by Student's *t*-test between the control and treated groups.

§ P values larger than 0.05 were considered not significant (NS).

‖ Statistical comparisons by Student's *t*-test between the ethanol and AT-ethanol groups.

¶ Statistical comparisons by Student's *t*-test between the ethanol and ethanol–AT group.

creased by a single injection of AT. When AT was injected 30 min before the administration of ethanol (AT–ethanol group), the catalase activity decreased 50 per cent of that of the ethanol group. However, the enzyme activity was not affected when AT was injected 30 min after the administration of ethanol (ethanol–AT group). On the other hand, the liver TG level decreased by 40 per cent of that of the ethanol group in both AT–ethanol and ethanol–AT groups. AT did not show any effect on the serum TG level.

DISCUSSION

Many reports have suggested that peroxisomes or catalase, a main compartment of peroxisomes, are involved in lipid metabolism [3-5, 10]. The fact that AT, a strong inhibitor of catalase, induced a marked decrease in liver triglyceride level has been established in our laboratory [7]. In our present work, the effect of AT on the TG level and the catalase activity in the hepatotoxin-induced fatty liver has been examined, and the relationship between TG metabolism and catalase activity in the liver was investigated.

AT inhibited the liver catalase activity and decreased the liver TG level. Little change was observed in the liver catalase activity when CCl_4, ethanol or ethionine, all of which are known to increase the TG level in the liver, was administered. There was only a slight decrease caused by the injection of CCl_4, which was 20 per cent of the activity when compared to the control (Tables 1 and 2). Thus, a parallel relationship between the increase of TG level and catalase activity in the liver was not indicated. Furthermore, the increase of liver TG level caused by ethanol was prevented without the inhibition of catalase activity (Table 3). This fact indicates that the decrease of liver TG level caused by AT was not dependent on the decrease of catalase activity in the liver. Therefore, it has been established that AT acts independently on catalase activity and TG metabolism.

It has been considered that the CCl_4-induced fatty liver is produced by inhibition of lipoprotein synthesis in the liver [11-13]. With the injection of CCl_4, serum TG levels showed a tendency to decrease and liver TG levels to increase. Pretreatment of AT markedly inhibited the increase of liver TG caused by CCl_4; however, the pretreatment did not show any effect on the serum TG level (Table 1). The ethionine-induced fatty liver is considered to be the cause of the inhibition of protein synthesis which is brought about by the decrease of ATP level in the liver [14, 15]. The effect of AT pretreatment on the ethionine-induced fatty liver was found to be similar to the result of CCl_4-induced fatty liver. These results suggest that the decrease in liver TG level caused by AT administration does not result from the accelerating release of liver TG into the blood stream. This fact has also been supported in a previous paper [7] which discusses a tendency of AT to decrease the serum TG level.

Therefore, it is thought that the ethanol-induced fatty liver was produced by a stimulation of fatty acid synthesis in the liver as proposed by Lieber and Schmid [16]. AT prevented the accumulation of ethanol-induced fatty liver to the same degree in both single and repeated injections of the material (Tables 1 and 3). Furthermore, the serum FFA level did not show any change after several injections which were given after a single injection of AT. It has been suggested that AT does not show an antilipolytic action on fat tissues [7]; the fact that AT does not show an inhibiting action on the increase of serum FFA caused by the administration of adrenalin, also supports this fact. In conclusion, all these facts which have been discussed indicate that AT acts directly on fatty acid synthesis and on fatty acid esterification to TG in the liver.

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