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Effect of 3-amino-1,2,4-triazole on lipid metabolism in the rat

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It is known that catalase is a major component of peroxisomes (microbodies) [1]. The intraparticulate localization of peroxisomal enzymes in rat liver was elucidated previously in this laboratory [2, 3]. It was reported that administration of ethyl-α-*p*-chlorophenoxyisobutyric acid* [4, 5] and 2-methyl-2-[*p*-(1,2,3,4-tetrahydro-1-naphthyl)-phenoxy]-propionic acid* [6], which are hypolipidemic drugs, to male rats and mice, results in proliferation of peroxisomes and a marked increase in catalase. An association between peroxisomes and lipid metabolism was suggested. In addition, it was reported that mutant 'acatalasemic' mice had an unstable catalase, which was readily inactivated [7], and had a low level of circulating sterol and triglyceride [8]. Thus it was proposed that the decrease of catalase activity was related to the inhibition of liver sterol and fatty acid synthesis [9].

On the other hand, it is known that 3-amino-1,2,4-triazole combines irreversibly with catalase in the hepatic cell and inactivates this enzyme without affecting catalase synthesis [10, 11]. In order to investigate the relationship between liver catalase and lipid metabolism, we have inquired into the effect of AT-administration on liver catalase activity and lipid content.

Animals and drugs—Male Wistar rats weighing about 150 g were fasted for 24 hr before sacrifice. 3-Amino-1,2,4-triazole (AT) was purchased at Tokyo Kasei Kogyo Co. It was dissolved in physiological saline in various concentrations. The rats were injected intraperitoneally (i.p.) at a dose of 10 ml of the solution per kg of body weight. The control group received only saline. After the rats were killed, the liver was perfused with approx 20 ml of ice-cold saline and a 30 per cent homogenate in saline was prepared.

Assay methods—Catalase activity was measured according to the method of Lück [12]. A Hitachi 323 recording spectrophotometer was used. Prior to measurement of enzyme activity, the homogenate was treated with Triton X-100 and then diluted with water. One unit of enzyme is defined as the amount with a k value of 1, where k is the decrease in extinction at 240 nm per second at 25°. Liver and serum triglyceride (TG) were assayed by the method of Van Handel-Kawade [13] with modification: phenylhydrazine hydrochloride was used instead of chromotropic acid. Free fatty acid (FFA) was assayed by the method of Kushiro *et al.* [14]. Cholesterol was assayed by the modified method of Zak-Henly [15]. Phospholipid was determined according to Fiske-Subbarow's ashing method [16], and the inorganic phosphate produced was measured according to the method of Lindberg and Ernster [17].

To study the effect of liver catalase activity on lipid metabolism, liver catalase activity and lipid content were determined after the injection of AT (1 g/kg). The correlation of these changes in the liver and the time relationship are shown in Fig. 1. Liver catalase activity decreased to 7 per cent of control level at 1 hr after AT-injection and this inhibition was continued for about 12 hr after the injection (Fig. 1-a). Liver TG level began to decrease at 1 hr after AT-injection and then reached 40 per cent of control level at 12 hr (Fig. 1-b). This decrease caused by AT-treatment was statistically significant ($P < 0.05$), while changes in the level of cholesterol, FFA or phospholipid were not significant (Fig. 1-c,d,e).

On the other hand, lipid content of the serum did not show a significant change, as shown in Fig. 2. Among the parameters measured, serum FFA showed a tendency to decrease, although this change was not a statistically significant one compared to that of the control (Fig. 2-c). TG, cholesterol and phospholipid levels in the serum did not vary significantly during this time (Fig. 2-a,b,d). Thus, only the liver TG content changed significantly by the injection of AT, and this change was found to occur promptly after AT-injection.

The dose-response of liver catalase and lipid content to AT was further investigated (Fig. 3). Liver catalase ac-

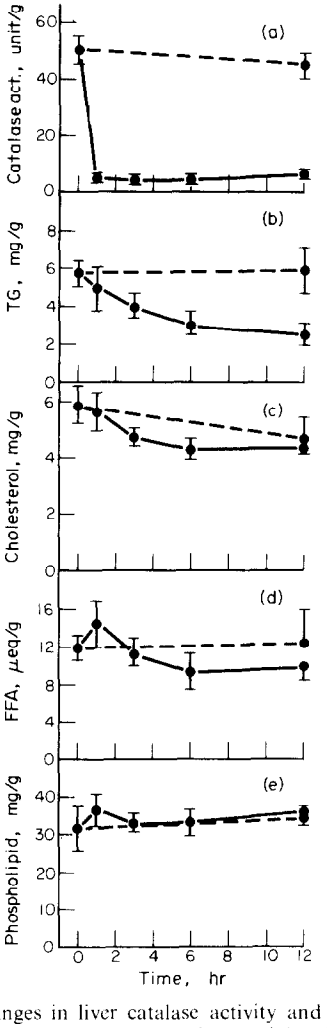

Fig. 1. Changes in liver catalase activity and lipids after a single injection of AT: a, catalase activity; b, triglyceride; c, cholesterol; d, free fatty acid; e, phospholipid. Rats received i.p. injections of either AT (1 g/kg) or an equal vol of 0.9 per cent NaCl and were killed at various time-intervals thereafter. Each group consisted of 5 animals and points represent the mean ± S.D. The solid line indicates the values after AT treatment and the dotted line indicates that of the control.

* Trade-names: Ethyl-α-*p*-chlorophenoxyisobutyrate, Clofibrate; 2-methyl-2-[*p*-(1,2,3,4-tetrahydro-1-naphthyl)-phenoxy]-propionate, Nafenopin.

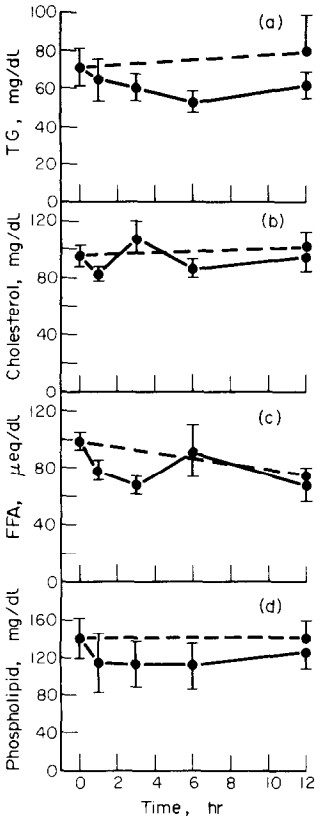

Fig. 2. Changes in serum lipids after a single injection of AT: a, triglyceride; b, cholesterol; c, free fatty acid; d, phospholipid. Rats received i.p. injection of either AT (1 g/kg) or an equal vol of 0.9 per cent NaCl and were killed at various time-intervals thereafter. Each group consisted of 5 animals and points represent the mean ± S.D. The solid line indicates the values after AT treatment and the dotted line indicates that of the control.

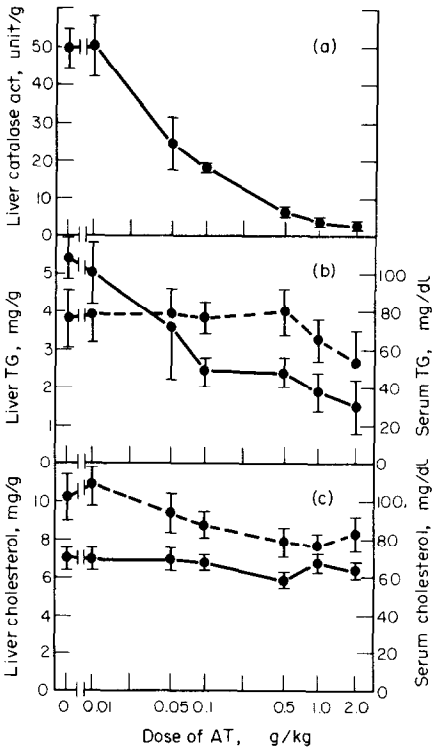

Fig. 3. Dose–response curves for liver catalase activity and lipids of liver and serum after a single injection of AT: a, catalase activity; b, triglyceride;, c, cholesterol. Rats were injected i.p. with various doses of AT and were killed at 12 hr after the injection. Each group consisted of 5 animals and plots represent the mean value ± S.D. The solid line indicates the values for the liver and the dotted line indicates that for the serum.

tivity was not affected when AT was administered at a dose of 0.01 g/kg body wt. However, the activity began to decrease at a dose of 0.05 g/kg body wt and showed a linear decrease until it reached a dose of 0.5 g/kg. Along with this change, the liver TG level also decreased and reached 28 per cent of control at a dose of 2 g AT/kg body wt. Changes in cholesterol and serum TG levels were not significant.

In order to investigate the change of TG level during the low level of catalase activity in the liver, the liver catalase activity was measured after the repeated injection of AT for 3 days. As shown in Table 1, liver catalase activity reached a markedly low level (9 per cent of control level). The TG level was found to decrease by 37 per cent of control level in the liver though decrease in the serum was not as significant as that in the liver (76 per cent of control). In addition, the liver weight increased in the AT-treatment group. These results corresponded with the results obtained by D'Acosta *et al.* [18] and Raisfeld *et al.* [19].

A relationship between liver catalase activity and lipid metabolism has been suggested on the basis of the alteration of catalase activity and lipid metabolism in acatalasemia [8, 21] and in rats dosed with hypolipidemic drugs [6, 20]. Liver catalase activity and several lipid components of the serum and the liver were measured after

Table 1. Effect of repeated injection of AT on liver weight, catalase activity and triglyceride, and serum triglyceride

Group	Number of rats	Liver weight (g/100 g b. w.)*	Liver catalase activity (Unit/g liver)*	Triglyceride	
				Liver (mg/g liver)*	Serum (mg/dl)*
Control	7	3.44 ± 0.21	49.9 ± 8.9	5.9 ± 1.1	71.4 ± 12.9
AT	7	4.28 ± 0.39	4.5 ± 1.0	2.2 ± 0.4	54.5 ± 14.9
		(P < 0.01)†	(P < 0.01)†	(P < 0.01)†	(P < 0.01)†

Rats received i.p. injection of either AT (1 g/kg) or an equal vol of 0.9% NaCl at 12-hr intervals from 72 hr before sacrifice. The rats were fasted for 24 hr prior to sacrifice and were sacrificed 12 hr after the final injection.

* The results are expressed as the mean ± S.D.

† Comparison of treatment with saline control by Student's *t*-test.

the injection of AT, and the results are described in this paper. Only the liver TG level decreased significantly after a single injection of AT (1 g/kg).

On the other hand, other lipids did not show any significant change until 12 hr after AT-treatment. Catalase activity showed minimum activity at 1 hr after AT-administration and this inhibition continued for 12 hr after AT-administration.

The parallel changes in liver catalase activity and TG level were again seen in the dose-response curve, though the other lipid contents did not show any significant change. In the present work, depression of the serum TG and cholesterol levels have not been found after a single injection of AT, which produces a condition similar to acatalasemia.

On the other hand, the serum TG level was decreased by repeated injection of AT, somewhat analogous to acatalasemia. Since the amount of decrease in the liver TG level was equal to that seen after the single injection of AT, it may be considered that the maintenance of lowered catalase activity is not necessary for the occurrence of this action. It is known that administration of ethyl-α-*p*-chlorophenoxyisobutyrate, a hypolipidemic drug, increases catalase activity. Furthermore, Reddy *et al.* suggested a connection between catalase activity and lipid metabolism because of the fact that the decrease of serum TG changed parallel to the increase of catalase activity [6]. TG level decreased along with liver catalase activity when AT was used, suggesting that perhaps catalase itself is directly related to TG metabolism. However, it appears that catalase itself is not directly related to lipid metabolism.

No report has previously been presented dealing with AT action and its effect on hepatic TG. It is assumed that the depression of liver TG caused by AT is not dependent on the acceleration of the release of liver TG into the blood stream, or on an antilypolytic action of AT. This assumption is supported by studies in progress which indicate that the increase of serum FFA caused by the administration of adrenalin was not inhibited by pretreatment with AT.

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