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EFFECT OF 3-AMINO-1,2,4-TRIAZOLE ON *IN VIVO* FORMATION OF LIVER TRIGLYCERIDE

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Abstract—The mechanics of the decrease in the liver triglyceride (TG) level caused by the administration of 3-amino-1,2,4-triazole (AT) were studied *in vivo*. The incorporation of [^{14}C]acetate (10 μCi/100 g, i.p.) into liver TG of rats injected with AT (100 mg/100 g, i.p.) decreased about 55 per cent when compared with the control group. Incorporation of [^{14}C]palmitate into liver and serum TG, did not differ in the AT and control group. Incorporation of [^{14}C]acetate into liver TG of rats administered ethanol (600 mg/100 g, p.o.) was 3 times that of the control, but with AT-pretreatment incorporation was 50 per cent less when compared with that of ethanol group. Serum free fatty acids of rats injected with adrenalin (200 μg/100 g, i.m.) increased two times above the control group. The increase was not affected by pretreatment with AT.

3-Amino-1,2,4-triazole (AT) is an herbicide which shows several effects on mammals, such as inhibition of catalase activity in liver and kidney [1], inhibition of δ-aminolevulinic acid dehydratase [2], inhibition of induced synthesis of cytochrome *p*-450 [3, 4], and an antithyroid effect [5]. In addition, it has been reported that AT pretreatment represses phenobarbital-induced changes in liver microsomes [6, 7] and CCl_4-induced necrosis of hepatocytes [8].

On the other hand, studies in our laboratory showed that AT treatment of rats markedly decreased the triglyceride (TG) level in liver [9]. Furthermore, the AT pretreatment repressed CCl_4, ethanol, and ethionine-induced fatty livers in the rats, as discussed in a previous paper [10].

For the purpose of investigating the mechanism of the decrease in liver TG level caused by the administration of AT, the effect of AT on incorporation *in vivo* of radioactive precursors into liver TG was studied.

MATERIALS AND METHODS

Materials. Chemical compounds were obtained from the following companies: 3-amino-1,2,4-triazole (AT) from Tokyo Kasei Co.; sodium acetate-2-[^{14}C] (42.0 mCi/m-mole) and palmitate-1[^{14}C] (53.2 mCi/m-mole) from Daiichi Pure Chemicals Co.; l-adrenalin from Fluka AG.; and crystallized bovine albumin from Nutritional Biochemical Corp. Albumin-bound palmitate-1-[^{14}C] was prepared by the method of Milstein and Driscoll [11].

Treatment of animals. Male Wistar rats weighing 120–170 g were used throughout these studies. In all cases, except when adrenalin was used, rats were not fed for 24 hrs prior to the experiment, but were given water ad lib. AT (1 g/kg), which had been dissolved in physiological saline, was injected i.p. 12 hr before sacrifice; control rats were injected with physiological saline. Ethanol was given by oral intubation 30 min after the injection of AT. The control group was given isocaloric glucose instead ethanol. [^{14}C]acetate (10 μCi/100 g, i.p.) and albumin-bound palmitate-[^{14}C] (10 μCi/100 g, i.v.) were injected into these rats 15 min before the experiment. In the experiment in which adrenalin was used, the rats which were fed received intramuscular injections of adrenalin (200 μg/100 g), which was dissolved in 0.01 N HCl-0.9 per cent NaCl, 30 min after the AT injection. The control group was injected with physiological saline. The rats were killed 2 hr after the adrenalin injection.

Separation of triglyceride (TG). Lipids were extracted from the serum and liver according to the method of Folch *et al.* [12]. The phase containing lipid was separated and evaporated. The residue was redissolved in chloroform, and aliquots were used for further analysis. In the experiment on the incorporation of radioactive precursors, lipids were separated by TLC on Kieselgel 60 F_{254} (Merck) with petroleum ether–diethyl ether–glacial acetic acid (80:25:1) as developing solvents. Each fraction was detected under iodine vapor and was identified by comparison with a simultaneously running standard. After the iodine had sublimed, the area containing TG was scraped and extracted in chloroform–methanol (2:1) three times. The extracts were used as samples to determine the TG content and radioactivity.

Assays. The uptake of radioactive precursors in the liver was determined by the following method. After the rats were killed, the weight of the liver was measured. About 0.1 g of the liver was subjected to combustion quantitatively to $^{14}CO_2$ by combustion apparatus (Aloka ASC-111). Radioactivity was determined with a liquid scintillation counter (Aloka LSC-502). The incorporation of radioactive precursors into TG was determined by the following method: one ml of methanol and 15 ml of scintillation medium were added to the TG fraction which was separated from TLC chromatograms. The scintillation medium which was used consisted of 100 g of naphthalin, 4 g of 2,5-diphenyloxazole (PPO) and 0.4 g of 1,4-bis-2-(4-methyl-5-phenyloxazole)-benzen (dimethyl POPOP) per liter of mixture of dioxane–toluene–methylcellosolve (750:150:150 v/v). Radio-

Table 1. Effect of aminotriazole (AT) treatment on *in vivo* incorporation of [^{14}C]acetate into liver triglyceride

	Radioactivity in liver (% of dose)	Triglyceride content (mg/g liver)	Radioactivity in triglyceride (dis/min/g liver) × 10^{-2}
Control	2.6 ± 0.2	4.96 ± 1.24	122 ± 49
Aminotriazole	3.6 ± 0.9	2.26 ± 0.93	56 ± 26
	$P < 0.05$*	$P < 0.01$*	$P < 0.05$*

The aminotriazole group received aminotriazole (1 g/kg, i.p.) 12 hr before sacrifice. The control group received physiological saline at the same time. [^{14}C]Acetate (10 μCi/100 g, i.p.) was injected into both groups 15 min before sacrifice. Values are represented as means ± S.D. in five animals.

* Comparison of treatment with control by *t*-test.

activity was determined with a liquid scintillation counter (Aloka LSC-502). Serum and liver TG were determined according to the method described previously [9]. Serum free fatty acids were determined by the method of Kushiro *et al.* [13].

RESULTS AND DISCUSSION

In order to investigate the effect of AT on fatty acid synthesis and fatty acid esterification to TG in the liver, incorporation *in vivo* of [^{14}C]acetate into liver TG was examined (Table 1). The liver TG level in AT-treated rats decreased by 55 per cent when compared to the control group. The incorporation of radioactivity into the liver of the AT-injected rat showed a tendency to increase rather than decrease when compared to that of the control. However, the incorporation of [^{14}C]acetate into the TG in the liver decreased to 45 per cent. In this experiment, the isotope dilution of [^{14}C]acetate caused by the endogenous precursors was negligible, and this was confirmed in subsequent experiments *in vitro*. Therefore, this result indicates that AT inhibits fatty acid synthesis or fatty acid esterification to TG in the liver.

The incorporation of [^{14}C]palmitate into liver and serum TG was measured in order to determine the effect of AT on fatty acid esterification to TG and on the release of liver TG into the blood stream (Table 2). The incorporation of [^{14}C]palmitate into TG was not inhibited by AT. Furthermore, AT did not affect the incorporation of radioactivity into serum TG in the short time after the injection of [^{14}C]palmitate. These facts suggest that AT did not affect the esterification of fatty acid to TG, the inhibition of fatty acid synthesis in the liver, and the release of liver TG into the blood stream. These facts are supported by the results shown in Table 3. In addition, this concept can be supported by the fact that serum TG level did not increase at any time after AT administration [9] and the increase of serum TG was not accompanied by the depressing action of AT on the development of hepatotoxine-induced fatty liver [10].

The effect of AT on the release of free fatty acid (FFA) from fat tissues into the blood stream is shown in Table 3. Serum FFA was increased about 2 times by the injection of adrenalin. This result supports Dole's review [14] that adrenalin accelerates the release of FFA from fat tissues by lipolytic action. Neither the level of serum FFA of the rats which were not injected with adrenalin nor the serum FFA which was increased by an injection of adrenalin was affected by AT. This indicates that AT did not inhibit the release of FFA from fat tissues, and that the decrease in the liver TG level caused by AT did not derive from the antilipolytic action of the material. In addition, this is supported by the fact that serum FFA did not show any change at any time after AT injection [9]. AT is widely distributed in liver, kidney and spleen but is not detectable in fat tissues of the rat [15]. On the other hand, liver TG levels decreased in rats which were injected with AT, and the TG level in the AT-pretreated adrenalin group did not decrease. As is clear from the results in Table 2, this phenomenon can be said to be due to the flow rate of FFA into the liver which was increased by the

Table 2. Effect of aminotriazole (AT) treatment on *in vivo* incorporation of [^{14}C]palmitate into the liver and serum triglyceride

	Liver			Serum	
	Radioactivity in liver (% of dose)	Triglyceride content (mg/g liver)	Radioactivity in triglyceride (dis/min/g liver) × 10^{-3}	Triglyceride content (mg/dl)	Radioactivity in triglyceride (dis/min/ml) × 10^{-2}
Control	19.9 ± 2.1	10.78 ± 1.59	550 ± 80	88.2 + 32.0	264 + 102
Aminotriazole	17.8 ± 1.6	6.91 ± 0.98	498 ± 118	90.6 ± 23.6	240 ± 192
	N.S.*,†	$P < 0.02$*	N.S.*,†	N.S.*,†	N.S.*,†

The aminotriazole group received aminotriazole (1 g/kg, i.p.) 12 hr before sacrifice. The control group received physiological saline at the same time. Albumin-bound palmitate[^{14}C] (10 μCi/100 g, i.v.) was injected in these groups 15 min before sacrifice. Values are represented as means ± S.D. in four animals.

* Comparison of treatment with control by *t*-test.

† P values larger than 0.05 were considered not significant (N.S.).

Table 3. Effect of aminotriazole (AT) pretreatment on serum free fatty acid and liver triglyceride contents in the adrenalin-treated rats

	No. of rats	Serum free fatty acid (μeq./l)	P	Liver triglyceride (mg/g)	P
Control	5	395 ± 72	—	5.59 ± 1.27	—
Aminotriazole	5	366 ± 87	N.S.*,‡	3.95 ± 0.59	< 0.05*
Adrenalin	4	778 ± 101	< 0.01*	6.25 ± 0.91	N.S.*,‡
Aminotriazole-Adrenalin	4	756 ± 87	N.S.†,‡	7.09 ± 0.96	N.S.†,‡

The first group received aminotriazole (1 g/KG. i.p.) 30 min before adrenalin injection. The second group received adrenalin (200 μg/100 g, i.m.) 2 hr before sacrifice. The third group received aminotriazole and adrenalin.

The control group received saline. Values are represented as means ± S.D.

* Comparison of treatment versus control by *t*-test.

† Comparison of aminotriazole-adrenalin group versus adrenalin group by *t*-test.

‡ P values larger than 0.05 were considered not significant (N.S.).

Table 4. Effect of aminotriazole (AT) pretreatment on the *in vivo* incorporation of [^{14}C]acetate into liver triglyceride in ethanol-induced fatty liver

	No. of rats	Radioactivity in liver (% of dose)	Triglyceride content (mg/g liver)	Radioactivity in triglyceride (dis/min/g liver) × 10^{-2}
Control	6	4.1 ± 0.7	3.50 ± 0.68	50.1 ± 10.2
Ethanol	6	2.9 ± 1.0	19.23 ± 6.79	168.0 ± 29.8
		N.S.*,†	P < 0.01*	P < 0.01*
Aminotriazole-Ethanol	5	3.5 ± 0.7	9.80 ± 1.82	78.6 ± 6.46
		N.S.†,‡	P < 0.05‡	P < 0.05‡

The ethanol group received ethanol (6 g/kg, p.o.) 12 hr before sacrifice. The aminotriazole-ethanol group received aminotriazole (1 g/kg, i.p.) 30 min before ethanol administration. The control group received physiological saline and isocaloric glucose. [^{14}C]acetate (10 μCi/100 g, i.p.) was injected in these animals 15 min before sacrifice. Values are represented as means ± S.D.

* Comparison of treatment with control by *t*-test.

† P values larger than 0.05 were considered not significant (N.S.).

‡ Comparison of aminotriazole-ethanol group versus ethanol group by *t*-test.

administration of adrenalin, and to the esterification of this fatty acid to TG which compensated for the decrease of fatty acid synthesis caused by AT.

The increase in the synthesis of endogenous fatty acid is thought to occur because of the development of ethanol-induced fatty liver [16]. The effect of AT pretreatment on the incorporation of [^{14}C]acetate into liver TG in the ethanol-induced fatty liver was studied (Table 4). The incorporation of radioactivity into liver TG in the ethanol group increased 3 times over the control group. However, the incorporation into the AT-ethanol group was reduced to about 45 per cent of the amount of the ethanol-group. Therefore, it is thought that AT inhibits fatty acid synthesis in the liver.

The decrease in liver TG level which is caused by AT is considered to occur for the following reasons; (1) decreased TG formation, resulting either from a decreased formation of fatty acid or a decreased esterification of fatty acids to the TG in the liver, (2) increased hepatic TG secretory mechanism; and (3) decreased mobilization of FFA from peripheral adipose tissue. AT inhibited the uptake of acetate into TG (Table 1), but did not inhibit the uptake of palmitate into TG (Table 2). Therefore, it has become apparent that AT inhibits the synthesis of hepatic fatty acid in its formation stage (Table 3, 4). On the other hand, it has been demonstrated that AT did not show any effect on the hepatic TG secretory mechanism and on mobilization of FFA from peripheral adipose tissue (Table 2, 3). In conclusion, the main cause of the decrease of liver TG caused by the administration of AT is the inhibition which affects fatty acid synthesis in the liver.

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