Chem. Pharm. Bull. **38**(9) 2532—2536 (1990) Vol. 38, No. 9

Inhibition of Cholesterol Synthesis from Mevalonate by Aminotriazole Treatment *in Vivo*

Fumie HASHIMOTO,* Chishio SUGIMOTO and Hidenori HAYASHI

Department of Physiological Chemistry, Faculty of Pharmaceutical Sciences, Josai University, Sakado, Saitama 350-02, Japan. *Received March 16, 1990*

3-Amino-1,2,4-triazole (aminotriazole) is an irreversible inhibitor of catalase which is a marker enzyme of peroxisomes. We studied the effect of aminotriazole treatment on biosyntheses of cholesterol and bile acid *in vivo*. When catalase activity of peroxisomes of rat liver was inhibited by aminotriazole treatment, bile acid content in the bile was significantly decreased to about 70% of the control, but that in the liver was not changed. Cholesterol content in the bile was significantly decreased to about 80% of the control, while in the liver and serum the content was not significantly changed. When [2-^{14}C]mevalonate was administered to rats, radioactivities of cholesterol in the liver, serum and bile were all drastically decreased by aminotriazole treatment, and an unidentified radioactive product was detected. Radioactivity of bile acid in the bile was also greatly decreased. In a similar experiment with [4-^{14}C]cholesterol, aminotriazole treatment had no effect on the radioactivity of either cholesterol or bile acid in the liver, serum and bile. In this case, the unidentified product could not be detected.

These results indicate that when catalase activity of liver peroxisomes is suppressed by aminotriazole treatment, biosynthesis of bile acid from exogenous cholesterol is not inhibited, but a step in the pathway of biosynthesis of endogenous cholesterol from mevalonate is inhibited.

Keywords aminotriazole; cholesterol; mevalonate; bile acid; peroxisome; catalase

Introduction

It is well known that peroxisomes participate in the β-oxidation of long chain fatty acid.[1] Since it was reported that 3α,7α,12α-trihydroxy-5β-cholestanoic acid (THCA), an intermediate of bile acid synthesis, was accumulated in biological fluids of patients with Zellweger syndrome,[2,3] which is caused by a defect in peroxisomes,[4] the relationship between peroxisomes and bile acid synthesis has become established.[5-8] Recently, it was found that peroxisomes are involved in the biosynthesis of cholesterol, which is the immediate precursor for bile acid synthesis. 3-Hydroxy-3-methyl glutaryl-coenzyme A (CoA) reductase, which is the rate-limiting enzyme for cholesterol synthesis and had been considered to be localized exclusively in microsomes, is also present in peroxisomes.[9,10] Cholesterol synthesis from mevalonate is carried out in peroxisomes in addition to microsomes in the presence of cytosolic fraction *in vitro*.[11] However, the association of peroxisomes with the biosynthesis of cholesterol *in vivo* is not yet established.

In a previous paper we reported that the bile acid content in rat bile was decreased by administration of 3-amino-1,2,4-triazole (aminotriazole)[12]; however, the mechanism of this reduction was not determined. Aminotriazole is an irreversible inhibitor of activity of catalase which is a marker enzyme of peroxisomes. Further, it was reported that in mutant 'acatalasemic' mice, which have unstable catalse that is readily degraded, cholesterol synthesis from ^{14}C-acetate is inhibited.[13] Therefore, we speculated that cholesterol biosynthesis may be inhibited by aminotriazole treatment, and consequently bile acid synthesis may be suppressed.

We then studied the effect of aminotriazole treatment on biosyntheses of cholesterol and bile acid *in vivo*. Bile acid is synthesized from both endogenous and exogenous cholesterol.[14,15] Endogenous cholesterol is synthesized within the liver, and exogenous cholesterol originates from the diet or extrahepatic organs. We used ^{14}C-mevalonate as a source for endogenous cholesterol, and ^{14}C-cholesterol as exogenous cholesterol. The result clarified that when catalase activity of liver peroxisomes was suppressed by aminotriazole treatment, biosynthesis of endogenous cholesterol from mevalonate was inhibited.

Materials and Methods

Materials Aminotriazole was purchased from Tokyo Chemical Industry (Japan). Lipids such as bile acid and cholesterol were obtained from Sigma Chemicals (U.S.A.). [2-^{14}C]Mevalonolactone (1.85 GBq/mmol) and [4-^{14}C]cholesterol (2.22 GBq/mmol) were purchased from NEN Research (U.S.A.). All other reagents were of analytical grade from Wako Pure Chemicals, Ltd. (Japan).

Treatment of the Rats Male Wistar rats (250—300 g) were maintained in a light- and temperature-controlled environment and fed *ad libitum* on chow pellets (Oriental MF, Japan). Bile duct cannulation was carried out at 10:00 a.m., and bile and urine were collected. Aminotriazole was intraperitoneally administered to the rats at a dose of 1 mg per 1 g body weight in physiological saline at 0, 6 and 12 h after the operation. Control rats were injected with the same volume of physiological saline instead of the aminotriazole solution. In the experiment on liver and serum, the liver was perfused with cold saline and excised immediately after exsanguination through the abdominal aorta under anesthesia at 6 h after the operation. The light mitochondrial fraction was obtained from liver homogenate according to the method of de Duve *et al.*[16]

In the radioisotope experiment, aminotriazole was intraperitoneally injected into the rats 2 h before the bile duct cannulation. Control rats were injected with saline instead of aminotriazole. ^{14}C-Mevalonolactone (29.6 kBq/0.2 ml of saline containing 2% Tween 80) or ^{14}C-cholesterol (14.8 kBq/0.2 ml of saline containing 2% Tween 80) was administered to the rats through the portal vein immediately after the operation, and bile was collected. At 6 h after the aminotriazole treatment, blood was collected under anesthesia and the liver was excised.

Determination of Contents of Bile Acid and Cholesterol Bile acid content in bile was determined according to the method of Eastwood *et al.*[17] Since liver, serum and urine contain only small amounts of bile acid, the quantities in these samples were assayed by gas chromatography.

Liver was homogenized with 20 volumes of Folch's solution (chloroform : methanol = 2 : 1), and lipids were extracted. The lipid fraction was mixed with 0.5 volume of 0.9% NaCl containing 10 mM NaOH. The methanol layer was evaporated to dryness and the residue was dissolved in 0.1 N NaOH. After acidification, bile acid was extracted with ethyl acetate, and then it was dried under nitrogen and hydrolyzed in 10% KOH/50% ethanol solution at 121 °C for 4 h. The hydrolysate was acidified and extracted with ethyl acetate, followed by addition of estriol (100 μg) as an internal standard. The mixture was allowed to react with hexafluoroisopropyl alcohol and trifluoroacetic anhydride at 60 °C for 45 min.[18] After evaporation of the reagents, the derivatives were dissolved in *n*-hexane. An aliquot was subjected to gas chromatography (Silicone DC QF-1 2%, Chromosorb WHP 80/100 glass column, i.d. 3 mm × 2 m,

injection temp. 240 °C, column temp. 220 °C, flow rate N_2 45 ml/min).

A serum sample was diluted with 4 volumes of 0.5 M phosphate buffer (pH 7.0) and heated at 60 °C for 20 min. The mixture was passed through a SEP PAK C_{18} cartridge (Waters, U.S.A.). A urine sample was diluted with an equal volume of 0.5 M phosphate buffer (pH 7.0) and passed through the cartridge. After successive washing of the cartridge with water and 1.5% ethanol, bile acid was eluted with 90% ethanol,[19-23] and then it was dried and dissolved in 0.1 N NaOH. After acidification, bile acid was extracted with ethyl acetate, and subjected to solvolysis[24] and hydrolysis. Bile acid extracted from the hydrolysate was derivatized, and subjected to gas chromatography as stated above.

Content of total cholesterol in the bile, liver and serum was assayed by the method of Zlatkis and Zak.[25]

Assay of Catalase Activity and Protein Content Catalase activity of the light mitochondrial fraction was determined according to the method of Leighton *et al.*,[26] but the procedure was performed manually.[27] Protein was estimated by the method of Lowry *et al.*[28]

Incorporation of Radioactivity into Cholesterol and Bile Acids Liver was homogenized in 20 volumes of Folch's solution. A bile or serum sample was diluted with 20 volumes of Folch's solution, and from these samples, lipids were extracted and concentrated. Cholesteryl palmitate, cholesterol and taurocholate (200 μg each) were added as markers of cholesteryl ester, free cholesterol and bile acid, respectively. These samples were subjected to thin-layer chromatography (TLC) using reversed-phase KC18F plates (20 × 20 cm, thickness 0.2 mm, Whatman, U.S.A.). After development with *n*-hexane–diethyl ether–formic acid (80 : 20 : 2) as a solvent, the plate was sprayed with 20% H_2SO_4 and heated. The areas corresponding to the positions of the standards were scraped from the plate into centrifuge tubes. The cholesteryl ester, free cholesterol and bile acid were extracted 3 times with Folch's solution and the solvent was evaporated off. The radioactivities were determined with an Aloka scintillation counter (Model LSC-700, Japan) in scintillation fluid consisting of 2,5-diphenyloxazole (4 g) and 1,4-bis-2-(5-phenyloxazole-2-yl)benzene (50 mg) in 1 l of toluene and Triton X-100 (2 : 1).

Results

Effects of Aminotriazole on Bile Acid Content Peroxisomes are contained in the light mitochondrial fraction. First, we determined the catalase activity of the light mitochondrial fraction of rat liver 6 h after aminotriazole treatment. Catalase activity was almost completely inhibited, while liver weight was hardly affected by aminotriazole (Table I).

Table II shows the effects of aminotriazole treatment on bile acid contents in bile, liver, serum and urine. Bile acid content in the bile collected during the 6 h after the injection of aminotriazole was reduced to about 70% of the control. In the liver at 6 h after the treatment the content was not significantly changed, but that in the serum was increased about 3 times. Bile acid content in the collected urine during the 24 h after the first injection of aminotriazole was increased about 2-fold. However, in the liver, serum and urine the contents were far lower than that in the bile, so the increases in the amounts of this acid in the serum and urine of the aminotriazole-treated rats did not correspond to the decrease of the amount in the bile.

These results suggest that the reduction of bile acid content in the bile by aminotriazole treatment is not due to the inhibition of excretion of bile acid into the bile, but to the inhibition of bile acid biosynthesis.

Effects of Aminotriazole on Cholesterol Content Bile acid is synthesized from cholesterol, and we studied the effect of aminotriazole on cholesterol contents in the bile, liver and serum. In the bile 6 h after aminotriazole treatment cholesterol content was significantly decreased to 84% of the control, while the contents in the liver and serum were not significantly changed (Table III).

Table I. Effects of Aminotriazole on Catalase Activity of Light Mitochondrial Fraction and on Liver Weight

		Aminotriazole	Control	Aminotriazole / Control
Catalase[a]	(3)	1.9±0.3	134.5±20.6	0.01[c]
Liver weight[b]	(10)	12.2±1.0	12.3± 1.0	0.99

Six hours after administration of aminotriazole to rats with a bile-duct fistula, the liver was isolated. The light mitochondrial fraction was prepared from the liver, and catalase activity in the fraction was determined. The same volume of saline was injected into control rats instead of aminotriazole. *a*) U/mg protein, *b*) g. Numbers in parentheses represent numbers of experimental animals. Data are means ±S.D. *c*) Represents a significant decrease ($p<0.005$).

Table II. Bile Acid Contents in Bile, Liver, Serum and Urine of Aminotriazole-Treated and Control Rats

	Total bile acid		Aminotriazole
	Aminotriazole	Control	Control
Bile[a]	35.4±5.3	49.9±4.2	0.71[e]
Liver[b]	0.382±0.057	0.449±0.073	0.85
Serum[c]	0.011±0.001	0.004±0.001	2.75[f]
Urine[d]	0.146±0.010	0.064±0.024	2.28[f]

Aminotriazole was immediately injected into rats after bile-duct cannulation. Bile was collected for 6 h after the operation, and then the liver and serum were isolated. Urine was collected for 24 h from rats which had received aminotriazole 3 times at 0, 6 and 12 h after the operation. *a*) mg/rat/6 h, *b*) mg/whole liver, *c*) mg/ml serum, *d*) mg/rat/24 h. Data are means±S.D. of 4 animals. *e,f*) Represent significant changes, (*e*) $p<0.01$; *f*) $p<0.005$).

Table III. Effects of Aminotriazole on Cholesterol Content in Bile, Liver and Serum

	Total cholesterol		Aminotriazole
	Aminotriazole	Control	Control
Bile[a]	7.27±0.60	8.61±0.57	0.84[d]
Liver[b]	28.0±2.5	31.0±1.2	0.90
Serum[c]	1.03±0.05	0.95±0.11	1.08

The samples of bile, liver preparation and serum were prepared from the rats with a bile duct fistula as described in Table II. *a*) mg/rat/6 h, *b*) mg/whole liver, *c*) mg/ml serum. Values are means±S.D. of 6 animals. *d*) Represents a significant decrease ($p<0.005$).

These results indicate that the inhibition of bile acid synthesis by aminotriazole expected from the experiment of Table II is due to the decrease in the quantity of cholesterol, which is the immediate precursor for bile acid synthesis.

Inhibition of Syntheses of Cholesterol and Bile Acid from ^{14}C-Mevalonate by Aminotriazole Effects of aminotriazole on the syntheses of endogenous cholesterol and bile acid from ^{14}C-mevalonate were investigated. In order to elucidate the effect of this inhibitor at a time when the radioactivities of cholesterol and bile acid synthesized from ^{14}C-mevalonate were high enough to permit precise evaluation, aminotriazole was injected into rats 2 h before the bile duct cannulation. ^{14}C-Mevalonate was administered through the portal vein immediately after the operation, and the bile was collected during two successive 2 h periods.

Table IV shows the radioactivities of cholesterol and bile acid synthesized from ^{14}C-mevalonate in the liver and serum. With respect to the liver, the radioactivity of free cholesterol was the highest in the control group, and was

TABLE IV. Effects of Aminotriazole on Synthesis of Cholesterol and Bile Acid from ^{14}C-Mevalonate

	Radioactivity		Aminotriazole / Control
	Aminotriazole	Control	
Liver[a]			
Cholesteryl ester	4700±740	2180±910	2.16[c]
Free cholesterol	2890±1340	12490±3390	0.23[c]
Bile acid	1700±300	1180±300	1.44
Serum[b]			
Cholesteryl ester	600±40	1400±130	0.43[c]
Free cholesterol	90±40	370±120	0.24[d]
Bile acid	170±40	330±40	0.52[c]

Two hours after the injection of aminotriazole, the rats were subjected to bile duct cannulation and ^{14}C-mevalonate was immediately administered through the portal vein. Control rats were given saline instead of aminotriazole. Four hours later, blood was taken from the animals under anesthesia, and the liver was excised. Total lipids extracted from the liver and serum were subjected to TLC. *a*) dpm/g liver, *b*) dpm/ml serum. Data are means±S.D. of 5 animals. *c, d*) Represent significant changes (*c*) $p<0.005$; *d*) $p<0.01$).

TABLE V. Decrease of Excretion Rate of Cholesterol and Bile Acid Synthesized from ^{14}C-Mevalonate into Bile by Aminotriazole Treatment

After injection of ^{14}C-mevalonate (h)		Radioactivity (dpm/h) Aminotriazole	Control	Aminotriazole / Control
Cholesteryl ester	0—2	Trace	Trace	—
	2—4	Trace	Trace	—
Free cholesterol	0—2	1120±320	3210±90	0.35[a]
	2—4	570±170	3120±140	0.18[a]
Bile acid	0—2	16080±2140	27240±4660	0.59[a]
	2—4	3870±890	9910±1300	0.39[a]

Bile was collected during two successive 2 h periods after the bile duct cannulation from the same rats as described in Table IV. Data are means±S.D. of 5 animals. *a*) Represents significant changes ($p<0.005$). "Trace" means below the limit of quantitation (60 dpm/h).

decreased to about 20% of the control by the aminotriazole treatment. Radioactivity of esterified cholesterol was increased about 2-fold by aminotriazole, but total radioactivities (free plus esterified cholesterol) amounted to only about 50% of the control. Radioactivity of bile acid amounted to about 9% that of free cholesterol, but was not significantly changed by aminotriazole. In the serum, the radioactivity of esterified cholesterol was the highest in the control group, and was remarkably decreased together with that of free cholesterol by aminotriazole treatment. Radioactivity of bile acid was very low, and reduced to about 50% of the control by aminotriazole treatment.

Table V shows the excretion rates of cholesterol and bile acid synthesized from ^{14}C-mevalonate into the bile. Little radioactivity of esterified cholesterol was detected. The excretion rate of radioactive free cholesterol into the bile of the control rats from 0 to 4 h was almost constant; however, this rate was remarkably decreased by aminotriazole treatment. Radioactivity of bile acid in the bile was very much higher than that of free cholesterol. In the control group, the excretion rate of radioactive bile acid into the bile from 2 to 4 h was decreased to about 40% of that from 0 to 2 h. The excretion rate of bile acid was greatly suppressed by aminotriazole.

Figures 1 and 2 show typical thin-layer chromatography

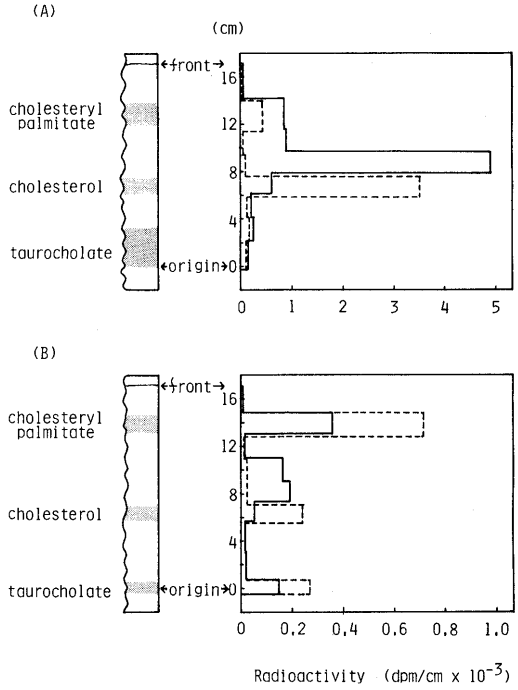

Fig. 1. Effects of Aminotriazole on the TLC Profile of Lipids Synthesized from ^{14}C-Mevalonate, in the Liver and Serum

Total lipids were extracted from the liver and serum of the aminotriazole-treated (——) or control (------) rats as described in Table IV. Lipids corresponding to 0.5 g of the liver or 1 ml of the serum were loaded on the TLC plate. (A) liver, (B) serum.

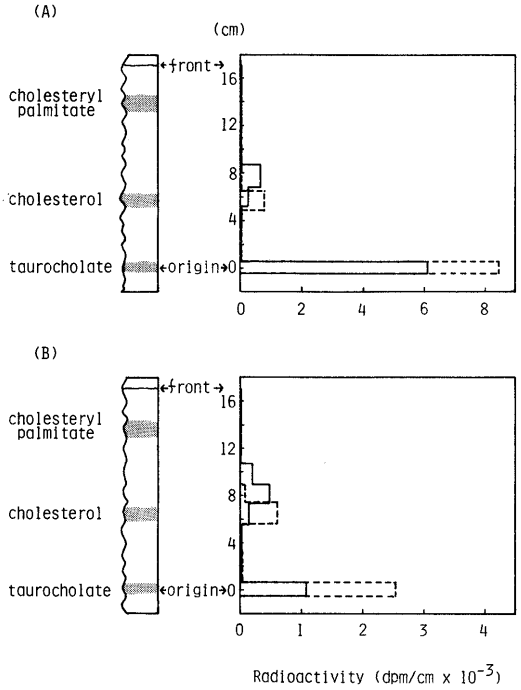

Fig. 2. Effect of Aminotriazole on the TLC Profile of Lipid Synthesized from ^{14}C-Mevalonate, in the Bile

Total lipids were extracted from the bile of the aminotriazole-treated (——) or control (------) rats as described in Table V. Lipids corresponding to 0.5 ml of the bile were subjected to the chromatography. (A) bile from 0 to 2 h after the injection of ^{14}C-mevalonate, (B) bile from 2 to 4 h after the injection.

profiles of lipid extract from the liver, serum and bile. The lipid was extracted from the whole fraction on the plate. In liver, serum and bile of the aminotriazole-treated rats, radioactivity due to some unidentified material(s) was detected in the area above the position of free cholesterol,

TABLE VI. Effects of Aminotriazole on Radioactivity of Cholesterol and Bile Acid in Liver and Serum after Injection of ^{14}C-Cholesterol

	Radioactivity		Aminotriazole
	Aminotriazole	Control	Control
Liver[a]			
Cholesteryl ester	1810±20	1730±300	1.05
Free cholesterol	23810±4250	25630±240	0.93
Bile acid	750±180	680±20	1.10
Serum[b]			
Cholesteryl ester	4880±290	4700±590	1.04
Free cholesterol	740±70	840±30	0.88
Bile acid	110±10	130±50	0.85

^{14}C-Cholesterol instead of ^{14}C-mevalonate was injected into rats as described in Table IV. *a*) dpm/g liver, *b*) dpm/ml serum. Data are means±S.D. of 5 animals.

TABLE VII. Effects of Aminotriazole on Excretion Rate of Radioactive Cholesterol and Bile Acid into Bile after Injection of ^{14}C-Cholesterol

After injection of ^{14}C-cholesterol (h)		Radioactivity Aminotriazole (dpm/h)	Control (dpm/h)	Aminotriazole Control
Cholesteryl ester	0—2	Trace	Trace	—
	2—4	Trace	Trace	—
Free cholesterol	0—2	4340±1090	3230±140	1.34
	2—4	3700±220	4300±1270	0.86
Bile acid	0—2	16160±1350	16220±1700	1.00
	2—4	19790±2100	17820±3040	1.16

Bile was collected during two successive 2 h periods after the bile duct cannulation from the same rat as described in Table VI. Data are means±S.D. of 5 animals. "Trace" means below the limit of quantitation (60 dpm/h).

and the radioactivity of this material(s) from the liver was higher than that of free cholesterol (Fig. 1). Even in the bile from 0 to 2 h, the radioactivity of the unidentified material(s) could be detected (Fig. 2).

These results suggest that the inhibition of endogenous cholesterol synthesis may at least partially contribute to the inhibition of bile acid synthesis from mevalonate by aminotriazole.

Effects of Aminotriazole on Bile Acid Synthesis from ^{14}C-Cholesterol We studied whether aminotriazole inhibits bile acid synthesis from exogenous cholesterol, by using ^{14}C-cholesterol instead of ^{14}C-mevalonate. Table VI shows the radioactivities of ^{14}C-cholesterol and bile acid synthesized *de novo* from ^{14}C-cholesterol in the liver and serum. Radioactivities of cholesteryl ester, free cholesterol and bile acid were little affected by aminotriazole.

Table VII shows the excretion rates of ^{14}C-cholesterol and bile acid synthesized *de novo* from ^{14}C-cholesterol into the bile. The excretion rate of radioactive cholesterol into the bile from 0 to 4 h was almost constant, and was little affected by aminotriazole. The excretion rate of radioactive bile acid into the bile from 2 to 4 h tended to be higher than that into the bile from 0 to 2 h, contrary to the data of Table V. However, aminotriazole had little effect on these excretion rates.

No unidentified radioactive material could be detected on the thin-layer chromatogram of the lipid extract from the liver, serum, and bile of the aminotriazole-treated rats after the injection of ^{14}C-cholesterol (data not shown).

These results suggest that the process of bile acid synthesis from exogenous cholesterol is not inhibited by aminotriazole treatment.

Discussion

We previously reported that bile acid content in the bile is decreased by the administration of aminotriazole to rats[12]; in this experiment, the reason for this was studied in detail. After aminotriazole treatment, the contents of bile acid and cholesterol in the bile were significantly decreased (Tables II and III). These decreases seemed due to the effect of the treatment not on the bile acid synthesis from exogenous cholesterol (Tables VI and VII), but on the synthesis of endogenous cholesterol from mevalonate (Tables IV and V). Namely, synthesis of endogenous cholesterol from mevalonate may be inhibited by aminotriazole treatment, leading to the suppression of bile acid synthesis.

When catalase activity of liver peroxisomes was almost completely inhibited by aminotriazole (Table I), cholesterol synthesis was suppressed. We do not deny the possibility that aminotriazole directly affects enzyme(s) of the cholesterol-synthetic pathway other than catalase in peroxisomes. However, this possibility may be weak, because the inhibition of cholesterol synthesis in mutant 'acatalasemic' mice has been reported.[13]

In the serum, the radioactivity of bile acid synthesized from ^{14}C-mevalonate was decreased by aminotriazole treatment (Table IV). However, bile acid content in both the serum and urine was increased, though the amount of the increases was far less than the amount of the decrease of bile acid in the bile (Table II). From these results it seems that the leakage of bile acid, which entered the so-called enterohepatic circulation before the operation, into the peripheral blood was increased by aminotriazole treatment.

Cholesterol content in the bile was significantly decreased by this treatment, while that in the liver was not significantly changed (Table III). Much cholesterol is already present in plasma membrane, *etc.* in the liver, and so the ratio of *de novo*-synthesized cholesterol to total cholesterol is small. Consequently, even though cholesterol synthesis was inhibited by aminotriazole treatment (Tables IV and V), there may be no apparent significant change.

Bile acid is synthesized from both endogenous and exogenous cholesterol; it has been reported that the former is more preferentially utilized for the formation of bile acid than the latter.[14,15] In this experiment, the excretion rate of bile acid synthesized from ^{14}C-mevalonate into the bile from 0 to 2 h was higher than that into the bile from 2 to 4 h (Table V). In a similar experiment with ^{14}C-cholesterol, however data of the excretion rate were contrary to those of Table V (Table VII). These results support the preferential utilization of endogenous cholesterol. Further, bile acid synthesis from ^{14}C-cholesterol was not inhibited by aminotriazole treatment (Tables VI and VII), but syntheses of cholesterol and bile acid from ^{14}C-mevalonate were inhibited (Tables IV and V). Therefore, it is suggested that aminotriazole does not affect bile acid synthesis from exogenous cholesterol, but suppresses the synthesis of endogenous cholesterol from mevalonate, thereby inhibiting bile acid synthesis. In a previous paper, we reported that aminotriazole inhibits the fatty acyl-CoA β-oxidation

system of peroxisomes without affecting the system of mitochondria.[29] The mechanism of this inhibition is as follows. When catalase activity of liver peroxisomes is inhibited by aminotriazole treatment, H_2O_2 is accumulated within the peroxisomes. The H_2O_2 affects thiolase, which is a component enzyme of the fatty acyl-CoA β-oxidation system, and its substrate (3-ketoacyl-CoA). Since thiolase reaction is coupled with the reaction of the fatty acyl-CoA β-oxidation system, the β-oxidation system is inhibited. In the pathway of bile acid synthesis, THCA is converted to cholic acid by an oxidative reaction similar to fatty acyl-CoA β-oxidation.[5-8] Therefore, aminotriazole may inhibit β-oxidation for bile acid synthesis as well as fatty acyl-CoA β-oxidation in peroxisomes. Namely, aminotriazole may inhibit the process of bile acid synthesis not from exogenous cholesterol, but from endogenous cholesterol, though we have not yet confirm this.

When ^{14}C-cholesterol was injected into aminotriazole-treated rats, no radioactive material other than cholesteryl ester, free cholesterol and bile acid could be found (data not shown). However, an unidentified radioactive material(s) was detected in the liver, serum and bile of the treated rats following the injection of ^{14}C-mevalonate, and was located in a more hydrophobic area than free cholesterol on the TLC plate (Figs. 1 and 2). In this area on the TLC, sterol intermediates of cholesterol synthesis such as a 4-methyl sterol are reportedly located.[30,31] Therefore, the unknown material(s) may be a sterol intermediate of cholesterol synthesis from mevalonate. That is, a step in the biosynthetic pathway to cholesterol from mevalonate was probably inhibited by aminotriazole treatment, and consequently some sterol intermediate might accumulate in the liver, serum and bile.

Peroxisomes may play an important role in cholesterol synthesis. It has been demonstrated that 3-hydroxy-3-methylglutaryl-CoA reductase, the rate-limiting enzyme of the biosynthetic pathway of cholesterol, is present not only in the membranes of the endoplasmic reticulum but also in peroxisomes.[9] Further, it has recently been shown that cholesterol is synthesized from mevalonate in peroxisomes in addition to microsomes in the presence of cytosolic fraction *in vitro*.[11] Sterol carrier protein-2 has a variety of carrier functions *in vitro*, all associated with cholesterol biochemistry. This nonenzymatic protein is required in the enzymatic conversion of sterol intermediate to cholesterol during cholesterol biosynthesis, and had been thought to be exclusively localized in cytoplasm; however it was recently found that it is localized almost entirely in peroxisomes.[32-34] This fact is very interesting. In our experiment, when catalase activity of the liver peroxisomes was almost completely inhibited by aminotriazole treatment (Table I), cholesterol synthesis from mevalonate *in vivo* was suppressed (Tables IV and V), and sterol intermediate(s) was accumulated in the liver, serum and bile (Figs. 1 and 2). These results support such a relationship between peroxisomes and cholesterol synthesis. However, at the present stage of the experiment, we can not ignore the possibility that aminotriazole affects not only catalase of peroxisomes, but also the cholesterol-synthetic pathway of the endoplasmic reticulum or cytoplasm. Therefore, further study may be necessary for conclusive proof of the association of peroxisomes with cholesterol synthesis *in vivo* using aminotriazole.

In conclusion, it was clarified that when catalase activity of liver peroxisomes is suppressed by aminotriazole treatment, this does not result in an inhibition of bile acid biosynthesis from exogenous cholesterol, but a step in the pathway of biosynthesis of endogenous cholesterol from mevalonate is inhibited.

References

1) P. B. Lazalow, *J. Biol. Chem.*, **253**, 1522 (1978).
2) R. F. Hanson, P. S. Van Leeuwen, G. C. Williams, G. Grobowski and H. L. Sharp, *Science*, **203**, 1107 (1978).
3) G. G. Parmentier, G. A. Janssen, E. A. Eggermant and H. J. Eyssen, *Eur. J. Biochem.*, **102**, 173 (1979).
4) S. Goldfischer, C. L. Moors, A. B. Johnson, A. J. Spiro, M. P. Valsamis, H. K. Wisniewsi, R. H. Ritch, W. T. Nortou, I. Rapin and L. M. Gartner, *Science*, **182**, 62 (1973).
5) K. Prydz, B. F. Kase, I. Björkhem and J. I. Pedersen, *J. Lipid Res.*, **27**, 622 (1986).
6) J. I. Pedersen and J. Gustafsson, *FEBS Lett.*, **121**, 345 (1980).
7) S. L. Thompson and S. K. Krisans, *Biochem. Biophys. Res. Commun.*, **130**, 708 (1985).
8) B. F. Kase, K. Prydz, I. Björkhem and J. I. Pedersen, *Biochim. Biophys. Acta*, **877**, 37 (1986).
9) G. A. Keller, M. C. Barton, D. J. Shapiro and S. L. Singer, *Proc. Natl. Acad. Sci. U.S.A.*, **82**, 770 (1985).
10) G. A. Keller, M. Parirandeh and S. Krisans, *J. Cell Biol.*, **103**, 875 (1986).
11) S. L. Thompson, R. Burrows, R. J. Laub and S. K. Krisans, *J. Biol. Chem.*, **262**, 17420 (1987).
12) H. Hayashi, K. Fukui and F. Yamasaki, *J. Biochem.* (Tokyo), **96**, 1713 (1984).
13) R. R. Cuadrado and L. A. Bricker, *Biochim. Biophys. Acta*, **306**, 168 (1973).
14) M. Ogura, Y. Ayaki and M. Goto, *J. Biochem.* (Tokyo), **80**, 537 (1976).
15) N. B. Myant and K. A. Mitropoulos, *J. Lipid Res.*, **18**, 135 (1977).
16) C. de Duve, B. C. Pressman, R. Gianetto, R. Watiaux and F. Appelmans, *Biochem. J.*, **60**, 604 (1955).
17) E. A. Eastwood, D. Hamilton and L. Mowbray, *J. Chromatogr.*, **65**, 407 (1972).
18) K. Imai and Z. Tamura, *J. Chromatogr.*, **120**, 181 (1976).
19) J. Goto, M. Saito, T. Chikai and T. Nambara, *J. Chromatogr.*, **276**, 289 (1983).
20) J. Goto, H. Kato, Y. Saruta and T. Nambara, *J. Chromatogr.*, **226**, 13 (1981).
21) J. Goto, H. Kato, Y. Saruta and T. Nambara, *J. Liquid Chromatogr.*, **3**, 991 (1980).
22) H. Takikawa, H. Otsuka, T. Beppu, Y. Seyama and T. Yamakawa, *J. Biochem.* (Tokyo), **92**, 985 (1982).
23) J. Goto, K. Suzaki, M. Ebihara and T. Nambara, *J. Chromatogr.*, **345**, 241 (1985).
24) N. Murata, T. Beppu, H. Takikawa, H. Otsuka, Y. Kasama and Y. Seyama, *Steroids*, **42**, 575 (1983).
25) A. Zlatkis and B. Zak, *Anal. Biochem.*, **29**, 143 (1969).
26) F. Leighton, B. Poole, H. Beaufay, P. Baudhuin, J. W. Coffey, S. Fowler and C. de Duve, *J. Cell Biol.*, **37**, 482 (1968).
27) H. Hayashi and T. Suga, *J. Biochem.* (Tokyo), **84**, 513 (1978).
28) O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).
29) F. Hashimoto and H. Hayashi, *Biochim. Biophys. Acta*, **921**, 142 (1987).
30) G. F. Gibbons and K. A. Mitropoulos, *Biochem. J.*, **132**, 439 (1973).
31) G. F. Gibbons, K. A. Mitropoulos and K. Ramananda, *J. Lipid Res.*, **14**, 587 (1973).
32) T. P. Van der Krift, J. Leunissen, T. Teerlink, G. P. H. Van Heusden, A. J. Verkleij and K. W. A. Wirtz, *Biochim. Biophys. Acta*, **812**, 387 (1985).
33) M. Tsuneoka, A. Yamamoto, Y. Fujiki and Y. Tashiro, *J. Biochem.* (Tokyo), **104**, 560 (1988).
34) G. A. Keller, T. J. Scallen, D. Clarke, P. A. Maher, S. K. Krisans and S. J. Singer, *J. Cell Biol.*, **108**, 1353 (1989).