Effects of Monatepil Maleate, a New Ca$^{2+}$ Channel Antagonist With $\alpha_1$-Adrenoceptor Antagonistic Activity, on Cholesterol Absorption and Catabolism in High Cholesterol Diet-Fed Rabbits

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Received May 11, 1998 Accepted August 20, 1998

ABSTRACT—The mechanism of the prophylactic effect against hyperlipidemia by monatepil maleate was investigated in animal models. Monatepil maleate is an antihypertensive agent with Ca$^{2+}$-channel antagonistic, $\alpha_1$-adrenergic receptor-blocking, and lipid peroxidation inhibitory activity. In high cholesterol diet-fed rabbits, monatepil maleate (30 mg/kg, p.o., once daily for 9 weeks) showed a prophylactic effect against increases in total cholesterol and $\beta$-lipoprotein. Monatepil maleate significantly accelerated the clearance of radioactivity from the blood after intravenous injection of low-density lipoprotein (LDL) labeled with [$\alpha_2$,2$\alpha$ (n)-$^3$H]cholesterol, increasing biliary excretion of [$^3$H]-bile acids without modifying bile acid composition. Furthermore, monatepil maleate tended to inhibit the absorption of orally administered [$\alpha_2$,2$\alpha$ (n)-$^3$H]cholesterol from the gastrointestinal tract in these rabbits. In Watanabe heritable hyperlipidemic (WHHL) rabbits, an animal model of hepatic LDL receptor deficiency, monatepil maleate (30 mg/kg, p.o., once daily for 6 months) did not suppress the increase in plasma lipids. These results suggest that the plasma lipid lowering effect of monatepil maleate requires the presence of hepatic LDL receptors. It is also suggested that monatepil maleate improves plasma lipid metabolism through two mechanisms: enhancement of clearance of plasma LDL, which may be mediated by up-regulation of hepatic LDL receptors, and acceleration of conversion of free cholesterol to bile acids in the liver.

Keywords: Monatepil maleate, Cholesterol absorption, Cholesterol catabolism, Low-density lipoprotein receptor

Hypertension plays an important role in the onset and progression of atherosclerosis, and clinical study results have shown that blood pressure in hypertensive patients can be well controlled with various antihypertensive agents (e.g., Ca$^{2+}$ channel antagonists, angiotensin-converting enzyme inhibitors, $\alpha_1$-adrenergic receptor blockers), resulting in decreased incidence of hypertension-related vascular complications such as apoplectic stroke. Nevertheless, antihypertensive therapy still does not reduce the incidence of atherosclerotic diseases such as myocardial infarction in hypertensive patients (1, 2). It is also accepted that atherosclerosis is closely correlated with abnormal blood lipid metabolism. Therefore, an antihypertensive agent that improves plasma lipid metabolism would be favorable in preventing the onset and progression of atherosclerosis.

Antihypertensive agents have various effects on blood lipid abnormality and atherosclerosis. Thiazides and $\beta$-adrenergic receptor blockers adversely affect blood lipid metabolism (3, 4). In contrast, $\alpha_1$-adrenergic receptor blockers improve plasma lipid metabolism (5, 6) and Ca$^{2+}$ channel antagonists have antiatherosclerotic actions (7, 8), and both these types of antihypertensive agents are expected to suppress the onset and progression of atherosclerotic diseases.

Monatepil maleate (±)-N-(6,11-dihydrodibenzo[b,e]thiepin-11-yl)-4-(4-fluorophenyl)-1-piperazine butyramide) is an antihypertensive agent synthesized on the basis of the above findings to have both Ca$^{2+}$-channel antagonistic and $\alpha_1$-adrenergic receptor blocking activities (9,
10), as well as anti-lipid peroxidation activity (11). Monatelip (monatelip) improved plasma lipid metabolism and reduced atherosclerotic lesions in high cholesterol diet-fed monkeys and rabbits (11, 12). Furthermore, monatelip decreased plasma cholesterol in mild to moderate hypertensive patients with high cholesterol levels in a clinical study (13). However, the mechanism of these effects has not yet been clarified.

In the present study, we investigated the effects of monatelip on cholesterol absorption at the gastrointestinal tract and on hepatic cholesterol catabolism in high cholesterol diet-fed rabbits to elucidate the mechanism by which monatelip improved plasma lipid metabolism in humans (13) and animals (11, 12) and prevented atherosclerosis in animal models (11, 12). We also investigated the role of low-density lipoprotein (LDL) receptors in the plasma cholesterol lowering effect of monatelip using Watanabe heritable hyperlipidemic (WHHL) rabbits (14-17) deficient in the hepatic LDL receptors, which play an important role in cholesterol catabolism.

MATERIALS AND METHODS

Animals

Male Japanese white rabbits and homozygous WHHL rabbits (Kitayama Labes, Inc., Nagano) were purchased at the age of 8 weeks and acclimatized for 2 weeks on a standard diet (RC-4; Oriental Yeast Co., Ltd., Tokyo). Throughout acclimatization and the study period, the rabbits were housed in individual cages at a room temperature of 23±2°C and lighting from 6:00 a.m. to 6:00 p.m. Animals were administered 100 g of the diet once daily.

Test compounds

Monatelip (lot No. T-91018) was synthesized at Dai-Nippon Research Laboratories. Nitrendipine (lot No. Y039) was purchased from Yoshitomi Pharmaceutical Industries, Ltd. (Osaka). The test compounds were suspended approximately every 10 days in a 0.5% tragacanth solution and then stored in a refrigerator. Nitrendipine was stored in the dark. Monatelip administered at a daily dose of 30 mg/kg improved plasma lipid metabolism and reduced atherosclerotic lesions in high cholesterol diet-fed rabbits (11). Furthermore, this dose of monatelip caused a 20-30% decrease in blood pressure in conscious normotensive rabbits (data not shown) and its degree was the same as that of the dose-dependent antihypertensive effect shown by experimental hypertensive animals (10). The dose of nitrendipine was set at 2 mg/kg, which reduced blood pressure to the same degree as observed with the administration of monatelip 30 mg/kg/day (data not shown).

Studies in high cholesterol diet-fed rabbits

Effects on plasma lipid content in high cholesterol diet-fed rabbits: High-cholesterol diet was prepared by adding 1% cholesterol and 6% coconut oil to the standard diet. Thirty-one Japanese white rabbits were given the high-cholesterol diet for 2 weeks. Twenty-eight of these rabbits did not exhibit abnormal behavior or leave food uneaten during the 2 weeks, and they were examined for the following parameters: body weight, plasma lipid contents (total cholesterol [TC], β-lipoprotein [β-LP] and high-density lipoprotein cholesterol [HDL-C]) and bile acid content. The 28 rabbits were then divided randomly into two groups: Group 1, the vehicle control group (high cholesterol diet + control, n=14) and Group 2, the monatelip group (high cholesterol diet + monatelip, n=14). Both groups were administered the high cholesterol diet for an additional 9 weeks. The vehicle control group was given a 0.5% tragacanth aqueous solution (2 ml/kg), and the monatelip group was given monatelip (30 mg/2 ml/kg) once daily at 9:00 a.m. via gavage. Blood samples were withdrawn from the ear artery before and 4, 6 and 9 weeks after the start of drug administration. Plasma samples were obtained by centrifugation and measured for lipid, lipoprotein and bile acid content. After the drug administration period of 9 weeks, 6 rabbits from the vehicle control group and 5 rabbits from the monatelip group were used for a cholesterol absorption study, and 7 rabbits from each group were used for a catabolism study. In addition, 3 other rabbits separately administered high cholesterol diet were used for the preparation of [1α,2α(n)-3H]cholesterol labeled LDL.

Effects on cholesterol absorption in the gastrointestinal tract: An emulsion containing [1α,2α(n)-3H]cholesterol and β-[4-14C]sitosterol was prepared by the method of Amorosa et al. (18). First, 750 μCi of [1α,2α(n)-3H]cholesterol and 15 μCi of β-[4-14C]sitosterol were mixed, and then toluene and ethanol were removed from the mixture with nitrogen gas. The residue was dissolved in 4 ml of corn oil and mixed with 16 ml of 6.8% skim milk, followed by sonication for 1 hr to prepare the emulsion.

The 6 vehicle control rabbits and 5 monatelip-treated rabbits, all of which had been fed a high-cholesterol diet, were fasted overnight. The next morning, they were orally administered vehicle solution or monatelip suspension via gavage. Fifteen minutes after administration, 1 ml of the emulsion containing 32 μCi of [1α,2α(n)-3H]cholesterol and 0.57 μCi of β-[4-14C]sitosterol was orally administered via gavage, followed by diet. The animals were administered test compounds and diet in the same way for the next 2 days. Feces were collected once daily and stored at −18°C until analysis.

After grinding the dried feces samples in a mortar to obtain feces powder, neutral steroids in feces were
extracted according to the procedure of Miettinen et al. (19), and 0.5 g of the pulverized feces was refluxed for 1.5 hr in 10 ml of 90% ethanol solution containing 1 N/NaOH, to which 5 ml of distilled water was added. Neutral steroids were extracted 3 times with 20 ml of hexane. The extract solution was evaporated under nitrogen gas, and the resultant residue was dissolved in 2 ml of hexane. Then 100 μl of the solution was evaporated under reduced pressure, and 0.5 ml of distilled water and 15 ml of a liquid scintillation cocktail (ACSII) were added to the residue. Radioactivity was measured with a liquid scintillation counter (Tri-Carb 2000CA; Packard Instrument Co., Meriden, CT, USA). The percentage of cholesterol absorption was calculated by the following equation:

Cholesterol absorption (%) = \(1 - \frac{\text{\(1^4\)H in fecal neutral steroid fraction/\(1^4\)C in fecal neutral steroid fraction}}{\text{\(4^4\)C in oral dose/\(3^4\)H in oral dose}}\) \times 100

Effects on cholesterol catabolism in liver: LDL labeled with [\(1\alpha,2\alpha\)(n)-\(3^3\)H])cholesterol was prepared according to a modified procedure by Schwarz et al. (20). The LDL fraction (1.019 < d < 1.063) was isolated by ultracentrifugation (21) from the plasma of high cholesterol diet-fed rabbits not treated with the test compounds, and then it was filtered (0.22 μm; Flow Laboratories, Costa Mesa, CA, USA) and dialyzed against a buffer solution containing 0.15 M NaCl and 0.3 mM EDTA (pH 7.4). In order to incorporate [\(1\alpha,2\alpha\)(n)-\(3^3\)H])cholesterol into LDL, the LDL fraction (3 ml containing 15 mg of protein) was incubated with a filter paper (No. 1; Whatman, Kent, UK) containing 300 μCi [\(1\alpha,2\alpha\)(n)-\(3^3\)H])cholesterol at 4°C for 48 hr. The solution was then filtered again and dialyzed against a 0.15 M NaCl–0.3 mM EDTA buffer solution (pH 7.4). The relative radioactivity of the labeled LDL was 6.27 μCi per milligram of protein. The protein content was determined by the method of Lowry et al. (22).

The labeled LDL was administered as follows. Seven rabbits from the vehicle control group and 7 rabbits from the monatepil group were anesthetized with Somnopentyl® (Pitman Moore, Muncelein, IL, USA), and the carotid artery and common bile duct were exposed. The carotid artery was cannulated with a polyethylene tube (Hibiki No 5; Kunii, Tokyo), and the cannula was led subcutaneously through the back of the neck, out of the body, and fixed. The common bile duct was also cannulated with a polyethylene tube (SP45; Natsume, Tokyo), and the other end of the cannula was passed under the skin of the back and then reinserted into the bile duct at the anterior portion of Oddi’s sphincter and fixed. On the next day, the bile-buct cannula in the back was cut and connected to a new cannula; after visual confirmation that bile was flowing, the animal was allowed to stabilize for about 1 hr. Test compounds were then administered via gavage, followed by administration of LDL labeled with [\(1\alpha,2\alpha\)(n)-\(3^3\)H])cholesterol (4.5 μCi/kg) via the ear vein. Blood samples were withdrawn from the carotid artery cannula at 5, 10, 20, 30, 60, 120, 240 and 480 min after administration of the labeled LDL. Bile samples were collected from 0 to 2, 2 to 4 and 4 to 8 hr after administration of the labeled LDL.

Radioactivity in the plasma was measured using a liquid scintillation counter. After drying the plasma samples (50 or 100 μl) under reduced pressure, 500 μl of distilled water and 15 ml of ACSII were added to the residue.

Neutral steroid and bile acid in bile were extracted by our modified procedure according to Imai et al. (23). One milliliter of bile was mixed with 5 ml of ethanol and heated at 100°C for about 5 min, cooled in an ice bath, and filtered (0.22 μm, Flow Laboratories). The filtrate was dried under nitrogen gas, and the residue was redissolved in 1.0 ml of distilled water. The solution was applied to an Extrelut® column (E. Merck, Darmstadt, Germany). After 10 min, 6 ml of diethylether/petroleum ether solution (1:1, v/v) was applied to the column twice to elute neutral steroids. The column was then washed three times with 6 ml of methanol to elute bile acid.

After drying under nitrogen gas, 15 ml of ACSII was added to the residue of neutral steroids, and radioactivity was measured. The bile acid eluate was dried under nitrogen gas, and hydrolyzed in 3 ml of 1.25 N NaOH for 10 hr at 120°C in an autoclave. The hydrolysate was acidified to pH 1 with 4 N HCl, and bile acid was extracted three times with 4 ml of ethyl acetate. The extract was dried under nitrogen gas and the residue was dissolved in 1.0 ml of methanol, and 15 ml of ACSII was added to an aliquot of 250 μl of the solution to measure radioactivity. Another aliquot of 120 μl was applied to thin layer chromatography (Silica Gel 60 Plate, E. Merck), and bile acids were separated with an isooctane / ethyl acetate / acetic acid system (10:10:2, v/v). Standard bile acids (cholic acid, hyodeoxycholic acid, ursodeoxycholic acid, chenodeoxycholic acid, deoxycholic acid, and lithocholic acid) were used for identification. After developing, a 10% molybosphosphoric acid ethanol solution was sprayed on the TLC plates. The area giving the same Rf value as the standard samples, the cholic acid position (cholic acid and deoxycholic acid), the chenodeoxycholic acid position (hyodeoxycholic acid, ursodeoxycholic acid, chenodeoxycholic acid, deoxycholic acid, and lithocholic acid), and the position containing other materials were scraped from the plate. Finally, 1 ml of methanol and 15 ml of ACSII were added to the scraped silica gel and the radioactivity was measured.
Effects on plasma lipid abnormality and atherosclerotic lesions in WHHL rabbits

Forty-five WHHL rabbits were weighed and the following plasma lipid contents were measured: TC, free cholesterol (FC), triglyceride (TG), HDL-C, lipid peroxide (LPO) and β-LP. The animals were then divided into three groups: 1: vehicle control (0.5%-traganach solution, n=15), 2: monatapril (30 mg/kg once daily, n=15) and 3: nitrendipine (2 mg/kg once daily, n=15). An additional control group of normal Japanese white rabbits of the same age receiving vehicle (0.5%-tragacanth solution, n=7) was also used.

Each rabbit was given 100 g of the standard diet (RC-4) daily, and the test drugs were administered once daily at 9:00 a.m. via gavage for 6 months. Blood samples were withdrawn from the ear artery before and every month after the start of study, and plasma lipid content was measured. Blood samples obtained at 1, 3 and 6 months (2 and 24 hr after each dosing) were also used for measurement of plasma monatapril concentration by the method of Kurono et al. (24). At the end of the 6-month treatment period, the rabbits were anesthetized with ketamine hydrochloride (approximately 10–15 mg, i.m.) and then exsanguinated via a carotid artery under thiobromyl sodium anesthesia (25 mg/kg, i.v.). The aorta (from the carotid arteries to the iliac arteries) was quickly isolated, and any adherent tissue was carefully cleaned on ice. The thoracic and abdominal aorta were bisected longitudinally; one half was used for measurement of aortic cholesterol content, and the other half was used for measurement of the sudanophilic area. Aortic cholesterol was extracted by the method of Folch et al. (25). For measurement of the sudanophilic area, aortic tissues were stained with Sudan-IV and photographed, and the photographs were analyzed by an image analyzer (SP1000; Olympus Co., Ltd., Tokyo); the sudanophilic area was calculated by the following formula:

\[ \text{Sudanophilic area} \% = \frac{\text{Sudan-stained areas}}{\text{Total area of intima}} \times 100 \]

Parts of the aorta (the aortic arch; proximal, intermediate, and distal parts of the thoracic aorta; and proximal and distal parts of the abdominal aorta) and the heart were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned (for the heart, 2-mm-thick transverse sections) and stained with hematoxylin and eosin.

Measurement of plasma lipid and lipoprotein content

Plasma TC content was measured using cholesterol oxidase and p-chlorophenol (26). The HDL-C and β-LP contents were measured by the same method used to measure TC content after precipitation with heparin and manganese (27) and after precipitation with heparin (28), respectively. For determination of bile acid content, the fraction containing cholesterol was removed by precipitation with dextran and magnesium, and then bile acid content in the supernatant was measured using 3α-hydroxy-steroid dehydrogenase and 3-oxo-5β-steroidΔ4-dehydrogenase (29). FC content was measured using cholesterol oxidase and phenol (30). TG content was measured using glycerol-3-phosphate oxidase and 3,5-dimethoxy-N-ethyl-N-(2'-hydroxy-3'-sulfopropyl)-sodium aniline (31). LPO content was measured by the method of Yagi (32).

Reagents

[1α,2α(n)-3H]Cholesterol (46 Ci/mmol), β-[4-14C]sitosterol (55.4 mCi/mmol) and ACSI were purchased from Amersham Japan, Ltd. (Tokyo). Cholic acid, deoxycholic acid, hyodeoxycholic acid, ursodeoxycholic acid, chenodeoxycholic acid and lithocholic acid were purchased from Nakalai Tesque, Inc. (Kyoto). Sudan-IV was purchased from Wako Pure Chemical Industries, Ltd. (Osaka).

Statistical analyses

Data are expressed as the mean±S.E.M. Data were analyzed by paired (Student’s t-test) and multiple-comparison (Dunnett’s test) tests. Differences at P<0.05 were considered to be statistically significant.

RESULTS

High cholesterol diet-fed rabbits

Plasma contents of lipid, lipoprotein and bile acid: Compared with values before treatment, plasma TC, β-LP and bile acid content were increased by the high cholesterol diet in the vehicle control group, but there was no change in HDL-C content. Monatapril significantly inhibited (P<0.05) the increases in TC and β-LP content, and it tended to inhibit (P<0.10) the increase in bile acid content after 9 weeks of administration. There was no difference in HDL-C content between the two groups (Fig. 1). These results were consistent with results obtained previously (11).

Cholesterol absorption in the gastrointestinal tract:
The weight of dried feces collected for 3 days was 53.5±8.5 g in the vehicle control group and 59.8±7.4 g in the monatapril group. The difference between the two groups was not significant.

Cholesterol absorption rate was 39.0±2.5% in the vehicle control group and 31.2±3.5% in the monatapril group. Monatapril tended to inhibit (P=0.056) cholesterol absorption in the gastrointestinal tract (Fig. 2).

Cholesterol catabolism in the liver: After intravenous administration of LDL labeled with [1α,2α(n)-3H]-cholesterol, plasma radioactivity decreased in
**Lipid-Lowering Mechanism of Monatepil**

![Graphs showing lipid and lipoprotein levels over weeks](image)

**Fig. 1.** Effects of monatepil on plasma lipid and lipoprotein contents in high cholesterol diet-fed rabbits. Values are each a mean and standard error. ○: vehicle control (1% cholesterol + 0.5%-tragacanth) group, n=13; ●: monatepil (1% cholesterol + monatepil, 30 mg/kg, once daily) group, n=11. *P<0.10, **P<0.05, ***P<0.01, compared with the vehicle control group.

![Bar graphs showing dried feces weight and cholesterol absorption](image)

**Fig. 2.** Effects of monatepil on cholesterol absorption in high cholesterol diet-fed rabbits. Values are each a mean and standard error. □: vehicle control (1% cholesterol + 0.5%-tragacanth) group, n=5; ■: monatepil (1% cholesterol + monatepil, 30 mg/kg, once daily) group, n=5. *P<0.10, compared with the vehicle control group.

The vehicle control group. Plasma radioactivity in the monatepil group was significantly lower (P<0.05) or tended to be lower (P<0.10) than that in the vehicle control group from 60 to 480 min after administration (Fig. 3). Total radioactivity in the bile acid fractions extracted from the bile collected between 0–2, 2–4 and 4–8 hr after the administration of labeled LDL in the monatepil group was 4.4, 3.6 and 2.4 times higher than that in the
vehicle control group at corresponding time intervals, respectively. All of these differences were statistically significant. Also, the radioactivity in the bile acid fraction between 0–8 hr after administration in the monatelip group was significantly higher than that in the vehicle control group (Fig. 4). Radioactivity in the neutral sterol fraction of the bile was not significantly different between the two groups, and bile volume and the composi-

Fig. 3. Effects of monatelip on elimination of LDL labeled with [1α,2α(n)-3H]cholesterol from the blood in high cholesterol diet-fed rabbits. Values are each a mean and standard error. ○: vehicle control (1% cholesterol + 0.5%-traganth) group, n=6; ●: monatelip (1% cholesterol + monatelip, 30 mg/kg, once daily) group, n=5. Plasma radioactivity is expressed as percent change from the value 10 min after intravenous administration of the labeled LDL. *P<0.10, *P<0.05, compared with the vehicle control group.

Fig. 4. Effects of monatelip on bile volume and biliary excretion of [3H]radioactivity derived from [1α,2α(n)-3H]cholesterol labeled LDL in high cholesterol diet-fed rabbits. Values are each a mean and standard error. A: ○, vehicle control group; ●, monatelip group. B: △, vehicle control group and ▲, monatelip group in bile acid fraction; □, vehicle control group and ■, monatelip group in neutral sterol fraction. C: △, vehicle control group; ■, monatelip group. *P<0.10, *P<0.05, compared with the vehicle control group.
Table 1. Effect of monatelip on bile acid composition in high cholesterol diet-fed rabbits

<table>
<thead>
<tr>
<th>Time (hr)</th>
<th>Group</th>
<th>Radioactivities of bile acid constituents (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Origin</td>
</tr>
<tr>
<td>0–8</td>
<td>Vehicle control (n=6)</td>
<td>23.2±7.0</td>
</tr>
<tr>
<td></td>
<td>Monatelip (n=4)</td>
<td>17.4±3.0</td>
</tr>
</tbody>
</table>

WHHL Rabbits

Plasma lipid and lipoprotein content: The pre-treatment values of plasma TC, FC, TG and LPO content in WHHL rabbits were higher than those in normal rabbits of the same age, whereas HDL-C content was lower in the former than in the latter. Throughout the period of administration of 0.5% tragacanth solution, plasma TC, FC, TG and β-LP content in WHHL rabbits remained high and HDL-C content remained low. LPO content markedly decreased 2 months after administration of the 0.5% tragacanth solution and remained low thereafter (Fig. 5). Monatelip and nitrendipine did not significantly alter plasma lipid and lipoprotein content (Fig. 5).

Plasma monatelip concentrations at 1, 3 and 6 months after drug administration were $9.1±2.2$, $8.9±0.9$ and $13.7±2.4$ ng/ml just before daily dosing, respectively, and $206±21.7$, $346±33.9$ and $277±29.7$ ng/ml at 2 hr after dosing, respectively. These plasma monatelip concentrations were 4–9 times higher compared with those in high cholesterol diet-fed monkeys (12).

Sudanophilic area and cholesterol content in the aorta: In normal rabbits, the sudanophilic areas in the thoracic and abdominal aortas were $1.1±1.0\%$ and $1.3±0.7\%$, respectively. In the vehicle control group of WHHL rabbits, the aortas were covered extensively with lipid

Fig. 5. Effects of monatelip on plasma lipid and lipoprotein contents in Watanabe heritable hyperlipidemis rabbits. Values are each mean and standard error. □: vehicle control (0.5%-tragacanth) group, n=15; ☆: monatelip (monatelip, 30 mg/kg, once daily) group, n=13–15; ●: nitrendipine (nitrendipine, 2 mg/kg, once daily) group, n=15; △: normal (Japanese white rabbit: 0.5%-tragacanth) group, n=7. *P<0.05, **P<0.01, compared with the vehicle control group.
Table 2. Effect of monatepil on the sudanophilic area in the aorta of Watanabe heritable hyperlipidemic rabbits

<table>
<thead>
<tr>
<th>Group</th>
<th>Thoracic aorta</th>
<th>Abdominal aorta</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle control</td>
<td>40.8±18.0</td>
<td>20.3±12.1</td>
</tr>
<tr>
<td>Monatepil</td>
<td>42.3±22.5</td>
<td>26.1±14.4</td>
</tr>
<tr>
<td>Nitrendipine</td>
<td>36.4±23.9</td>
<td>22.4±16.8</td>
</tr>
<tr>
<td>Normal (Japanese white)</td>
<td>1.1±1.0**</td>
<td>1.3±0.7***</td>
</tr>
</tbody>
</table>

Sudanophilic area (%) = Sudan IV-stained areas / Total area of intima × 100. **P<0.01, compared with the vehicle control group.

deposits, and their sudanophilic areas were 40.8±18.0% and 20.3±12.1%, respectively. In the monatepil group, sudanophilic areas in the thoracic and abdominal aortas were 42.3±22.5% and 26.1±14.4%, respectively. In the nitrendipine group, they were 36.4±23.9% and 22.4±16.8%, respectively. No significant difference was found in the size of sudanophilic area between the monatepil or nitrendipine group and the vehicle control group of WHHL rabbits (Table 2).

Cholesterol content in the thoracic and abdominal aortas in the vehicle control group of WHHL rabbits was approximately 17 to 19 times higher than that in the control group of normal rabbits. Neither monatepil nor nitrendipine significantly reduced the increased cholesterol content in the aortas of WHHL rabbits (Fig. 6).

**Histological examinations in atherosclerotic lesions:** In normal rabbits, no abnormal lesions of the aorta or coronary arteries were found. In the vehicle control group of WHHL rabbits, foam cells containing lipids were widespread in the aorta and coronary arteries. The lesions were most severe in the aortic arch and increasingly less severe toward the abdominal aorta. There were no differences in the degree or characteristics of lesions in the aorta and coronary arteries between the monatepil or nitrendipine groups and the vehicle control group of WHHL rabbits.

**DISCUSSION**

We have already reported that monatepil, having Ca²⁺ entry blocking activity and α₁-adrenoceptor blocking activity as well as an inhibitory activity on lipid peroxidation, improves plasma lipid metabolism and atherosclerotic lesions in high cholesterol diet-fed monkeys and rabbits (11, 12). It has also been reported that these effects could be attributed to the increased mRNA expression of hepatic LDL receptors (33). In the present study, in an attempt to elucidate the mechanism of the plasma lipid lowering effect of monatepil, we studied the effects of monatepil on cholesterol absorption in the gastrointestinal tract and cholesterol catabolism in the liver. Furthermore, the effects on plasma lipid metabolism and the characteristics of atherosclerotic lesions were investigated using WHHL rabbits, an animal model of hepatic LDL receptor deficiency (14–17).

Monatepil significantly accelerated the decay after intravenous injection of LDL labeled with [1α,2α(n)-³H]cholesterol in plasma. LDL levels in the blood greatly depend on uptake by hepatic LDL receptors (34). Therefore, this result suggests that monatepil enhances cholesterol uptake by increasing the activity or number of hepatic LDL receptors. Notake et al. reported that monatepil significantly increased mRNA expression of hepatic LDL receptors in high cholesterol diet-fed monkeys (33). In high cholesterol diet-fed monkeys and rabbits, hepatic LDL receptors are down-regulated (33,

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**Fig. 6.** Effects of monatepil on cholesterol content in thoracic and abdominal aorta in Watanabe heritable hyperlipidemic rabbits. Values are each a mean and standard error. ■: vehicle control (0.5%-tragananthi) group, n=15; □: monatepil (monatepil, 30 mg/kg, once daily) group, n=13; △: nitrendipine (nitrendipine, 2 mg/kg, once daily) group, n=14; ◻: normal (Japanese white rabbit: 0.5%-tragananthi) group, n=7. **P<0.01, compared with the vehicle control group.
35), and acceleration of the decay from plasma by monatepil is thought to be due to inhibition of the down-regulation, or enhancement of the up-regulation, of hepatic LDL receptors. Furthermore, monatepil did not improve plasma lipid metabolism in WHHL rabbits. This result suggests that hepatic LDL receptors are essential for the plasma lipid lowering effect of monatepil. To date, no Ca²⁺ channel antagonists have been reported to increase the number or activity of LDL receptors, but α₁-adrenoreceptor blocking blockers have been reported to increase the number of LDL receptors and to inhibit the synthesis of endogenous cholesterol (36, 37). Thus, the possible role of α₁-adrenoreceptor blocking activity in the lipid lowering mechanism of monatepil cannot be ruled out, although monatepil increased the binding of 125I-LDL in HepG2 cells in vitro, while prazosin, an α₁-adrenoreceptor blocker, did not (M. Hashimoto et al., unpublished data).

Monatepil increased biotransformation of plasma LDL to bile acid without affecting bile acid composition. Also, it has been reported that monatepil did not increase hepatic free cholesterol contents even though it decreased hepatic ester cholesterol contents in cholesterol diet-fed monkey (38). The rate of bile acid formation is determined by the balance among the rate of uptake of LDL (and free cholesterol) into the liver by LDL receptors, the rate of esterification of incorporated free cholesterol by ACAT, and the rate of conversion of free cholesterol to 7-α-hydroxycholesterol by 7-α-hydroxylase, which is a rate-limiting step in the formation of bile acids. It is speculated that by facilitating the uptake of LDL via hepatic LDL receptors, monatepil indirectly increases the inflow of free cholesterol into the bile acid-forming system and accelerates the catabolic excretion of cholesterol. However, it cannot be excluded that monatepil also acts directly on 7-α-hydroxylase and further studies will be required to clarify this point.

Monatepil tended to inhibit cholesterol absorption at the gastrointestinal tract in high cholesterol diet-fed rabbits. Exogenous (dietary) cholesterol is absorbed into the body by the following series of processes: cholesterol in the small intestine forms micelles with bile acid, which are then incorporated into the intestinal epithelial cells; within the cells, cholesterol in the micelles is esterified by acylcoenzyme A:cholesterol acyltransferase (ACAT), followed by incorporation into chylomicrons and secretion into the lymphatic ducts (39). It has been reported that monatepil tended to inhibit hepatic ACAT activity in monkeys (38). However, monatepil did not improve plasma lipid metabolism and reduce atherosclerotic lesions in WHHL rabbits. Therefore, the inhibition of intestinal cholesterol absorption by monatepil seems to have only a minor role in its plasma-cholesterol lowering effect in high cholesterol diet-fed rabbits in this study.

In conclusion, the present study revealed that monatepil lowered plasma lipids mainly through the following mechanism: 1) enhancement of clearance of plasma LDL, which may be mediated by up-reguration of hepatic LDL receptors, and 2) acceleration of conversion of free cholesterol to bile acids in the liver. The inhibitory tendency of monatepil in cholesterol absorption in the gastrointestinal tract may have some additional effect on its amelioration of lipid metabolism.

Acknowledgment
We thank Dr. T. Karasawa, Senior Director of Discovery Research Laboratories, Dainippon Pharmaceutical Co., Ltd., for helpful advice and encouragement, and Ms Jody Schaffran, Dainippon Pharmaceutical Co., Ltd., for her English revision.

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