NTE-122, an Acyl-CoA:Cholesterol Acyltransferase Inhibitor, Prevents the Progression of Atherogenesis in Cholesterol-Fed Rabbits

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ABSTRACT—The cholesterol-lowering and anti-atherosclerotic effects of NTE-122 (trans-1,4-bis[[1-cyclohexyl-3-(4-dimethylaminophenyl)ureido[methyl]cyclohexane), an acyl-CoA:cholesterol acyltransferase (ACAT) inhibitor, were evaluated in 1% cholesterol diet-fed rabbits. NTE-122 (1, 3 and 10 mg/kg per day) lowered the total cholesterol levels in both plasma and liver dose-dependently (by 99% and 94% at 10 mg/kg per day, respectively). In the aortic wall of the rabbits given NTE-122, the atherosclerotic lesion area in both aortic arch and thoracic aorta were dose-dependently reduced (by 100% at 10 mg/kg per day), and the total cholesterol content in aortic arch was also lowered significantly at more than 3 mg/kg per day. These results suggest that NTE-122 is capable of exhibiting anti-atherosclerotic effects.

Keywords: NTE-122, Acyl-CoA:cholesterol acyltransferase (ACAT) inhibitor, Atherosclerosis

Acyl-CoA:cholesterol acyltransferase (ACAT, EC 2.3.1.26) is a microsomal enzyme catalyzing the intracellular formation of cholesteryl esters from acyl-coenzyme A and free cholesterol in such tissues as intestine, liver and arterial wall (1). In the intestine, ACAT facilitates the absorption of exogenous cholesterol, which is incorporated into chylomicron (1). In the liver, ACAT is thought to play an important role in the assembly of very low-density lipoprotein (VLDL), which is secreted into the blood (1, 2). In the arterial wall, ACAT is thought to be involved in the progression of atherosclerosis since accumulation of cholesteryl esters in the macrophages and smooth muscle cells is an essential step in formation of atherosclerotic lesions (1, 3). Therefore, ACAT inhibitor may exhibit cholesterol-lowering and anti-atherosclerotic activities by blocking intestinal absorption of dietary cholesterol, inhibiting hepatic secretion of VLDL and preventing formation of foam cells in the arterial wall (1, 4).

We have previously reported that NTE-122, trans-1,4-bis[[1-cyclohexyl-3-(4-dimethylaminophenyl)ureido[methyl]cyclohexane, is a potent and selective inhibitor of ACAT; and it inhibited the microsomal ACAT activities in intestine, liver and aorta of cholesterol-fed rabbits with the IC$_{50}$ value of 7.6, 4.4 and 9.6 nM, respectively (5). Recently, two ACAT genes have been identified in mammals (1, 6). ACAT-1 is highly expressed in macrophages and macrophage-rich tissues, such as atherosclerotic lesions, and ACAT-2 is mainly expressed in liver and small intestine (1, 6). Therefore, NTE-122 would potently inhibit both ACAT-1 and ACAT-2 in the rabbit. Furthermore, NTE-122 suppressed the cholesterol absorption in rats and the lipid secretion from CaCo-2 cells, a human intestinal cell line (7), and exhibited strong cholesterol-lowering effects in cholesterol-fed rats and rabbits (5). Cholesterol-diet fed rabbits have frequently been used to make experimental atherosclerotic models due to hypercholesterolemia for evaluating anti-atherosclerotic effects of some drugs. It has been reported that ACAT inhibitors, such as E5324 (8) and HL-004 (9), prevented the progression of atherosclerosis in cholesterol-fed rabbits. In the present study, we investigated the effect of NTE-122 on the progression of atherosclerosis in cholesterol-fed rabbits.

NTE-122 was synthesized at Central Research Institute, Nissin Food Products Co., Ltd., Kusatsu. Male Japanese White rabbits (Kbl:JW; Kitayama Labes Co., Ltd., Nagano), 10-week-old, were individually housed in metal cages in a room with controlled temperature (23.5 ± 2°C), humidity (55 ± 10%) and light (8:00 – 20:00 h). This study was performed in accordance with the “Guiding Principles for the Care and Use of Laboratory Animals” approved by The Japanese Pharmacological Society. Rabbits were fed 100 g/day of a standard rabbit chow, RC-4 (Oriental Yeast Co., Ltd., Tokyo) or a high cholesterol diet (RC-4 contain-
ing 1% cholesterol) for 2 weeks. Then the rabbits fed a high cholesterol diet were divided into groups and fed a high cholesterol diet with or without NTE-122 (1, 3 and 10 mg/kg per day) for 10 weeks. Blood samples were obtained from the marginal ear vein at 9:30 – 10:30 before feeding. Plasma concentration of total cholesterol was determined by enzymatic methods using commercial kit. After final blood sampling, rabbits were anesthetized with pentobarbital (Nembutal® sodium solution; Abbott Labs., North Chicago, IL, USA), followed by exsanguinating from the carotid artery. Immediately after laparotomy, the aorta and liver were excised, and adventitial tissue grossly adherent to the aorta was removed. The aorta was opened longitudinally and the atherosclerotic lesioned (atheroma) areas in the aortic arch and thoracic aorta were measured by an image analyzer (SP500F; Olympus Optical Co., Ltd., Tokyo). Lesion index was expressed as percentage of the lesioned area. The lipids were extracted from the intimal and medial tissue of the aortic arch by the method of Bligh and Dyer (10). The lipids of the liver were extracted with isopropanol. The concentrations of total and free cholesterol were determined by enzymatic methods using commercial kits. Cholesteryl ester mass was calculated as the difference between total and free cholesterol.

The data represent the mean ± S.E.M. of 7 – 8 animals. Statistical significance was evaluated by Dunnett’s multiple comparison test, Student’s t-test or Aspin-Welch’s t-test, at P<0.05.

During the experiments, body weight and food consumption (100 g/day) were not different among all groups, and rabbits given NTE-122 did not shown diarrhea (data not shown), although it was recently reported that an ACAT inhibitor caused diarrhea (11). The changes in plasma total cholesterol levels and liver total cholesterol content are shown in Fig. 1. Since the rabbits in cholesterol-fed (control) and NTE-122-treated groups were fed a high cholesterol diet for 2 weeks before drug treatment, plasma total cholesterol levels had increased about 34-fold compared with the value of the normal group (cholesterol-fed group: 927 mg/dl, normal group: 27 mg/dl) before the initiation of drug treatment. In the cholesterol-fed group, plasma total cholesterol levels continued to increase during the treatment period, whereas in the NTE-122-treated groups, the plasma total cholesterol levels were reduced dose-dependently. NTE-122 at the doses of 1, 3 and 10 mg/kg per day lowered plasma total cholesterol levels by 55%, 94% and 99% at end of the experiment compared with the control, respectively (Fig. 1a). Liver total cholesterol content also showed significant increases by feeding of the cholesterol diet, followed by decreases by NTE-122. NTE-122 reduced total cholesterol content at 1, 3 and 10 mg/kg per day by 11%, 82% and 94%, respectively (Fig. 1b).

Figure 2 shows the percentage area of atherosclerotic lesion (atheroma) of the aortic arch and thoracic aorta. In the cholesterol-fed control group, 72.5% ± 6.5% of the aortic arch was covered with atherosclerotic plaque (Fig. 2a). The degree of atherosclerosis in the aortic arch was greater than that in the thoracic aorta (lesion index: 11.8 ± 1.8%,

![Plots](image_url)

**Fig. 1.** Effect of NTE-122 on plasma (a) and liver (b) cholesterol content in cholesterol diet-fed rabbits. Rabbits were fed a chow diet or a high cholesterol diet for 2 weeks. Then the rabbits fed a high cholesterol diet were divided into groups and fed a high cholesterol diet with or without NTE-122 for 10 weeks. Liver total cholesterol content was measured after 10-week treatment. Each value represents the mean ± S.E.M. (N = 7 – 8). **Significantly different from normal, P<0.01. * ***Significantly different from the cholesterol-fed control (control), P<0.05, P<0.01.
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Fig. 2a. Effect of NTE-122 on atherosclerotic lesions in aortic arch (a) and thoracic aorta (b) of cholesterol-fed rabbits. Percent area involved with atherosclerotic lesions was measured after 10-week treatment. Each value represents the mean ± S.E.M. (N = 7–8). **Significantly different from the cholesterol-fed control (control), P < 0.01.

Fig. 3. Effect of NTE-122 on the contents of total cholesterol (a), free cholesterol (b) and cholesteryl esters (c) in aortic arch of cholesterol-fed rabbits. The cholesterol contents in aortic arch were measured after 10-week treatment. Each value represents the mean ± S.E.M. (N = 7–8). *Significantly different from normal, P < 0.05, **Significantly different from the cholesterol-fed control (control), P < 0.01.
the lipid secretion from CaCo-2 cells (7). Furthermore, the action of NTE-122 relative to the control group in the liver and aorta in this experiment almost correlates with the plasma cholesterol levels. These results suggest that the anti-atherosclerotic effect of NTE-122 mainly depended on the cholesterol-lowering effect due to the inhibition of cholesterol absorption in the intestine.

However, we reported that in HepG2 cells, a human hepatic cell line, NTE-122 inhibited hepatic ACAT activity and consequently decreased the secretion of apolipoprotein B-containing lipoprotein (VLDL), including cholesteryl esters from the liver (13). We also found that NTE-122 prevented the accumulation of cholesteryl esters and stimulated the efflux of cholesterol from foam cells derived from human THP-1 macrophages in the presence of high-density lipoprotein, the cholesterol acceptor (14). Therefore, NTE-122 would be expected to exhibit a cholesterol-lowering and anti-atherosclerotic effect not only through inhibiting cholesterol absorption via the intestine but also through direct action in the liver and arterial wall. Further investigations are needed to clarify the direct effect in arterial walls in some other atherosclerotic models, such as Watanabe heritable hyperlipidemic (WHHL) rabbits that lack the low density lipoprotein receptor (15).

In conclusion, NTE-122 markedly prevented both the increase of plasma and liver cholesterol and the progression of atherosclerosis in cholesterol-fed rabbits. Therefore, we suggest that NTE-122 should be effective as an anti-atherosclerotic agent.

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