The Novel Compound NO-1886 Elevates Plasma High-Density Lipoprotein Cholesterol Levels in Hamsters and Rabbits by Increasing Lipoprotein Lipase Without Any Effect on Cholesteryl Ester Transfer Protein Activity

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Lipoprotein lipase (LPL) and cholesteryl ester transfer protein (CETP) are determinants of high-density lipoprotein (HDL) cholesterol concentrations in plasma. We have previously reported that NO-1886, by increasing LPL activity, causes elevation of HDL cholesterol levels in rats. In the present study, we studied the effect of NO-1886 on CETP activity in experimental animals. Since previous reports suggest that rats may lack CETP, we examined hamsters and rabbits, as well as rats. We found that NO-1886 increased LPL activity, resulting in elevation of plasma HDL cholesterol in all three animals. We confirmed that rats lack CETP and that both hamsters and rabbits have high CETP activity. NO-1886 had no effect on CETP activity in hamsters and rabbits. These results demonstrate that the compound NO-1886 elevates HDL cholesterol in experimental animals by selectively increasing LPL activity without any effect on CETP. Animals with low CETP and high LPL activities appear to be more sensitive to NO-1886 than those with high CETP and low LPL activities.

A NUMBER OF STUDIES have shown a significant inverse relationship between plasma high-density lipoprotein (HDL) cholesterol and coronary heart disease,[1] and HDL cholesterol is now known to be a strong protector against coronary artery sclerosis. Plasma HDL originates from three sources: the liver, small intestine, and lipoprotein lipase (LPL)-mediated lipolysis of chylomicrons and very–low-density lipoproteins.[2] Although production of HDL by the liver and small intestine may be important for the plasma HDL cholesterol level, other factors contribute to the determination of plasma HDL cholesterol. Among them, two major factors are LPL and cholesteryl ester transfer protein (CETP).[3,4]

We have shown previously that the novel compound NO-1886, which increases LPL activity, causes an elevation of HDL cholesterol in rats.[5,6] We are now interested in knowing whether NO-1886 has any effect on CETP activity, since an increasing number of reports have shown a marked elevation of plasma HDL cholesterol in CETP-deficient patients.[7,8] We have learned from the literature that rats may lack CETP,[9] and hence we have studied the effect of NO-1886 on CETP activity in other animals, as well as rats.

MATERIALS AND METHODS

Materials

NO-1886, diethyl 4-[(4-bromo-2-cyanophenyl)carbamoyl]benzylphosphonate, was synthesized at the New Drug Research Laboratory of Otsuka Pharmaceutical Factory (Tokushima, Japan). Glycerol tri[1-^{14}C]oleate (2.2 GBq/mmol) was obtained from Amersham International (Amersham, UK) and [1,2,6,7-^{3}H(*N*)]-cholesteryl oleate from DuPont-NEN (Wilmington, DE). Apolipoprotein A-I was obtained from Cosmo Bio (Tokyo, Japan). All other chemicals used were high-grade commercially available products.

Animal Experiments

Male Syrian hamsters weighing 140 to 160 g were obtained from Inoue Experimental Animal Center (Kumamoto, Japan). Male New Zealand white rabbits weighing 2.0 to 2.5 kg were obtained from Kitayama Labes (Nagano, Japan). Male SD rats weighing 200 to 230 g were obtained from Charles River Japan (Yokohama, Japan). Animals were maintained under a 12-hour light-dark cycle at a constant temperature of 23° ± 2°C and acclimatized for at least 2 weeks before the start of the experiment.

Effects of NO-1886 in Hamsters

The compound NO-1886 was suspended in 5% gum arabic and administered to hamsters via a gastric tube at a dose of 30 mg/kg body weight. Four hours later, blood samples were drawn from the vena cava into tubes containing EDTA 1 mg/mL and plasma was isolated using a refrigerated centrifuge. The animals were then injected with heparin (100 U/kg body weight) via the vena cava, and blood samples collected 5 minutes later were used to determine LPL activity. Whenever blood samples were collected, animals were anesthetized with sodium pentobarbital (50 mg/kg body weight).

Effects of NO-1886 in Rabbits

Single administration. In our preliminary experiments, NO-1886 at a dose of 30 mg/kg body weight did not have any effects on plasma lipids and LPL activity in rabbits. We therefore increased the dose to 70 mg/kg body weight in this animal. Plasma lipid concentrations, CETP activity, and postheparin plasma LPL activity were determined under the same conditions used in hamsters.

Repeated administration. The NO-1886 group was fed 35 g/kg body weight rabbit chow (RC-4; Oriental Yeast, Tokyo, Japan) containing 0.2% NO-1886, equivalent to 70 mg/kg body weight, once per day for 7 days. The control group was fed a placebo diet that did not contain NO-1886. Blood samples were collected 4 hours after the final dose from an artery of the ear for determination of lipids and CETP activity. The animals were then injected with heparin (100 U/kg body weight) via marginal ear veins, and blood samples collected 5 minutes later were used to determine LPL activity.

Effects of NO-1886 in Rats

NO-1886 suspended in 5% gum arabic was administered to rats via a gastric tube at a dose of 30 mg/kg body weight. Plasma lipid concentrations, CETP activity, and postheparin plasma LPL activity were determined under the same conditions used in hamsters.

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Analytical Methods

Plasma Lipids

Plasma total cholesterol, HDL cholesterol, and triglycerides were determined by conventional enzymatic methods. The cholesterol C-test Wako (Wako Pure Chemical Industries, Osaka, Japan) was used for cholesterol, the Nescote HDL-C kit N (heparin calcium precipitation; Nippon Shoji, Osaka, Japan) for HDL cholesterol, and the triglyceride G-test Wako (Wako Pure Chemical Industries) for triglycerides.

LPL Activity in Postheparin Plasma

LPL activity in postheparin plasma was measured by the method described previously using glycerol tri[1-^{14}C]oleate as substrate.[10] Assay of postheparin plasma was performed in the presence or absence of 1 mol/L NaCl to estimate both LPL and hepatic triglyceride lipase activity in hamsters and rabbits. Lipase activity in the presence of 1 mol/L NaCl represented hepatic triglyceride lipase activity. LPL activity was calculated by subtraction of the salt-resistant fraction (hepatic triglyceride lipase activity) from total lipase activity.[11] In rats, two lipase activities in plasma were measured separately using the antiserum prepared against hepatic triglyceride lipase.[10]

CETP Activity

CETP activity was measured by the method of Kato et al[12] with slight modification. A preparation of a discoidal bilayer particle, 240 μL ethanol containing 4.5 μmol egg phosphatidylcholine, 1.13 μmol cholesterol, and 134 nmol (4.4 μCi) [^{3}H]-cholesteryl oleate, was rapidly injected into 1.0 mL 39-mmol/L phosphate buffer containing 0.025% EDTA and 60 mmol/L NaCl (phosphate-buffered saline [PBS]) through a glass syringe with a 20-gauge needle. After mixing for 5 minutes under a stream of nitrogen gas, 380 μL 200-mmol/L sodium cholate and 3.0 mg human apolipoprotein A-I in PBS were added to the lipid mixture while stirring. After mixing for 2 minutes under a stream of nitrogen gas, the mixture was diluted to 5.0 mL with PBS. The solution was dialyzed at 4°C against PBS to remove ethanol.

Assay procedures. The assay mixture for CETP activity consisted of 60 μL discoidal bilayer particle as cholesteryl ester donor, 100 μL human low-density lipoprotein (LDL) as the acceptor, 60 μL 7-mmol/L 5,5′-dithiobis-2-nitrobenzoic acid, 80 μL PBS, and 5 μL sample plasma. The incubation was performed at 37°C for 30 minutes. After incubation, the assay tubes were immediately placed in an ice bath, and LDL present in the assay mixture was then precipitated by adding 30 μL 60-mmol/L magnesium chloride and 30 μL 0.1% dextran sulfate in PBS. After standing for another 20 minutes on ice, the assay mixture was centrifuged at 2,900 × *g* for 15 minutes, after which supernatant containing discoidal bilayer particles was carefully removed. An aliquot of 300 μL was counted for radioactivity. The LDL precipitate was dissolved in 120 μL 0.1N sodium hydroxide, and an aliquot (100 μL) was counted for radioactivity. The cholesteryl ester transfer activity was expressed as a percentage of the [^{3}H]-cholesteryl oleate transferred from discoidal bilayer particles to LDL.

Statistical Analysis

The results are expressed as the mean ± SD. Comparisons between two groups were analyzed for statistical significance by Student's *t* test or the Aspin-Welch *t* test.

RESULTS

Effects of NO-1886 in Hamsters

Plasma lipids. Hamsters treated with NO-1886 had 34.4% lower plasma triglyceride levels than control animals. Animals treated with NO-1886 had slightly but nonsignificantly higher plasma cholesterol levels. Plasma HDL cholesterol concentrations in NO-1886–treated hamsters were significantly higher by 29.7% than those in controls (Table 1).

Postheparin plasma LPL activity and plasma CETP activity. LPL activity in postheparin plasma was 28.2% higher in animals treated with NO-1886 than in controls. Hepatic triglyceride lipase activity did not differ significantly. NO-1886 showed no effect on CETP activity (Table 1).

Effects of NO-1886 in Rabbits

Single administration. Plasma lipids in rabbits treated with NO-1886 did not differ from those in control animals. The compound had no significant effects on both postheparin plasma LPL and CETP activities (Table 1).

Repeated administration. Rabbits treated with NO-1886 had 33.7% lower plasma triglyceride levels and 65.0% higher HDL cholesterol levels than control animals. Plasma cholesterol

Table 1. Effects of NO-1886 on Plasma Lipid Levels, Postheparin Plasma LPL Activity, and CETP Activity in Various Animals

Animal	No.	Plasma Lipids (mg/dL)			Postheparin Lipase Activity (μmol FFA/mL/min)	Plasma CETP Activity (%)
		Cholesterol	HDL Cholesterol	Triglycerides		
Hamsters, single administration (30 mg/kg body weight)						
Control	10	148 ± 24	74 ± 10	189 ± 11	0.696 ± 0.145	7.4 ± 2.0
NO-1886	10	162 ± 13	96 ± 4*	124 ± 23*	0.892 ± 0.110*	8.4 ± 1.8
Rabbits						
Single administration (70 mg/kg body weight)						
Control	7	63 ± 8	22 ± 4	82 ± 19	0.341 ± 0.071	ND
NO-1886	8	61 ± 9	21 ± 4	75 ± 8	0.378 ± 0.051	ND
Repeated administration (70 mg/kg body weight/d for 7 days)						
Control	7	60 ± 16	20 ± 4	80 ± 27	0.430 ± 0.050	24.1 ± 6.9
NO-1886	8	66 ± 14	33 ± 7*	53 ± 13†	0.547 ± 0.121‡	24.1 ± 6.6
Rats, single administration (30 mg/kg body weight)						
Control	8	68 ± 2	59 ± 6	140 ± 17	0.909 ± 0.065	<1.0
NO-1886	8	101 ± 16*	89 ± 15*	83 ± 16*	1.165 ± 0.073*	<1.0

NOTE. Data are expressed as the mean ± SD (n = 7 to 10).

Abbreviation: ND, not determined.

*$P < .001$, †$P < .01$, ‡$P < .05$: *v* controls.

levels did not differ between experimental and control animals. LPL activity in postheparin plasma was 27.2% higher in animals treated with NO-1886 than in controls. NO-1886 showed no effect on CETP activity (Table 1).

Effects of NO-1886 in Rats

Plasma lipids. Rats treated with NO-1886 had 40.7% lower plasma triglyceride levels than control animals. Animals treated with NO-1886 had 48.5% higher plasma cholesterol levels than control animals. Plasma HDL cholesterol concentrations in NO-1886–treated rats were 50.8% higher than in controls (Table 1). The HDL level of treated rats is indeed higher than the control level, but because their total cholesterol is also higher, the percentage of HDL is the same in both groups. The increase in plasma total cholesterol is obviously a result of the increase in HDL cholesterol in NO-1886–treated rats.

Postheparin plasma LPL activity and plasma CETP activity. LPL activity in postheparin plasma was 28.2% higher in animals treated with NO-1886 than in controls. Hepatic triglyceride lipase activity did not differ significantly. Plasma CETP activity was undetectable (<1.0%) in both NO-1886–treated and nontreated rats (Table 1).

DISCUSSION

The present study demonstrates that the novel compound NO-1886 causes an elevation of plasma HDL cholesterol by increasing LPL activity and has no effect on CETP activity in experimental animals. In our previous reports,[5,6] we have shown that NO-1886 elevated HDL cholesterol in rats. Since this compound has been shown to increase LPL activity, we concluded that the elevation was solely a result of increased production of HDL cholesterol through the enhanced LPL-mediated lipolysis of triglyceride-rich lipoproteins. However, recently, an increasing number of clinical studies have indicated an association of a marked elevation of HDL cholesterol in patients with CETP deficiency.[7,8] This raises the possibility that besides LPL stimulation, NO-1886 may have some effect on CETP activity. We have learned from the literature that rats may lack CETP activity.[9] If so, then two questions may arise: Does NO-1886 cause an elevation of HDL cholesterol in selective animals that are deficient in CETP activity? And does NO-1886 have no effect in animals that, like humans, have CETP? These questions are of particular importance, because if NO-1886 has HDL cholesterol–elevating action only in animals lacking CETP activity, we cannot anticipate a favorable effect of this compound in humans.

As in previous experiments with rats,[5,6] we observed in the present study that the compound NO-1886 has the same action in both rabbits and hamsters: it increases LPL activity, resulting in a reduction of plasma triglyceride with concomitant elevation of HDL cholesterol, without any effects on CETP activity.

CETP is a protein that transfers cholesteryl esters from HDL to other lipoproteins.[13] Inhibition of CETP activity retards the metabolism of HDL cholesterol, causing an increase in HDL cholesterol levels, as supported by the case of CETP deficiency.[7,8] Therefore, in CETP-deficient humans and animals, an increase in plasma cholesterol is accompanied by an increase in HDL cholesterol.[5,7,8] CETP activity varies greatly among species, being high in humans, rabbits, and hamsters but almost undetectable in dogs and rats.[9] In the present study, we have confirmed that CETP activity is very low in rats (<1%), high in rabbits (24.1% ± 6.9%), and intermediate in hamsters (7.4% ± 2.0%). Plasma CETP activity in humans (16.7% ± 3.7%) is between that in rabbits and hamsters (unpublished observation, March 1996). Our present study showed that NO-1886 had no effect on CETP activity in both rabbits and hamsters.

It is interesting to compare the magnitude of the NO-1886 effect in different species. A single administration of NO-1886 caused a marked elevation of HDL cholesterol and a reduction of plasma triglycerides in rats, which have low CETP and high postheparin plasma LPL activities, whereas NO-1886 even in a larger dosage did not cause an apparent increase of HDL cholesterol or a reduction of plasma triglycerides in rabbits, which have high CETP and low postheparin plasma LPL activities. The important observation is that repeated administration of the compound caused an apparent increase of LPL activity, resulting in a significant elevation of HDL cholesterol in rabbits.

In the current study, administration of NO-1886 caused a significant increase in plasma cholesterol in rats and a slight but nonsignificant increase in hamsters. The increase in cholesterol in these animals was primarily a reflection of an increase in HDL cholesterol, as shown in our previous report.[5] There were no changes in non-HDL cholesterol in both animals.

In summary, the present study showed that the novel compound NO-1886 elevated HDL cholesterol by selectively increasing LPL activity without any effect on CETP activity in all three animals examined, although sensitivity to the compound differed among species. Animals with low CETP and high LPL activities, ie, rats, appear to be more sensitive to NO-1886 than those with high CETP and low LPL activities, ie, rabbits.

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