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Review Article

Lipoprotein lipase activator: efficacy in lipid metabolism and related diseases

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CONTENTS

Abstract 53
Introduction 53
Background to lipoprotein lipase 54
Relationship between lipoprotein lipase and atherosclerosis 54
Effect of NO-1886 on lipoprotein lipase 55
Antihyperlipidemic effects 55
Normal animals 55
Streptozotocin (STZ)-induced diabetic rats 56
Antiatherosclerotic effects 56
Endothelium-dependent relaxation of rat aorta 56
Coronary arteries 57
Aorta 57
Anticachexic effects 58
Antiobesity effects 59
Effects on insulin resistance 60
Conclusions 60
References 60

Abstract

Lipoprotein lipase (LPL) is a rate-limiting enzyme that hydrolyzes circulating triglyceride (TG)-rich lipoproteins such as very low-density lipoproteins (VLDL) and chylomicrons. A decrease in LPL activity is associated with an increase in plasma TG and a decrease in plasma high-density lipoprotein cholesterol (HDL-C). The increase in plasma TG and decrease in plasma HDL-C are risk factors for cardiovascular disease (CVD). Tsutsumi *et al.* hypothesized that elevating LPL activity would cause a reduction in plasma TG and an increase in plasma HDL-C, resulting in protection against the development of atherosclerosis. To test this hypothesis, Otsuka synthesized the LPL activator NO-1886. The effects of NO-1886 in animals have been extensively studied. NO-1886 has been shown to increase LPL mRNA and LPL activity in adipose tissue, myocardium and skeletal muscle, resulting in an elevation of post-heparin plasma LPL activity and LPL mass in rats. NO-1886 has also been shown to decrease plasma TG concentration and to cause a concomitant rise in plasma HDL-C. Long-term administration of NO-1886 to rats and rabbits with experimental atherosclerosis inhibited the development of atherosclerotic lesions in coronary arteries and aorta. The results of multiple regression analysis in these studies suggested that the increase in plasma HDL-C and the decrease in plasma TG protected against atherosclerosis. These results show that the atherogenic lipid profile is changed to an antiatherogenic lipid profile by increasing LPL activity, resulting in protection against the development of atherosclerosis. Therefore, the LPL activator NO-1886 is potentially beneficial for the treatment of hypertriglyceridemia and hypo-HDL-cholesterolemia, and for protection against atherosclerosis. Furthermore, we hypothesized that elevation of LPL activity in adipose tissue would cause an improvement in cachexia, and elevation of LPL activity in skeletal muscle would lead to an improvement in obesity, because the LPL in adipose tissue is related to fat storage and LPL in skeletal muscle is related to free fatty acid (FFA) oxidation. From the many published studies, we confirmed that NO-1886 improved cachexia by elevating LPL activity in adipose tissue and improved obesity by elevating LPL activity in skeletal muscle. It is concluded that NO-1886, and possibly other LPL-activating agents, protect against atherosclerosis, as well as cachexia and obesity.

Introduction

HMG-CoA reductase inhibitors are widely used for treating hypercholesterolemia. However, few high-density lipoprotein cholesterol (HDL-C)-increasing agents or triglyceride (TG)-lowering agents exist. Lipoprotein lipase (LPL) is a rate-limiting enzyme that hydrolyzes circulating

TG-rich lipoproteins. A decrease in LPL activity is associated with an increase in plasma TG levels. Therefore, dysfunction of LPL contributes to hypertriglyceridemia (1, 2). Increasing evidence points to an association between TG and an increased risk of cardiovascular disease (CVD) (3). Hypertriglyceridemia *per se* is an independent risk factor, and this is especially true in certain groups of individuals, such as women, those with high plasma low-density lipoprotein cholesterol (LDL-C) (4, 5), and those with diabetes (6, 7). Dysfunction of LPL also causes decreased plasma HDL-C concentrations. Low plasma HDL-C concentrations are an important risk factor for CVD. Therefore, Tsutsumi *et al*. hypothesized that lowering plasma TG concentrations and elevating plasma HDL-C by activating LPL may prevent the development of atherosclerosis.

Furthermore, the enzyme LPL acts as a potential gatekeeper for partitioning lipoprotein-derived free fatty acids (FFA) between adipose tissue and skeletal muscle (8). Lipoprotein lipase in adipose tissue is involved in fat storage, and LPL in skeletal muscle is related to FFA oxidation. Therefore, it may be possible to create novel drugs for specific syndromes by stimulating tissue-specific elevation of LPL. For example, a drug that elevates LPL activity in adipose tissue may exert anticachexic effects, while a drug that elevates LPL activity in skeletal muscle may be useful as an antiobesity agent or for improving insulin resistance. To test this hypothesis, a group of scientists studied the LPL activator NO-1886 in animals.

Background to lipoprotein lipase

Lipoprotein lipase is a glycoprotein located on the luminal surface of capillary endothelial cells. The active enzyme is a noncovalent homodimer (9). The enzyme has an apparent monometric molecular mass of 60,000 daltons on SDS-PAGE. The human LPL gene is approximately 30 kb in length (10).

Lipoprotein lipase mRNA has been found in human adipose tissue, and also in muscle, adrenals, kidneys, intestine and neonatal, but not adult, liver. The mRNA for LPL in humans is highly homologous with that of mice, rats and cows (11, 12).

Lipoprotein lipase binds to heparin sulfate (13) on the surface of endothelial cells via the heparin-binding site, which allows the enzyme to be extended into the plasma (14). Following intravenous administration of heparin, LPL can be displaced from the endothelial surface into plasma (post-heparin plasma), where enzyme activity can be measured. The active enzyme bound to heparin sulfate on the capillary endothelium is predominantly in the dimeric form.

Triglycerides and monoglycerides are preferred substrates for LPL, which preferentially hydrolyzes 1- and 3-ester bonds in TGs, generating 2-monoglycerides, which are converted to 1-monoglycerides by isomerization for further hydrolysis (15).

A small portion of the core TG from chylomicrons and very-low-density lipoprotein (VLDL) can be transferred to HDL. More important contributors to HDL are the surface remnants of the TG-rich lipoproteins that occur as a result of hydrolysis of core TG. Nikkila *et al.* have noted a relationship between LPL activity and HDL-C, especially HDL2 cholesterol, in many clinical situations (16). Tsutsumi *et al.* (17) reported that plasma TG levels were inversely correlated with post-heparin plasma LPL activity, while HDL-C levels were positively correlated with the activity of the enzyme in rats.

Insulin increases LPL activity, rates of LPL synthesis and LPL mRNA levels in adipocytes (18). Since insulin does not stimulate LPL gene transcription (18), the increases in steady-state LPL mRNA levels must be due to changes in mRNA stability (post-transcriptional mechanism).

Insulin-deficient diabetes results in a reduced degradation of VLDL by the reduction of functional (endothelium-bound) LPL activity in myocardium and adipose tissue (19). Short-term administration of insulin *in vivo* restores the effects of LPL activity in adipose tissue, but not in myocardium (19).

Relationship between lipoprotein lipase and atherosclerosis

Whether LPL directly or indirectly promotes or protects against atherosclerosis remains controversial. Miesenbock *et al.* (20) reported that LPL +/– humans have atherogenic lipoproteins, especially in the postprandial state. Katzel *et al.* (21) found that older, normocholesterolemic, nondiabetic athletic individuals with silent myocardial ischemia have increased insulin resistance, increased post-heparin plasma hepatic triglyceride lipase (HTGL) activity and a reduced postprandial response of abdominal adipose tissue LPL activity to feeding. These conditions are associated with low HDL2-C levels and increased postprandial lipemia. The abnormalities in plasma HDL-C and postprandial TG metabolism may increase the risk for coronary artery disease in these subjects.

Reymer *et al.* (22) studied human LPL mutations. They showed that in approximately 1 in 20 males with proven atherosclerosis, an Asn291Ser mutation in the human LPL gene is associated with significantly reduced plasma HDL-C concentrations and results in a significant decrease in LPL catalytic activity. They showed the relationship between LPL activity and plasma HDL-C concentrations, and suggested that a specific LPL mutation may be a factor in the development of atherosclerosis.

Higher levels of post-heparin plasma LPL activity are associated with decreased plasma TG and increased HDL-C (17). People who are heterozygous for LPL deficiency have increased plasma TG and decreased plasma HDL-C concentrations, a profile associated with increased atherogenic risk (23). These reports suggest that increased post-heparin plasma LPL activity is associated with protection against atherosclerosis in humans.

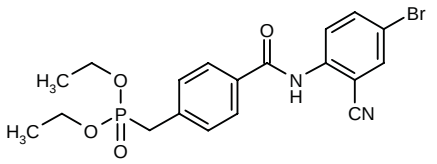
Fan *et al.* (24) generated transgenic rabbits expressing human LPL to elucidate the physiological roles of LPL in lipid and lipoprotein metabolism. When the transgenic rabbits were fed a cholesterol-rich diet, the development of hypercholesterolemia and aortic atherosclerosis was dramatically suppressed. Using another model, Shimada *et al.* (25) established an overexpressed human LPL gene in the heart, skeletal muscle and adipose tissue of mice. These transgenic mice had 5- and 1.7-fold higher LPL activity in adipose tissue and post-heparin plasma, respectively. Also, VLDL triglycerides were greatly reduced and HDL2-C was increased 1.4-fold. These results demonstrated that the lipid profile in these LPL transgenic mice is antiatherogenic.

Shimada *et al.* (26) also created LDL receptor knockout mice (LDLRKO) that overexpressd LPL (LPL/LDLRKO) by mating LPL transgenic mice to LDLRKO mice, and compared their plasma lipoprotein profiles and atherosclerosis with those in nonexpressing LDLRKO mice. LPL/LDLRKO mice showed marked suppression of mean plasma TG concentrations and a modest decrease in cholesterol concentrations compared to LDLRKO mice. Remnant lipoprotein was selectively reduced in LPL/LDLRKO mice. The atherosclerotic lesion area in the aorta of LDLRKO mice was 18-fold larger than in LPL/LDLRKO mice. Shimada *et al.* (26) showed that the altered lipoprotein profile, in particular the reduced level of remnant lipoproteins, is mainly responsible for protection by LPL against atherosclerosis.

These published papers show that activation of LPL protects against the development of atherosclerosis. To further test this hypothesis, an LPL activator, NO-1886 [**I**], was synthesized and evaluated at Otsuka.

Effect of NO-1886 on lipoprotein lipase

Single doses of NO-1886 were followed by significant dose-dependent increases in post-heparin plasma LPL activity in normal rats (Table I) (17). On the other hand, NO-1886 did not affect post-heparin plasma HTGL activity. NO-1886 also significantly and dose-dependently increased tissue LPL activity in normal rats (Table II). NO-1886 enhanced expression of LPL mRNA in adipose tissue and myocardium, and increased LPL protein mass and LPL activity in post-heparin plasma (17).



[**I**]

Table I: Post-heparin plasma lipase activity in normal rats after single administration of NO-1886.

			Lipase activity (μmol FFA/ml/min)	
	Dose	n	LPL	HTGL
Control		7	0.909 ± 0.065	0.455 ± 0.144
NO-1886	1 mg/kg	7	0.983 ± 0.083	0.460 ± 0.099
	3 mg/kg	7	1.934 ± 0.071*	0.492 ± 0.126
	10 mg/kg	7	1.107 ± 0.098**	0.520 ± 0.154
	30 mg/kg	7	1.165 ± 0.073**	0.519 ± 0.110

Data are expressed as means ± SD. *$p < 0.05$, **$p < 0.01$ compared with the corresponding values in control rats. FFA: free fatty acids.

Table II: Tissue LPL activity after 7-day administration of NO-1886 in normal rats.

			LPL activity (nmol FFA/g tissue/min)	
	Dose	n	Adipose tissue	Myocardium
Control		8	198 ± 74	555 ± 102
NO-1886	1 mg/kg	8	361 ± 262	563 ± 101
	3 mg/kg	8	384 ± 217	647 ± 53
	10 mg/kg	8	381 ± 140	784 ± 88*
	30 mg/kg	8	534 ± 240*	716 ± 73*

Data are expressed as means ± SD. *$p < 0.01$ compared with corresponding values in control rats. FFA: free fatty acids.

Antihyperlipidemic effects

Normal animals

Single doses of NO-1886 were followed by significant, dose-dependent decreases in plasma TG levels, with concomitant increases in plasma HDL-C in rats (Fig. 1) (17). NO-1886 administration for 7 days also significantly decreased plasma TG concentrations and increased HDL-C in hamsters and rabbits (27). NO-1886 resulted in increased plasma total cholesterol concentrations and concomitantly increased plasma HDL-C in rats, but this phenomenon was not observed in hamsters, rabbits or monkeys (Table III). NO-1886 caused a marked elevation of plasma HDL-C, especially HDL2-C. Previous reports have clearly demonstrated that enhanced lipolysis of TG-rich lipoproteins results in an increase in HDL2 particles, and therefore, a precursor–product relationship exists between the two (28). The transfer of cholesterol from newly formed HDL2 particles to VLDL is mediated by cholesteryl ester transfer protein (CETP) (29). However, rats, mice and dogs lack CETP (30). Because of this, the number of HDL particles following enhanced VLDL degradation by LPL was increased and accumulated in the circulation, resulting in a marked elevation of HDL-C. The increases in plasma total cholesterol are obviously a result of the increases in HDL2, as there was no change in cholesterol in the LDL fraction after

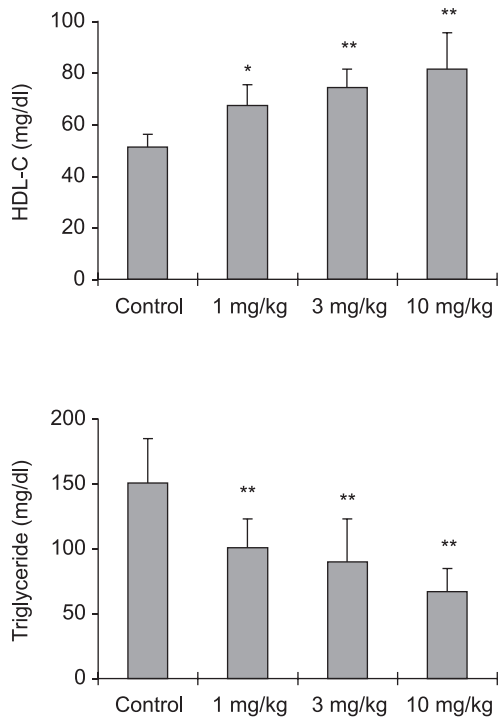


Fig. 1. Plasma HDL-C and triglyceride levels after single oral administration of NO-1886 in normal rats. *$p < 0.05$, **$p < 0.01$ compared with corresponding values in control rats.

Table III: Effects of NO-1886 on plasma lipids in various animals.

	Total cholesterol	HDL-C	Triglyceride	CETP
Rat	↑	↑	↓	–
Mouse	↑	↑	↓	–
Dog	↑	↑	↓	–
Hamster	↔	↑	↓	+
Rabbit	↔	↑	↓	+
Monkey	↔	↑	↓	+

↑: increased, ↓: decreased, ↔: unchanged, +: CETP is present, -: CETP is absent.

NO-1886 administration. Hamsters, rabbits and monkeys have CETP, and therefore plasma total cholesterol did not increase. These results indicate that NO-1886 may not increase plasma total cholesterol levels in humans because of the presence of CETP (31).

NO-1886 was shown to have potentially strong pharmacological efficacy in rats, dogs, rabbits, hamsters and monkeys, but weak efficacy in mice. NO-1886 is easily hydrolyzed by amidase, which is naturally present in high levels in the plasma of mice.

Streptozotocin (STZ)-induced diabetic rats

Diabetes is frequently associated with hypertriglyceridemia, and this is often combined with reduced plasma HDL-C (32). Therefore, diabetes is thought to be a risk factor for CVD. Tsutsumi *et al.* performed experiments in rats with STZ-induced diabetes to see whether NO-1886 corrected the hypertriglyceridemia associated with low plasma HDL-C in diabetes (19).

These animals showed a marked elevation in plasma TG concentrations and reduction in plasma HDL-C concentrations. Marked hypertriglyceridemia may be a consequence of either overproduction of VLDL by the liver, defective removal of TG-rich lipoproteins from the circulation, or both. The latter possibility can be explained by the fact that LPL, a key enzyme for TG removal, is an insulin-dependent enzyme (28). Tsutsumi *et al.* (19) confirmed that rats with STZ-induced diabetes had reduced post-heparin plasma LPL activity, and reduced LPL activity and LPL mRNA in adipose tissue, myocardium and skeletal muscle. Both single- and multiple-dose experiments showed that NO-1886 increased post-heparin plasma LPL activity in diabetic rats. Multiple doses increased the amount of LPL mRNA. Thus, the mode of action of NO-1886 is thought to be stimulation of tissue LPL synthesis.

NO-1886 and insulin should act on tissue LPL in different ways. Insulin preferentially increases adipose tissue LPL, while NO-1886 causes approximately a 3-fold increase of LPL in adipose tissue, myocardium and skeletal muscle. Furthermore, insulin increases post-heparin plasma HTGL activity, whereas NO-1886 has no effect on HTGL. Consequently, NO-1886 shows different effects from insulin on plasma lipids. An important difference is that NO-1886 treatment causes a marked elevation in plasma HDL-C, especially cholesterol in the HDL2 fraction. The reason for this is that NO-1886 does not affect HTGL. Low HTGL in diabetic rats persists even after NO-1886 treatment (19). HTGL is thought to mediate the catabolism of remnant lipoproteins by the liver (33). In addition, this enzyme appears to be involved in the conversion of HDL2 to HDL3 (28). Thus, low HTGL activity may result in the accumulation of circulating HDL2. These results indicate that NO-1886 increases LPL activity, leading to a reduction in plasma TG and a concomitant elevation in plasma HDL-C in STZ-induced diabetic rats (Table IV). Therefore, the LPL activator NO-1886 is suggested to be potentially beneficial for the treatment of hypertriglyceridemia associated with low plasma HDL-C, which is commonly associated with diabetes.

Antiatherosclerotic effects

Endothelium-dependent relaxation of rat aorta

Endothelial function is closely related to the development of atherosclerosis and is impaired before the development of initial lesions in hypercholesterolemic animals (34). Aging is associated with a progressive development of dyslipidemia, insulin resistance and obesity, all of which are risk factors for cardiovascular disease and atherosclerosis (35). It is known that endothelium-dependent

Table IV: Post-heparin plasma lipases and lipids of STZ-induced rats after administration of NO-1886.

			Lipase activity (μmol FFA/ml/min)		Lipids (mg/dl)	
Group	Dose	n	LPL	HTGL	HDL-C	TG
Normal rats		7	1.018 ± 0.152**	1.095 ± 0.178**	66 ± 10*	132 ± 19**
Diabetic rats		7				
Control			0.473 ± 0.168	0.328 ± 0.180	53 ± 8	752 ± 151
NO-1886	25 mg/kg	7	0.724 ± 0.043*	0.344 ± 0.065	120 ± 18**	368 ± 86**
Insulin	30 U/kg	7	1.248 ± 0.270**	0.651 ± 0.167*	69 ± 5*	182 ± 60**

Data are expressed as mean ± SD. *$p < 0.05$, **$p < 0.01$ compared with the corresponding values in diabetic control rats.

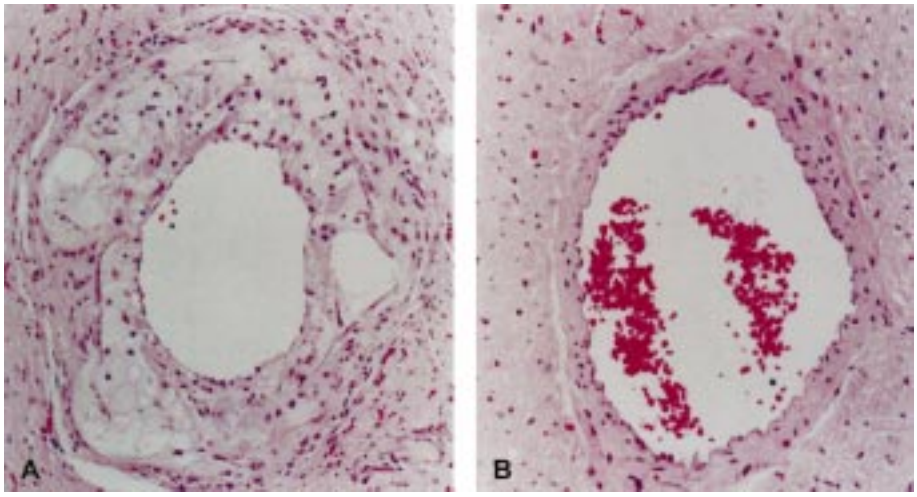


Fig. 2. NO-1886 was orally administered to cholesterol diet-fed male Wistar rats via a gastric tube for 90 days. A: Coronary artery section stained with hematoxylin and eosin, showing marked thickening of tunica intima with foam cell accumulation and fibrous proliferation from cholesterol diet-fed rats. Magnification, X175. B: Section of corresponding portion of a coronary artery from a chole sterol diet-fed, NO-1886 (30 mg/kg)-treated rat showing no atherosclerotic lesions. Magnification, X231.

relaxation decreases with age (36). Hara *et al.* (37) reported that NO-1886 ameliorated the aging-related deterioration of endothelium-dependent relaxation in thoracic aorta in 10-month-old male rats. Kusunoki *et al.* (38) also reported that NO-1886 prevented the development of impaired endothelium-dependent relaxation of rat thoracic aorta in 2-year-old male rats. These groups speculated that NO-1886 might have improved the endothelium-dependent relaxation by normalizing the lipid disorder, in particular by elevating plasma HDL-C, which possesses antioxidant effects (39) and is very important in exercised old rats due to elevated plasma lipid peroxide levels caused by exercise (38).

Coronary arteries

Long-term administration of NO-1886 to rats with experimental atherosclerosis caused by a high-cholesterol diet significantly inhibited the development of atherosclerotic lesions in the coronary arteries (17) (Fig. 2). The results of multiple regression analysis in the studies suggest that plasma HDL-C is a strong protective factor against atherosclerosis in coronary arteries.

Aorta

Chiba *et al.* (40) administered NO-1886 to cholesterol-fed New Zealand White rabbits for 20 weeks. NO-1886 increased post-heparin plasma LPL activity 30-40% compared with the control group. Plasma HDL-C concentrations were 2-fold greater in the NO-1886 group compared to in the controls, and plasma TG was reduced to the level of normal controls. Post-heparin plasma LPL activity was positively correlated with plasma HDL-C and inversely correlated with plasma TG. The relative atheromatous area in the aorta was reduced to 11-14% in the NO-1886 group compared to 51% in the control group (Fig. 3). Multiple regression analysis of post-heparin plasma LPL activity, plasma HDL-C and TG indicated that plasma HDL-C was the most powerful protector against aortic cholesterol accumulation. A decrease in plasma TG also protected against atherosclerosis, though not as strongly as plasma HDL-C. They concluded that NO-1886 prevented the development of atherosclerosis by increasing LPL activity, resulting in an increase in plasma HDL-C and a decrease in plasma TG, without a significant influence on plasma total cholesterol concentrations.

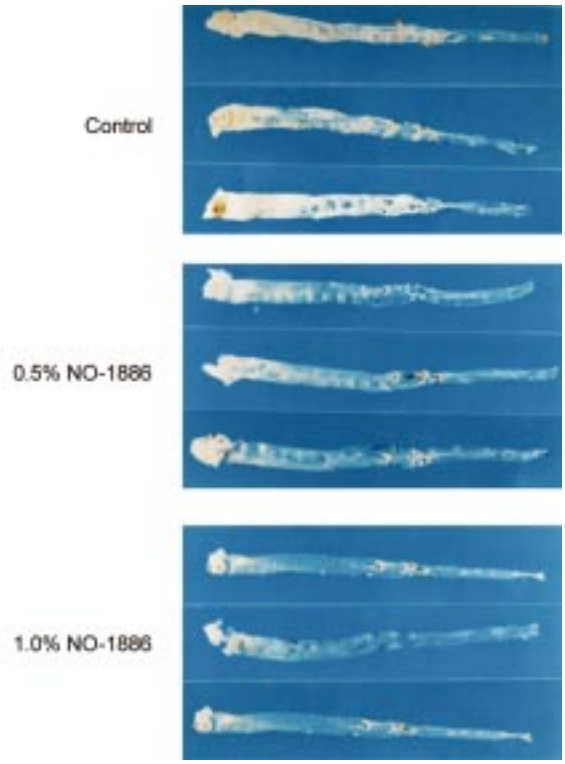


Fig. 3. A comparison of atheromatous plaque in aortae from control, 0.5% NO-1886 group and 1.0% NO-1886 group. For 20 weeks, 4 groups of male New Zealand White rabbits received regular rabbit chow (normal group), 0.25% cholesterol-containing chow (control group) and cholesterol chow supplemented with 0.5% and 1.0% NO-1886. At the end of the experimental period, the aorta was dissected from the heart, divided and rinsed with normal saline, and the periaortic tissue was removed. Relative atheromatous area (% of whole area) measured by a dot-counting method was 51% in the control group, and the area was reduced to 14% and 11% in the 0.5% and 1.0% NO-1886 groups, respectively.

Recently, Yin *et al.* (41) created a diabetic rabbit model with atherosclerosis in the aorta by feeding a high-fat/high-sucrose diet. They administered NO-1886 to these rabbits to determine whether the LPL activator had an antiatherogenic effect. NO-1886 decreased plasma cholesterol and TG, and increased plasma HDL-C. Interestingly, NO-1886 also lowered plasma glucose. NO-1886 provided protection against the development of atherosclerosis in the aorta. These results suggest that NO-1886 not only ameliorates the lipid disorder, but also lowers plasma glucose levels and suppresses atherosclerosis in the aorta of diabetic rabbits.

Anticachexic effects

Cachexia is defined as an extreme wasting condition with marked weight loss, anorexia and lassitude (42). It is observed in patients with cancer and severe infectious diseases, and is a terminal manifestation of these diseases. Cachexia is associated not only with the deterioration of quality of life, but also with shortened survival. However, the causes of the body weight loss, anorexia and wasting condition that occur during cachexia have not been adequately elucidated. Although it is important to improve cachexia, no appropriate method has been satisfactorily established, and the search for an effective treatment continues.

Adipose tissue atrophy is marked in cachectic patients and animals. Fat deposition is accomplished by the action of LPL in adipose tissue and by *de novo* lipogenesis in the liver and adipose tissue (43). Lipoprotein lipase activity in adipose tissue has been reported to be depressed in tumor-bearing animals (43-45). Vlassara *et al.* reported that LPL activity in cancer patients was lower than in healthy persons and that the degree of the decrease was closely correlated with the degree of weight loss when LPL activity was determined in the post-heparin plasma of these patients (46).

Research on cytokines and cachexia has advanced, and it has become clear that certain cytokines are involved in the onset of cachexia (47, 48). Tumor necrosis factor (TNF) (47, 48), IL-1 (49) and IL-6 (50), in particular, are thought to inhibit the activity of LPL, thereby suppressing hydrolysis of VLDL-TG, decreasing the supply of FFA to adipose tissue, and eventually inducing weight loss as a result of a reduction of fat accumulation in the tissues. These findings indicate that LPL activation in adipose tissue may improve cachexia.

Obeid *et al.* have reported that the Leydig cell tumor is a model that resembles human cachexia rather well, because the tumor induces slow progression of anorexia, as well as marked weight loss (51). Sabatini *et al.* have reported that Leydig cell tumors produce TNF and that TNF induces cachexia (52). Therefore, NO-1886 administered to Leydig cell tumor-bearing rats may have beneficial effects. When Leydig cells were inoculated into rats, there was an early decrease in plasma total protein and albumin levels after inoculation, followed by a decrease in plasma glucose and HDL-C, with the animals showing signs of malnutrition throughout. Food consumption decreased after tumor inoculation, and thereafter the rats rapidly grew leaner. Lipoprotein lipase activity in rat adipose tissue and adipose tissue weight were decreased by Leydig cell inoculation. NO-1886 increased the LPL activity in adipose tissue and the adipose tissue weight that had been decreased by Leydig cell inoculation. NO-1886 prevented the decrease in carcass weight and malnutrition resulting from the appetite suppression attributable to Leydig cell tumors (Figs. 4 and 5). From these results, the LPL activator NO-1886 is considered to be potentially beneficial for the treatment of cancer cachexia and other wasting syndromes.

Also, anticancer drugs have side effects such as appetite suppression and reduction of body weight. Therefore, combination therapy with anticancer drugs and an LPL activator may result in suppression of the side effects.

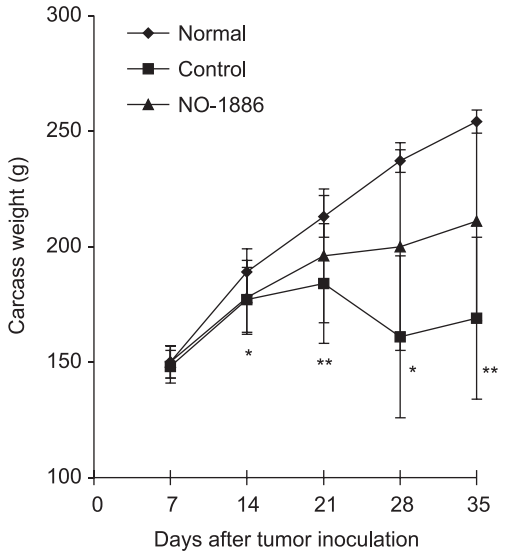


Fig. 4. Effects of NO-1886 on carcass weight in Leydig cell tumor-bearing rats. NO-1886 was administered to the rats at a daily dose of 100 mg/kg for 35 days. Carcass weight was measured as the difference between the weight of whole body and tumor. *$p < 0.05$, **$p < 0.01$ compared with corresponding values in normal rats.

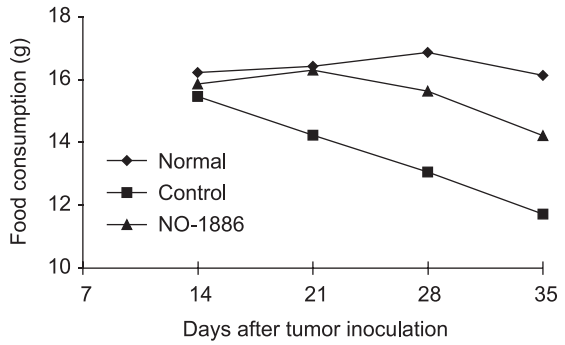


Fig. 5. Effects of administration of NO-1886 on food consumption in Leydig cell tumor-bearing rats. NO-1886 was administered to the rats at a daily dose of 100 mg/kg for 35 days.

Antiobesity effects

Jensen *et al.* have reported that overexpression of human LPL in skeletal muscle prevents diet-induced obesity in transgenic mice (53). According to Ferraro *et al.*, the respiratory quotient (RQ) is inversely correlated with LPL activity in skeletal muscle in Pima Indians, and Pima Indians have a high RQ, which is a risk factor for body weight gain (54). Hara *et al.* have also reported that long-term administration of NO-1886 causes a reduction in RQ in high-fructose-induced diabetic rats without fat accumulation in tissues (55). The RQ is the steady-state ratio of carbon dioxide production to oxygen consumption by whole-body tissue metabolism. Therefore, in general, a decrease in RQ means an increase in fatty oxidation. Based on this information, we hypothesized that an LPL activator may improve obesity by activating LPL in skeletal muscle.

NO-1886 was administered to rats rendered obese with a high-fat diet. NO-1886 suppressed the body weight gain and accumulation of visceral and subcutaneous fat (Figs. 6 and 7). NO-1886 also increased skeletal muscle LPL activity without affecting adipose tissue LPL activity, and lowered the RQ in rats fed a high-fat fed (56). These

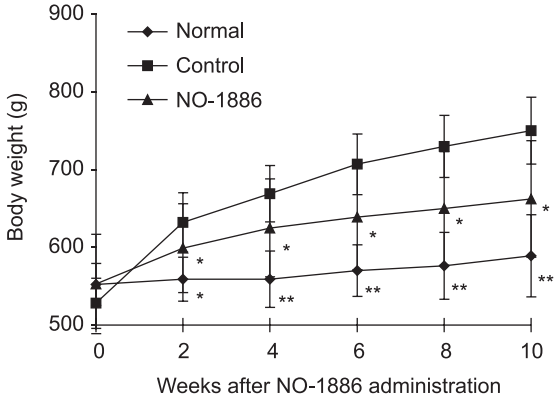


Fig. 6. Effects of chow or a high-fat diet with and without NO-1886 (50 mg/kg) for 10 weeks on body weight gain. *$p < 0.05$, **$p < 0.01$ compared with corresponding values in control rats.

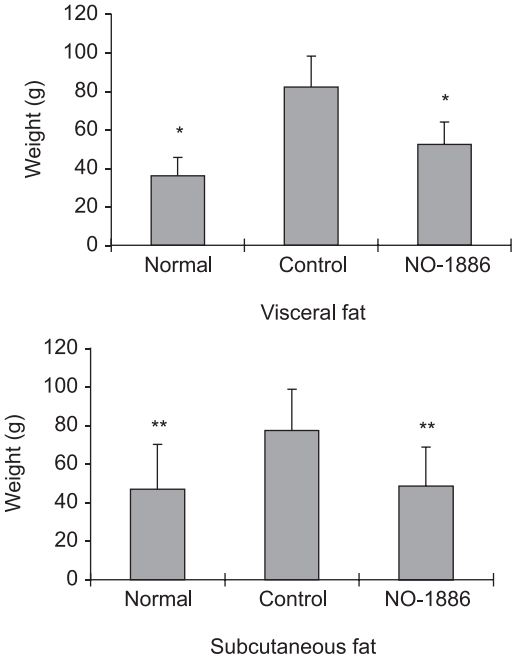


Fig. 7. Effects of chow or high-fat diet with and without NO-1886 (50 mg/kg) for 10 weeks on visceral fat and subcutaneous fat accumulation. *$p < 0.05$, **$p < 0.01$ compared with corresponding values in control rats.

combined effects might have led to the suppression in fat accumulation. The LPL activator NO-1886 is therefore considered to be potentially beneficial for the treatment of obesity.

Effects on insulin resistance

Kusunoki *et al.* reported that NO-1886 improved insulin resistance in rats with obesity due to a high-fat diet by improving the obesity (56).

Lipid metabolism and glucose metabolism share a close relationship. Therefore, improvement of the lipid disorder may improve the glucose disorder. Recently, Yin *et al.* hypothesized that the activation of LPL, a key enzyme in lipid metabolism, may improve glucose disorders. NO-1886 was administered to high-fat/high-sucrose-fed rabbits and pigs, which are insulin-resistant animal models (41, 57). NO-1886 inhibited fat accumulation and improved insulin resistance, resulting in reduced plasma TG levels, elevated HDL-C levels and reduced glucose levels. Furthermore, NO-1886 decreased plasma FFA and TNF-α levels, which cause insulin resistance in pigs. NO-1886 may therefore have potential in the treatment of insulin resistance.

Conclusions

It is known that lipid disorders, particularly plasma hypercholesterolemia, hypertriglyceridemia and hypo-HDL-cholesterolemia, are risk factors for cardiovascular disease. Many scientists have evaluated the utility of reducing plasma cholesterol levels for protecting against the development of atherosclerosis. As a result, HMG-CoA reductase inhibitors (statins) were invented. Today, statins are widely used for protection against atherosclerosis. However, few effective medicines are available for hypertriglyceridemia and hypo-HDL-C.

Tsutsumi *et al.* proposed that elevating LPL activity would cause a reduction in plasma TG and an increase in plasma HDL-C, resulting in protection against the development of atherosclerosis. To test this hypothesis, Otsuka researchers synthesized the LPL activator NO-1886. Using NO-1886, we studied whether this hypothesis was correct.

NO-1886 has been shown to increase LPL mRNA in adipose tissue, myocardium and skeletal muscle, resulting in an elevation of post-heparin plasma LPL activity and LPL mass in animals. Long-term administration of NO-1886 to rats and rabbits with experimental atherosclerosis inhibited the development of atherosclerotic lesions in coronary arteries and aorta. The results of multiple regression analysis in these studies suggest that the increase in plasma HDL-C and the decrease in plasma TG protect against atherosclerosis. These results show that LPL activation changes the atherogenic lipid profile to an antiatherogenic lipid profile by increasing LPL activity, resulting in protection against the development of atherosclerosis. Therefore, these results show that LPL-activating agents, such as NO-1886, are potentially beneficial for the treatment of hypertriglyceridemia and hypo-HDL-cholesterolemia, and for protecting against atherosclerosis. Further evaluation of LPL activators may demonstrate a clinically relevant benefit in the treatment of various lipid-associated diseases.

The main LPL synthetic tissues are adipose tissue and muscle. Lipoprotein lipase in adipose tissue has a role in fat storage, whereas LPL in skeletal muscle has a role in fatty oxidation. Therefore, if adipose tissue-specific LPL activators and skeletal muscle-specific LPL activators are developed, we may be able to design anti-cachexic and antiobesity drugs. NO-1886 improved cancer cachexia by elevating adipose tissue LPL activity, and it improved obesity by elevating skeletal muscle LPL activity. We expect further evaluation of tissue-specific LPL activators may also show a clinically relevant benefit in the treatment of lipid-associated and non-lipid-associated diseases.

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