Lipoprotein Lipase Activation Improves the Cachexia and Obesity

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Abstract

Cachexia is defined as an extreme wasting condition with marked weight loss. It is observed in patients with cancer and severe infectious disease. Obesity is one of the fastest growing major diseases in developed and developing countries. As has been persuasively argued, long-term imbalance between intake and expenditure of fat is a central factor in the etiology of obesity. We hypothesized that elevating lipoprotein lipase (LPL) activity would cause an improvement of cachexia and obesity. To test this hypothesis, we studied the effects of the LPL activator NO-1886 in cachexia and obese animals.

Keywords: Lipoprotein lipase; Insulin resistance; Lipid metabolism; Obesity; Weight loss

Introduction

Cachexia is defined as an extreme wasting condition with marked weight loss, anorexia, and lassitude [1]. About half of all cancer patients show a syndrome of cachexia characterized by loss of adipose tissue and skeletal muscle mass. Such patients have a decreased survival time compared with that of patients without weight loss [2]. Abnormal metabolism is thought to be the basis of the cachexia status in advanced cancer patients. Cachexia induces the cytokines, in particular tumor necrosis factor (TNF)-α, IL-1, and IL-6 [3-9], is thought to inhibit the activity of lipoprotein lipase (LPL), and thereby induces weight loss as a result of reduced fat accumulation in the tissues. Therefore, it is thought that emaciation of cancer patients might be prevented and cachexia improved by increasing LPL activity.

On the other hand, obesity in adulthood is characterized by adipocyte hypertrophy. Adipose tissue participates in the regulation of energy homeostasis. High-fat diet-induced insulin resistance associated with obesity is a major risk factor for diabetes and cardiovascular disease. Adipose tissue itself serves as the site of triglyceride (TG) storage and free fatty acid release in response to changing energy demands [10].

LPL plays a pivotal role in lipids and the metabolism of lipoprotein [11]. Major functions of LPL include the hydrolysis of TG-rich lipoproteins and release of non-esterified fatty acid (NEFA), which are taken up and used for metabolic energy in peripheral tissue such as muscle, or are re-esterified into TG and stored in adipose tissue. The balance between these competing effects could determine whether increased LPL activity will lead to a reduced rate of weight gain or to increased adiposity through increased rates of adipose tissue storage of TG. An imbalance of LPL activity may alter the partitions of plasma TG between muscle and adipose tissue, and thus influence insulin resistance and obesity.

Institute of Otsuka Pharmaceutical Factory, Inc. synthesized the LPL activator NO-1886 ([4-(4-bromo-2-cyano-phenylcarbamoyl)-benzyl]-phosphonic acid diethyl ester, CAS133208-93-2, generic name: ibrolipim).

Hara et al. reported that LPL activator NO-1886 treatment in high-fructose diet induced insulin resistance rats decreases the respiratory quotient (RQ) and plasma TG [12]. These results may indicate the elevation of LPL activity ameliorate obesity.

Therefore, we hypothesized that elevating LPL activity would cause an improvement of cachexia and obesity. To test this hypothesis, we studied the effects of the LPL activator NO-1886 in cachexia and obese animals.

Lipoprotein Lipase (LPL)

LPL is a glycoprotein located on the luminal surface of capillary endothelial cells. The active enzyme is a nonconvalent homodimer [13]. The enzyme has an apparent monomeric molecular mass of 60,000 daltons on SDS-PAGE. The human LPL gene is approximately 30 kb in length [14].

LPL mRNA has been found in human adipose tissue, and also in muscle, adrenal, kidneys, intestine and neonatal, but not adult liver. The mRNA for LPL in humans is highly homologous with that of mice, rats and cows [15,16].

LPL binds to heparin sulfate [17] on the surface of endothelial cells via the heparin-binding site, which allows the enzyme to be extended into the plasma [18]. 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.

TGs and monoglycerides are preferred substrates for LPL, which preferentially hydrolyzes 1- and 3- ester bounds in TGs, generating 2-monoglycerides, which are converted to 1-monoglycerides by isomerization for further hydrolysis [19]. A small portion of the core TG from chylomicron and very-low-density lipoprotein (VLDL) can be transferred to HDL. More important contributors to high density lipoprotein (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 HDL₄ cholesterol (HDL₄-C), in many clinical situations [20]. Tsutsumi et al. [21] 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 [22]. Since insulin does not stimulate LPL gene transcription [13], the increases in steady-state LPL mRNA 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, and short-term administration of insulin in vivo restores the effects of LPL activity in adipose tissue, but not in myocardium [23].

Whether LPL directly or indirectly promotes or protects against atherosclerosis remains controversial. Misenbock et al. [24] reported that LPL +/- humans have atherogenic lipoproteins, especially in the postprandial state. Katzel et al. [25] 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 reduced postprandial response of abdominal adipose tissue LPL activity to feeding. These conditions are associated with low HDL-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.

Reymert et al. [26] 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 decrease plasma TG and increased HDL-C [21]. People who are heterozygous for LPL deficiency have increased plasma TG and decreased plasma HDL-C concentrations, a profile associated with increased atherogenic risk [27]. These reports suggest that increased post-heparin plasma LPL activity is associated post-heparin plasma LPL activity is associated with protection against atherosclerosis in humans.

Fan et al. [28] 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. [29] established an over expressed 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 HDL, was increased 1.4-fold. These results demonstrated that the lipid profile in these LPL transgenic mice is antiatherogenic.

Shimada et al. [30] also created LDL receptor knockout mice (LDLRKO) that overexpressed 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. Thus, it was shown that the altered lipoprotein profile, in particular the reduced level of remnant lipoproteins exerts protection by LPL against atherosclerosis.

Regarding the LPL activator NO-1886, NO-1886 were significantly dose-dependent increases in post-heparin plasma LPL activity in normal rats [21]. On the other hand, NO-1886 did not affect post-heparin plasma HTGL. NO-1886 also significantly and dose-dependently increased tissue LPL activity in normal rats. NO-1886 enhanced expression of LPL mRNA in adipose tissue and myocardium, and increased LPL protein mass and LPL activity in post-heparin plasma [21].

NO-1886 was significantly dose-dependent decrease in plasma TG levels, with concomitant increase in plasma HDL-C in rats, hamsters and rabbits [31]. Endothelial function is closely related to the development of atherosclerosis and is impaired before the development of initial lesions in hypercholesterolemic animals [32]. Aging is associated with a progressive development of dyslipidemia, insulin resistance and obesity, all of which are risk factors for cardiovascular disease and atherosclerosis [33]. It is known that endothelium-dependent relaxation decreases with age [34]. Previously, we reported that NO-1886 ameliorated the aging-related deterioration of endothelium-dependent relaxation in thoracic aorta in 10-month-old male rats [35]. We also reported that NO-1886 prevented the development of impaired endothelium-dependent relaxation of rat thoracic aorta in 2-year-old male rats [36]. 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 [37] and is very important in exercised older rats due to elevated plasma lipid peroxide levels caused by exercise [36]. Long-term administration of NO-1886 to rats with experimental atherosclerosis caused by a high-cholesterol diet significantly inhibited the development of atheromatous lesions in the coronary arteries [21]. The results of multiple regression analysis in the studies suggest that plasma HDL-C is a strong protective factor against atherosclerosis in coronary arteries. Chiba et al. [38] administered NO-1886 to cholesteral-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 atherosomatous area in the aorta was reduced to 11-14% in the NO-1886 group compared to 51% in the control group. 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 of plasma total cholesterol concentrations.

Yin et al. [39] 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 provided protection against the development of atherosclerosis in the aorta. These results suggest that NO-1886 not only ameliorates the lipid disorder but also lower plasma glucose levels and suppresses atherosclerosis in the aorta of diabetic rabbits.

These published papers show that activation of LPL protects against the development of atherosclerosis.

Cachexia

Cachexia is defined as an extreme wasting condition with marked weight loss, anorexia, and lassitude [1]. It is observed in patients with cancer and severe infectious disease, and is a terminal manifestation of these diseases. About half of all cancer patients show a syndrome of cachexia characterized by loss of adipose tissue and skeletal muscle mass. Such patients have a decreased survival time compared with that of patients without weight loss [2]. 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 [40]. LPL activity in adipose tissue has been reported to be depressed in tumor-bearing animals [40–42]. 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 [43].

Research on cytokines and cachexia has advanced, and it has become clear that certain cytokines are involved in the onset of cachexia [3,44]. TNF [3,44], IL-1 [45] and IL-6 [46], in particular, are thought to inhibit the activity of LPL, thereby suppressing hydrolysis of VLDL-TG, decreasing the supply of NEFA to adipose tissue, and eventually inducing weight loss as a result of a reduction of fat accumulation in the tissue. LPL activity is reported to decrease as the tumor burden increases in tumor-bearing animals and patients with lung cancer; thus LPL is suggested to be the mechanism inducing the decrease in fat depots in cancer patients [41,42,47].

Cachexia patients and cancer-bearing animals show decrease in LPL activity in postheparin plasma and adipose tissue, and a number of reports have indicated that the decrease in LPL activity is presumably attributable to the emaciation in cachexia [40–43]. This suggests that cachexia can be prevented and cachexia improved by suppressing the decrease in LPL activity. Ohara et al. [48] therefore administered an LPL activator NO-1886, to a rat model of cachexia to investigate its effects [48].

Obeid et al. have reported that the Leydig cell tumor is a model that resembles human cachexia rather well, because the tumor induced slow progression of anorexia, as well as marked weight loss [49]. Sabatini et al. [50] have reported that Leydig cell tumors produce TNF and that TNF induces cachexia. Therefore, LPL activator 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. LPL activity in rat adipose tissue and adipose tissue weight were 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. From these results, the LPL activator is considered to be potentially beneficial for the treatment of cancer cachexia and other wasting syndromes.

Also, anticancer drugs have side effect 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.

Obesity

Obesity in adulthood is characterized by adipocyte hypertrophy. Adipose tissue participates in the regulation of energy homeostasis. High-fat diet-induced insulin resistance associated with obesity is a major risk factor for diabetes and cardiovascular disease [10]. Adipose tissue itself serves as the site of triglyceride (TG) storage and free fatty acid release in response to changing energy demands [10]. Adipose tissue also participates in the regulation of energy homeostasis as an important endocrine organ that secretes a number of biologically active adipokines such as adipin [51], leptin [52], plasminogen activator inhibitor-1 [53], resistin [54], TNF-α [55], and adiponectin [56]. LPL is one such adipokine.

LPL plays a pivotal role in lipids and the metabolism of lipoprotein [11]. Major functions of LPL include the hydrolysis of TG-rich lipoproteins and release of NEFA, which are taken up and used for metabolic energy in peripheral tissue such as muscle, or are re-esterified into TG and stored in adipose tissue. The balance between these competing effects could determine whether increased LPL activity will lead to a reduced rate of weight gain or to increased adiposity through increased rates of adipose tissue storage of TG.

Some reports have suggested that LPL activity in adipose tissue is high in obesity [57,58]. An imbalance of LPL activity may alter the partitions of plasma TG between muscle and adipose tissue, and thus influence insulin resistance and obesity.

Shimada et al. [59] have reported that none of the mice in which human LPL gene expression was induced became obese, and that storage and decomposition of fat were balanced in mice as a result of increased activity of hormone-sensitive lipase in adipose tissue. In other words, because of homeostasis body weight may not be increased in normal animals even by elevation of LPL activity.

Jensen et al. [60] have reported that overexpression of human LPL in skeletal muscle prevents diet-induced obesity in transgenic mice. Accordingly to Ferraro et al. [61] the 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.

Hara et al. [12] have also reported that long term administration of LPL activator NO-1886 causes a reduction in RQ in high-fructose-induced diabetic rats without fat accumulation in tissues. The RQ is the steady-state ration 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. NO-1886 also increased skeletal muscle LPL activity without affecting adipose tissue LPL activity, and lowered the RQ in obese rats [62].

It has long been known that uncoupling proteins (UCPs) are responsible for facultative thermogenesis in rodents. UCPs play an important role in energy metabolism and obesity [63]. UCP1 expression is restricted to brown adipose tissue (BAT), UCP2 is widely...
expressed, and UCP3 is found mainly in skeletal muscle [64]. Doi et al. [65] have reported that NO-1886 accelerates the expression of fatty acid oxidation-related enzymes, resulting in a reduction of RQ. However, the mechanism for antihyperglycemic effects of NO-1886 remained unclear. To clarify the mechanism, we studied the effects of NO-1886 on the expression of UCP1, UCP2, and UCP3 in rats [66]. NO-1886 did not affect the expression of UCP1 and UCP2 in BAT, mesenteric adipose tissue, and skeletal muscle, but NO-1886 increased the expression of UCP3 mRNA only in skeletal muscle. Therefore, a possible mechanism for NO-1886’s antihyperglycemic effects in rats may be the enhancement of LPL activity in skeletal muscle and the accompanying increase in UCP3 expression.

The antidiabetic agent pioglitazone is thought to promote the differentiation of adipocytes, convert large-type hypertrophic adipocytes into small-type adipocytes, and to increase insulin activity through peroxisome proliferator-activated receptor-gamma activation [67]. In addition, thiazolidinediones have also been shown to improve the serum levels of several adipokines, such as adiponectin and TNF-α, in type 2 diabetic patients [68]. However, as a result of enhanced adipocyte differentiation, pioglitazone treatment has been shown to be associated with body weight gain in obese animals and type 2 diabetic patients [68-70]. As the mechanism underlying the body weight gain, Hallakou et al. [71] explained that pioglitazone stimulated the expression of genes involved in lipid metabolism and induced a large increase in glucose utilization in the adipose tissue. Obesity aggravates diabetes and promotes cardiovascular diseases and atherosclerosis, and the body-weight-increasing action of pioglitazone is a disadvantage in diabetic patients. On the other hand, the LPL activator is known to improve both obesity and insulin resistance in obese animals [62,66].

Recently, we investigated the effect of the simultaneous administration of pioglitazone, which induces a body weight gain, and NO-1886, which has an anti-obesity action, on the body weight and insulin resistance of obese rats [72]. The concomitant administration of pioglitazone and NO-1886 suppressed the body weight gain in animals fed a high-fat diet, confirming that NO-1886 mitigates the body weight-increasing action of pioglitazone. Since an assessment of their effects on insulin resistance showed that both pioglitazone and NO-1886 treated fed a high-fat diet, confirming that NO-1886 mitigates the body weight-increasing action of pioglitazone and NO-1886, compared with that observed following the administration of either drug alone. Thus, the combined administration of pioglitazone and LPL activator may be of great benefit for the treatment of type 2 diabetic patients.

Profile of LPL activator NO-1886 (ibrolipim) is shown in Table 1.

Conclusions
The main LPL synthetic tissues are adipose tissue and muscle. LPL in adipose tissue has a role in fat storage, where 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 antitumoral and antiobesity drugs. LPL activator 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.

Table 1: Profile of LPL activator NO-1886 (ibrolipim) in animals.

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<tr>
<th>LPL activity</th>
<th>Post-heparin plasma</th>
<th>Activation</th>
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<td>Adipose tissue</td>
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<td>Myocardium</td>
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| LPL mRNA | Enhancing |
| LPL mass | Increasing |
| Plasma lipid | HDL cholesterol | Increasing |
| | Triglyceride | Reduction |
| | Total cholesterol | No effects of elevation |
| | Free fatty acid | No effects of reduction |

| Atherosclerosis | Coronary artery protection |
| Fatty liver in diabetes | Lipids contents improvement |
| Obesity | Body weight reduction |
| | Respiratory quotient reduction |
| | Insulin resistance improvement |
| Uncoupling protein (UCP) | UCP1 mRNA No effects |
| | UCP2 mRNA No effects |
| | UCP3 mRNA Increasing |
| Cachexia | Body weight Increasing |
| | Food consumption Increasing |

References


