Lipoprotein Lipase Activator NO-1886 Improves Fatty Liver Caused by High-Fat Feeding in Streptozotocin-Induced Diabetic Rats

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NO-1886 is a lipoprotein lipase (LPL) activator. Administration of NO-1886 results in an increase in plasma high-density lipoprotein cholesterol (HDL-C) and a decrease in plasma triglyceride (TG) levels. The aim of this study was to ascertain whether NO-1886 improves fatty liver caused by high-fat feeding in streptozotocin (STZ)-induced diabetic rats. Administration of NO-1886 resulted in increased plasma HDL-C levels and decreased TG levels without affecting total cholesterol and glucose levels in the diabetic rats. NO-1886 dose-dependently decreased liver TG contents and cholesterol contents, resulting in improvement of fatty liver. NO-1886 also reduced plasma aspartate aminotransferase (AST) and alanine aminotransferase (ALT) that accompany fatty liver. The liver cholesterol contents were inversely correlated with plasma HDL-C levels ($r = -0.5862$, $P < .001$) and were positively correlated with plasma TG levels ($r = 0.4083$, $P < .003$). The liver TG contents were inversely correlated with plasma HDL-C levels ($r = -0.6195$, $P < .001$) and were positively correlated with plasma TG levels ($r = 0.5837$, $P < .001$). There was no correlation between plasma cholesterol levels, and cholesterol and TG contents in liver. These results indicate that reducing plasma TG levels and elevating in HDL-C levels may result in improving fatty liver.

WE HAVE BEEN interested in the relationship between plasma lipids and fatty liver. However, no animal model existed for us to study this relationship. Fatty liver may result from raised levels of plasma free fatty acids (FFAs). The quantity of triglyceride (TG) present in the liver is significantly increased during starvation and after consuming of a high-fat diet. This may be due to low insulin levels. In uncontrolled diabetes mellitus, pregnancy toxemia in ewes, and ketosis in cattle, fatty infiltration is sufficiently severe to cause visible pallor (fatty appearance) and enlargement of the liver with possible liver dysfunction.[1] In this study, we produced fatty liver by feeding a high-fat diet for 32 weeks to streptozotocin (STZ)-induced diabetic rats. We have previously reported that STZ-induced diabetic rats fed a high-fat diet caused diabetic cataracts after a 10-week feeding period[2]; however, we were unable to visibly confirm the presence of fatty liver. We successfully created fatty liver by long-term high-fat feeding.

NO-1886 administration results in increased LPL activity, with resulting elevated plasma high density lipoprotein cholesterol (HDL-C) and decreased TG levels in animals.[3-6] The aim of this study was to ascertain whether the LPL activator NO-1886 improved fatty liver and to ascertain the relationship between plasma lipids and fatty liver in STZ-induced diabetic rats fed a high-fat diet.

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MATERIALS AND METHODS

Materials

NO-1886, 4-diethoxyphosphorylmethyl-N-(4-bromo-2-cyanophenyl) benzamide, was synthesized in the New Drug Research Laboratory of Otsuka Pharmaceutical Factory, Inc, Naruto, Tokushima, Japan. STZ was obtained from Sigma (St Louis, MO). All other chemicals used were high-grade, commercially available products.

Animal Experiments

Male Wistar rats weighing 180 to 200 g at the age of 6 to 7 weeks were obtained from Japan SLC, Inc, Shizuoka, Japan. The animals were maintained under a 12-hour light-dark cycle (light cycle from 7 AM to 7 PM) at a constant temperature of 23 ± 2°C.

Rats were initially allocated to 2 groups: a diabetic group comprising 40 animals and a normal group comprising 10 animals.

STZ, freshly dissolved in 0.01 mol/L citrate buffer, pH 4.5, was administered to 40 rats at a dose of 85 mg/kg body weight via the tail vein.[7] Seven days later, blood was drawn from the tail vein, and plasma glucose concentrations were measured. STZ-treated rats were subdivided into 4 groups (3 groups treated with NO-1886 and a control group) of 10 each stratified by baseline plasma glucose levels.

Normal group rats were fed standard laboratory chow (CRF-1, Oriental Yeast Co, Tokyo, Japan). STZ-treated rats were fed high-fat chow (0.25% cholesterol, 0.4% cholic acid sodium salt, and 2.5% olive oil in standard laboratory chow CRF-1, Oriental Yeast Co). The animals were given free access to food and tap water.

NO-1886 was suspended in 5% gum arabic and administered once daily to STZ-induced diabetic rats fed a high-fat diet at a dose of 30 mg/kg, 100 mg/kg, or 300 mg/kg via a gastric tube for 32 weeks. Control rats received 5% gum arabic only.

At the end of the experimental period, blood samples were collected from the posterior vena cava after a 12-hour overnight fast for determination of plasma lipids and glucose at 9 AM. The liver samples were maintained in liquid nitrogen for measurement of lipids.

All animal experiments were approved by the local animal ethics committee of Aichi Medical University.

Analytical Methods

Plasma lipids and glucose. Plasma total cholesterol, HDL-C, TG, and glucose were determined by conventional enzymatic methods. The cholesterol C-test Wako (Wako Pure Chemical Industries, Osaka, Ja-

Table 1. Body and Liver Weights in STZ-Induced Diabetic Rats After NO-1886 Administration

	n	Body Weight (g)	Liver Weight (g)
Normal	10	546 ± 15*	19.7 ± 2.5*
STZ control	10	329 ± 36	30.1 ± 4.7
NO-1886			
30 mg/kg	10	330 ± 44	29.9 ± 3.9
100 mg/kg	10	329 ± 21	27.7 ± 6.9
300 mg/kg	10	329 ± 29	26.1 ± 3.7

NOTE. Data are expressed as means ± SD.

Significantly different from the values in respective control rats: *$P < .01$.

pan) was used for cholesterol, the Nescote HDL-C kit N (Nippon Shoji, Osaka Japan) for HDL-C, the triglyceride G-test Wako (Wako Pure Chemical Industries) for TG, and the glucose CII test Wako for glucose.

Tissue lipids. Tissue samples of approximately 0.2 g, together with 2 mL of chloroform-methanol (2:1) solution,[8] were added into Pyrex centrifuge tubes and homogenized by Polytron (PCU-2-110, Kinematica, Luzern, Switzerland). The tubes were then centrifuged at 3,000 rpm. An aliquot of chloroform-methanol extract was transferred to another Pyrex tube and dried under a stream of nitrogen gas. These samples were redissolved in 100 μL isopropyl alcohol, after which cholesterol and TG levels in the isopropyl alcohol were measured by conventional enzymatic methods.

Statistical Analysis The results are expressed as means ± SD. Comparisons between the 3 groups were analyzed for statistical significance using 1-way analysis of variance (ANOVA), followed by Dunnett's test multiple comparisons. Correlation analysis was done with Spearman's test. *P* values less than .05 were considered significant.

RESULTS

Body Weight and Liver Weight After NO-1886 Administration

At the end of the experiment, the body weights of the STZ-induced diabetic rats were significantly lower than those of the normal group. However, there was no significant difference in weights among rats in the 4 diabetic groups (Table 1). At the end of the experiment, the liver weights of the diabetic rats were significantly higher than the normal group. The liver weights of rats in the 4 diabetic groups were not significantly different from each other (Table 1).

Plasma Lipid and Glucose Levels After NO-1886 Administration

Plasma lipid and glucose levels are shown in Table 2. Plasma total cholesterol, TG, and glucose levels in the control group were higher than those in the normal group. Plasma HDL-C levels in the control group were lower than in the normal group. NO-1886 dose-dependently increased plasma HDL-C, and dose-dependently decreased TG levels in the STZ- induced diabetic rats with high fat. NO-1886 had no effect on plasma total cholesterol and glucose levels.

Plasma ALT and AST Levels After NO-1886 Administration

Plasma ALT and AST levels are shown in Table 3. Plasma ALT and AST levels in the control group were higher than the normal group. NO-1886 decreased plasma ALT and AST levels.

Table 2. Plasma Lipid and Glucose Levels in STZ-Induced Diabetic Rats After NO-1886 Administration

		Plasma Lipid and Glucose Levels (mg/mL)			
	n	Total Cholesterol	HDL-C	Triglycerides	Glucose
Normal	10	129 ± 11†	97 ± 8†	162 ± 21†	132 ± 6†
STZ control	10	578 ± 157	55 ± 7	230 ± 46	474 ± 40
NO-1886					
30 mg/kg	10	753 ± 238	67 ± 21	148 ± 46*	458 ± 40
100 mg/kg	10	682 ± 245	112 ± 27†	168 ± 41*	518 ± 25
300 mg/kg	10	607 ± 165	195 ± 19†	157 ± 24*	477 ± 37

NOTE. Data are expressed as means ± SD.

Significantly different from the values in respective control rats: *$P < .05$, †$P < .01$.

Liver After NO-1886 Administration

Photographs of the representative liver of each group are shown in Fig 1. The photographs show that NO-1886 dose-dependently improved fatty liver.

TG and Cholesterol Contents in Rat Liver After NO-1886 Administration

TG and cholesterol contents in liver are shown in Table 4. Liver TG and cholesterol contents in the control group were higher than in the normal group. NO-1886 dose-dependently decreased liver TG and cholesterol contents.

Relationship Between Lipid Contents in Liver and Plasma Lipid Levels

The relationship between liver lipid contents and plasma lipid levels was assessed in experimental animals (normal rats and STZ-induced diabetic rats fed a high-fat diet). The cholesterol contents were inversely correlated with plasma HDL-C levels ($r = -0.5862$, $P < .001$, Fig 2A) and were positively correlated with plasma TG levels ($r = 0.4083$, $P < .003$, Fig 2B). TG contents were inversely correlated with plasma HDL-C levels ($r = -0.6195$, $P < .001$, Fig 2C) and were positively correlated with plasma TG levels ($r = 0.5837$, $P < .001$, Fig 2D). There was no noticeable relationship between plasma cholesterol levels, and liver cholesterol and TG contents.

Table 3. Plasma ALT and AST Levels in STZ-Induced Diabetic Rats After NO-1886 Administration

	n	ALT (IU/L)	AST (IU/L)
Normal	10	49.1 ± 11.4†	102.8 ± 18.2†
STZ control	10	144.6 ± 80.0	206.2 ± 89.4
NO-1886			
30 mg/kg	10	116.2 ± 59.7†	177.9 ± 66.7*
100 mg/kg	10	84.7 ± 26.5†	145.1 ± 35.4*
300 mg/kg	10	82.3 ± 20.9†	143.4 ± 40.7†

NOTE. Data are expressed as means ± SD.

Significantly different from the values in respective control rats: *$P < .05$, †$P < .01$.

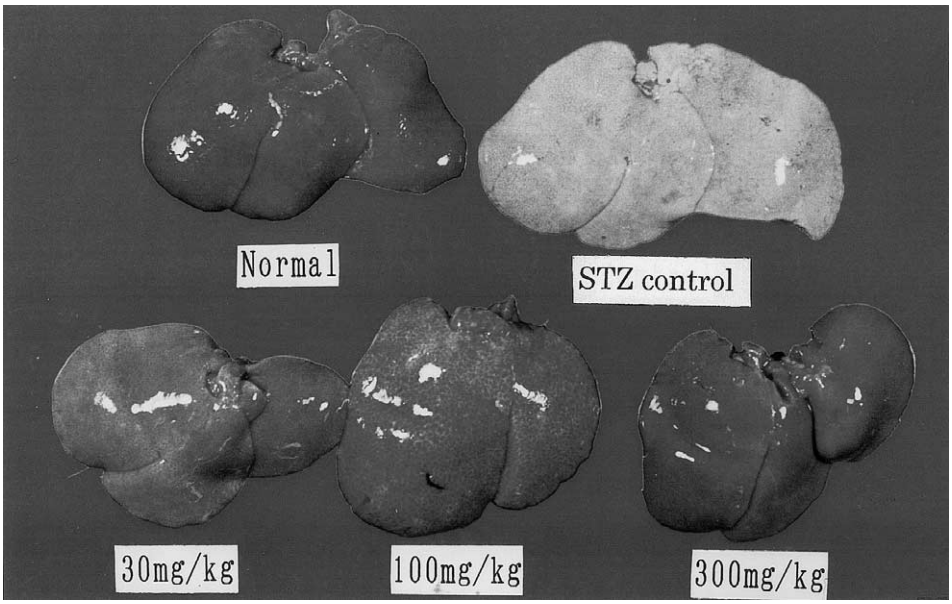

Fig 1. Photograph of liver after NO-1886 administration for 32 weeks in STZ-induced diabetic rats fed a high-fat diet.

DISCUSSION

We have been interested in the relationship between plasma lipids and fatty liver. However, few reports exist that discuss the relationship between plasma lipids and liver lipid contents. In this study, we observed the relationship between plasma lipids and liver lipid contents, and we also observed the effects of the LPL activator NO-1886 on improving the fatty liver in STZ-induced diabetic rats fed a high-fat diet.

Insulin resistance has been reported to cause fatty liver, but to mild degree only.[9,10] We attempted to create a severe fatty liver animal model.

Long-term high-fat feeding in STZ-induced diabetic rats resulted in increased liver cholesterol contents and plasma TG contents, and caused fatty liver (observed visually). STZ-induced diabetic rats are characterized by low plasma insulin levels and low LPL activity.[4] Therefore, low insulin levels cause high plasma TG levels and FFA levels and low HDL-C levels, and high FFA levels cause fatty liver. Also, low insulin levels suppress secretion of very-low-density lipoprotein (VLDL), which may result in fatty liver. The high-fat feeding may accelerate fatty liver in STZ-induced diabetic rats. The liver weights in these rats were heavier than the liver weights in normal rats, and the AST and ALT values, which are indicators of abnormal liver, were elevated compared to normal rats. This indicates that fatty liver causes liver dysfunction.

Table 4. Liver Lipid Levels in STZ-Induced Diabetic Rats After NO-1886 Administration

		Liver Lipid Levels (mg/g wet tissue)	
	n	Cholesterol	Triglycerides
Normal	10	12.78 ± 2.22*	53.08 ± 14.79*
STZ control	10	228.80 ± 51.82	376.38 ± 39.48
NO-1886			
30 mg/kg	10	165.79 ± 44.86*	213.20 ± 33.80*
100 mg/kg	10	75.76 ± 40.00*	143.19 ± 40.38*
300 mg/kg	10	37.66 ± 16.94*	101.39 ± 25.51*

NOTE. Data are expressed as means ± SD.

Significantly different from the values in respective control rats: *$P < .01$.

We have previously reported that NO-1886 increased plasma HDL-C and decreased TG levels,[2-4] and decreases FFA levels in rats.[11] In this study, NO-1886 increased HDL-C and decreased TG levels, and decreased the liver cholesterol contents and TG contents, resulting in improvement of the fatty liver (visual observation). Furthermore, NO-1886 decreased the plasma AST and ALT levels. These results indicate that NO-1886 improved fatty liver and the associated liver dysfunction.

In this study, we observed the relationship between plasma lipid levels and liver lipid contents in STZ-induced diabetic rats fed a high-fat diet. The liver cholesterol contents were inversely correlated with plasma HDL-C levels ($r = -0.5862$, $P < .001$) and were positively correlated with plasma TG levels ($r = 0.4083$, $P < .003$). The liver TG contents were inversely correlated with plasma HDL-C levels ($r = -0.6195$, $P < .001$) and were positively correlated with plasma TG levels ($r = 0.5837$, $P < .001$). There was no noticeable relationship between plasma cholesterol levels, and cholesterol and TG contents in liver. These results may indicate that the improvement of fatty liver is associated with a reduction of plasma TG levels and an elevation of HDL-C.

In summary, administration of NO-1886 resulted in increased plasma HDL-C levels and decreased TG levels and dose-dependently decreased liver TG contents and cholesterol contents, resulting in improvement of fatty liver in STZ-induced diabetic rat fed a high-fat diet. The liver cholesterol contents were inversely correlated with plasma HDL-C levels and were positively correlated with plasma

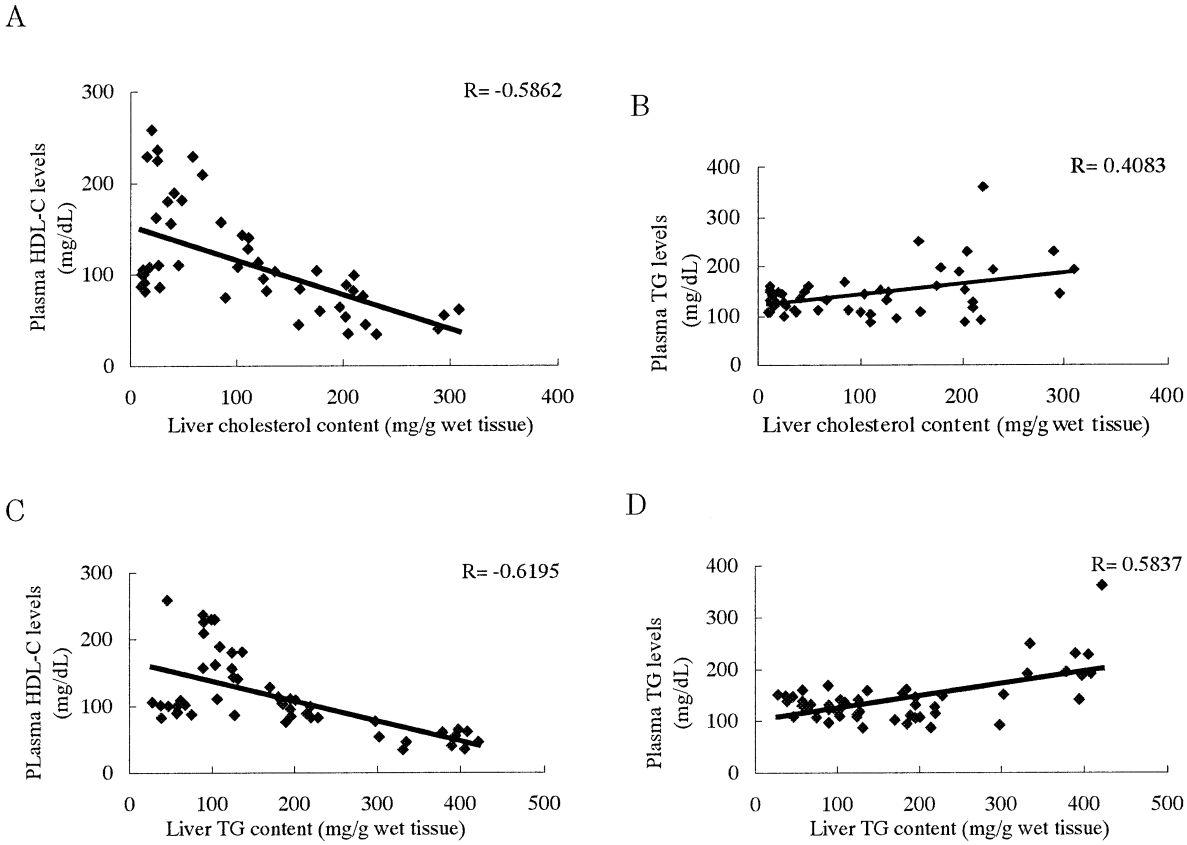

Fig 2. Relationship between plasma lipid levels and liver lipid contents in STZ-induced diabetic rats fed a high-fat diet. (A) Plasma HDL-C levels and liver cholesterol contents; (B) plasma TG levels and liver cholesterol contents; (C) plasma HDL-C levels and liver TG contents; (D) plasma TG contents and liver TG contents.

TG levels. The liver TG contents were inversely correlated with plasma HDL-C levels and were positively correlated with plasma TG levels. These results are compatible with the possibility that in order to improve fatty liver, a reduction in plasma TG levels and an elevation in HDL-C may be necessary.

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