Streptozotocin-Induced Diabetic Cynomolgus Monkey Is a Model of Hypertriglyceridemia with Low High-Density Lipoprotein Cholesterol

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Hypertriglyceridemia with low high-density lipoprotein (HDL) cholesterol is a risk factor of cardiovascular disease. We attempted to create an animal model of hypertriglyceridemia with low HDL cholesterol by intravenously injecting 30 mg/kg body weight streptozotocin (STZ) to cynomolgus monkeys. This induced hypoinsulinemia and resulted in a decrease in postheparin plasma lipoprotein lipase (LPL) activity and LPL enzyme mass, reduction of plasma HDL cholesterol and elevation of triglycerides. Low HDL cholesterol subsequently caused a reduction of HDL₂₀ cholesterol, while hypertriglyceridemia caused an elevation of very low-density lipoprotein (VLDL) triglyceridemia. Apolipoprotein CII, a co-factor of LPL, was not affected by STZ administration. These results show that hypertriglyceridemia with low HDL cholesterol results from a reduction of LPL activity without affecting apolipoprotein CII after STZ administration. The STZ-induced diabetic cynomolgus monkey is a model of hypertriglyceridemia with low HDL cholesterol, and may be potentially beneficial for studying atherosclerosis caused by hypertriglyceridemia with low HDL cholesterol.

Key words cynomolgus monkey; streptozotocin; low high-density lipoprotein cholesterol; hypertriglyceridemia; lipoprotein lipase

In the past several years, there has been increasing evidence of an association between elevated plasma triglyceride levels and increased risk of cardiovascular disease,¹² a risk that is especially high in subjects with low high-density lipoprotein (HDL) cholesterol.动物 Animal models are necessary to study atherosclerosis and hyperlipidemia, however, none exist that adequately represent a model of hypertriglyceridemia with low HDL cholesterol. Reymert and colleagues reported that injection of HDL cholesterol into mice restored the metabolism of HDL cholesterol by reduced lipoprotein lipase (LPL) activity in the LPL mutation model (asparagine291→serine) is an important risk factor for coronary artery disease.⁵ Fisher and colleagues reported similar results.⁶ Diabetes is a risk factor for cardiovascular disease. Therefore, an association between hypertriglyceridemia and low HDL cholesterol in diabetic subjects should increase the risk for cardiovascular disease. One of the major causes of hypertriglyceridemia is the increase of LPL activity due to insulin deficiency, as LPL is known to be an insulin-dependent enzyme.⁸ Ninety percent of LPL is an enzyme that plays a crucial role in both triglyceride removal and HDL cholesterol production.¹⁰,¹¹ These reports indicate that hypertriglyceridemia with low HDL cholesterol is caused by LPL activity. Streptozotocin (STZ) is well known as a diabetogenic agent that acts through selective destruction of pancreatic β cells. The aim of this study was to ascertain whether it was possible to create an animal model of hypertriglyceridemia with low HDL cholesterol by administering STZ to cynomolgus monkeys.

MATERIALS AND METHODS

Materials STZ was obtained from Sigma (St. Louis, MO, U.S.A.). Glycerol tri[¹⁴C]oleate (2.2 GBq/mmol) was obtained from Amersham International (Amersham, UK) and heparin from Novo Nordisk (Bagsvaerd, Denmark). All other chemicals were used at a high grade commercially available products.

Animal Experiments Male cynomolgus monkeys weighing 4.5 to 5.5 kg were obtained from Keari Co., Ltd. (Kawagami, Japan). Animals were maintained under a 12 h light-dark cycle at a constant temperature of 23±2°C and acclimatized for at least 2 weeks before the start of the experiment. Monkeys were fed 100 g of monkey chow (Monkey BIT, Nihon Nosan Kogyo Co., Ltd., Yokohama, Japan) once a day at 10:00 am. All animal experiments were approved by the local animal ethics committee of Otsuka Pharmaceutical Factory Inc.

Preparation of STZ-Induced Diabetic Cynomolgus Monkey: STZ, freshly dissolved in 0.01 mol/l citrate buffer, pH 4.5, was administered to monkeys at doses of 20, 30 or 40 mg/kg body weight per group (3 groups, 2 monkeys per group), via the cephalic vein at 9:00 am (day 1). Blood was drawn from the cephalic vein at 9:00 am (fasting) and 13:00 (post-prandial), and plasma glucose, lipid and insulin concentrations were measured.

Plasma Lipoproteins, Apolipoprotein CII, Postheparin Plasma LPL Activity and LPL Mass in STZ 30 mg/kg Treated Cynomolgus Monkey: STZ was administered to three monkeys at 9:00 am. Three other monkeys were administered vehicle and used as control. Blood was drawn from the cephalic vein on day 4 at 9:00 am, and plasma lipoproteins and apolipoprotein CII concentrations were measured. For the measurement of postheparin plasma LPL activity and LPL enzyme mass, the animals were injected with heparin (200 U/kg body weight) via the cephalic vein and blood samples were collected 10 min later at 9:00 am and 13:00 on day 4. Plasma samples were used to determine LPL activity and LPL enzyme mass.

Analytical Methods Plasma Glucose, Lipids and Insulin: Plasma glucose, total cholesterol, HDL cholesterol, triglycerides and non esterified free fatty acid (NEFA) were determined by conventional enzymatic methods. The glucose CII test Wako (Wako Pure Chemical Industries, Osaka, Japan) was used for glucose, the cholesterol C-test Wako (Wako Pure Chemical Industries) for cholesterol, the Nescopte HDL-C kit N (heparin calcium precipitation; Nippon Shoji, © 1998 Pharmaceutical Society of Japan

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Osaka, Japan) for HDL cholesterol, the triglyceride G-test Wako (Wako Pure Chemical Industries) for triglycerides, and NEFA C-test Wako (Wako Pure Chemical Industries) for NEFA. Glazyme Insulin-EIA test (Wako Pure Chemical Industries) was used for insulin.

Plasma Lipoprotein Analysis: Plasma lipoprotein analysis was performed in plasma on day 4 at 9:00 am. Ultracentrifugation of plasma lipoproteins was carried out using a rotor (L42T; Hitachi Koki Co., Ltd., Tokyo, Japan). The densities of the samples were adjusted with KBr and KBr solutions of known density. Plasma samples (100 µl) were ultracentrifuged at densities of 1.006, 1.019, 1.063, 1.100 and 1.125 g/ml, and the lipid concentration of each fraction was measured. The lipid levels of five lipoprotein fractions were calculated as: very low-density lipoprotein (VLDL), \( \text{d}<1.006 \text{ g/ml} \); intermediate density lipoprotein (IDL), \( \text{d} 1.006-1.019 \); low density lipoprotein (LDL), \( \text{d} 1.019-1.063 \); HDL2b, \( \text{d} 1.063-1.100 \); HDL2a, \( \text{d} 1.100-1.125 \) and HDL3, \( \text{d}>1.125 \).

Apolipoprotein CII: Apolipoprotein CII was measured by immunonephelometry. Measurements were performed by Osaka Assay Laboratories Inc. (Tokyo).

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. Assay of postheparin plasma was performed in the presence or absence of 1 mol/l NaCl to estimate both LPL and hepatic triglyceride lipase (HTGL) activity in rabbits. Lipase activity in the presence of 1 mol/l NaCl represented HTGL activity. LPL activity was calculated by subtraction of the salt resistant fraction (HTGL activity) from total lipase activity.

LPL Enzyme Mass in Postheparin Plasma: LPL enzyme mass in postheparin plasma was measured by conventional sandwich enzyme immunoassay. Markit-F LPL (Dainippon Pharmaceutical Co., Osaka) was used for LPL mass.

Statistical Analysis: The results are expressed as means ±S.D. Comparisons between the two groups were analyzed for statistical significance by Student's *t*-test or Aspin-Welch's *t*-test.

### RESULTS

**Clinical Signs**

Monkeys were fed 100 g of monkey chow once a day before injection of STZ, but were not fed on the day of STZ administration. They were again fed 100 g of chow the day following administration. The 40 mg/kg administered monkeys grew weak at 6 after STZ administration, and died on day 7.

**Effects of STZ on Plasma Glucose and Insulin-Dose Relationship**

STZ was administered to monkeys on day 1 at 9:00 am. Plasma insulin levels decreased at 4 h after STZ administration (day 1, 13:00), and increased 24 h (day 2, 9:00 am) after administration in 30 and 40 mg/kg STZ administered monkeys. STZ administration caused a dose dependent decrease in plasma insulin from day 3 at 9:00 am. In monkeys administered with 40 mg/kg, insulin levels were not detected from day 3 at 9:00 am (Fig. 1A).

Plasma glucose levels increased 4 h after STZ administration (day 1, 13:00), and decreased 24 h (day 2, 9:00 am) after administration in 30 and 40 mg/kg STZ administered monkeys. STZ administration caused a dose dependent increase in plasma glucose from day 2 at 13:00 (Fig. 1B). Plasma glucose levels were inversely correlated with plasma insulin. Administration of 20 mg/kg STZ did not affect insulin or glucose.

**Effects of STZ on Plasma Lipid-Dose Relationship**

Plasma total cholesterol increased in 40 mg/kg STZ administered monkeys (Fig. 2A). STZ administration caused a reduction in HDL cholesterol in 40 mg/kg administered monkeys, and in 30 mg/kg administered monkeys values were lower than pre-treatment (day 1, 9:00 am) from day 3 (Fig. 2B). Administration with 40 mg/kg STZ caused an elevation of triglycerides compared with pre-treatment from day 2 (Fig. 2C). NEFA increased in 40 mg/kg administered monkeys, whereas 30 mg/kg administered monkeys showed a slight increasing tendency compared with pre-treatment (Fig. 2D). Administration with 20 mg/kg STZ had no effect on plasma lipids.

**Effects of STZ 30 mg/kg Administration on Plasma Lipoproteins**

STZ treatment caused a marked decrease in HDL cholesterol (reduction of 50% compared with con-

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![Fig 1](image-url)  
**Fig 1.** Plasma Insulin (A) and Plasma Glucose (B) of Cynomolgus Monkeys after a Single Intravenous Administration of STZ. STZ, freshly dissolved in 0.01 mol/l citrate buffer, pH 4.5, was administered to monkeys via the cephalic vein on day 1 at 9:00 am. Blood was drawn from the cephalic vein at 9:00 and 13:00. Data are expressed as means (n=2).
Fig. 2. Plasma Total Cholesterol (A), HDL Cholesterol (B), Triglycerides (C) and NEFA (D) of Cynomolgus Monkeys after a Single Intravenous Administration of STZ

STZ was administered on day 1 at 9:00 am. Blood was drawn from the cephalic vein at 9:00 am and 13:00. Data are expressed as means (n=2).

crol), but did not affect HDL2a cholesterol or HDL3 cholesterol. On the other hand, VLDL cholesterol increased in STZ-treated monkeys. IDL and LDL cholesterol increased slightly but not significantly compared with control (Fig. 3A). STZ treatment caused a marked increase in VLDL triglyceride. IDL, LDL and HDL triglycerides increased slightly but not significantly compared with control (Fig. 3B).

Effects of STZ 30 mg/kg Administration on Apolipoprotein CII STZ treatment had no effect on apolipoprotein CII (STZ treatment: 2.4±0.2 mg/dl, control: 2.3±0.5 mg/dl).

Effects of STZ 30 mg/kg Administration on Postheparin Plasma LPL Activity and LPL Mass STZ treatment caused a decrease in postheparin plasma LPL activity and LPL mass (Table 1). Fasting LPL activity was 57% lower in
animals treated with STZ than control, and postprandial LPL activity was 32% lower than control. Fasting LPL protein mass was 58% lower in animals following STZ treatment than control, and postprandial LPL mass was 45% lower than control.

DISCUSSION

We attempted to create an animal model of hypertriglyceridemia with low HDL cholesterol by STZ administration to cynomolgus monkeys. Administration with 20 mg/kg STZ did not cause diabetes or hypertriglyceridemia with low HDL cholesterol. Administration with 40 mg/kg STZ caused severe hypertriglyceridemia with low HDL cholesterol (Fig. 2B, C), but all test monkeys died 7 after administration. Administration with 30 mg/kg STZ caused mild hypertriglyceridemia with low HDL cholesterol, and all test monkeys survived for over 6 months after administration. From these data, we concluded that the optimal dose for creating a model of hypertriglyceridemia with low HDL cholesterol in cynomolgus monkeys was 30 mg/kg.

In this study, plasma insulin levels decreased after the first 4 h following STZ treatment, but increased after 24 h. Forty-eight hours after STZ treatment, insulin again fell to lower levels than pre-treatment and became stable (Fig. 1A). Changes in plasma glucose were opposite to those of insulin (Fig. 1B). Takimoto et al. reported that after the first 12 h following STZ treatment, insulin release from the pancreas is inhibited by the action of STZ, and 24 h after STZ treatment, insulin levels markedly increase due to the transient release of large amounts of insulin during extensive β-cell destruction. Our data show a similar pattern, possibly due to the same mechanism.

STZ-induced diabetic animals, a model of insulin-dependent diabetes mellitus (IDDM), showed an elevation in plasma triglyceride levels and reduction of HDL cholesterol. Hypertriglyceridemia may be a consequence of either overproduction of VLDL by the liver (high values of plasma NEFA), defective removal of triglyceride-rich lipoproteins from the circulation, or both. STZ administration caused reduction of LPL activity due to insulin deficiency because LPL is an insulin-dependent enzyme. LPL is an enzyme that plays crucial roles in both VLDL triglyceride removal and HDL cholesterol production. STZ treated monkeys have higher plasma triglyceride levels, with VLDL levels being the highest, and lower HDL cholesterol, with HDL-25 levels being the lowest compared with control monkeys (Fig. 3). Previous reports have demonstrated that enhanced LPL activity results in an increase in HDL2 particles, thus a precursor-product relationship exists between the two. STZ administration had no effect on apolipoprotein CII, a co-factor of LPL. Apolipoprotein CII deficiency causes an absence of postheparin lipolytic activity, resulting in gross hypertriglyceridemia. Our data show a reduction of LPL activity causing hypertriglyceridemia with low HDL cholesterol with no effect on apolipoprotein CII.

STZ administration decreased not only LPL activity but also LPL mass (Table 1). STZ may inhibit LPL synthesis by reducing plasma insulin levels. LPL protein is known to be present as an isoform, the catalytically active LPL a homodimer, and the inactive LPL a monomer. In this study, we calculated LPL specific activity (activity-to-mass ratio) in each group. The LPL specific activity was 0.029 in the fasting group and 0.032 in the post prandial group in control monkeys, and 0.029 in the fasting group and 0.040 in the post prandial group in STZ treated monkeys. These data show that a reduction of LPL mass in STZ treated monkeys may simply decrease LPL activity during fasting, and LPL specific activity may increase during feeding in both control and diabetic animals. Bergo et al. reported that the decrease in LPL specific activity in adipose tissue during fasting is caused by a shift in the distribution of lipase protein toward an inactive, monomeric form. On the other hand, in the postprandial condition, LPL specific activity was higher than control. This indicates that the active LPL increase in STZ treated monkeys was greater than in control monkeys in the postprandial condition. We have no data to explain why these phenomena occurred, although they may be due to the inactive LPL being insufficient in STZ treated monkeys, resulting in facilitation of transformation to the active form from the inactive, or to the increase in active form to compensate for hypertriglyceridemia.

Weinstock et al. created an LPL knockout mouse, but this animal dies as a result of hypertriglyceridemia within 18 h after birth, therefore this model cannot be used for the study of atherosclerosis. STZ treated cynomolgus monkeys, however, can potentially be used to study this condition as their life span is long.

In summary, we attempted to create an animal model of hypertriglyceridemia with low HDL cholesterol by administering STZ to cynomolgus monkeys. Administration with 30 mg/kg STZ caused a reduction of LPL activity and LPL mass in postheparin plasma, and produced a rise in plasma VLDL triglyceride levels and a reduction of HDL2 cholesterol. The STZ-induced diabetic cynomolgus monkey is a model of hypertriglyceridemia with low HDL cholesterol, and may be potentially beneficial for studying atherosclerosis caused by this condition.

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<td>Fasting</td>
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<td>Control monkeys</td>
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<td>STZ-induced diabetic</td>
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For the measurement of postheparin plasma LPL activity and LPL enzyme mass, the monkeys were injected with heparin (200 U/kg) via the cephalic vein and blood samples were collected 10 min later on day 4 at 9:00 am and 13:00. Plasma samples were used to determine LPL activity and LPL mass. LPL activity was measured by the method of Murane and Uchimura, and LPL mass was measured by Sandwich enzyme immunoassay. Data are expressed as means±S.D. Significantly different from the value in the respective control monkeys: *p<0.05, **p<0.01.
REFERENCES