The Effects of Streptozotocin-Induced Hypoinsulinemia on Serum Lipid Levels in Spontaneously Hyperlipidemic Rats

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We compared the effects of streptozotocin (STZ) treatment on serum cholesterol and lipoprotein levels in spontaneously hyperlipidemic rats (HLR), a hereditary hyperlipidemic model animal, with those in Sprague-Dawley rats (SDR). The body weight of control SDR and HLR were increased continuously for 30 days. Both SDR and HLR lost their body weight after STZ administration. Glucose levels of SDR and HLR were significantly increased by STZ treatment. Insulin levels were markedly decreased in HLR compared with those in SDR. Serum cholesterol and triglyceride levels of HLR treated with STZ were significantly higher than those of untreated HLR. The increment of both levels in HLR was much larger than that in SDR. The high density lipoprotein (HDL) cholesterol level of the STZ-treated HLR was significantly lower than that of untreated HLR. In the STZ-treated HLR the intensities of both bands of the very low density lipoprotein (VLDL) and the low density lipoprotein (LDL) were higher than those in untreated HLR, while the intensity of any lipoprotein band remained unchanged between SZT-treated and control SDR. The atherogenic index (the ratio of total cholesterol level minus HDL cholesterol level to HDL cholesterol level) in the STZ-treated HLR was significantly high compared with that in other groups. The STZ-treated HLR showed the extremely hyperlipidemic state and this animal might be useful in experiments for the development of atherosclerosis or the drug evaluation for the agents used in hyperlipidemia.

Key words: Cholesterol — Hyperglycemia — Hyperlipidemia — LDL — HDL — Atherogenic index — Streptozotocin — Lipoprotein

Introduction

Hyperlipidemia is well known to play a main role in the development of atherosclerosis (1,2,3). Lipids research has been carried out using rabbit as an experimental animal model, since hyperlipidemia is easily obtained in a rabbit with high-cholesterol diet (4,5). The Watanabe heritable hyperlipidemic (WHHL) rabbit is a typical experimental animal model for lipid research (6). A rat, however, is not relevant because the serum cholesterol level is much lower than that of WHHL and total lipoprotein consists of a very small fraction of LDL and a large amount of HDL (7,8), which is quite different from hyperlipidemia in WHHL or humans.

Spontaneously hyperlipidemic rat (HLR) was originally raised in our laboratory based on the high response of the serum cholesterol level to high cholesterol diet from Sprague-Dawley rats (SDR) as an origin and bred over 60 generations. HLR has extremely high serum cholesterol and triglyceride levels, even on the normal diet, compared with those in SDR. The mechanisms of congenital elevation in serum cholesterol of HLR are still being elucidated in our laboratory (9,10,11). In the present study, we examined the characteristics of hyperlipidemia in the streptozotocin (STZ)-treated HLR and demonstrated whether the treatment of STZ produced an extremely hyperlipidemic state because diabetes mellitus is known as an important factor in hyperlipidemia deterioration.

Materials and Methods

Animals

Eight-week old male SDR and HLR weighing from 248—296 g were raised in our breeding colony by brother-sister mating. They were maintained in air-conditioned quarters with 12 hr light-dark-cycles and given laboratory chow (CE-2) of Clea Japan (Tokyo, Japan) and water ad libitum. A single intraperitoneal administration of STZ (65 mg/kg, Wako Pure Chem., Tokyo, Japan) dissolved in 0.05 M citrate buffer (pH 4.5, 25 mg/ml) was given in STZ treatment group. Untreated SDR and HLR were used as control groups. All rats were sacrificed on the 30th day after STZ administration. All procedures were carried out in accordance with the guiding principles for the care and use of laboratory animals approved by the Japanese Pharmacological Society.

Measurements

Blood was drawn into tubes and centrifuged at 1,500 x g for 15 min at room temperature to separate serum. The concentrations of serum glucose, cholesterol and triglyceride were measured enzymatically using commercially available kits, Glucose Test Wako, Cholesterol E-test Wako and Triglyceride E-
test. Wako (Wako Pure Chem., Tokyo, Japan). HDL cholesterol level was measured with dextran sulfate-Mg<sup>2+</sup> using HDL fractionating kit (Daichi Pure Chem., Tokyo, Japan) after LDL and VLDL particles were precipitated with polyethylene glycol. The atherogenic index was calculated from the following equation: (total cholesterol level - HDL cholesterol level)/HDL cholesterol level. Serum insulin concentration was determined with a double antibody radioimmunoassay technique using anti-rat insulin serum, rat insulin for a standard, and porcine<sup>[125I]labeled insulin as a tracer. Polyacrylamide disk gel electrophoresis was carried out with Lipophor System (Jookou Co., Tokyo, Japan). The serum was applied (25 µl) to each disk gel. The electrophoresis (gel size 0.5 × 30 mm) was conducted at 5 mA/ tube for 20 min. The intensity of each lipoprotein band was measured with a densitometer AE-6920-MF-Densitograph ver. 2.5 (ATTO, Tokyo, Japan).

Statistical analysis

The data are presented as mean ± standard error. Data were compared using an unpaired t-test. A p-value of less than 0.05 was considered significant.

Results

Body weight of each group was measured every three days for 30 days and is shown in Fig. 1. A control group of SDR or HLR gained weight continuously for 30 days, while the STZ-treated group lost weight gradually in both SDR and HLR. Serum glucose, insulin, triglyceride and cholesterol levels of the control (n = 9) or STZ-treated SDR and HLR (n = 8) are shown in Table 1. Serum glucose levels were 4.7-fold and 7.9-fold increased in the STZ-treated SDR and HLR, compared with those in the control group of SDR and HLR, respectively. On the other hand, serum insulin level of the STZ-treated HLR was significantly lower than that of the STZ-treated SDR. Serum triglyceride and total cholesterol levels of the HLR were much higher than those of the SDR. The increments of triglyceride and total cholesterol levels of the STZ-treated HLR were much larger than those of the STZ-treated SDR. HDL cholesterol levels of the STZ-treated HLR significantly decreased to 42% of the control HLR, while those of the STZ-treated SDR increased by 36% to the control SDR. In addition, the serum non-HDL cholesterol levels were significantly increased in SDR (1.6-fold) and HLR (4.9-fold), compared with those of the control SDR and HLR, respectively. In the STZ-treated HLR, the atherogenic index was significantly high, compared with the index ratio in other groups. The contents of serum lipoprotein were measured by sudan black B staining polyacrylamide gel electrophoresis and the intensity of each band was densitometrically compared (Fig. 2). In the STZ-treated HLR, the intensity of HDL fraction was decreased and that of VLDL or LDL fractions was increased significantly, compared with that in control HLR, while the intensity of any lipoprotein fraction remained unchanged between STZ-treated and control SDR.

Discussion

The serum cholesterol concentrations of rats were reported to be lower than those of rabbits or humans because of the rapid feedback regulation of cholesterol metabolism in rats (12). Although high cholesterol diet hardly changes the plasma cholesterol concentrations of rats (5), the HLR shows spontaneous hypercholesterolemia on the normal diet. We are now investigating the mechanism to maintain the serum cholesterol level of HLR remarkably high. The activity and expression level of the hepatic microsomal cytochrome P450 cholesterol 7α-hydroxylase (CYP7A1), the major catabolic enzyme for cholesterol (13,14), were low in HLR, compared with those in SDR (15). The reduction in the activity of CYP7A1 may contribute to the decrease in cholesterol elimination and induce hypercholesterolemia in HLR.

Since diabetes mellitus is known as an important factor in hyperlipidemia deterioration (16,17), we examined the characteristics of the hyperlipidemia in the STZ-treated HLR and evaluated whether the treatment of STZ produced an extremely hyperlipidemic state in HLR and this animal would be relevant to lipid research. As reported before (10), serum cholesterol and triglyceride levels of HLR were significantly higher than those of SDR in the present study. However, the atherogenic index in the control HLR was not different from that in the control or STZ-treated SDR because HDL cholesterol consists of one third of total cholesterol in HLR. The treatment of STZ affected the triglyceride and total cholesterol levels of HLR much more than those of SDR. The decrease in lipoprotein lipase (LPL) activity is reported to contribute to the development of hyperlipidemia in maturity-onset diabetes.

Reduction in the LPL activity was restored by the replacement of insulin (18). Therefore, the lower level of insulin observed in this study might contribute to the alteration of lipoprotein catabolism in the STZ-treated HLR.

In the STZ-treated HLR, the atherogenic index was significantly high, compared with those in the other groups. The composition of serum lipoprotein, analyzed by sudan black B staining polyacrylamide disk gel electrophoresis, also showed that the intensity of HDL decreased and that of VLDL or LDL increased significantly in the STZ-treated HLR, compared with that in the other groups. The LDL band of the STZ-treated HLR seemed to migrate towards VLDL in the electrophoresis. As Witztum et al.
(19) have reported that the diabetic and synthetic glucosylated LDL has greater mobility on lipoprotein electrophoresis than normal LDL, the LDL fraction band having migrated to a higher molecular weight was considered to be glucosylated LDL. Glucosylated LDL is reported to interact with endothelial cell and initiate atherogenic changes in vessel wall (20). In addition, it was reported that glucosylated apoprotein B in LDL blocks receptor-mediated LDL catabolism in vivo, which reduces LDL clearance in hyperglycemic patients (21, 22). Alloxan treatment develops atherosclerosis in diabetic rats on the high-cholesterol diet. These rats showed a significant increase in the serum total cholesterol and atherogenic index [(total cholesterol — HDL cholesterol)/HDL cholesterol] (23). Although pathological changes in cardiovascular system were not evaluated yet in the STZ-treated HLR, atherosclerosis is likely to be generated by the extreme elevation of the atherogenic index and both VLDL and LDL, which is also observed in WHHL rabbits.

From this study, the remarkable decrease in the serum insulin concentration in the SZT-treated HLR might contribute to the alteration of lipoprotein catabolism. Naturally, STZ might inhibit CYP7A1 directly and induce significant hyperlipidemia. Further studies are needed to clear this point. The results of the present study suggested that the STZ-treated HLR might be useful in experiments for the development of atherosclerosis or drug evaluation for the agents used in hyperlipidemia.

### References


Lipid Level of Hyperglycemia induced Hyperlipidemic Rats

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