Suppression of Hyperlipidemia-Associated Cataracts in Diabetic Rats with the Lipoprotein Lipase Activator NO-1886

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Diabetic cataracts are thought to be caused by hyperglycemia associated with disturbed glucose metabolism. Diabetes mellitus often involves abnormal lipid metabolism in addition to abnormal glucose metabolism. To date, however, very few studies have counted hyperlipidemia as a risk factor for diabetic cataracts. The present study was undertaken to determine whether this actually is a risk factor for diabetic cataracts and to confirm that the onset of cataracts associated with diabetes mellitus can be suppressed by correction of hyperlipidemia.

When rats with streptozotocin (STZ)-induced diabetes mellitus were fed an ordinary diet, cataracts became evident at 9 weeks in 26.7% of animals, and increased to an incidence of 73.3% after 10 weeks of STZ treatment. However, in rats with STZ-induced diabetes mellitus that were fed a cholesterol rich diet to induce severe hyperlipidemia, cataracts were observed one week earlier, after 8 weeks of treatment, in 36.0% of animals, with an increase to a 52.0% incidence and a 76.0% incidence after 9 and 10 weeks of STZ treatment, respectively. Hyperlipidemia was therefore associated with an earlier onset and an elevated incidence of diabetic cataracts. When the lipoprotein lipase (LPL) activator NO-1886 was administered to diabetic rats which had developed severe hyperlipidemia, they showed a decrease in plasma total cholesterol, triglyceride and non-high density lipoprotein (non-HDL)-cholesterol levels and an increase in high density lipoprotein (HDL)-cholesterol level, and the onset of diabetic cataracts was markedly suppressed. The results of this study suggest that hyperlipidemia and low HDL-cholesterol levels may be risk factors for the onset of diabetic cataracts, and that this onset can be suppressed if measures are taken to alleviate these risk factors. The LPL activator NO-1886 may be useful in preventing the onset of diabetic cataracts.

Key words: diabetes; cataract; NO-1886; lipoprotein lipase; lipid

Diabetic cataracts have been attributed to hyperglycemia due to a disturbed glucose metabolism.1) However, since diabetes mellitus also involves abnormal lipid metabolism associated with a shortage of insulin, these changes may also be responsible for the development of diabetic cataracts. To date, however, very few papers have dealt with the relationship between diabetic cataracts and abnormal lipid metabolism in detail. This study was performed to determine whether abnormal lipid metabolism is a risk factor for onset of these cataracts, and whether correction of hyperlipidemia could lead to suppression of their onset. We recently described discovering a novel compound NO-1886 which activates lipoprotein lipase (LPL) (a rate-limiting factor in lipid metabolism), reduces plasma triglyceride levels, elevates high density lipoprotein (HDL)-cholesterol levels and suppresses the development of arteriosclerosis.2,3) We have also previously reported that this compound elevated the LPL activity in rats with streptozotocin (STZ)-induced diabetes mellitus, reducing the abnormal lipid metabolism associated with diabetes.4) In the present study, NO-1886 was used as a means of correcting hyperlipidemia.

Diabetic cataracts can be induced in rats by treating them with STZ,5) however, this method cannot induce severe hyperlipidemia. We therefore fed a cholesterol rich diet to the STZ-treated rats to produce diabetic rats with severe hyperlipidemia (cholesterol rich diet group). The onset and frequency of diabetic cataracts following induction of hyperlipidemia was compared to cataract development in control rats with STZ-induced diabetes mellitus fed an ordinary diet (ordinary diet group). We also attempted to identify risk factors for the onset of diabetic cataracts by analyzing the correlation of cataract incidence in the cholesterol rich diet group with plasma total cholesterol, triglyceride, HDL-cholesterol, non-HDL-cholesterol and glucose levels. Treatment with an LPL activator NO-1886 corrected abnormal lipid metabolism in diabetic rats with hyperlipidemia and suppressed the onset of diabetic cataracts in these rats. This suggests the importance of correcting hyperlipidemia to prevent the onset of diabetic cataracts.

MATERIALS AND METHODS

Materials 4-[(4-Bromo-2-cyanophenyl)carbamoyl]benzylphosphonate (NO-1886) was synthesized in the New Drug Research Laboratory of Otsuka Pharmaceutical Factory, Inc., Naruto, Tokushima, Japan. STZ was obtained from Sigma, St. Louis, MO, U.S.A. All other chemicals used were high grade commercially available products.

Animal Experiments Male Wistar rats, 6−7 weeks old and weighing 160−180 g, were obtained from the Nissin Tokushima Institute for Animal Reproduction, Tokushima, Japan. The animals were maintained in a 12-h light−dark cycle at a constant temperature of 23 ± 2 °C. The ordinary diet group was fed a standard laboratory chow. The cholesterol rich diet group was fed a standard laboratory chow supplemented with 0.25% cholesterol, 0.4% cholic acid sodium salt, and 2.5% olive oil. The animals were given free access to food and tap water. Food consumption was measured daily, and body weight was recorded weekly. STZ, freshly dissolved in 0.01 mol/l citrate buffer, pH 4.5, was administered to rats at a dose

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of 65 mg/kg body weight via the tail vein. NO-1886 was suspended in 5% gum arabic and administered to STZ-induced diabetic rats in daily doses of 30 mg/kg for 10 weeks via a gastric tube beginning 5 days after STZ administration. Control animals received 5% gum arabic, a diluent of NO-1886. Each rat was checked for cataracts once a week using a fundus camera (RC-2621, KOWA Co., Ltd., Osaka, Japan). A diagnosis of cataract was made upon detection of lens opacity. The same examiner checked for and diagnosed cataracts throughout the study. At the end of the experimental period, the animals were killed by exsanguination under sodium pentobarbital anesthesia. Blood samples were collected from the posterior vena cava for plasma lipid and glucose measurements.

**Analytical Methods** Plasma total cholesterol, HDL-cholesterol, triglycerides and glucose were determined by conventional enzymatic methods. The cholesterol C-test Wako (Wako Pure Chemical Industries, Osaka, Japan) was used in the case of total cholesterol, Nescote HDL-C Kit N (Nippson Shoji, Osaka, Japan) for HDL-cholesterol, the triglyceride G-test Wako (Wako Pure Chemical Industries) for triglycerides and the glucose C-II test Wako (Wako Pure Chemical Industries) for glucose. Non-HDL-cholesterol was measured as total cholesterol excluding HDL-cholesterol; it includes very low density lipoprotein (VLDL), low density lipoprotein (LDL), remnant and beta-VLDL.

**Histological Analysis** The 50 rats in the cholesterol rich diet group (NO-1886 treated group and untreated control group, 25 rats per group) were analyzed histologically to determine the relationship between the incidence of cataracts and plasma total cholesterol, triglyceride, HDL-cholesterol, non-HDL-cholesterol and glucose levels.

**Statistical Analysis** The results are expressed as means ± S.D. Two groups were compared and analyzed for statistical significance by Student’s t-test or Aspin–Welch’s t-test. Analysis among more than two groups for statistical significance was done using Dunnet’s test.

**RESULTS**

**Plasma Lipid and Glucose Levels in STZ-Induced Diabetic Rats** Rats injected with STZ developed diabetes. They had high glucose levels with high triglyceride, high non-HDL-cholesterol and low HDL-cholesterol levels. The cholesterol rich diet group had significantly higher total cholesterol, non-HDL-cholesterol and slightly higher triglyceride levels than the ordinary diet group. HDL-cholesterol levels were lower in animals receiving a cholesterol rich diet, although glucose levels did not differ (Table 1).

Administration of NO-1886 to the cholesterol rich diet group decreased total cholesterol, triglyceride and non-HDL-cholesterol, and increased HDL-cholesterol levels. NO-1886 had no effects on glucose levels (Table 1).

**Diabetic Cataracts** Diabetic cataracts in the ordinary diet group became evident at 9 weeks in 26.7% of animals, and increased to an incidence of 73.3% after 10 weeks of STZ administration. In the cholesterol rich diet group, cataracts were observed one week earlier, after 8 weeks of STZ treatment, in 36.0% of animals, with an increase to a 52.0% incidence and a 76.0% incidence after 9 and 10 weeks of STZ treatment (Fig. 1).

After administration of NO-1886 to the cholesterol rich diet group, cataracts were apparent at 8 weeks in 8.0%, at 9 weeks in 25.0% and at 10 weeks in 41.7% after STZ treatment (Fig. 1).

**Histogram Showing the Relationship between the Incidence of Cataracts and Plasma Lipid and Glucose Levels in Cholesterol Rich Diet Group** No cataracts developed when total cholesterol levels were below 200 mg/dl. As the level rose above that, the incidence of cataracts increased.

<table>
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<tr>
<th>Table 1. Effect of NO-1886 on Plasma Lipid and Glucose Levels in STZ Induced Diabetic Rats</th>
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<tr>
<td>Normal rats (15)</td>
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<td>STZ induced diabetic rats (15)</td>
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<td>Ordinary diet group</td>
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<td>Control (25)</td>
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<td>NO-1886 (25)</td>
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Data are expressed as means ± S.D. Significantly different from the value in the respective rats: * p < 0.001 vs. normal rats; a) p < 0.001, b) p < 0.01, c) p < 0.05 vs. high cholesterol fed STZ induced diabetic control rats.
progressively and at over 500 mg/dl reached 90% (Fig. 2).

As non-HDL-cholesterol levels increased, the incidence of cataracts progressively increased. The incidence was 100% when non-HDL-cholesterol levels were over 500 mg/dl (Fig. 2).

No cataracts developed when triglyceride levels were below 100 mg/dl, but as they rose over 100 mg/dl, cataract incidence increased progressively and at over 400 mg/dl was 100% (Fig. 2).

As HDL-cholesterol levels decreased, the incidence of cataracts progressively increased. The incidence was 100% when HDL-cholesterol levels were below 30 mg/dl. No cataracts developed when these levels were over 90 mg/dl (Fig. 2).

In animals with glucose levels below 400 mg/dl, no cataracts were observed, although they were seen when levels were over 400 mg/dl, at a maximum incidence of 50% (Fig. 2).

DISCUSSION

Diabetic cataracts are currently believed to be caused by hyperglycemia due to disrupted glucose metabolism. Kinoshita has proposed a polyol osmotic theory to explain the pathogenesis of diabetic cataracts. According to his view, hyperglycemia activates aldose reductase and pro-
motates the formation of sorbitol from glucose; accumulation of sorbitol in the lens leads to the onset of cataracts. Based on this, a number of research facilities have worked to develop aldose reductase inhibitors and have attempted to use these inhibitors to prevent or treat diabetic cataracts. Other investigators have proposed a view that diabetic cataracts are attributable to glycation of protein within the lens. All these theories proposed to explain the pathogenesis of diabetic cataracts assume that hyperglycemia is associated with diabetic cataracts. However, since diabetes mellitus involves abnormal glucose and lipid metabolism due to insulin shortage, it is possible that disrupted lipid metabolism is also involved in the onset of diabetic cataracts. Diabetes mellitus is often accompanied by hypercholesterolemia, hypertriglyceridemia and low HDL-cholesterol levels, and the incidence of arteriosclerosis is higher in diabetic patients than in non-diabetic individuals. Assuming that diabetic complications are caused by arteriosclerosis-associated vascular disease or damage, it is possible that hyperlipidemia, which is a known risk factor for arteriosclerosis, also serves as a risk factor for diabetic cataracts. The present study was undertaken to test this hypothesis. When rats with STZ-induced diabetes mellitus were fed an ordinary diet, cataracts developed in the 9th week of STZ treatment. In the cholesterol rich diet group, cataracts were first seen in the 8th week of this treatment. Thus, the onset of cataracts was earlier in diabetic rats with severe hyperlipidemia. The incidence of diabetic cataracts after 9 weeks of STZ treatment for the cholesterol rich diet group was about twice that in the ordinary diet group, suggesting that severe hyperlipidemia increases the incidence of diabetic cataracts. In the cholesterol rich diet group, treatment with the LPL activator NO-1886 resulted in a reduction in plasma total cholesterol, non-HDL-cholesterol and triglyceride levels, an increase in HDL-cholesterol levels, and no change in plasma glucose levels. As a result, the onset of cataracts was suppressed in this group. The 50 rats in the cholesterol rich diet group (NO-1886 treated group and untreated control group, 25 rats per group) were analyzed histographically to determine the relationship between the incidence of cataracts and plasma total cholesterol, triglyceride, HDL-cholesterol, non-HDL-cholesterol and glucose levels. The results showed that the onset of cataracts correlated positively with plasma total cholesterol, triglyceride, non-HDL-cholesterol and glucose levels and negatively with the HDL-cholesterol levels. They thus suggest that elevated plasma levels of total cholesterol, triglyceride and non-HDL-cholesterol and reduced HDL-cholesterol levels serve as risk factors for the onset of diabetic cataracts. The histograms indicate that cataracts developed when plasma glucose levels exceeded 400 mg/ml, however, cataract incidence did not go above 50% even when the plasma glucose level was over 400 mg/ml. This suggests that the onset of diabetic cataracts cannot be attributed to elevated plasma glucose level alone, but that a combination of hyperglycemia with hyperlipidemia or low HDL-cholesterol level precipitates the onset of these cataracts. In other words, hyperglycemia and hyperlipidemia or low HDL-cholesterol levels may be involved in diabetic cataract onset.

The same study was performed using several derivatives of NO-1886 and the improved lipid metabolism, found the degree of which was directly related to the prevention of the development of cataracts (unpublished data). It therefore seems possible that the onset of diabetic cataracts may be suppressed by correction of lipid metabolism. Hyperlipidemia and reduced HDL-cholesterol levels are known to serve as risk factors for arteriosclerosis. The results of the present study suggest that arteriosclerosis may be responsible for the onset of diabetic cataracts. The lens has no vascular supply and receives oxygen and nutrients from the aqueous humor. In diabetic rats with severe hyperlipidemia, the choroidal microvessels which supply oxygen and nutrients to the aqueous humor may be disturbed.

In summary, the incidence of diabetic cataracts was found to be elevated by hyperlipidemia and low blood HDL-cholesterol levels. When lipid metabolism was corrected by treatment with NO-1886, the onset of cataracts was suppressed. Hyperlipidemia and low HDL-cholesterol levels thus may serve as risk factors for the onset of diabetic cataracts, and NO-1886 shows promise as a means of preventing them.

REFERENCES