Acceleration of Development of Diabetic Cataract by Hyperlipidemia and Low High-Density Lipoprotein in Rats

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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 abnormal lipid metabolism is a risk factor for diabetic cataracts in rats. Cataracts were caused by streptozotocin (STZ) administration in the ordinary diet or cholesterol rich diet fed rats. When rats with STZ (65 mg/kg)-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 53.3% 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 and low high density lipoprotein (HDL) cholesterol, cataracts were observed one week earlier, after 8 weeks of treatment, in 40.0% of animals, with an increase to a 53.3% incidence and an 86.7% incidence after 9 and 10 weeks of STZ treatment, respectively. Plasma glucose levels did not differ between the groups. These results suggest that hyperlipidemia and low HDL cholesterol are associated with an earlier onset and an elevated incidence of diabetic cataracts. We then investigated the relationship between plasma lipids and cataracts by STZ (45–85 mg/kg) administration. The results showed that the onset of cataracts correlated positively with plasma total cholesterol, triglyceride, non-HDL cholesterol and glucose levels, and negatively with HDL cholesterol levels. 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 diabetic cataracts may be accelerated by hyperlipidemia and low HDL cholesterol in rats.

Key words diabetes; cataract; hyperlipidemia; low HDL cholesterol; rat

Diabetic cataracts have been attributed to hyperglycemia due to 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. Therefore, we studied the relationship between diabetic cataracts and abnormal lipid metabolism in detail by determining whether abnormal lipid metabolism is a risk factor for the onset of these cataracts, and whether diabetic cataracts are accelerated by hyperlipidemia.

Diabetic cataracts can be induced in rats by treating them with STZ;2 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. The onset and frequency of diabetic cataracts following the induction of hyperlipidemia was compared to cataract development in control rats with STZ induced diabetes mellitus fed an ordinary diet. 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, high density lipoprotein (HDL) cholesterol, non HDL-cholesterol and glucose levels.

MATERIALS AND METHODS

Materials 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 (normal group) was fed a standard laboratory chow (CRF-1, Oriental Yeast Co., Ltd., Yokohama, Japan). 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. Food consumption was determined in each cage and divided by the number of rats housed per cage to calculate food consumption per animal. STZ, freshly dissolved in 0.01 mol/l citrate buffer, pH 4.5, was administered to rats via the tail vein. Each rat was checked for cataracts once a week using a fundus camera (RC-2621, KOWA Co., Ltd., Osaka, Japan) until 10 weeks after STZ administration. A diagnosis of cataracts was made upon detection of lens opacity. The same examiner checked for and diagnosed cataracts throughout the study in a blind fashion. 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. Aqueous humors were collected using a micro syringe.

All animal experiments were approved by the local animal ethics committee at Otsuka Pharmaceutical Factory, Inc.

Differences in Incidence of Cataracts in STZ Treated Ordinary Diet Fed Rats or Cholesterol Rich Diet Fed Rats STZ was administered to ordinary diet fed rats and cholesterol rich diet fed rats at a dose of 65 mg/kg body weight. Each rat was checked for cataracts once a week.

Effects of STZ on Body Weight, Food Consumption, Plasma Lipid, Glucose and Incidence of Cataracts in

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Cholesterol Rich Diet Fed Rats STZ was administered to cholesterol rich diet fed rats at doses of 45 mg/kg, 65 mg/kg, 75 mg/kg and 85 mg/kg. Ten weeks after STZ administration, cataracts were diagnosed, and blood samples and aqueous humors were collected at the end of the experimental period.

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 (Nippon 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 the total cholesterol, excluding HDL cholesterol and including very low density lipoprotein (VLDL), low density lipoprotein (LDL), remnant and beta VLDL. Plasma albumin and aqueous humor albumin were determined using DRY CHEM 5000 (FUJI FILM, Kanagawa, Japan) by the Methods of Doumas E.T., et al.2) 3)

Histogramical Analysis The 57 rats in the cholesterol rich diet 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.

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 performed using Dunnett’s test. Regarding cataract incidence, two groups were compared for statistical significance by the chi-square test.

RESULTS

Differences in Incidence of Cataracts in STZ Treated Ordinary Diet Fed Rats or Cholesterol Rich Diet Fed Rats Plasma total cholesterol, non-HDL cholesterol and triglyceride levels in the cholesterol rich diet group were higher than the ordinary diet group, but HDL cholesterol levels were lower than the ordinary diet group. On the other hand, plasma glucose levels were similar in both groups (Table 1).

Diabetic cataracts in the ordinary diet group became evident at 9 weeks in 26.7% of animals, and increased to an incidence of 53.3% 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 40.0% of animals, with an increase to a 53.3% incidence and 86.7% incidence after 9 and 10 weeks of STZ treatment respectively. The Incidence of cataracts in the cholesterol rich diet group was significantly higher than in the ordinary diet group (Fig. 1).

Changes of Body Weight and Food Consumption in STZ Treated Diabetic Rats Body weight gain did not differ between the normal diet fed rats (normal group) and the cholesterol rich diet fed rats (control group). Body weights of STZ 45 mg/kg treated cholesterol rich diet fed rats (STZ 45 mg/kg) decreased slightly compared with the control group. Body weight of STZ 65 mg/kg, 75 mg/kg and 85 mg/kg

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Total cholesterol (mg/dl)</th>
<th>HDL-C (mg/dl)</th>
<th>Non-HDL-C (mg/dl)</th>
<th>Triglycerides (mg/dl)</th>
<th>Glucose (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetic rats</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ordinary diet</td>
<td>15</td>
<td>94±13</td>
<td>51±5</td>
<td>43±10</td>
<td>217±60</td>
<td>619±47</td>
</tr>
<tr>
<td>Cholesterol rich diet</td>
<td>15</td>
<td>626±295**</td>
<td>37±6**</td>
<td>562±299*</td>
<td>277±75*</td>
<td>642±34</td>
</tr>
</tbody>
</table>

STZ was administered to rats at a dose of 65 mg/kg body weight. Plasma samples were collected at 10 weeks after STZ administration, and plasma lipids and glucose were measured. Data are expressed as means±S.D. Significantly different from the value in the respective control rats: * p<0.05; ** p<0.01.

![Fig. 1](image1) Differences in the Incidence of Diabetic Cataracts in Ordinary Diet or Cholesterol Rich Diet Fed Rats

STZ was administered to rats at a dose of 65 mg/kg body weight. Each rat was checked for cataracts once a week using a fundus camera. Significantly different from the value in the respective control group: * p<0.05; ** p<0.001.

![Fig. 2](image2) Changes in Body Weight in Cholesterol Rich Diet Fed Rats after the Administration of STZ

STZ was administered to rats at a dose of 45–65 mg/kg body weight. Data are expressed as means±S.D. Significantly different from the value in the respective control group: * p<0.01.
treated cholesterol rich diet fed rats significantly decreased compared with the control group (Fig. 2).

Food consumption did not differ between the normal group, the control group and the STZ 45 mg/kg group. Food consumption in the STZ 65 mg/kg, 75 mg/kg and 85 mg/kg groups increased compared with the control group (Fig. 3).

Changes in Plasma Lipid, Glucose and Incidence of Cataracts in STZ Treated Diabetic Rats Plasma total cholesterol and non-HDL cholesterol levels in the control group were higher than the normal group, but HDL cholesterol levels were lower than the normal group. Plasma total cholesterol, non-HDL cholesterol, triglyceride and glucose levels in the STZ 65 mg/kg, 75 mg/kg and 85 mg/kg treated rats were significantly higher than the control group. However, HDL cholesterol levels were lower than the control group. Changes in plasma lipids were dose dependent in the 65—85 mg/kg body weight range. Plasma glucose showed a sharp increase after 65 mg/kg administration.

Administration of 65 mg/kg, 75 mg/kg and 85 mg/kg STZ caused cataracts in a dose dependent manner, but administration of 45 mg/kg STZ did not cause cataracts (Table 2).

Changes of Aqueous Humor Glucose and Albumin in STZ Treated Diabetic Rats Aqueous humor glucose levels in the control group were slightly higher than the normal group. Aqueous humor glucose levels in the STZ treated rats were higher than the control group. Aqueous humor albumin levels in the STZ 65 mg/kg, 75 mg/kg and 85 mg/kg treated rats were higher than the control group and increased in a dose dependent manner (Table 3).

**Histogram Showing the Relationship between the Incidence of Cataracts and Plasma Lipid and Glucose Levels in STZ Treated Cholesterol Rich Diet Fed Rats**

No cataracts developed when total cholesterol levels were below 200 mg/dl. As the level rose above 200 mg/dl, the incidence of cataracts increased progressively and at over 400 mg/dl reached 75% (Fig. 4A).

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 above 50 mg/dl (Fig. 4B).

As non-HDL cholesterol levels increased, the incidence of cataracts progressively increased. The incidence was 100% when non-HDL cholesterol levels were above 1000 mg/dl (Fig. 4C).

No cataracts developed when triglyceride levels were below 100 mg/dl, but as they rose above 100 mg/dl, the cataract incidence increased progressively and at over 200 mg/dl was 75% (Fig. 4D).

In animals with glucose levels below 500 mg/dl, no cataracts were observed. Cataracts were observed when levels were above 500 mg/dl, at a maximum incidence of 75% (Fig. 4E).

**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 promotes the formation of sorbitol from glucose; the 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 atherosclerosis is higher in diabetic patients than in non-diabetic.

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**Table 2. Plasma Lipids, Glucose and Incidence of Cataracts in STZ-Induced Cholesterol Rich Diet Fed Diabetic Rats**

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Total cholesterol (mg/dl)</th>
<th>HDL-C (mg/dl)</th>
<th>non-HDL-C (mg/dl)</th>
<th>Triglycerides (mg/dl)</th>
<th>Glucose (mg/dl)</th>
<th>Incidence of cataracts (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>10</td>
<td>91±7**</td>
<td>71±8*</td>
<td>18±9*</td>
<td>110±28</td>
<td>152±8</td>
<td>0</td>
</tr>
<tr>
<td>Control</td>
<td>12</td>
<td>166±19</td>
<td>63±9</td>
<td>103±14</td>
<td>97±31</td>
<td>148±12</td>
<td>0</td>
</tr>
<tr>
<td>STZ 45 mg/kg</td>
<td>12</td>
<td>176±24</td>
<td>59±4</td>
<td>118±22</td>
<td>113±20</td>
<td>172±23*</td>
<td>0</td>
</tr>
<tr>
<td>STZ 65 mg/kg</td>
<td>12</td>
<td>651±361**</td>
<td>34±5**</td>
<td>617±363</td>
<td>294±186</td>
<td>683±57</td>
<td>58.3</td>
</tr>
<tr>
<td>STZ 75 mg/kg</td>
<td>11</td>
<td>1051±553**</td>
<td>34±4**</td>
<td>1015±558**</td>
<td>428±231**</td>
<td>649±59</td>
<td>81.8</td>
</tr>
<tr>
<td>STZ 85 mg/kg</td>
<td>12</td>
<td>1369±496**</td>
<td>33±3**</td>
<td>1335±497**</td>
<td>542±224**</td>
<td>633±34</td>
<td>83.3</td>
</tr>
</tbody>
</table>

Plasma samples collected at 10 weeks after STZ administration, plasma lipids and glucose were measured. Data are expressed as means±S.D. Significantly different from the value in the respective control rats: *, p<0.05; **, p<0.01.
Table 3. Aqueous Humor Glucose and Albumin Levels in STZ-Induced Cholesterol Rich Diet Fed Diabetic Rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Aqueous humor</th>
<th>Plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Glucose (mg/dl)</td>
<td>Albumin (g/dl)</td>
</tr>
<tr>
<td>Normal</td>
<td>9</td>
<td>113±26*</td>
<td>0.5±0.1</td>
</tr>
<tr>
<td>Control</td>
<td>11</td>
<td>137±8</td>
<td>0.5±0.1</td>
</tr>
<tr>
<td>STZ 45 mg/kg</td>
<td>12</td>
<td>152±12**</td>
<td>0.5±0.1</td>
</tr>
<tr>
<td>STZ 65 mg/kg</td>
<td>12</td>
<td>577±27**</td>
<td>2.3±1.3**</td>
</tr>
<tr>
<td>STZ 75 mg/kg</td>
<td>10</td>
<td>561±72**</td>
<td>3.5±1.5**</td>
</tr>
<tr>
<td>STZ 85 mg/kg</td>
<td>12</td>
<td>573±62**</td>
<td>3.5±1.5**</td>
</tr>
</tbody>
</table>

Aqueous humor and plasma samples collected at 10 weeks after STZ administration, glucose and albumin levels in aqueous humor and plasma were measured. Data are expressed as mean±S.D. Significantly different from the value in the respective control rats: *, p<0.05; **, p<0.01.

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.

The same study was performed using ordinary diet fed rats and cholesterol rich diet fed rats. When rats with STZ (65 mg/kg body weight)-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 (p<0.001) of treatment. Thus, the onset of cataracts was earlier in diabetic rats with severe hyperlipidemia. The incidence of diabetic cataracts after 9 weeks and 10 weeks (p<0.05) of STZ treatment for the cho-

Fig. 4. Histogram Showing the Relationship between the Incidence of Cataracts with Plasma Lipid and Glucose Levels in STZ Treated Cholesterol Rich Diet Fed Rats

Histographic analysis was performed using the 57 rats in the cholesterol rich diet group to determine the relationship between the incidence of cataracts and plasma lipid and glucose levels. Number of incidences of cataract/total number of rats.
listerol rich diet group was about twice that in the ordinary diet group, suggesting that severe hyperlipidemia increases the incidence of diabetic cataracts. It therefore seems that the onset of diabetic cataracts may be accelerated by abnormal lipid metabolism.

We then investigated the relationship between plasma lipids and cataracts by STZ (45—85 mg/kg) administration. STZ administration caused a significant decrease in body weight and increased food consumption. These results may be because of a compensation for the increase of urinary excretion of glucose and reduction of glucose utilization in diabetic rats.

The STZ treated cholesterol rich diet fed rats caused cataracts in a dose dependent manner. The 57 rats in the STZ treated cholesterol rich diet group were analyzed statistically to determine the relationship between the incidence of cataracts and plasma total cholesterol, triglyceride, HDL cholesterol, non-HDL cholesterol and glucose levels. The results show that the onset of cataracts correlated positively with plasma total cholesterol, triglyceride, non-HDL cholesterol and glucose levels and negatively with HDL cholesterol levels. They thus suggested 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 500 mg/ml. However, cataract incidence did not go above 75% even when the plasma glucose level was over 500 mg/ml, indicating that a combination of hyperglycemia with hyperlipidemia or low HDL cholesterol level precipitates the onset of these cataracts. Hyperglycemia and hyperlipidemia or low HDL cholesterol levels may be involved in the onset of diabetic cataracts. Of particular importance is that below 20 mg/dl of HDL cholesterol and over 1000 mg/dl of non-HDL cholesterol, a 100% incidence of cataracts was observed. These data suggest that low HDL cholesterol and high non-HDL cholesterol with hyperglycemia are significant risk factors in diabetic cataracts.

Hyperlipidemia and low HDL cholesterol levels are known to serve as risk factors for arteriosclerosis.11—13) 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 this study, albumin increased in aqueous humor. This may suggest that the blood-lens barrier or tight junction of choroidal microvessels is disturbed in diabetes. On the other hand, the glucose levels of the aqueous humor was similar to plasma glucose levels. However, the glucose levels of the aqueous humor in the cholesterol rich diet control group were much higher than in the ordinary diet group, although we feel that this difference does not have a specific meaning.

In summary, the incidence of diabetic cataracts was found to be elevated by hyperlipidemia and low HDL cholesterol levels. Hyperlipidemia and low HDL cholesterol levels thus may serve as risk factors for the onset of diabetic cataracts, and cataracts may be accelerated by hyperlipidemia and low HDL cholesterol in STZ induced diabetic rats. Furthermore, these results suggest the importance of correcting hyperlipidemia and low HDL cholesterol to prevent the onset of diabetic cataracts.

REFERENCES