GP-1447, an Inhibitor of Aldose Reductase, Prevents the Progression of Diabetic Cataract in Rats

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We examined the effects of GP-1447 (3-[(4,5,7-trifluorobenzothiazol-2-yl)methyl]-5-methylphenyl acetic acid) on existing cataracts and sorbitol content in the lens in rats with streptozotocin-induced diabetes. GP-1447 is an inhibitor of aldose reductase, which is the first enzyme in the polyol pathway. Cataracts in the central region of the lens were observed in 7 of 14 eyes (50%) by the fifth week after induction of diabetes, and development of mature cataracts was observed in most lenses by the ninth week. In diabetic rats that received GP-1447 treatment beginning in the fifth week after induction of diabetes, progression of cataracts was observed for 1 week after initiation of treatment. Thereafter, the severity of cataracts did not change substantially. Sorbitol levels in the lens peaked during the first week of diabetes, and this increase was maintained during the 9-week observation period. Elevated sorbitol levels in the lenses of diabetic rats gradually declined after GP-1447 treatment was started on the fifth week after induction of diabetes. Cataracts and sorbitol elevation were not observed in the lenses of controls or diabetic rats treated with GP-1447 immediately after induction of diabetes. These results suggest that the polyol pathway plays an important role in both the appearance and progression of cataracts in diabetic rats. Inhibition of aldose reductase could significantly prevent progression of existing cataracts.

Key words  aldose reductase; cataract; diabetes; hyperglycemia; sorbitol

One of the most common complications of diabetes mellitus is cataracts, characterized by opacification or cloudiness of the lens of the eye. The risk of appearance and progression of a cataract depends upon the level of hyperglycemia and duration of diabetes.1–3) Surgery, consisting of removal of the opaque fibers from the lens and replacement with an intraocular implant, is generally accepted as the best method to treat cataract. Despite recent improvements in the quality of artificial lenses and surgical techniques, secondary deterioration in vision can occur through opacification of the posterior capsule.4) Therefore, it is important to establish non-surgical approaches for the prevention and treatment of cataracts.

Several mechanisms that may promote formation of diabetic cataracts have been suggested: activation of the polyol pathway,5–11) non-enzymatic glycosylation (glycation) of lens proteins,12–14) increased oxidative stress,15–18) and elevated Ca2+ concentrations in the lens.19–21) These molecules and biochemical pathways may be therapeutic targets for treatment of diabetic cataracts. Potential therapeutic agents that prevent or slow the progression of cataract must be carefully evaluated in vivo.

Aldose reductase is the first enzyme in the polyol pathway, which converts excess glucose to sorbitol. Sorbitol is further metabolized to fructose by sorbitol dehydrogenase. Previous studies using inhibitors of aldose reductase showed that the polyol pathway plays a critical role in cataract formation in the early stages of diabetes22–25) in rats. However, in most studies, treatment with aldose reductase inhibitors was started immediately after induction of diabetes and then the effects of these inhibitors on cataract formation were evaluated. Therefore, the question of how the polyol pathway contributes to progression of diabetic cataracts remains to be elucidated.

Recently, we developed a lens imaging system for capturing full-lens images of the horizontal plane, without the artifacts of corneal reflection and shadow26) in vivo. The lens imaging device is completely different from those used for clinical and basic research purposes. In most cases, these imaging devices are based on the slit lamp system. However, our system allows us to display cataracts of the entire lens in a single image, and even small vesicles and opacities at the peripheral part of the lens can be precisely identified with high reproducibility.24,26) Using this lens imaging system, we examined the effect of the aldose reductase inhibitor GP-1447 (3-[(4,5,7-trifluorobenzothiazol-2-yl)methyl]-5-methylphenyl acetic acid) (Fig. 1)7) on central opacities observed in diabetic rats in order to determine whether the polyol pathway contributes to the progression of existing cataracts. We also examined effects of this inhibitor on the contents of sorbitol in the lens and retina.

MATERIALS AND METHODS

Animals and Induction of Diabetes All experiments were performed in accordance with the Guidelines for Animal Experiments at Kitasato University as adopted by the University’s Committee on the Care and Use of Laboratory Animals. These guidelines follow the tenets of the Association for Research in Vision and Ophthalmology statement regarding the Use of Animals in Ophthalmic and Vision Research.

Male Wistar rats weighing 160–170 g were maintained in an...
animal room with controlled temperature and humidity under a 12-h light/dark cycle. Animals had free access to standard food (Japan SLC, Inc., Shizuoka, Japan) and water. After 7 d of acclimation, diabetes was induced by a single intravenous injection of streptozotocin (65 mg/kg; Nacalai Tesque Inc., Kyoto, Japan) dissolved in citrate buffer (pH 4.5). Control rats were treated with an equal volume of vehicle alone. Induction of diabetes was confirmed by elevated non-fasting plasma glucose levels (>350 mg/dL) 2 d after streptozotocin injection. The rats were given drinking water containing 5% D-glucose following treatment with streptozotocin to minimize variability and to hasten the development of diabetic cataracts by maintaining extremely high plasma glucose levels. Plasma glucose concentration was determined using a commercially available enzyme kit in accordance with manufacturer’s instructions (Glucose Test Wako; Wako Pure Chemical, Osaka, Japan).

**Treatment of GP-1447** The aldose reductase inhibitor GP-1447 (0.625 mg/mL in stock solution) was diluted in 5% D-glucose solution (final concentration, 0.01 mg/mL) and provided *ad libitum* in the drinking water. The treatment with GP-1447 started immediately or 5 weeks after confirmation of induction of diabetes. The doses of GP-1447 were selected based on the results of previous studies.

**Evaluation of Cataracts** The animals were divided into 4 groups: control (Control; n=6), diabetic rats (DM; n=7), diabetic rats with immediate GP-1447 treatment (DM+GP 0 week; n=4), and diabetic rats with treatment beginning in the fifth week after induction of diabetes (DM+GP 5 week; n=6).

The progression of cataracts was assessed on a weekly basis, as described previously. In brief, high-resolution images of the entire lens in the horizontal plane were captured using our original digital camera system equipped with a non-reflecting illuminator. The severity of diabetic cataracts was assessed by an observer-based scoring method and by quantitative analysis of the digital images of lenses. For the observer-based scoring method, the status of the lens was scored according to classification of lens opacification: score 0, clear; score 1, peripheral vesiaces and opacities; score 2, central opacities; score 3, diffused opacities; score 4, mature cataract; and score 5, hypermature cataract. The cataract score for each animal was obtained by averaging the scores of the right and left lenses. For quantitative image analysis, the opaque area in the central region of the lens (diameter: 30% of the eyeball diameter), representing the region that directly affects vision, was evaluated using Adobe Photoshop CS4 (Adobe Systems, San Jose, CA, U.S.A.) software as reported in our previous studies. The opacity was calculated as the ratio of number of pixels in the opaque area and the total number of pixels in the selected central region of the lens. These values were expressed as percentages. The right and left lens opacities were averaged in each animal.

**Measurement of Sorbitol in the Lens and Retina** For measurement of sorbitol content in the lens and retina, control and diabetic rats were sacrificed at 1 week (Control, n=3; DM, n=3), 5 weeks (Control, n=3; DM, n=3), 7 weeks (Control, n=3; DM, n=3), and 9 weeks (Control, n=3; DM, n=4) after induction of diabetes. In the groups that received immediate GP-1447 treatment or treatment starting at 5 weeks after induction of diabetes, animals were sacrificed at 7 weeks (DM+GP 0 week, n=3; DM+GP 5 week, n=4) or 9 weeks (DM+GP 0 week, n=4; DM+GP 5 week, n=3) after induction of diabetes.

The content of sorbitol in the lens and retina was measured as described previously. In brief, tissues were homogenized in 16% perchloric acid (0.04 mL/mg tissue) and then neutralized with 2 M potassium carbonate. The homogenates were centrifuged (5500×g for 10 min) at room temperature. The content of sorbitol in the supernatants was measured using a commercially available kit according to the manufacturer’s instructions (F-kit, τ-sorbitol/xylitol; R-Biopharm AG, Darmstadt, Germany). The tissue sorbitol content was expressed as micromoles of sorbitol per gram of wet weight of tissue (μmol/g).

**Data Analyses** Unpaired *t*-test and Tukey’s test were used for the comparisons between 2 groups and among more than 2 groups, respectively (GraphPad, San Diego, CA, U.S.A.). For comparing the time course of changes in blood glucose and body weight in the experimental groups, two-way analysis of variance (ANOVA) was used. A *p*-value of less than 0.05 was considered statistically significant. All values are presented as the mean±S.E.M.

**RESULTS** Non-fasting plasma glucose levels were significantly higher in streptozotocin-treated rats than in control rats (Fig. 2A), whereas body weights were significantly lower in diabetic rats than in controls (Fig. 2B). There were no significant differences in plasma glucose levels and body weights between DM and DM+GP 0 week or DM+GP 5 week groups. The average water intakes in Control, DM, DM+GP 0 week, and DM+GP 5 week groups were 196±10 (n=6), 2164±65 (n=7), 1788±58 (n=4), and 1962±50 (n=6) mL·kg−1·d−1, respectively. Water intakes were significantly (*p<0.05) increased in diabetic rats. GP-1447 treatment did not affect water intakes. Based on water intake data, the daily intake of GP-1447 in the DM+GP 0 week group was estimated to be 17.9±0.6 mg·kg−1·d−1. In the DM+GP 5 week group, the daily intake of GP-1447 was 18.9±0.5 mg·kg−1·d−1. These values were not significantly different. The food intake also increased in diabetic rats, but the increased food intake was unaffected by the GP-1447 treatment (data not shown).

All lenses in Control and DM+GP 0 week groups were clear throughout the experimental period (Fig. 3, Table 1). In the DM group, the cataracts progressed depending on the length of the diabetic period, and 71% of lenses (10/14) had developed hypermature cataracts 9 weeks after induction of diabetes (Table 1). Cataracts in the central region were observed at week 7 or later. The results obtained from quantitative analyses are summarized in Fig. 4. Similar results were obtained with the scoring method (Fig. 4A) and the measurement of the opaque area in the central region (Fig. 4B). These clearly show that the severity of cataracts increased with the length of the diabetic period. In the DM+GP 5 week group, no progression of existing cataracts was observed at week 7 or later.

In the lenses of diabetic rats, the maximum increase in sorbitol content was observed during the first week after
induction of diabetes. This increased level was maintained throughout the observation period (Fig. 5A). In contrast, the sorbitol content in the retina increased in a time-dependent manner in diabetic rats (Fig. 5B). There was no increase in sorbitol content in the lenses and retinas of Control and DM+GP 0 week groups. Sorbitol levels in the lenses of diabetic rats declined gradually after GP-1447 treatment beginning in the fifth week after induction of diabetes. As a result, in the seventh and ninth weeks, the levels of sorbitol in the lenses in the DM+GP 5 week group were significantly lower than those in the DM group, but still higher than those in the Control group. The rate of decline in sorbitol content was slower in the retina than in the lens.

DISCUSSION

The present study demonstrates that cataract formation was completely prevented by treatment with the aldose reductase
inhibitor GP-1447 immediately after induction of streptozotocin-induced diabetes in rats. In addition, GP-1447 treatment that was started after central opacities had become apparent in the lens almost completely blocked further progression of the cataracts. These results strongly suggest that the polyol pathway plays a crucial role in both the appearance and...

### Table 1. Effect of GP-1447 on the Formation and Progression of Cataracts in Rats with Streptozotocin-Induced Diabetes

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Each lens was monitored once a week and scored according to an established scoring scale. Score 0, clear lens (no cataract); score 1, peripheral vesicles and opacities; score 2, central opacities; score 3, diffuse central opacities; score 4, mature cataract; and score 5, hypermature cataract. The data represent the number of lenses. After 2 weeks of hyperglycemia, onset of cataracts was observed in 71% of lenses of diabetic rats (DM). After 9 weeks of hyperglycemia, all lenses of diabetic rats had mature or hypermature cataracts, whereas mature cataract were observed in only 1 lens of a diabetic rat whose GP-1447 treatment was started 5 weeks (DM+GP 5 week) after induction of diabetes. Lenses of control rats (Control) and diabetic rats receiving immediate treatment with GP-1447 (DM+GP 0 week) were clear throughout the experimental period.

**Fig. 4. Changes in the Cataract Score and Opacity in the Central Region of the Lens in Each Experimental Group**

The progression of cataracts until 6 weeks after induction of diabetes was practically identical between untreated diabetic rats (DM) and diabetic rats receiving GP-1447 treatment beginning 5 weeks after induction of diabetes (DM+GP 5 week). In control rats (Control) and diabetic rats treated with GP-1447 immediately after induction of diabetes (DM+GP 0 week), formation of cataracts was not observed. Each point with a vertical bar represents the mean±S.E.M. from 4–7 animals. *p<0.05, compared with the DM group.
progression of cataracts in diabetic rats.

Under diabetic conditions, glucose is present in high concentration in the aqueous humor and can enter the lens by passive, facilitated, and insulin-independent transport mechanisms. The enzyme aldose reductase converts glucose to sorbitol, which accumulates because it cannot diffuse passively out of the lens. Indeed, we found that sorbitol contents were higher in the lenses of diabetic rats. This increase reached a maximum during the first week after induction of diabetes and remained constant throughout the observation period. Despite the rapid elevation of sorbitol level within 1 week, several weeks were required to establish mature cataracts. The finding indicates that multiple steps may be involved in the process of cataract formation. At present, the role of the activated polyol pathway and elevated sorbitol levels in the pathogenesis of diabetic cataracts are not fully understood, but several roles have been suggested. For example, increased sorbitol levels could cause osmotic changes, which lead to morphological changes in the lens, such as hydration and swelling. Osmotic stress induced by accumulated sorbitol has long been suspected as the major factor in the formation of diabetic cataracts. However, several recent findings indicated that the activation of the polyol pathway generates oxidative stress in the lens, and there is strong evidence suggesting that the development of diabetic cataracts is associated with increased oxidative stress. Furthermore, glycation of lens proteins may play a role in lens opacification because sorbitol dehydrogenase metabolizes sorbitol to fructose and consequently fructose 3-phosphate. Fructose and fructose 3-phosphate are glycation agents. Thus, activation of the polyol pathway is likely to contribute to diabetic cataract formation through osmotic and other mechanisms.

GP-1447 treatment beginning in the fifth week after induction of diabetes gradually reduced sorbitol contents in lenses in diabetic rats. However, sorbitol levels in the seventh and ninth weeks were still much higher in lenses of diabetic rats than in those of controls. The slower clearance of sorbitol may be explained by the low activity of sorbitol dehydrogenase, which catalyzes the conversion of sorbitol to fructose. A previous report demonstrated that cataract formation was accelerated in diabetic rats treated with sorbitol dehydrogenase inhibitors, whereas no difference in cataract formation was observed in galactose-fed rats treated or rats not treated with sorbitol dehydrogenase inhibitors. On the other hand, aldose reductase inhibitors prevented cataract formation in both diabetic and galactosemic rats. Therefore, the activity of aldose reductase, rather than that of sorbitol dehydrogenase, may play an important role in determining the sorbitol content of the lens. Although the sorbitol contents in lenses in the seventh and ninth weeks were still much higher in diabetic rats than in control animals, the progression of existing cataracts was completely blocked. These data imply that sorbitol concentrations must rise above a certain threshold level for cataract formation.

GP-1447 treatment almost completely blocked progression of central opacities in lenses, but the cataracts did not disappear. Under prolonged hyperglycemic conditions, irreversible changes occur in the long-lived molecules, extracellular matrix, eye lens crystallins, and chromosomal DNA in lenses. Therefore, reduction of sorbitol levels through inhibition of aldose reductase may not ameliorate diabetic cataracts. However, it is unclear whether prolonged GP-1447 treatment can reduce the severity of diabetic cataracts. Further studies are required to determine the effect of longer treatment with GP-1447 on existing cataracts.

Diabetic retinopathy is a major complication of diabetes mellitus, and several studies on animal diabetic models have demonstrated the potential of aldose reductase inhibitors as therapeutic agents for diabetic retinopathy. We therefore measured sorbitol contents in the retinas. Surprisingly, the content of sorbitol in the retina increased slowly and reached a maximum at 9 weeks after induction of diabetes. Thus, there are substantial differences between the retina and the lens in the rates and levels of sorbitol accumulation, probably due to the differences in activities of aldose reductase and sorbitol dehydrogenase. We previously demonstrated that morphological changes are detected in the retina 2 weeks after induction of diabetes, as indicated by the reduction in thickness of the inner plexus layer, and that these changes are prevented by GP-1447 treatment in the same experimental model used in this study. The retinal damage observed in this model could therefore be mediated by activation of the polyol pathway. However, retinal neurons may be more vulnerable than lens cells to the effects of accumulated sorbitol.

In the present study, no significant difference was observed in blood glucose levels between diabetic rats that were treated
with GP-1447 and those that were not treated. These results indicate that the effects of GP-1447 on cataracts and changes in sorbitol content were not due to changes in blood glucose levels. Furthermore, GP-1447 treatment had no effect on body weight and food/water intake, suggesting that non-selective, secondary effects did not contribute to the control or progression of cataracts.

In many studies, treatment with potential therapeutic agents for diabetic cataracts is started immediately after induction of diabetes. Therefore, effects of these agents on existing cataracts cannot be determined. One explanation for this lack of data may be that conventional lens imaging methods, including slit lamp imaging, result in large variations in data due to corneal reflection of the illuminating light. In addition, entire lens images are difficult to obtain with conventional methods. These points limit the reliability of the assessment of opacity \textit{in vivo}. In contrast, the imaging system used in the present study allows us to non-invasively capture horizontal full lens images without reflection artifacts.\textsuperscript{24} These images can be used to prepare a precise planar map of the locations of opacities in the entire lens and repeatedly compare pathological characteristics of the same lens. The present study clearly demonstrates that our \textit{in vivo} cataract imaging method is helpful for accurately evaluating effects of therapeutic drugs on the progression of existing cataracts.

In summary, we found that the aldose reductase inhibitor GP-1447 showed preventive effects on diabetic cataract formation and completely prevented an increase in sorbitol accumulation in the lens. Furthermore, we showed that GP-1447 prevented the progression of existing cataracts. Although the cataracts did not disappear during the treatment period, agents that can prevent the progression of existing cataracts are useful for treatment of this complication. Thus, aldose reductase inhibitors have the potential to prevent the appearance of cataracts and the progression of existing cataracts under diabetic conditions.

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