A Novel High Resolution In Vivo Digital Imaging System for the Evaluation of Experimental Cataract in Diabetic Rats

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Abstract. The purpose of this study was to establish a novel imaging system for evaluation of cataract in small animals. Diabetic cataract was induced in male Wistar rats by a combination of a bolus injection of streptozotocin (65 mg/kg, i.v.) and 5% D-glucose given as drinking water. To assess cataract development, we designed a digital camera system equipped with a non-reflecting illuminator for capturing clear high-resolution full lens images in the horizontal plane. Cataract was evaluated from the resulting images using both an observer-based scoring system and quantitative digital image-analysis techniques. The onset of cataract was detected in the peripheral section of the lens in 57% of cases 2 weeks after induction of hyperglycemia. Central opacities were visible following 3 weeks in hyperglycemic conditions. The cataract increased in severity with time, so that by week 9 in hyperglycemic conditions, mature cataracts were developed in over 90% of lenses. Treatment of diabetic rats with GP-1447, an aldose reductase inhibitor, completely prevented the formation of diabetic cataracts. These results indicate that the digital imaging system established in the present study permits an assessment of all stages of cataract development and it is helpful for accurately evaluating the effects of therapeutic drugs on cataracts.

Keywords: cataract, diabetes, lens, streptozotocin, imaging

Introduction

A cataract is characterized by opacification or cloudiness of the lens of an eye and this disease is one of the most common complications of diabetes mellitus (about 20% of the patients are considered to suffer from cataracts). It is in fact the leading cause of blindness worldwide (1). The risk of formation and progression of a cataract increases depending upon the level of hyperglycemia and duration of diabetes (2 – 6). Presently, surgery is generally accepted as the best way to treat cataract and the procedure consists of removing the opaque fibers from the lens and replacing them with an intraocular lens implant. Despite recent improvements in the quality of artificial lenses and surgical techniques, a secondary deterioration in vision can occur through posterior capsule opacification (PCO) (7). Therefore, it is important to establish non-surgical approaches for the prevention or treatment of cataracts.

Imaging of the lens plays a vital role in the understanding of cataract formation and subsequent evaluation of the benefits of putative therapies. The most common method of imaging the lens in vivo is by a slit lamp technique. Since the invention of the Scheimpflug camera, a slit lamp has been combined with the camera (8 – 10). While this method has many merits and greatly aided our evaluation of cataract in both patients and small animal studies (11 – 15), it is by no means a perfect system. Using Scheimpflug slit imaging devices, it is very difficult to obtain the entire lens image since it is necessary to move the equipment around the lens and take multiple photographs.

Another confounding problem with slit lamp imaging systems is the corneal reflection of the illuminating light. These reflection artifacts can limit the reliability in assessment of the opacity. Moreover, quantification of
Materials and Methods

Animals and induction of diabetes

All experiments were performed in accordance with the Guidelines for Animal Experiments in Kitasato University adopted by the Committee on the Care and Use of Laboratory Animals of Kitasato University and tenets of the ARVO statement for the Use of Animals in Ophthalmic and Vision Research.

Prior to the experiment, male Wistar rats weighing 160 – 170 g were maintained at least 1 week on standard rat chow and tap water ad libitum under a 12:12-h dark cycle in a quiet environment. Diabetes was induced by a single intravenous injection of streptozotocin (65 mg/kg) (Sigma, St. Louis, MO, USA) dissolved in citrate buffer (pH 4.5). Control rats were treated with an equal volume of vehicle. Induction of diabetes was confirmed with high plasma glucose levels (>350 mg/dl) on the third day after streptozotocin injection. To accelerate the formation of diabetic cataract by keeping extremely high concentrations of plasma glucose (>700 mg/dl), rats were given 5% D-glucose in their drinking water following treatment with streptozotocin. Plasma glucose levels were determined with a commercially available kit (Glucose Test Wako; Wako Pure Chemical, Osaka).

Assessment of cataracts

The severity of diabetic cataract was assessed by an observer-based scoring system and by quantitative digital image-analysis techniques. According to the classification of lens opacification as reported previously (16 – 19), the status of lens was scored as follows: score 0: clear (no cataract), score 1: peripheral vesicles and opacities, score 2: central opacities, score 3: diffused opacities, score 4: mature cataract, and score 5: hypermature cataract (Fig. 2). Quantitative assessment of the cataract was performed by determination of the opaque area in the central region of the lens representing the region that directly affects vision. As shown in Fig. 3, we selected the central region that is outlined by a broken circle (its diameter is 30% of that of the eyeball) (Fig. 3A) and converted the image from full-color to grayscale using the software Adobe Photoshop 7.0 (Adobe Systems, Tokyo) (Fig. 3B). After intensifying the contrast of the image, the opaque regions were distinguished from background by determining a certain threshold level for each image (Fig. 3C). The threshold level was determined by several experts to minimize the inter-observer variation. The numbers of pixels of the

Lens photography

Figure 1A shows an original digital camera system we developed for capturing rat lens images. The digital still camera (CoolPix 5000; Nikon, Tokyo) and the macro lens were coupled with the relay lens in order to obtain sharp images with an appropriate size for assessment of cataract. The illuminating device was placed at the tip of the macro lens and a positioning ring attached inside the device (Fig. 1: B and C). Several positioning rings with different inner diameters (6.1 – 7.1 mm) and lengths were prepared for eyes of varying sizes. Illuminating lights are horizontally projected to the rat lens from 16 directions around the positioning ring (Fig. 1C).

To take photographs of the lenses, the rats were anesthetized with diethyl ether. The pupils were dilated with one drop of 1% atropine sulfate (Nihon Tenganyaku Institute, Nagoya). To protect the cornea surface, hydroxyethyl cellulose solution (SCOPISOL 15®; Senju Pharmaceutical, Osaka) was placed at the interface between the positioning ring and the eyeball (Fig. 1D). The rat eyelid was slightly pressed with the positioning ring and the rat position was adjusted, if necessary, for exact focusing. The digital camera allowed us to take photographs while observing the lens appearing on the 14-inch display. The appropriate shutter speed (1/60) and aperture value (F6.1, F6.8, or F7.5) of the camera was chosen. Digital images of lenses were saved on a built-in compact-flash memory and transferred to a personal computer for analysis of the opaque part of the lens. Figure 1E shows a representative lens image captured by the digital camera system. The light reflected from the surface of the cornea is not observed in the image. The entire lens that develops hypermature cataract and the iris vasculature are clearly shown. For comparison, the vertical-sectional lens image was captured with the slit lamp-type imaging system (SL-1800; Nidek, Nagoya) (Fig. 1F).
Opaque region and the selected region were counted using the software NIH Image 1.63 (National Institute of Health, Bethesda, MD, USA). The opacity was calculated as percentage of the number of opaque area pixels to the total number of the pixels in the selected region of the lens. The right and left lens opacities in each animal were averaged.

Drug treatment
We observed the effect of the aldose reductase
inhibitor GP-1447, which has been reported to prevent completely the diabetic cataract (20), in order to determine whether the drug can inhibit cataract formation even under our severe experimental conditions. Seven diabetic rats were fed on chow containing 0.01% w/w GP-1447 for 9 weeks from the third day of streptozo-
tocin injection. The concentration was chosen on the basis of the previous report (20).

Statistical analyses

Data are presented as means ± S.E.M. The significance of the difference between mean values was evaluated by the Bonferroni-Dunn test for multiple comparisons after analysis of variance (ANOVA). A P value smaller than 0.05 was considered to be statistically significant.

Results

Body weights, plasma glucose levels, and food/water intakes

Table 1 summarizes body weights, plasma glucose levels, and food/water intakes in each experimental group. Plasma glucose levels of streptozotocin-treated rats were significantly higher than those of control rats. Body weights of diabetic rats were significantly lower than those of control rats, whereas intakes of food and water were significantly increased in diabetic rats. There was no difference in plasma glucose levels, body weights, and food/water intakes between diabetic rats treated with and without GP-1447. The averaged food intake in GP-1447–treated diabetic rats was 172 ± 5 g/kg per day (n = 7); therefore, it is estimated that the daily intake of GP-1447 was 17.2 ± 0.5 mg/kg.

Assessment of diabetic cataracts

All lenses in control and GP-1447-treated diabetic rats were clear throughout the experimental period. In diabetic rats, cataracts were observed in 8 of 14 lenses (57%) 2 weeks after diabetes induction (Table 2). The cataracts progressed depending on the length of the diabetic period. Nine weeks after induction of hyperglycemia, 93% of lenses (13 of 14 lenses) developed mature cataracts. The relationship between the averaged cataract score and duration of diabetes in each experimental group is shown in Fig. 4.

Figure 3D shows the time-course of opacity developed in the central region of lens quantified by image analysis methods. The digital image of rat lens 6 weeks after induction of diabetes (A) and the processed images (B and C). A central region outlined by the broken circle (radius = 220 pixels) was selected (A) and converted to a grayscale image (B). After intensifying the contrast of the image, the opaque regions were determined with a certain threshold level (C). The numbers of pixels of the opaque region and the selected central region were counted in the binary image. The opacity was calculated as a percentage of the opaque area in the area of the selected region. Numbers of pixels of the central region outlined by the broken circle and of the opaque parts are 152,053 pixels and 26,514 pixels, respectively, in this case. Thus, the value of opacity is 17%. Since the peripheral vesicles and cortical opacities are observed in this lens, the score based on the scaling method is 2. Panel D shows time-dependent progression of opacity in the central region of the lens in diabetic rats (DM). After 2 weeks of hyperglycemia, the opacity increased depending on the length of the diabetic period; however, cataract formation at early stages of diabetes was not detected. In control rats and diabetic rats treated with GP-1447 (DM + GP), the opacification of lenses was not observed. Values are each expressed as the mean ± S.E.M., number of animals = 5 - 7.
Evidence of opacification was observed from 3 weeks after hyperglycemia induction, and from this point, the opacity became more severe with time for the hyperglycemic animals. Treatment with GP-1447 totally prevented cataract formation in the central region of the lens of hyperglycemic animals.

Discussion

We report here a new digital imaging system for assessment of cataract in vivo, which is not based on any slit lamp system, and allows us to capture clear high-resolution full lens images in the horizontal plane without obstructive light reflection from the corneal surface. Using the horizontal full lens images without reflection artifacts, we can make a precise planar map of the location of the opacity in the entire lens and compared pathological characteristics of the same lens repeatedly. Therefore, it is possible to determine the onset and follow progression of a cataract precisely in the same animal. Moreover, the quantitative assessment of the opacity of the lens by using digital image-analysis techniques is also possible. Thus, the novel in vivo

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**Table 1.** Body weight (BW), plasma glucose levels, and intakes of food and water in each experimental group

<table>
<thead>
<tr>
<th></th>
<th>Initial BW (g)</th>
<th>Final BW (g)</th>
<th>Plasma glucose (mg/dL)</th>
<th>Food intake (g/kg per day)</th>
<th>Water intake (L/kg per day)</th>
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<tbody>
<tr>
<td>Control</td>
<td>175 ± 4</td>
<td>446 ± 24</td>
<td>153 ± 4</td>
<td>73 ± 3</td>
<td>0.086 ± 0.004</td>
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<tr>
<td>DM</td>
<td>170 ± 3</td>
<td>231 ± 13*</td>
<td>818 ± 14*</td>
<td>177 ± 16*</td>
<td>1.8 ± 0.07*</td>
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<tr>
<td>DM+GP</td>
<td>169 ± 2</td>
<td>184 ± 7*</td>
<td>902 ± 17*</td>
<td>172 ± 10*</td>
<td>1.5 ± 0.07*</td>
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Control: control rats, DM: diabetic rats, DM + GP: diabetic rats treated with GP-1447. GP-1447 was administered using chows containing 0.01% w/w of the drug. Values are each expressed as the mean ± S.E.M., number of animals = 5 – 7. *P<0.001, compared with the control group.

**Table 2.** Cataract formation in the lenses of streptozotocin-treated rats given 5% D-glucose in their drinking water

<table>
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<tr>
<th>Week(s)</th>
<th>Score</th>
<th>Control</th>
<th>DM</th>
<th>DM + GP</th>
<th>Control</th>
<th>DM</th>
<th>DM + GP</th>
<th>Control</th>
<th>DM</th>
<th>DM + GP</th>
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<th>Control</th>
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Each lens was monitored once a week and scored according to an established scoring scale. Score 0: clear (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 the lens. After 2 weeks of hyperglycemia, the onset of cataract was observed in 57% of lenses of diabetic rats (DM). Lenses of control rats and GP-1447-treated diabetic rats (DM + GP) were clear throughout the experimental period.
cataract imaging method permits an assessment of all stages of cataract development and it is helpful for accurately evaluating the effects of therapeutic drugs on cataracts.

One of the biggest problems for lens photography is the corneal reflection of the illuminating light. In fact, the light reflected from the surface of the cornea prevents the recording of a precise image of the opaque part of the lens, when the illuminating light is projected from the upper side of the eye. However, if we project the light from the horizontal direction, the light reflected from the surface of the cornea will go outside the image of the lens. Therefore, we designed an illuminator that horizontally projects the light on the lens. To illuminate all parts of the lens equally, the light is projected from 16 directions around the positioning ring. Using the digital camera system equipped with the non-reflecting illuminator, we succeeded in capturing clear high-resolution full lens images without corneal reflection artifacts and any shadows. The imaging system, which overcomes the limitation due to the reflection artifacts, allows us to estimate the severity of cataract with much greater precision.

The severity of cataract could be assessed by two methods: 1) the observer-based scoring method using standardized photographs and a grading system and 2) the quantitative method using digital image-analysis techniques. The assessment of cataract based on the scoring system is frequently used either for clinical or basic research purposes. This method is a useful tool to identify the morphology, density, and location of the cataract within the lens; however, considerable inter-observer variability exists even when evaluations are performed by experts in the field. The inconsistency may be, at least in part, related to the quality of lens photographs taken. Therefore, inter- and intra-observer variation would be reduced by using clear high-resolution lens images without any reflection artifacts. With the new imaging system that captures the full lens image in the horizontal plane, a single image is sufficient to evaluate the morphological changes of cataract in the entire lens. This is also an advantage of our imaging system and it enables us to obtain more accurate results with good reproducibility. However, because the results are subjective and qualitative, it is important to assess the severity of cataract quantitatively.

One quantitative method for assessing the severity of cataract is to measure the opaque area in the lens using digital image-analysis techniques. The high-resolution digital images without any reflection artifacts allow us to adopt the quantitative method. In the present study, we evaluated the opacity in the central region of the lens because the region is the most important part that directly affects vision. Diabetic cataracts are detected in the periphery of the lens at the early stage of the disease; therefore, the measurement of opacity in the central regions does not provide exact information on the onset of cataracts. Indeed, subtle cataract, such as peripheral vesicles and opacities, was detected in 57% of lenses after 2 weeks of hyperglycemia, whereas the values of opacity measured at the same time in the central regions were nearly 0. However, the data clearly indicate that, after the central opacity had begun to form, the opacity increased depending on the length of diabetic period. Apparently, changes in opacity in the central region of the lens could be a measure of the progression of visual disorder induced by diabetic cataracts.

As shown in the lens images presented in this paper, the blood in the choroidal/retinal circulation can be seen through the lens because of the absence of choroidal and retinal epithelial pigmentation in the albino rats. The influence of the blood on the lens image is different among lens images that are captured on different occasions. In the present study, the lens images were captured at certain conditions that minimize the influence of blood on the central region of the lens because we intended to assess the cataract in the region. Therefore, the images were not suitable for quantification of opacity at the other regions (e.g., peripheral region). However, the images, which are suitable for application of digital image analysis to the other regions, are captured by simply selecting the appropriate camera setting. Combining images captured under several different conditions, it is possible to assess quantitatively the opacities in all regions of the lens.

A single image in the horizontal plane captured with our novel imaging system allows us to make a precise planar map of the location of the opacity and to assess quantitatively the severity of cataract. However, it does not provide three-dimensional information on the opacity in the lens. One approach to make a detailed three dimensional-map of the opacity would be to combine information obtained from the vertical-sectional lens images that are taken with the slit lamp-type imaging system and horizontal lens images that are captured by the imaging system presented in this paper. This approach should be established in future studies.

We used streptozotocin-induced diabetic rats, which were given 5% D-glucose in their drinking water, as an animal model of diabetes mellitus. The rats maintained plasma glucose at extremely high levels (>700 mg/dl) and developed mature cataracts within 2 months. Because the cataracts induced by prolonged exposure to the severe hyperglycemia were completely prevented by treatment with GP-1447, an inhibitor of aldose reductase, the aldose reductase-dependent polyol pathway...
plays a critical role in the cataract formation also in our animal model, as demonstrated in the previous studies carried out under milder experimental conditions (18, 20 – 24). For screening potential therapeutic agents that prevent or slow the progression of cataract, it is important to make reproducible cataracts in a time period as short as possible. Therefore, the combination of streptozotocin treatment and D-glucose feeding would be a useful procedure to minimize the variability and the term in the development of diabetic cataracts in rats.

In conclusion, we have established a novel method for non-invasively and precisely assessing the opacity of the rat lens by developing an in vivo cataract digital imaging system for capturing high-resolution lens images of the horizontal plane without any corneal reflection artifacts. The present results demonstrate that the high-resolution horizontal lens imaging is a valuable method for in vivo studies of cataract in small animals.

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References