Full Paper

Effect of Nifedipine on Severe Experimental Cataract in Diabetic Rats

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Abstract. We examined the effects of Ca²⁺-channel blockers on sugar cataract formation in streptozotocin (65 mg/kg, i.v.)-induced diabetic rats that were given 5% D-glucose as drinking water. The diabetic rats were treated with an L-type Ca²⁺-channel blocker, nifedipine or verapamil, for 9 weeks from the 3rd day of streptozotocin injection. Using the full lens images of the horizontal plane captured with the new digital camera system that we developed recently, the cataract formation was quantitatively assessed in parallel with the conventional scaling method. In the animal model of diabetes mellitus, the cataracts at the peripheral region of the lens were detected 2 weeks after induction of hyperglycemia and progressed depending on the length of the diabetic period. The majority of them developed mature cataracts after 9 weeks of hyperglycemia. Nifedipine slowed the progression rate of diabetic cataracts without affecting the period of time required for the onset of this disease, whereas verapamil had no significant inhibitory effect on the diabetic cataract. These findings suggest that nifedipine may be considered as a candidate drug to suppress the progression of diabetic cataracts.

Keywords: cataract, diabetes, lens, L-type Ca²⁺ channel, streptozotocin

Introduction

Cataract is one of the most common complications of diabetes mellitus; about 20% of the patients suffer from cataracts. The risk of formation and progression of a cataract increases depending upon the level of hyperglycemia and duration of diabetes (1 – 5). Multiple mechanisms, such as activation of the polyl pathway in glucose disposition (6 – 11), non-enzymatic glycation of lens proteins in the eye (12 – 14), increases in oxidative stress (15 – 18), and increases in Ca²⁺ concentrations in the lens (19 – 21), and so on, are considered to be involved in development of diabetic cataracts. Therefore, drugs targeting the molecules and biochemical pathways present in the lens may be candidates for drug therapy of cataracts.

Blockade of L-type Ca²⁺ channels in the lens is one of the possible pharmacological interventions to prevent diabetic cataracts (20, 22 – 24). Since both systemic and topical administration of verapamil, an L-type Ca²⁺-channel blocker, have been reported to prevent cataract formation in diabetic rats (20, 22, 23), Ca²⁺ influx into lens cells via L-type Ca²⁺ channels has been suggested to be involved in the mechanism of this process. Although the detailed mechanism for how elevated Ca²⁺ in the lens induces cataracts is not understood, activation of Ca²⁺-dependent proteins, such as calpain (25); impairment of cell communication among lens fibers (26); changes in lens permeability (27); loss and/or cleavage of some proteins (28, 29); and formation of high molecular weight proteins (30) have been suggested. Furthermore, it also may be possible that verapamil inhibits diabetic cataract as an anti-oxidant drug (31). Thus, we sought to obtain more information on the effect of other L-type Ca²⁺-channel blockers on diabetic cataracts.

The purpose of this study, therefore, was to determine whether the L-type Ca²⁺-channel blocker nifedipine exerts an inhibitory effect against diabetic cataract in rats. Using the non-invasive and repeatable in vivo digital camera system designed for capturing full lens images of small animals, we assessed the effect of nifedipine and verapamil on the onset and progression of...
diabetic cataracts by comparing the severity of cataracts with two methods based on different principles (32). This work has been partly presented at the 114th Kanto Area Regional Meeting of The Japanese Pharmaceutical Society.

**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 experiments, 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 with fluorescent lamp covered with a 605-nm sharp-cut filter (Annaka Tokusyu Glass, Osaka) 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. Rats were given drinking water containing 5% D-glucose following treatment with streptozotocin to minimize the variability and to shorten the term in the development of diabetic cataracts by keeping extremely high plasma glucose levels (32). Plasma glucose levels were determined with a commercially available kit (Glucose Test Wako; Wako Pure Chemical, Osaka).

**Evaluation of cataracts**

The progression of a cataract was assessed on a weekly basis as described previously (32). In brief, the high-resolution full lens images in the horizontal plane were captured with the digital camera system equipped with the non-reflecting illuminator. Using the digital images of lenses, the severity of diabetic cataract was assessed by an observer-based scoring method and by a quantitative image-analysis method (32).

**The observer-based scoring method**

The status of the lens was scored according to the classification of lens opacification (33 – 36): score 0: clear, score 1: peripheral vesicles and opacities, score 2: central opacities, score 3: diffused opacities, score 4: mature cataract, and score 5: hypermature cataract.

**The quantitative image-analysis method**

We determined the opaque area in the central region of the lens representing the region that directly affects vision using the software Adobe Photoshop 7.0 (Adobe Systems, Tokyo) and the software NIH Image 1.63 (National Institute of Health, Bethesda, MD, USA) as shown in Fig. 4. We selected the central region outlined by a broken circle (its diameter is 30% of the eyeball diameter) (Fig. 4A) and converted the image from full-color to grayscale (Fig. 4B). The contrast of the image was intensified and then the opaque regions were distinguished from background by empirically determining a certain threshold level for each image (Fig. 4C). The numbers of pixels of the opaque region and the selected central region were counted. The opacity was calculated as the percentage of the number of opaque area pixels to the total number of the pixels in the selected central region of the lens. The right and left lens opacities in each animal were averaged.

**Drug treatment**

To examine the effects of nifedipine and verapamil on the cataract formation in diabetic rats, 9 diabetic rats were fed on chow containing 0.1% w/w nifedipine (Bayer Yakuhin, Ltd., Osaka) and 9 diabetic rats were injected with verapamil (Sigma) twice a day at 9:00 am (3 mg/kg, s.c.) and 5:00 pm (6 mg/kg, s.c) (20). To determine the concentration of nifedipine, we tested the effects of chows containing three different concentrations of nifedipine (0.03%, 0.1%, and 0.3% w/w) in a pilot study. Significant inhibitory effects on cataracts were observed in 0.1% nifedipine-treated group, but the effects were inconsistent in the lower dose (0.03%) group and apparent toxic effects were observed in some rats in the higher dose (0.3%) group. Based on the results, we chose the dose tested in this study. Nifedipine and verapamil were administered for 9 weeks from the third day of streptozotocin injection. Rats were bred under illumination with a fluorescent lamp covered with a 605-nm sharp-cut filter (Annaka Tokusyu Glass). This was necessary to avoid break down of nifedipine with light of short wavelength, and all rats were kept under the same housing condition.

**Measurements of systolic blood pressure and heart rate**

The systolic blood pressure and heart rate were measured under conscious conditions once a week with a tail-cuff sphygmomanometer (BP-98 A; Softron, Tokyo).

**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**

**Plasma glucose levels, body weights, food/water intakes, blood pressure, and heart rate**

Plasma glucose levels of streptozotocin-treated rats were significantly higher than those of control rats (Fig. 1A), whereas body weights of diabetic rats were significantly lower than those of control rats (Fig. 1B). There was no significant difference in plasma glucose levels and body weights among diabetic rats and diabetic rats treated with nifedipine or verapamil. Although intakes of food and water were significantly increased in diabetic rats, neither nifedipine nor verapamil affected the increased food/water intakes (data not shown). The averaged food intake in nifedipine-treated diabetic rats was $147 \pm 3$ g/kg per day ($n = 9$); therefore, it is estimated that the daily intake of nifedipine was $147 \pm 3$ mg/kg. Unexpectedly, neither nifedipine nor verapamil had an apparent effect on systolic blood pressure (Fig. 1C). Diabetes lowered the heart rate and the decreased heart rate was not affected by either nifedipine or verapamil (Fig. 1D).

**Cataract formation**

Figure 2 shows the representative lens images of the horizontal plane in control rats, diabetic rats, and diabetic rats treated with nifedipine or verapamil; and Table I summarizes the cataract scores in each experimental group. The lenses of control rats were clear throughout the experimental period. In diabetic rats, cataracts were observed in 9 of 18 lenses (50%) 2 weeks after diabetes induction. The cataracts progressed depending on the length of the diabetic period. Nine weeks after induction of hyperglycemia, 78% of lenses (14 of 18 lenses) had developed hypermature cataracts. In diabetic rats treated with nifedipine, the appearance of cataracts was detected in 9 of 18 lenses (50%) after 2 weeks of hyperglycemia. On the other hand, in diabetic rats treated with verapamil, 61% of lenses (11 of 18 lenses) were still clear. Thus, although a significant difference was not detected, verapamil showed a tendency to delay the onset of diabetic cataracts. After 9 weeks of hyperglycemia, 22% of lenses (4 of 18 lenses) of nifedipine-treated diabetic rats and 61% of lenses (11 of 18 lenses) of verapamil-treated diabetic rats developed hypermature cataracts. These results clearly indicate that nifedipine, but not verapamil, slows the progression of diabetic cataracts induced by the hyperglycemia.

Figure 3 shows the relationships between the average cataract score and duration of diabetes in each experimental group. The formations of cataracts at early stages of diabetes (<3 weeks after induction of hyperglycemia)
were practically identical between diabetic rats and diabetic rats treated with nifedipine (DM + Nif), or verapamil (DM + Ver). However, the progression of diabetic cataracts was significantly slowed by nifedipine, but not by verapamil.

Figure 4D shows the effects of the Ca\textsuperscript{2+}-channel blockers on the opacity of central region of lens assessed by measuring the opaque area in the region. The opacity increased depending on the length of the diabetic period after the central opacity had begun to form. Nifedipine, but not verapamil, slowed the rate of increase in opacification of central regions of diabetic lenses.

**Discussion**

In a previous study, we reported a novel in vivo digital camera system for capturing clear high-resolution
images of the whole lens of small animals and showed that the combination of streptozotocin treatment and D-glucose feeding was a useful procedure to minimize the variability and to shorten the period required for the development of diabetic cataract in rats (32). Using the digital camera system and the procedure, we found that nifedipine significantly slows the progression, but not the onset, of diabetic cataracts. However, although verapamil tended to delay the onset of cataract, it did not influence progression of cataracts due to the hyperglycemia.

Presently, the lens imaging devices used for clinical and basic research purposes are based on the slit lamp system in most cases. The digital camera system we employed is completely different from them and allows us to capture the full lens images of the horizontal plane. The resolution of the lens image is high enough to estimate cataract status and contains no artifacts of corneal reflection and shadow. Therefore, using this system, the cataracts of the entire lens are displayed in a single image and even small vesicles and opacities at the peripheral part of the lens are precisely identified with high reproducibility. Also, the high-quality lens images provide the opportunity for quantitative evaluation of the lens opacity using the digital image analysis techniques.

In the present study, the effects of Ca\textsuperscript{2+}-channel blockers on formation of diabetic cataracts were evaluated by two different methods: the observer-based scoring method and the quantitative digital image-analysis method. In the scoring method, the severity of diabetic cataracts was assessed using standardized photographs and a grading system. The method is useful to identify the morphology, density, and location of the cataract within the lens, but the results are subjective and qualita-
positive. On the other hand, the digital image-analysis method allows us to assess the cataracts quantitatively, whereas it does not provide the detailed information on the morphology and location of cataract. Thus, these two methods are complementary, indicating that both methods are necessary for a precise evaluation of drug effects on cataracts.

As a quantitative approach, we evaluated the opacity in the central region of lens by determining the opaque area in the region. The central region of the lens is most important for normal vision; therefore, it is possible to predict the adverse influence of cataract on visual quality and moreover assess beneficial effects of drugs by determining opacity in this location. Although the appearance of cataract at the peripheral part of the lens was detected after 2 weeks of hyperglycemia, the values of opacity measured at the same time in the central regions were nearly 0. Therefore, such subtle cataracts may not disturb vision practically. According to the results obtained by the scoring method, the period of time required for the onset of diabetic cataract was not affected significantly by treatment of diabetic rats with either nifedipine or verapamil. After central opacities appeared, the effects of drugs on cataracts could be

![Image](image.png)

**Fig. 3.** The relationships between the averaged cataract score and duration of diabetes in each experimental group. The formations of cataracts at early stages of diabetes (<3 weeks after induction of hyperglycemia) were practically identical among diabetic rats, nifedipine-treated diabetic rats, and verapamil-treated diabetic rats; however, the progression of diabetic cataracts was significantly prevented by nifedipine but not by verapamil. A cataract score for each animal was obtained by averaging the score of right and left lenses. Values are each expressed as the mean ± S.E.M. of 5–9 animals. *P<0.05, compared with the DM group; †P<0.05, compared with the DM + Ver group.

![Image](image.png)

**Fig. 4.** Effects of nifedipine and verapamil on the opacity in the central region of the lens in diabetic rats. The digital image of rat lens 4 weeks after induction of diabetes (A) and the processed images (B and C). A central region outlined by broken circle 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 percentage of the opaque area in the area of the selected region. 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 lens in diabetic rats (DM) and diabetic rats treated with nifedipine (DM+Nif) or verapamil (DM+Ver). 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. Nifedipine, but not verapamil, slowed the opacification of central regions of diabetic lenses. Values are each expressed as the mean ± S.E.M. of 5–9 animals. *P<0.05, compared with the DM group; †P<0.05 and ‡P<0.01, compared with the DM + Ver group.
evaluated by either the scoring method or the digital imaging analysis method. The results indicate that nifedipine, but not verapamil, slows the progression of diabetic cataracts with both these evaluation methods.

The estimated dose of nifedipine used in this study was 147.4 ± 3.4 mg/kg per day, which was much greater than doses in clinical usage (10 – 60 mg/day). The higher doses of nifedipine did not affect plasma glucose levels, body weight, food and water intakes, blood pressure, and heart rate. Therefore, the inhibitory effects of nifedipine on cataracts cannot be explained by amelioration of hyperglycemia. With regard to the effect on blood pressure, we found that, in a preliminary study using anesthetized diabetic rats, intravenously administered nifedipine (100 mg/kg bolus) markedly decreased blood pressure (41%), whereas oral administration of the same dose of nifedipine induced much smaller depressor responses (12%). These results suggest that orally administered nifedipine may be less effective in lowering blood pressure at normal levels, probably because of the slow and mild elevation of nifedipine concentrations in blood.

Previous studies have demonstrated that verapamil exerts an inhibitory effect on diabetic cataracts in rats (20, 22, 23). The concentration of verapamil we adopted has been shown to reduce the incidence of diabetic cataracts measured after 8 weeks of hyperglycemia from 90% to 20% in a previous study (20). However, in the present study, verapamil failed to prevent significantly the onset and progression of diabetic cataracts. The discrepancy might be due in part to the differences in severity of hyperglycemia in animal models used. We used an animal model of diabetes mellitus with severe hyperglycemia that was induced by the combination of streptozotocin treatment and D-glucose feeding in order to make reproducible cataracts in a relatively short period of time, whereas in the previous study, diabetic rats were not given any additional D-glucose. Therefore, it seems possible that the dose of verapamil used in the present study was not sufficient to prevent cataracts in diabetic rats with severe hyperglycemia.

Another possibility is that nifedipine may slow the progression of diabetic cataracts through mechanisms other than blockade of L-type Ca\(^{2+}\) channels. Both nifedipine and verapamil not only block Ca\(^{2+}\) influx into lens cells (20, 37–39) but also attenuate the oxidative stress (31, 40, 41). Because the increased oxidative stress in the lens could contribute to the development of diabetic cataracts (15–18), nifedipine might exhibit an inhibitory effect on diabetic cataracts as an anti-oxidant drug. Interestingly, it has been demonstrated that nifedipine is 5-fold more potent than verapamil in inhibiting sarcolemmal membrane lipid peroxidation (31).

In conclusion, using the in vivo digital camera system designed for capturing images of the horizontal lens of small animals, we found that nifedipine shows the inhibitory effects on progression of cataracts induced with very high plasma glucose levels in rats. These results suggest that nifedipine may be considered as a candidate drug to suppress the progression of diabetic cataracts. However, at present, molecular mechanisms underlying the inhibitory action of nifedipine on diabetic cataracts remain unclear. Also, it is unknown whether nifedipine exhibits the similar inhibitory effect on diabetic cataracts in patients with type 1 and/or type 2 diabetes mellitus. Furthermore, the effect of nifedipine on lens must also be carefully evaluated under in vivo conditions since nifedipine may increase cataract risk (42). These issues should be addressed in future studies.

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