Attenuation of Cataract Progression by A-3922, a Dihydrobenzofuran Derivative, in Streptozotocin-Induced Diabetic Rats

Maki Saito, Mayumi Ueo, Sokichi Kametaka, Orii Saigo, Seiichi Uchida, Hideo Hosaka, Kenji Sakamoto, Tsutomu Nakahara, Asami Mori, and Kunio Ishii

*Department of Molecular Pharmacology, School of Pharmaceutical Sciences, Kitasato University; 5–9–1 Shirokanedai, Minato-ku, Tokyo 108–8641, Japan; b Department of Biological Research, Division 2, Odawara Research Center, Nippon Soda Co., Ltd.; 345 Takada, Odawara 250–0280, Japan; and c Department of Molecular and Cellular Pharmacology, School of Pharmaceutical Sciences, Iwate Medical University; 2–1–1 Nishitokuta, Yahaba, Iwate 028–3694, Japan.

Received May 7, 2008; accepted August 1, 2008; published online August 7, 2008

The present study was undertaken to assess whether A-3922, a dihydrobenzofuran derivative that possesses antioxidative effects, had any preventive effect on the onset and/or progression of diabetic cataract. Male Wistar rats were received a bolus intravenous injection of streptozotocin (65 mg/kg) and were given 5% glucose in drinking water for 10 weeks. The diabetic rats were divided into two groups and treated with 30 mg/kg/d A-3922 or vehicle during the experimental period. The opacities of eye lenses were observed by using both our original device and a slit lamp microscope. The lens opacities were initially detected as early as the 2nd week and the cataracts were developed in similar fashion in both A-3922-treated and untreated diabetic rats until 7th week, suggesting that A-3922 did not show any appreciable effect on the onset of diabetic cataract. In the later period (8th week or later), however, progression of cataract was retarded and significant reductions in both the total cataract score and the degree of opacity were apparently observed on 10th week of A-3922-treated diabetic rats. These results suggest that A-3922 can delay the progression but not the onset of diabetic cataract, and it has a possibility to be a candidate for drugs of cataract associated with diabetes.

Key words: cataract; diabetes; antioxidant; streptozotocin; lens; dihydrobenzofuran

Cataract, which is clouding of the intraocular lens, is a major cause of blindness. It is predicted the current 20 million people in the world with severely reduced visual acuity by the cataract is going to swell to 40 million by the year 2020. In order to reduce a potential risk for blindness, it is expected to clarify pathologic mechanisms of cataract formation for development of anti-cataract drugs. Since we have established a precise method for evaluating rat cataract by using two types of digital camera systems that are equipped with a non-reflecting illuminator or a slit lamp.

Oxidative stress is now recognized as a major factor involved in the cataract formation induced by a variety of factors including impairment of systemic metabolism such as diabetes mellitus, aging, UV irradiation, and photochemical insults. Although the most frequent cause of cataracts is the usual aging process, it often occurs earlier in life with diabetes mellitus. In the diabetic status, it is now well known that activation of polyol pathway, alteration of protein kinase C (PKC) activity, and increase in advanced glycation end-products occur. All these mechanisms have been thought to be involved in generation of oxidative stress. In fact, signs of oxidative stress were augmented in various tissues including retinae, peripheral nerve system, plasma and red blood cells in diabetic model animals; increase in the levels of malondialdehyde (MDA) and 8-hydroxy-2′-deoxyguanosine (8-OHdG), decrease in the level and activity of superoxide dismutase (SOD), and decrease in contents of glutathione (GSH) were observed. Obviously, oxidative stress is increased in the diabetic lenses, while natural antioxidant defenses such as GSH are compromised. These reports strongly suggest a deep relationship between augmentation of oxidative stress and the onset and/or progression of cataract.

Dihydrobenzofuran derivatives known as potent antioxidative derivatives could stimulate pseudoperoxidase activity of 5-lipoxigenase, that catalyses the degradation of lipid hydroperoxides. A recent report indicated that a dihydrobenzofuran derivative A-3922 effectively inhibited corneal neovascularization induced by linoleic acid hydroperoxide in rabbits.

Here, we examined the effect of A-3922 on the cataract formation in streptozotocin-induced (STZ-induced) diabetic rats in vivo. Our present findings suggest that A-3922 can delay the progression of cataract associated with diabetes mellitus.

MATERIALS AND METHODS

Chemicals A-3922 [4-(±)-(5-amino-2,4,6,7-tetramethyldihydrobenzofuran-2-ylmethyl)-1-(3-imidazol-1-ylphenyl)piperazine] was synthesized at Odawara Research Center of Nippon Soda Co., Ltd. All other chemicals were purchased from Nacalai Tesque (Kyoto, Japan) or Wako Pure Chemical (Osaka, Japan).

A-3922 was suspended in 1% methylcellulose 100CP/saline. Streptozotocin (STZ) was dissolved in 10 mM citrate buffer (pH 4.5).

Care of Experimental Animals All experiments were performed in accordance with the Guidelines for Animal Experiments in Kitasato University and the Guiding Principles for the Care and Use of Laboratory Animals, approved by Japanese Pharmacological Society.

Male Wistar rats (6 weeks of age) were purchased from Japan Laboratory Animals Inc. (Tokyo, Japan). All rats were housed in hanging, stainless steel cages, individually, under controlled temperature (23±2°C) and light with a reverse 12 h light–dark cycle (8:00–20:00). The rats were divided into 3 groups; i.e., healthy control, diabetic, A-
3922-treated diabetic, respectively. Normal chow (MF; Oriental Yeast, Tokyo, Japan) was given to all groups. Rats received daily oral administration of 30 mg/kg A-3922 or vehicle for 10 weeks. All diabetic rats were given 5% glucose aqueous solution to maintain their blood glucose levels >700 mg/dl throughout the experimental period. Tap water was given to healthy control group. Each chow and water was freely available.

For induction of diabetes, rats were initially fasted overnight, and then they received a single injection of 65 mg/kg of STZ via tail vein. Healthy control rats were injected the same volume of the citrate-buffer. Two days after STZ injection, blood samples were collected via tail vein, and then blood glucose levels were determined using a commercially available kit (Glucose C2; Wako Pure Chemical) and the presence of diabetes was confirmed by blood glucose levels above 350 mg/dl at 2 d after STZ injection. Body weights and blood glucose levels were estimated once a week throughout the experimental period. There were no differences in viability of rats irrespective of the injection of STZ during the experimental period up to 10th week.

Evaluation of Cataract Score  Front view images of rat lenses were taken as described in our previous report.2) In brief, the pupils were dilated with one drop of 1% atropine sulfate (Nihon Tenganyaku Institute, Nagoya, Japan) prior to take photographs. The rats were anesthetized with diethyl ether. To protect the cornea from dryness and block the light reflecting from the cornea surface, hydroxyethyl cellulose solution (SCOPISOL 15%; Senju Pharmaceutical, Osaka, Japan) was applied. Digital photographs were taken from vertically upwards of rat lenses so that the optic disc might come right in the middle of each image. The score of distribution of lens opacities in front view was determined as follows according to the classification as described previously2,3): F0, clear; F1, peripheral vacuolation and/or opacification; F2, cortical opacification in central region; F3, diffuse cortical opacification; F4, mature cataract; F5, hypermetropic cataract.2) The prefix “F” of each score expresses the score by “Front view”. The right and left lens opacities in each animal were averaged.

The cross-sectional images were also obtained with a slit-lamp microscope (model SL-1800; Nidek, Nagoya, Japan) connected with a digital still camera (CoolPix5000; Nikon, Tokyo, Japan) via an adaptor (NYpixS5000S2; Micronet, Saitama, Japan). The slit-lamp photographs were taken following pupil dilation under anesthesia described as above. The score of cataract progression in slit view was determined as follows according to the classifications of Chylack et al.2,25) and Cotlier26) with minor modifications: S0, clear; S1, subcapsular cortical opacification; S2, moderate cortical opacification; S3, advanced cortical opacification; S4, nuclear opacification; S5, complete opacity. The prefix “S” of each score expresses the score by “Slit view”. The right and left lens opacities in each animal were averaged.

The progression of cataract was monitored every week. To evaluate the degree of cataract progression, we calculated the sum of the scores based on the front and slit views as the total score of cataract (e.g., if the scores of an animal are F3 and S4, total score would be calculated as 7). For information, the maximum value of the total cataract score is 10.

Quantification of Opacities in Central Region of Lenses  We also determined the opaque area in the central region of the lens representing the region that directly affects vision using front view images. Further detail was published by Kametaka et al.2) In brief, we selected the central region that was outlined by a circle that gave 10% of area of the eye ball and converted the image from full-color to grayscale using Adobe Photoshop CS (Adobe, Tokyo, Japan). After intensifying the contrast of the image, the opaque regions were distinguished from background by empirically determining a certain threshold level for each image. The numbers of pixels of the opaque region and the selected central region were counted by use of NIH image software (version 1.63; National Institution of Health, Bethesda, MD, U.S.A.). The opacity was calculated as 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.

Statistical Analysis  The data are expressed as the mean±S.E.M. except for the total cataract score. Total cataract scores are expressed in box plots. The box plot shows a rectangle stretching from the first to the third quartile of the distribution. Therefore, the box contains 50% of the data in the distribution. A line inside the box shows the approximate position of the median. The whisker lines extending from the box express the maximum and minimum values, respectively.

Statistical analysis was done by Bonferroni/Dunn test for multiple comparisons after analysis of variance. In case of non-parametric analysis for total cataract score, Mann–Whitney U-test was performed. Differences were considered significant when the p value was less than 0.05.

RESULTS  Body Weights and Blood Glucose Levels  The initial body weights were similar in healthy control and diabetic groups (Fig. 1A). However, during the course of experiments, the body weight gain was retarded, and the weights of both diabetic groups (A-3922-treated diabetic, 267.8±9.5 g, n=17; untreated diabetic, 263.3±9.3 g, n=14) significantly lower as compared with those in healthy control group (391.1±11.6 g, n=4) (p<0.01).

Blood glucose levels remained high in both A-3922-treated (905.1±28.7 mg/dl) and untreated diabetic rats (904.4±30.1 mg/dl) as compared with those in controls (149.7±8.74 mg/dl, p<0.01, Fig. 1B). The administration of A-3922 showed no appreciable effect on the hyperglycemia in the diabetic rats (Fig. 1B).

Effect of A-3922 on the Cataract Formation  The formation of cataract in both of the diabetic groups was observed from 2 weeks after STZ injection, and total cataract scores were increased in a similar fashion until the 7th week of diabetic duration; at the 7th week, median value of untreated diabetic was 4.5 (n=14) and the value of A-3922-treated diabetic was 4.0 (n=17), respectively (Fig. 2A). In A-3922-treated diabetic rats, further increase in total cataract score was prevented as compared with untreated diabetic group from the 8th week of diabetic duration. At 10 weeks after the administration of STZ, the median value of A-3922-treated diabetic group stayed at 6.5, whereas that of untreated diabetic group reached approximately 9.8 (p<0.05).
healthy control group \((n = 4)\) showed no cataract formation during the observation period of 10 weeks.

We estimated the opaque area in center region of the lenses, which seems to affect the visual acuity, by densitometric analysis (Fig. 2B). As in the case of evaluation by cataract score, A-3922 treatment tended to inhibit the increase in opaque area in central region from the 8th week. From 9 to 10 weeks after STZ injection, the area in the A-3922-treated rats was significantly smaller than those of untreated diabetic rats \(i.e., \) at the 9th week, A-3922-treated diabetic, 51.8\(\pm 6.6\)%, untreated diabetic, 80.8\(\pm 5.3\)\% \((p < 0.01)\); at the 10th week, A-3922-treated diabetic, 67.4\(\pm 6.8\)%, untreated diabetic, 89.4\(\pm 3.9\)\% \((p < 0.05)\).

DISCUSSION

It has been suggested that oxidative stress is deeply involved in the onset and/or progression of cataract\(^{27,28}\) caused by aging\(^{10,11}\) and UV irradiation,\(^{12}\) as well as diabetes mellitus.\(^{5-9}\) The present study shows that a dihydrobenzofuran derivative antioxidant, A-3922, has preventive effect on the progression of cataract in 5% glucose-induced diabetic rats.

STZ treatment, a model for type 1 diabetes, is well-established method for the studies in various diabetic complications.\(^{29}\) However, in our experiences, degree of hyperglycemia was varied by administration of STZ alone, which may be due to a short biologic half-life of STZ and/or injury-induced regeneration of pancreatic \(\beta\) cells through induction of PDX-1 transcription factor.\(^{30-32}\) Anyhow, the blood glucose levels did not always go over the threshold by STZ injection alone, and the incidence of cataractogenesis largely depends on the severity of diabetes in each animals. To overcome the varying degree of hyperglycemia, the STZ-injected rats were also given 5% glucose solution as a drinking water.\(^{33}\) This modification actually improved the variation of blood glucose levels among the animals, and the blood glucose level was maintained higher than 700 mg/dl, which is considered as critical levels to induce mature cataract in short term.\(^{2,33}\) According to this protocol, we made cataract model in diabetic rats, and we reproducibly observed the cataractogenesis within 2 weeks, and the opacities in almost all lenses of untreated diabetic were reached to nuclear cataract at the end of observation period (Fig. 2A).

In our preliminary experiment using A-3922 of up to 100 mg/kg/d, the retardation of cataract progression was...
equivalent in doses of 30 mg/kg/d or more, so that the dose of 30 mg/kg/d was employed to examine the effect of A-3922 on cataract in the present study. It has been also reported that A-3922 of 10—30 mg/kg/d successfully inhibited peroxylipid-induced angiogenesis on cornea of rabbits. This also supports the validity of dose of this drug to examine the inhibitory effect on the progression of cataract. We did not observe any appreciable effect of A-3922 on body weight change (Fig. 1A), blood glucose levels (Fig. 1B), and feeding behaviors (not shown) in STZ-treated diabetic rats. Therefore, it can be considered that the mechanisms for cataract formation and progression would be similar irrespective of the different cause of diabetes.

The intraocular lens has lamella structure consisted by lens fiber cells and a sheet of cubicoid epithelial cells covers the anterior surface of the lens. The structure is roughly divided into subcapsular region (closest to the cornea), nuclear region (central of the lens), and cortical region, which located between subcapsular and nuclear regions. In our evaluation system, total cataract score of 7 is the border value of nuclear cataract, whereas the value not less than 7 express the opacities reach nuclear region of the lenses.

Cataract formation in STZ-injected rats, a model for type 1-diabetes, is likely to be dependent on the long-lasting hyperglycemia; this dependency was not limited in this model, but also observed in other rat strains for type 2-diabetes (SDT and SDT fatty rats). Therefore, it can be considered that the mechanisms for cataract formation and progression were studied irrespective of the different cause of diabetes.

Osmotic swelling of lens is considered to be an initial step for cataract formation. As to the mechanisms for the osmotic swelling in sugar cataract, the aldose reductase-initiated accumulation of polyols plays an important role during the early stage of sugar cataract formation. Therefore, aldose reductase inhibitors such as SG-210 and GP-1447 effectively inhibit the onset of sugar cataract formation. However, it has been suggested that the endogenous aldose reductase is involved in both cataractogenesis and protection from oxidative stress-mediated apoptosis of lens epithelial cells. Therefore, complete inhibition of aldose reductase might not be a safe strategy for prevention of cataract.

In contrast, A-3922 did not show any appreciable effect on the onset of the cataract formation (Figs. 2A, B) until 6th week of diabetes duration probably due to lack of property of aldose reductase inhibitor. However, the progression of cataracts was significantly delayed by treatment of A-3922 from 8th week, i.e., the median value of the total cataract score of A-3922-treated diabetic rats stayed at 6.5, whereas that of untreated diabetic rats finally reached to 9.75 (Fig. 2A). There was a good concordance between changes in the total cataract score (Fig. 2A) and in the area of opacity (Fig. 2B). Opacities in the central region of lens are thought to affect visual acuity. Moreover, opacity of the lens is a direct result of oxidative stress and hyperglycemia is a major cause of elevated production of superoxide in mitochondria. Therefore, it is expected that antioxidant activity of A-3922 might reduce possible risk for blindness in diabetes irrespective of genetic differences of either type 1 or type 2. In this regard, Olofsson et al. have reported that nitric oxide (NO) can accelerates opacification of lenses in excessive reactive oxygen species-producing animals, SOD1-null mice. On the other hand, A-3922 has radical scavenging activity especially for NO radical (our unpublished observation). Taken together, it is suggested that the NO radical scavenging activities, including A-3922, may play a central role in the inhibitory effect on sugar cataract progression.

We did not yet determine whether A-3922 actually decreases signs of oxidative stress in the lesion sites. Therefore MDA and 8-OHdG in lenses as well as the visual acuity of the diabetic rats should be absolutely measured.

In conclusion, the present study demonstrated that an administration of antioxidant A-3922, having a dihydrobenzofuran skeleton, could delay cataract progression in 5% glucose-given STZ-induced diabetic rats without appreciable influences on other parameters, such as body weights and blood glucose levels. These findings would provide valuable information that can be used to design new drugs for preventing cataracts.

Acknowledgements Authors are grateful to Drs. A. Yamanaka of Kobe Kaisei Hospital and K. Abe of Konan Medical, Inc. for their valuable suggestion to establish the cataract score evaluating system. We thank Drs. K. Nakayama and Y. Tanabe of Iwate Medical University for their helpful discussions. We also thank Mr. K. Ueda, Mss. K. Hayashi, H. Nakatanai, A. Kawakami, H. Ozaki, R. Katayama, T. Takino, and A. Inoue for their technical assistance with the animal handling. This work was supported in part by Suzuki Memorial Foundation (MS) in 2005 and Kitasato University Research Grant for Young Researchers (MS) from 2004 to 2007.

REFERENCES


