

Aldose Reductase Inhibitor Fidarestat Prevents Diabetic Ocular Complications in Spontaneously Diabetic Torii Rats

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Abstract: We evaluated the effect of an aldose reductase inhibitor, fidarestat, on diabetic retinopathy (DR) and cataract in spontaneously diabetic Torii (SDT) rats. Four rat groups were included: untreated, low- and high-dose (8 and 32 mg/kg/day) fidarestat-treated SDT rats, and nondiabetic control Sprague-Dawley rats. DR and cataract were evaluated and retinal and lens sorbitol, reduced glutathione (GSH), ocular fluid vascular endothelial growth factor (VEGF), and urinary 8-hydroxy-2'-deoxyguanosine (8-OHdG) was measured. The incidence rates of DR and cataract were significantly lower in the low- and high-dose fidarestat groups vs the untreated group ($p < 0.001/p < 0.001$). Retinal and lens sorbitol levels were lower in the control ($1.1 \pm 0.1/3.1 \pm 0.2$ nmol/mg protein) ($p < 0.05/p < 0.01$) and low- ($2.7 \pm 1.1/30.0 \pm 3.3$ nmol/mg protein) ($p < 0.01/p < 0.01$) and high-dose groups ($0.7 \pm 0.2/5.9 \pm 0.6$ nmol/mg protein) ($p < 0.001/p < 0.001$) vs the untreated group ($23.2 \pm 4.7/123.9 \pm 29.6$ nmol/mg protein). Retinal and lens GSH levels were higher in the nondiabetic control ($52.2 \pm 5.8/29.0 \pm 2.7$ μ mol/mg protein) ($p < 0.01/p < 0.001$) and the low- ($46.8 \pm 8.2/24.7 \pm 2.8$ μ mol/mg protein) (not significant (NS)/ $p < 0.001$) and high-dose groups ($63.3 \pm 14.6/26.9 \pm 3.6$ μ mol/mg protein) ($p < 0.05/p < 0.001$) vs the untreated group ($30.3 \pm 2.0/1.6 \pm 0.4$ μ mol/mg protein). VEGF levels were lower in the nondiabetic control (40.4 ± 10.0 pg/ml) ($p < 0.01$) and low- (65.3 ± 4.5 pg/ml) ($p < 0.05$) and high-dose groups (47.7 ± 10 pg/ml) ($p < 0.001$) vs the untreated group (324.7 ± 76.4 pg/ml). 8-OHdG levels were lower in the nondiabetic control (0.73 ± 0.11 ng/mg creatinine) ($p < 0.01$) and low- (4.57 ± 0.42 ng/mg creatinine) (NS) and high-dose groups (3.58 ± 0.70 ng/mg creatinine) (NS) vs the untreated group (6.04 ± 1.28 ng/ml). Fidarestat inhibited activation of the polyol pathway, reduced oxidative stress and VEGF, and prevented DR and cataract in SDT rats.

Keywords: Fidarestat, spontaneously diabetic Torii (SDT) rat, diabetic retinopathy, cataract, vascular endothelial growth factor (VEGF).

INTRODUCTION

Diabetic retinopathy (DR), cataracts, and neovascular glaucoma are three major vision-threatening diabetic ocular complications. Although cataract can be treated surgically, DR eventually progresses to proliferative DR (PDR) accompanied by tractional retinal detachment and/or neovascular glaucoma. PDR and neovascular glaucoma can be treated by laser and vitreous surgery in certain cases, but the postoperative visual function is usually unsatisfactory. Prevention of DR is ideal for preventing blindness in patients with diabetes.

Clinical trials have shown that intensive glycemic control reduces the incidence and progression of DR [1-3]. However, achieving normal glucose homeostasis is not accomplished easily even in patients who are highly compliant. Furthermore, DR continues to develop and progress in well-controlled patients [1-3].

It is necessary to find treatments in addition to those that address glycemic control. Among the metabolic changes accompanying hyperglycemia are increased polyol pathway activity [4, 5], activation of protein kinase C (PKC) [6-8], increased oxidative stress [9-11], and accumulation of advanced glycation end products (AGEs) [12, 13] that are related to the development and progression of diabetic ocular complications. In particular, the polyol pathway is correlated strongly with oxidative stress, activation of PKC [7], and accumulation of AGEs that lead to induction of vascular endothelial growth factor (VEGF) [13]. A key enzyme of the polyol pathway, aldose reductase (AR), is found in the retina and lens. AR inhibitors (ARIs) slow thickening of the basement membrane of the retinal capillaries and progression of diabetic cataract in experimental studies [14-21]. The polyol pathway is the most attractive target for adjunctive treatment to prevent diabetic ocular complications. Based on favorable *in vivo* experiments using the ARI sorbinil [22, 23], a clinical trial of sorbinil was conducted, but the drug did not affect the development of DR [24], and enthusiasm for the clinical application of ARI waned.

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The spontaneously diabetic Torii (SDT) rat, a substrain of the Sprague-Dawley (SD) rat, spontaneously develops diabetes mellitus and exhibits the three major diabetic ocular complications, cataracts, advanced DR, and rubeotic glaucoma. In 1988, five male rats with polyuria and glucosuria were identified among 305 rats from an outbred colony of the Crj:CD (SD) strain (Charles River Japan, Inc., Kanagawa, Japan) of SD rats. After the 20th generation of sister-brother matings, the diabetic strain was established in 1997. The characteristics of this rat are described in this issue. The ARI, fidarestat, has a stronger AR inhibitory effect, longer half-life, and fewer adverse effects on the peripheral nerves than other ARIs [25]. Fidarestat showed strong AR inhibition and prevented diabetic retinal changes in streptozotocin (STZ)-induced diabetic rats, which have early DR [19-21]. Therefore, we evaluated the effects of fidarestat in SDT rats, which develop advanced diabetic ocular complications.

MATERIALS AND METHODS

The care and handling of the animals were in accordance with the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Visual Research and approved by the Jichi Medical University Animal Care and Use Committee. The male SDT rats and SD rats were obtained from CLEA, Inc., Tokyo, Japan. All SDT rats were confirmed to be diabetic based on a non-fasting blood glucose concentration exceeding 19.4 mmol/l. Four groups of rats were included in the study: untreated SDT rats (18 rats), low-dose fidarestat-treated SDT rats (8 mg/kg/day, 14 rats), high-dose fidarestat-treated SDT rats (32 mg/kg/day, 21 rats), and non-diabetic normal control SD rats (10 rats). The high- and low-dose fidarestat-treated SDT rats were fed standard rat chow (CRF-1, Oriental Yeast, Inc., Tokyo, Japan) containing the ARI fidarestat at the onset of diabetes. Other rats were fed unsupplemented rat chow. DR was evaluated using fluorescein angiomicroscopy and histopathology.

Measurement of Plasma Glucose and Glycated Hemoglobin

Blood samples were collected from the abdominal aorta with the animals anesthetized with diethyl ether to measure plasma glucose and glycated hemoglobin. Blood samples were centrifuged at 1,700 \times g for 10 minutes at 4°C to obtain plasma for measurement by the mutarotase-glucose oxidase method (Glucose CII-test Wako, Wako, Osaka, Japan). Glycated hemoglobin was measured using an automated glycohemoglobin analyzer (HLC-723GHb V, Tosoh Corporation, Tokyo, Japan).

Fluorescein Angiomicrography

Fluorescein-dextran microscopy was performed after intracardiac injection of fluorescein-dextran (fluorescein isothiocyanate-dextran, Sigma, St. Louis, MO), using a modification of a previously reported method [26]. Under deep anesthesia induced by intraperitoneal injection of pentobarbital sodium (25 mg/kg body weight), 1 ml of phosphate buffered saline containing 50 mg of fluorescein-dextran was injected into the left ventricle of each animal. After 5 minutes, the eyes were enucleated for fluorescein microscopy. The retina was peeled from the eyecup and the

entire retina was flat-mounted on a slide glass without fixation. A drop of aqueous mounting medium (Crystal/Mount, Biomedica Corp., Foster City, CA, USA) was applied over the retina and allowed to dry. The flat-mounted retina was examined by fluorescence microscopy (Nikon SMZ1500 with P-FLA fluorescence attachment, Nikon, Tokyo, Japan).

Histopathology

Under deep anesthesia induced by intraperitoneal injection of pentobarbital sodium (25 mg/kg body weight, Nembutal, Dainihonseiyaku, Osaka, Japan), the eyes were enucleated for conventional histopathologic studies and placed in a fixative (mixture of 2.5% paraformaldehyde and 1% glutaraldehyde in 0.15 M phosphate buffer) to avoid artificial retinal detachment. The fixed eyes were washed in 0.1% mol/l cacodylate buffer and embedded in paraffin. The paraffin block was sectioned to 4 μ m and stained with hematoxylin and eosin for conventional histopathologic examination. As reported previously [27], DR was identified when large retinal folds mimicking tractional retinal detachment and/or extensive leakage of fluorescein around the optic disc were observed. In this study, the left eyes were examined by fluorescein angiomicroscopy or histopathology.

Biomicroscopy of Cataract

The pupils were fully dilated with a topical ophthalmic solution containing tropicamide 5% and phenylephrine hydrochloride 5%, and the anterior segment including the lens was observed and photographed in both eyes of all rats.

Measurement of Sorbitol and Fructose

The retina and lens were separated from the enucleated eye and homogenized in distilled water. Trichloro-acetic acid (TCA) and internal standard (D-sorbitol-d4 and D-fructose-d2) were added to the homogenate followed by centrifugation at 10,000 \times g for 5 minutes at 4°C to obtain the supernatant fraction. To remove the TCA, the supernatant fraction was washed with ethyl ether. Sorbitol and fructose in the supernatant fraction were converted to sorbitol acetate derivative and fructose acetate derivative according to the method described by Guerrant and Moss [28] (high-performance liquid chromatography with the HP1050, Hewlett Packard, Palo Alto, CA, USA) and mass spectrometry (TSQ, Finnigan Mat, San Jose, CA, USA) using a Cadenza CD-C18 column (75 \times 2.0 mm, internal diameter 3 mm, Imtakt, Kyoto, Japan).

Measurement of Reduced Glutathione (GSH)

The retinas and lenses were separated from the enucleated eyes and homogenized after the addition of ice-cold 5% metaphosphoric acid. The tissue homogenates were centrifuged at 10,000 \times g for 20 min at 4°C. Aliquots of the supernatant fractions were used to determine the GSH with a colorimetric assay kit (Bioxytech GSH-400) supplied by Oxis Research (Portland, OR, USA).

Measurement of VEGF

Ocular fluid (aqueous and vitreous) was collected from the enucleated eyes and the VEGF levels were measured with a sandwich enzyme immunoassay technique (ELISA) using a mouse VEGF immunoassay (R&D System,

Minneapolis, MN, USA), according to the manufacturer's instruction.

Measurement of 8-Hydroxy-2'-Deoxyguanosine (8-OHdG)

The rats were housed in metabolic cages 39 weeks after the onset of diabetes. Urine was collected over 24 h, the samples were centrifuged at 1,700 xg for 10 min at 4°C, and the 8-OHdG levels in the supernatant were measured with an ELISA kit (8-OHdG check; Japan Institute for the Control of Aging, Fukuroi, Japan), according to the manufacturer's instructions.

The retina, lens, and ocular fluid samples were obtained from enucleated eyes except for eyes in the histopathology study and allocated for the previously mentioned analysis. Therefore, the numbers of eyes in each analysis were smaller than the number of rats used.

Statistical Analysis

The prevalence rates of DR and cataract were evaluated by the chi-square for independence test. The Student t test was used to compare the normal SD rats and untreated SDT rats. Analysis of variance and Dunnett's multiple comparison test were used to compare the differences among the three groups of SDT rats. The values are expressed as the mean±SE. $p < 0.05$ was considered to be statistically significant.

RESULTS

Body Weight, Plasma Glucose, and Glycated Hemoglobin

Table 1 shows the weights, plasma glucose, and glycated hemoglobin values. The mean body weight of the non-diabetic normal control SD rats (mean±SE, 900±24 g) was significantly ($p < 0.001$) higher than that of the untreated SDT rats (364±14 g). Fidarestat had no significant effect on body weight among the three groups of SDT rats.

Table 1. Body Weight, Plasma Glucose, and Glycated Hemoglobin

	Body Weight (g)	Plasma Glucose (mmol/l)	Glycated Hemoglobin (%)
Normal SD rat	900±24	9.3±0.4	3.5±0.1
Untreated	364±14*	42.8±3.4*	13.8±0.7*
Low-dose fidarestat group	366±10	48.6±3.9	16.4±0.4
High-dose fidarestat group	332±15	46.2±2.8	14.7±0.5

* $p < 0.001$ vs normal SD rats.

The mean plasma glucose level of the non-diabetic normal SD rats (mean±SE, 9.3±0.4 mmol/l) was significantly ($p < 0.001$) lower than that of the untreated SDT rats (42.8±3.4 mmol/l). Fidarestat had no significant effect on the plasma glucose level among the three groups of SDT rats.

The mean glycated hemoglobin concentration of the non-diabetic normal SD rats (mean±SE, 3.5±0.1%) was significantly ($p < 0.001$) lower than that of the untreated SDT

rats (13.8±0.7%). The mean glycated hemoglobin concentration in the low-dose fidarestat SDT rats (16.4±0.4%), but not the high-dose fidarestat SDT rats (14.7±0.5%), was higher than that of the untreated SDT rats (13.8±0.7%) ($p < 0.05$).

Prevalence of DR

DR developed less often in the low- (0/14 eyes, 0%) ($p < 0.001$) and high-dose groups (0/21 eyes, 0%) ($p < 0.001$) than in the untreated SDT rats (12/18 eyes, 66.6%) (Table 2). The typical pathological changes of DR, i.e., the large retinal folds mimicking tractional retinal detachment, were observed in most untreated SDT rats but not in the low-dose and high-dose fidarestat-treated SDT rats (Fig. 1A). The typical angiomic changes of DR, i.e., extensive leakage of fluorescein around the optic disc, were observed in most untreated SDT rats but not in the low- or high-dose groups (Fig. 1B).

Table 2. Prevalence of DR

	Retinopathy (+)	Retinopathy (-)
Normal SD rat	0% (0/10 eyes)	100% (10/10 eyes)
Untreated	66.6% (12/18 eyes)	33.3% (6/18 eyes)
Low-dose fidarestat group	0% (0/14 eyes)*	100% (14/14 eyes)
High-dose fidarestat group	0% (0/21 eyes)*	100% (21/21 eyes)

* $p < 0.001$ vs untreated SDT rats.

Prevalence of Cataract

Cataract developed less frequently in the low-dose (3/28 eyes, 10.7%) ($p < 0.001$) and high-dose fidarestat SDT rats (2/42 eyes, 4.8%) ($p < 0.001$) than in the untreated SDT rats (36/36 eyes, 100%) (Table 3). Typical mature diabetic cataracts were observed in most untreated SDT rats but not in the low- and high-dose groups (Fig. 1C).

Sorbitol and Fructose

The retinal sorbitol levels were lower in the non-diabetic normal SD rats (mean±SE, 1.1±0.1 nmol/mg protein, $n=4$) ($p < 0.05$), low-dose fidarestat SDT rats (2.7±1.1 nmol/mg protein, $n=3$) ($p < 0.01$) and high-dose fidarestat SDT rats (0.7±0.2 nmol/mg protein, $n=4$) ($p < 0.001$) than in the untreated SDT rats (23.2±4.7 nmol/mg protein, $n=3$) (Fig. 2A).

The lens sorbitol levels were lower in the non-diabetic normal SD rats (3.1±0.2 nmol/mg protein, $n=4$) ($p < 0.01$), low-dose fidarestat SDT rats (30.0±3.3 nmol/mg protein, $n=3$) ($p < 0.01$), and high-dose fidarestat SDT rats (5.9±0.6 nmol/mg protein, $n=4$) ($p < 0.001$) than in the untreated SDT rats (123.9±29.6 nmol/mg protein, $n=3$) (Fig. 2B).

The retinal fructose levels were lower in the non-diabetic normal SD rats (3.7±0.9 nmol/mg protein, $n=4$) ($p < 0.001$) but not in the low-dose fidarestat SDT rats (6.8±5.0 nmol/mg protein, $n=3$) (not significant (NS)) and high-dose fidarestat SDT rats (6.3±2.5 nmol/mg protein, $n=4$) (NS) compared

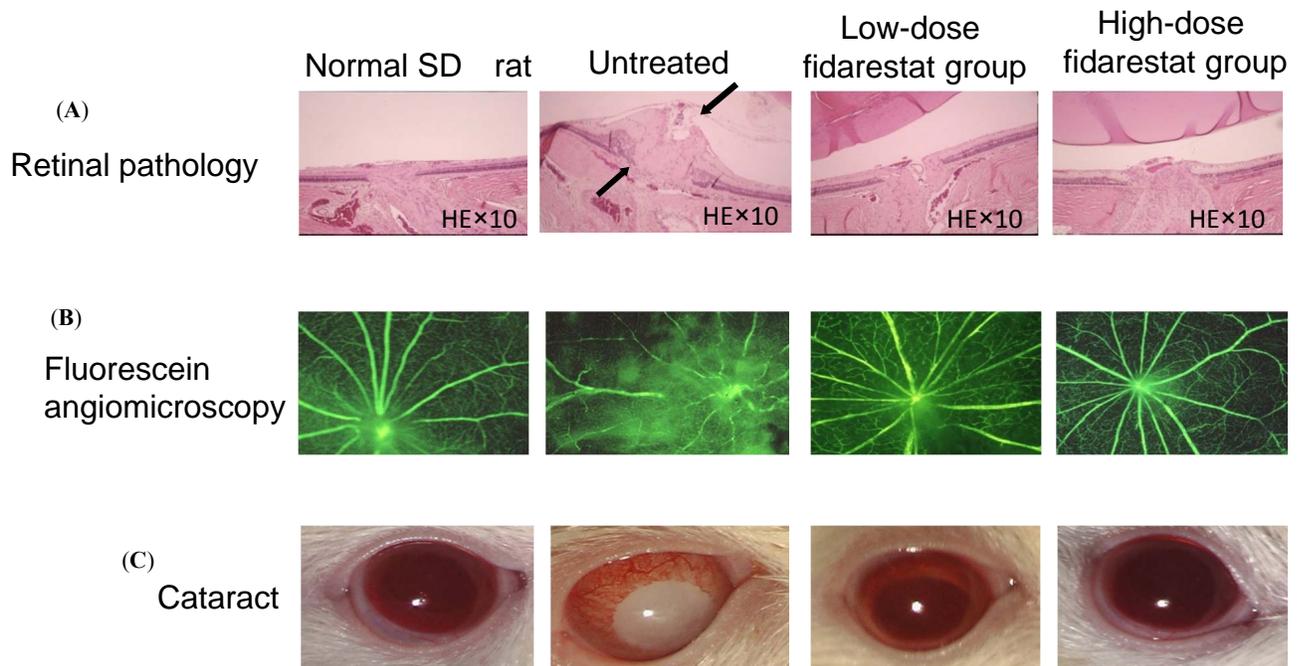


Fig. (1). Effect of fidarestat on DR and cataract. First row (A): Large retinal folds mimic tractional retinal detachment (arrows) in untreated SDT rats but not in low-dose fidarestat-treated SDT rats, high-dose fidarestat-treated SDT rats, and non-diabetic normal control SD rats. Second row: (B) Extensive leakage of fluorescein around the optic disc in an untreated SDT rat but not in a low-dose fidarestat-treated SDT rat, high-dose fidarestat-treated SDT rat, and non-diabetic normal control SD rat. Third row: (C) Mature cataract in an untreated SDT rat but not in a low-dose fidarestat-treated SDT rat, high-dose fidarestat-treated SDT rat, and non-diabetic normal control SD rat.

with the untreated SDT rats (13.7 ± 1.2 nmol/mg protein, $n=3$) (Fig. 3A).

The lens fructose levels were lower in the normal non-diabetic SD rat group (8.2 ± 0.4 nmol/mg protein, $n=4$) ($p < 0.01$), the low-dose fidarestat SDT rats (53.3 ± 2.6 nmol/mg protein, $n=3$) ($p < 0.01$), and the high-dose fidarestat SDT rats (21.4 ± 0.8 nmol/mg protein, $n=4$) ($p < 0.001$) than in the untreated SDT rats (154.6 ± 35.9 nmol/mg protein, $n=3$) (Fig. 3B).

Table 3. Prevalence of Cataract

	Cataract (+)	Cataract (-)
Normal SD rat	0% (0/20 eyes)	100% (20/20 eyes)
Untreated	100% (36/36 eyes)	0% (0/36 eyes)
Low-dose Fidarestat group	10.7% (3/28 eyes) *	89.3% (25/28 eyes)
High-dose Fidarestat group	4.8% (2/42 eyes) *	95.2% (40/42 eyes)

* $p < 0.001$ vs untreated SDT rats.

GSH

The retinal GSH levels were higher in the non-diabetic normal SD rats (mean \pm SE, 52.2 ± 5.8 μ mol/mg protein, $n=10$) ($p < 0.01$) and the high-dose fidarestat SDT rats (63.3 ± 14.6 μ mol/mg protein, $n=9$) ($p < 0.05$) but not in the low-dose

fidarestat SDT rats (46.8 ± 8.2 μ mol/mg protein, $n=8$) (NS) compared with the untreated SDT rats (30.3 ± 2.0 μ mol/mg protein, $n=10$) (Fig. 4A).

The lens GSH levels were higher in the non-diabetic normal SD rats (29.0 ± 2.7 μ mol/mg protein, $n=10$) ($p < 0.001$),

the low-dose fidarestat SDT rats (24.7 ± 2.8 μ mol/mg protein, $n=8$) ($p < 0.001$), and the high-dose fidarestat SDT rats (26.9 ± 3.6 μ mol/mg protein, $n=10$) ($p < 0.001$) compared with the untreated SDT rats (1.6 ± 0.4 μ mol/mg protein, $n=10$) (Fig. 4B).

VEGF

The VEGF levels in the ocular fluid were lower in the non-diabetic normal SD rats (mean \pm SE, 40.4 ± 10.0 pg/ml, $n=8$) ($p < 0.01$), the low-dose fidarestat SDT rats (65.3 ± 4.5 pg/ml, $n=6$) ($p < 0.05$), and the high-dose fidarestat SDT rats (47.7 ± 10 pg/ml, $n=9$) ($p < 0.001$) than in the untreated SDT rats (324.7 ± 76.4 pg/ml, $n=8$) (Fig. 5).

8-OHdG

The urinary 8-OHdG levels were lower in the non-diabetic normal SD rats (mean \pm SE, 0.73 ± 0.11 ng/mg creatinine, $n=8$) ($p < 0.01$) than in the low-dose fidarestat SDT rats (4.57 ± 0.42 ng/mg creatinine, $n=6$) (NS) and high-dose fidarestat SDT rats (3.58 ± 0.70 ng/mg creatinine, $n=8$) ($p = \text{NS}$) compared with the untreated SDT rats (6.04 ± 1.28 ng/ml, $n=8$) (Fig. 6).

DISCUSSION

The favorable effects of fidarestat on early DR in STZ-induced diabetic rats have been reported previously [21]. Fidarestat decreased the number of microaneurysms and pericytes and basement membrane thickness. However, that report confirmed only the decreased retinal sorbitol and coexisting vascular retinal changes. Subsequent experimental

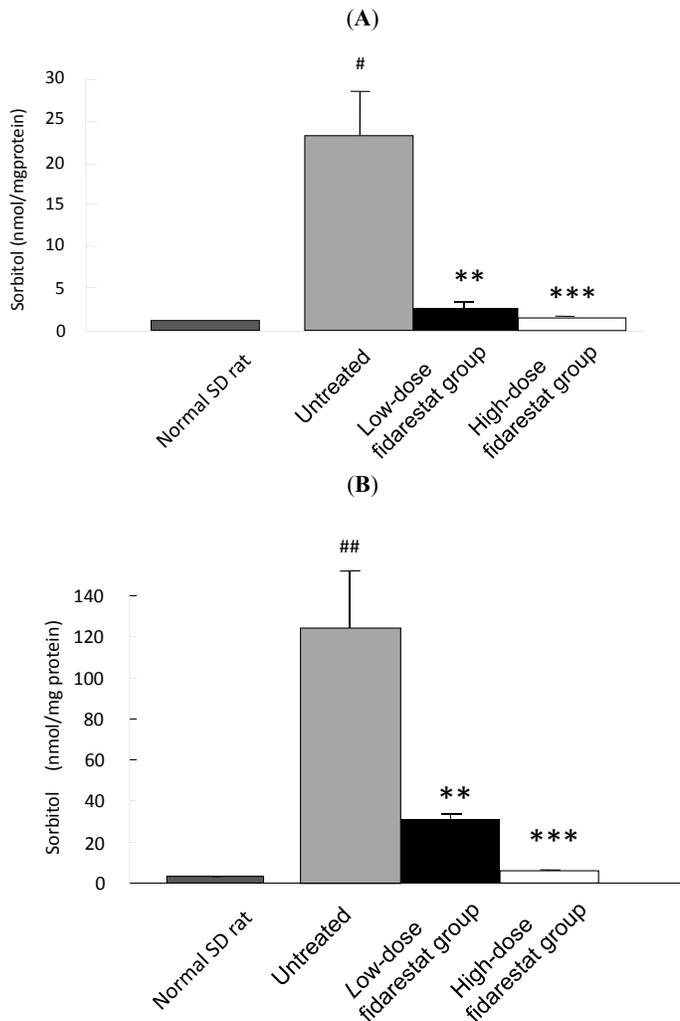


Fig. (2). Retinal (A) and lens (B) sorbitol levels. A: # $p < 0.05$ vs normal SD rats. **, *** $p < 0.01$, $p < 0.001$ vs untreated SDT rats. B: ## $p < 0.01$ vs normal SD rat. **, *** $p < 0.01$, $p < 0.001$ vs untreated SDT rats.

studies of STZ-induced diabetic rats have suggested that fidarestat prevents oxidative stress and VEGF production in tissues that are targets for diabetic complications [19, 20]. The limitation of these studies regarding ocular diabetic complications is that STZ-induced rats develop only early DR with microaneurysms and thickening of the basement membranes of the retinal capillaries without proliferative changes. In contrast, the SDT rats have more advanced changes with extensive vascular hyperpermeability and tractional retinal detachment with retinal thickening around the optic disc [27, 29, 30]. Hyperglycemia is also more severe in the SDT rats than the STZ-induced diabetic rats [29, 30]. Therefore, activation of the polyol pathway is

presumably more extensive and oxidative stress and VEGF production much higher in the SDT rats than in the STZ-induced diabetic rats. In the current study, we tested the prophylactic effect of fidarestat on diabetic ocular complications in SDT rats, which had very high glucose and glycated hemoglobin levels with and without fidarestat. Therefore, inhibition of cataract and retinopathy with fidarestat could not be attributed to improved glycemic

control. The retinal and lens sorbitol levels in the fidarestat-treated SDT rats were significantly lower than in the untreated SDT rats. Although reduction of fructose was not as significant, the effect of AR inhibition was marked in the current *in vivo* study.

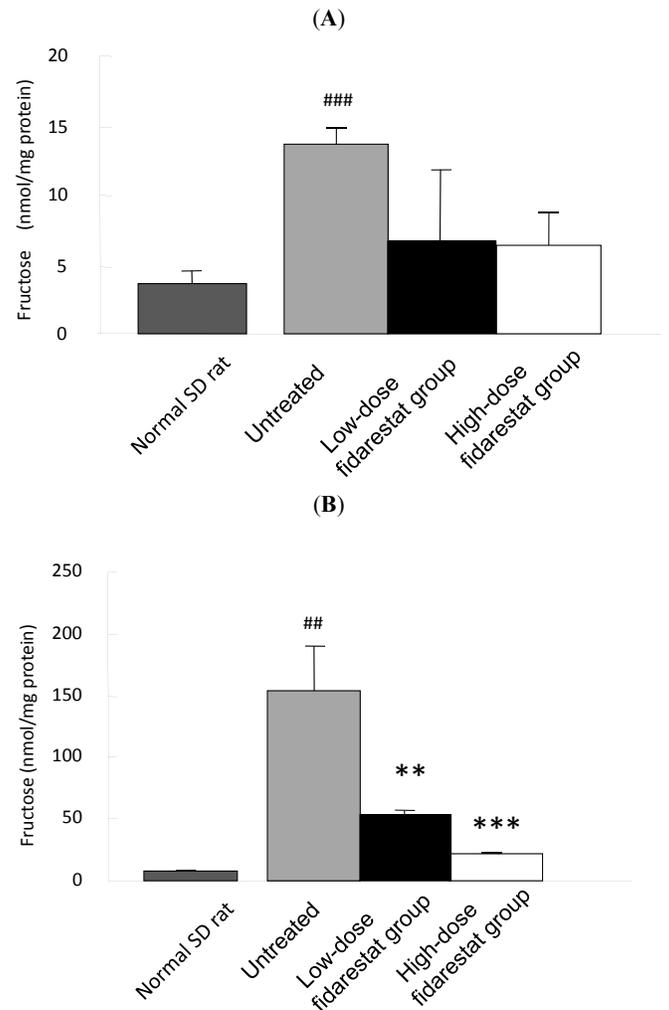


Fig. (3). Retinal (A) and lens (B) fructose levels. A: ### $p < 0.001$ vs normal SD rats. B: ## $p < 0.01$ vs normal SD rats. **, *** $p < 0.01$, $p < 0.001$ vs untreated SDT rats.

Oxidative stress is an important factor in the pathogenesis of diabetic complications [31-33]. GSH plays an important role in polyol pathway activation. In particular, GSH consumption is the most important factor in the development of diabetic cataract [34, 35]. GSH levels also contribute to the pathogenesis of DR [36]. It also has been reported that fidarestat prevents GSH consumption in retinal pericytes cultured in a high concentration of glucose [37].

The SDT rats had low GSH levels in the lens, and fidarestat raised those levels significantly. This antioxidative effect of fidarestat also was seen in the retina of rats treated with the high-dose fidarestat. 8-OHdG, also a good marker of systemic oxidative stress in diabetes [38-40], is excreted in the urine; the urinary 8-OHdG level was significantly higher in untreated SDT rats than in normal SD rats. However, the effect of fidarestat treatment on 8-OHdG was not significant.

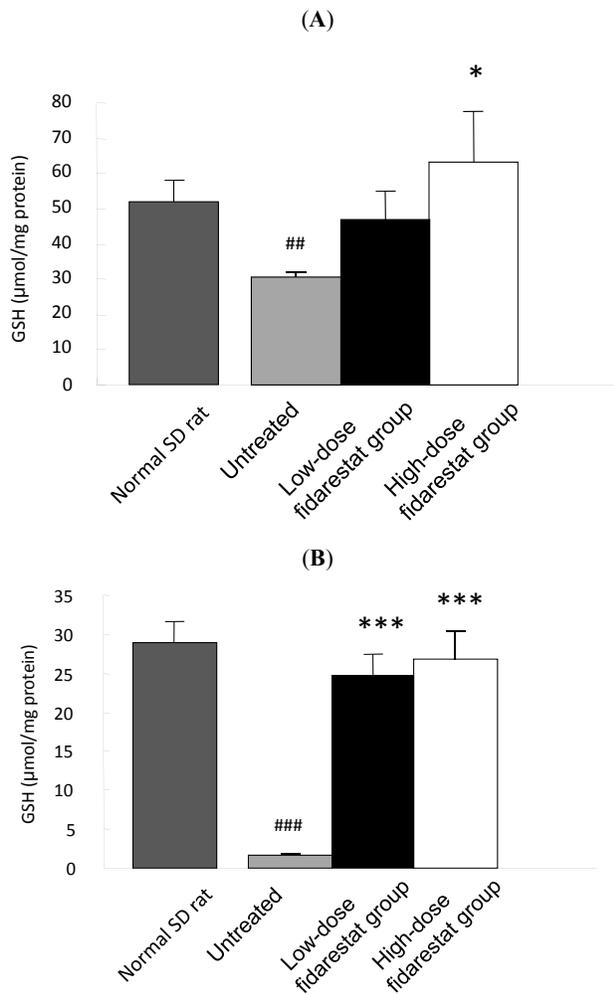


Fig. (4). Retinal (A) and lens (B) GSH levels. A: ##P<0.01 vs normal SD rats. *p<0.05 vs untreated SDT rats. B: ###p<0.001 vs normal SD rats. ***p<0.001 vs untreated SDT rats.

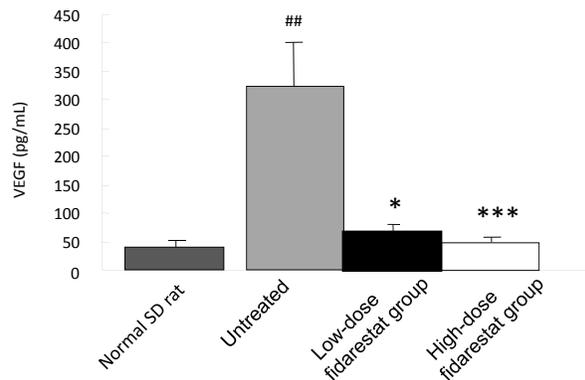


Fig. (5). VEGF levels in the ocular fluid. ##p<0.01 vs normal SD rats. * **p<0.05, p<0.001 vs untreated SDT rats.

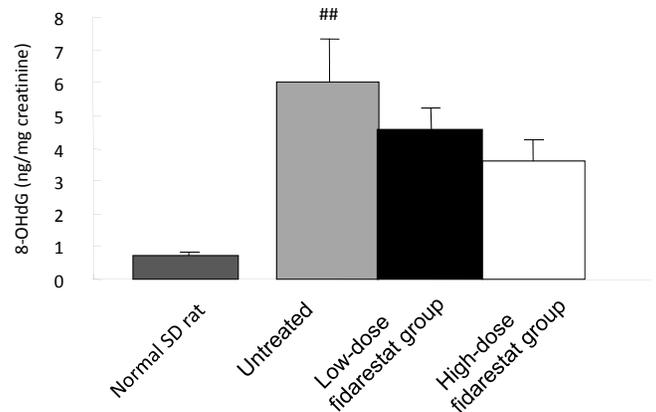


Fig. (6). Urinary 8-OHdG levels. ##p<0.01 vs normal SD rats.

VEGF is the most important molecule that induces PDR [41, 42]. VEGF induces vascular hyperpermeability and retinal neovascularization and eventually neovascular glaucoma [43]. Retinal neovascularization is the main cause of vitreous hemorrhage and diabetic tractional retinal detachment. Neovascular glaucoma complicated with PDR is the most tragic consequence of VEGF production. Although the untreated SDT rats did not have marked retinal neovascularization, the vascular hyperpermeability was prominent (Fig. 1B). The VEGF levels in the ocular fluid were inhibited significantly to almost normal levels in the low- and high-dose groups.

In conclusion, activation of the polyol pathway induces many biologic effects, such as oxidative stress, activation of PKC, and AGE accumulation, that induces VEGF. AR is a key enzyme in the polyol pathway. In the current study, fidarestat clearly inhibited AR and prevented diabetic ocular complications because of the antioxidative stress and anti-VEGF mechanisms in SDT rats. Fidarestat seems to prevent diabetic ocular complications, which warrants a clinical trial.

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