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ScienceDirect
Experimental Eye Research 85 (2007) 406–412
EXPERIMENTAL EYE RESEARCH
www.elsevier.com/locate/yexer

Angiotensin II receptor blocker inhibits abnormal accumulation of advanced glycation end products and retinal damage in a rat model of type 2 diabetes

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Received 28 February 2007; accepted in revised form 6 June 2007
Available online 19 June 2007

Abstract

The effects of an angiotensin II receptor blocker (ARB) on the accumulation of one of advanced glycation end products (AGEs), pentosidine, expression of vascular endothelial growth factor (VEGF) and retinal function were investigated in Spontaneously Diabetic Torii (SDT) rats. Candesartan, an ARB, was administered to SDT rats from 10 to 44 weeks of age and the results compared with untreated SDT rats and SD rats. Electroretinograms (ERGs) were recorded to evaluate retinal function. At 44 weeks of age, pentosidine was quantified in the vitreous, lens and plasma using high-performance liquid chromatography (HPLC). Real-time reverse transcription-PCR (RT-PCR) analysis was also performed in order to measure VEGF mRNA expression in the retina. Histological changes were examined and immunohistochemistry for pentosidine performed on the retina and retinal microvasculature. In untreated SDT rats, the amplitudes of a- and b-waves, oscillatory potentials were reduced significantly at 44 weeks of age compared with the 10-week levels, whereas they remained unchanged in SDT rats treated with candesartan. The concentration of pentosidine in the vitreous and lens did not change in treated SDT rats but increased in untreated SDT rats. Retinal VEGF mRNA expression was inhibited in treated SDT rats. Histologically, proliferative tissue was detected around the optic disc, with pentosidine being detected only in untreated SDT rats. Our findings indicate the ARB may inhibit the development of diabetic retinopathy by reducing the accumulation of pentosidine, one of AGEs and expression of VEGF in the retina.

Keywords: angiotensin II receptor blocker; advanced glycation end products; vascular endothelial growth factor; spontaneously diabetic torii rats; electroretinogram; high-performance liquid chromatography; real-time reverse transcription-PCR; immunohistochemistry

1. Introduction

In diabetic patients, advanced glycation end products (AGEs) are overproduced and accumulate in increasing amounts in various tissues. AGEs have been demonstrated to induce vascular dysfunction by increasing the activity of factors such as vascular endothelial growth factor (VEGF) and reactive oxygen species (Ido et al., 2001). Several studies have reported elevated levels of AGEs in the vitreous of patients with proliferative diabetic retinopathy (PDR) (Sebag et al., 1992; Hirata et al., 1997; Stitt et al., 1998). There is also evidence that AGEs play an important role in the development of diabetic retinopathy, by accelerating the expression of intercellular adhesion molecule-1 (Moore et al., 2003). This results

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doi:10.1016/j.exer.2007.06.008

in increased attachment of macrophages or monocytes to vessel walls, which is one of the causes to release VEGF, tissue necrotic factor (TNF)-α and interleukins that cause damage to the vascular endothelial cells and pericytes.

Recently, it was found that angiotensin II receptor blockers (ARBs) inhibited the formation of AGEs in an animal model of type 2 diabetic nephropathy (Nangaku et al., 2003). On the basis of this result, we undertook the present study to investigate the effects of ARB administration on retinal function and structure, AGE accumulation and expression of VEGF in the eyes of Spontaneously Diabetic Torii (SDT) rats (Shinohara et al., 2000).

2. Materials and methods

2.1. Animals

Twelve male SDT rats, aged 10 weeks were used in this study. Six age-matched Sprague–Dawley (SD) rats were used as control animals. The animals were housed in a climate-controlled room (temperature 22 ± 2 °C, humidity 45 ± 10% and 12 h lighting cycle) with free access to food and water. Twelve male SDT rats were randomized to treatment with candesartan cilexetil (2.5 mg/kg per day in drinking water) or placebo vehicle (0.12 v/v % ethanol/polyethyleneglycol).

The experimental procedures on the animals were conducted in accordance with the ARVO Statement for Use of Animals in Ophthalmic and Vision Research.

2.2. Measurement of biological parameters

The concentration of blood glucose was determined using Glutest Neo (Sanwa Kagaku Kenkyusho Co. Ltd., Nagoya, Japan), while mean blood pressure was measured using an automatic sphygmomanometer (BP Monitor for Rats and Mice Model MK-2000, Muramachi Kikai Co. Ltd., Tokyo, Japan). They are measured at 10, 15, 25, 35 and 44 weeks of age.

2.3. Recording of electroretinogram (ERG)

An ERG was used to evaluate changes in retinal function. The measurements were carried out after the rats had adapted to darkness for 13 h, followed by sedation with intramuscular xylazine (5 mg/kg) and ketamine (55 mg/kg). Monocular recordings were obtained with the pupil maximally dilated by instillation of 0.5% tropicamide, with the other eye being patched carefully in order to avoid all stimulation. For the recordings, we used a photic stimulator (SLS 4100; Nihon-Koden, Osaka, Japan), a biophysical amplifier (AVM-10; Nihon-Koden) and low-cut (0.5 Hz) and high-cut (100 Hz) filters. A ganzfeld white 60 msec stimuli with a maximum intensity of 690 candela/m^2 was flashed once and recordings made from the active electrode (Kyoto Contact Lens Laboratories, Kyoto, Japan) on the cornea. The first and second oscillatory potentials measured were referred to as OP_1 and OP_2, respectively. The amplitudes and the latencies of a-, b-, OP_1 and OP_2 waves were then measured. ERGs were recorded when the rats were aged 10 and 44 weeks and the data of the two time points then compared.

2.4. Pentosidine measurement by high-performance liquid chromatography (HPLC)

At 50 weeks of age, blood samples were collected from the hearts of the rats under diethyl ether anesthesia (Wako, Tokyo, Japan) and the plasma separated by centrifugation. The animals were then killed with increasing concentrations of carbon dioxide and the eyes enucleated. The eyes were cut open at the corneal limbus and the lenses, vitreous and retina collected. The plasma, lens and vitreous samples were used for pentosidine measurement, while the retina was used for real-time RT-PCR analysis. Tissues (lens and vitreous) were homogenized with 1.5 ml of chloroform/methanol (2:1) and with 1.0 ml of methanol to remove lipid. The homogenized tissues and plasma were then lyophilized under vacuum. Pentosidine measurement was performed using the HPLC assay described by Miyata et al. (1998). Briefly, each lyophilized sample was hydrolyzed in 50 μl of 6 N HCl for 16 h at 110 °C under nitrogen, followed by neutralization with 50 μl of 6 N NaOH and 100 μl of 0.5 M phosphate buffer (pH 7.4). The resulting solutions were then filtered through a 0.45-μm filter and diluted with phosphate buffered saline (PBS) (pH 7.4). The pentosidine levels in these specimens (corresponding to approximately 20 μg of protein) were measured by injecting into an HPLC system followed by fractionation on a C18 reversed-phase column (Waters, Tokyo, Japan). The effluent was monitored at an excitation-emission wave-length of 335/385 nm using a fluorescence detector (RF-10A, Shimadzu, Kyoto, Japan). Synthetic pentosidine was used to obtain a standard curve. The identity of the substance in the specimens, detected at the same retention time as authentic pentosidine, was confirmed as pentosidine by fast-atom bombardment-mass spectrometry (379.4 Daltons). The limits of detection were 0.1 pmol of pentosidine per milligram of protein.

2.5. Real-time reverse transcription-PCR (RT-PCR) for VEGF

Quantitative real-time RT-PCR analyses were performed in a fluorescent temperature cycler (Light Cycler, Roche Molecular Biochemicals, Mannheim, Germany) according to the manufacturer's instructions. Briefly, total RNA was isolated from retina using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). A 1 μg aliquot of the total RNA in a volume of 20-μl was then reverse transcribed using oligodT_{12-18} according to the protocol of the SuperScript first-strand cDNA synthesis system (Invitrogen). A 1 μl aliquot of each RT reaction served as the template in a 20 μl PCR that contained 4.0 mmol/l $MgCl_2$, 0.3 μmol/l of each primer (forward primer, 5′-AGA AAG CCC AAT GAA GTG GTG-3′ and reverse primer, 5′-ACT CCA GGG CTT CAT CAT TG-3′) and 1 × LyteCycler DNA Master SYBR Green I mix (Roche Molecular Biochemicals). The cycling reactions were as follows: an initial