Inhibitory effect of losartan, an AT1 angiotensin II receptor antagonist, on increased leucocyte entrapment in retinal microcirculation of diabetic rats

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Background: The effectiveness of losartan for the treatment of leucocyte entrapment in the retinal microcirculation of diabetic rats was evaluated quantitatively.

Methods: After diabetes was induced by injection of streptozotocin (STZ), the rats were divided into two subgroups. The first subgroup (n = 6), received no medications; the second subgroup (n = 6) was given fresh drinking water supplemented with losartan (5 mg/kg/day) for 4 weeks. Six rats that were not injected with STZ or given medications served as controls. 4 weeks after intervention, leucocyte dynamics in the retina were observed using acridine orange digital fluorography. Leucocyte entrapment in the retina was compared among the three groups.

Results: In the untreated diabetic rats, the number of trapped leucocytes (6.1 (SD 1.4) cells/mm²) increased significantly compared with control rats (2.8 (1.2) cells/mm²; p = 0.005) and diabetic rats treated with losartan (3.1 (0.9) cells/mm²; p = 0.0002).

Conclusions: Losartan, an AT1 angiotensin II receptor antagonist, inhibited increased leucocyte entrapment in the diabetic retina. The authors demonstrated that losartan may have therapeutic efficacy in preventing development of diabetic retinopathy. Further clinical studies of the effect of the angiotensin receptor antagonist on preventing development of diabetic retinopathy are needed.

Diabetes can result in retinopathy, which causes vision loss and blindness. The currently available treatments for diabetic retinopathy are laser photocoagulation and vitrectomy. However, the number of patients who recover good visual function after treatment is limited. Thus, there is a need for a drug that can be used from the early stage of diabetes to prevent retinopathy.

Abnormalities of retinal haemodynamics precede the appearance of clinical retinopathy and may be associated with development of diabetic retinopathy.¹ Microvascular occlusion and endothelial cell damage in the diabetic retina are primary events in the pathogenesis of diabetic retinopathy and were associated with the presence of leucocytes.² Several investigators reported that increased leucocyte entrapment in the retina with early stage diabetes may be associated with vascular non-perfusion and vascular leakage and may initiate a series of events leading to diabetic retinopathy.³⁻⁵

Vascular endothelial growth factor (VEGF) plays a key part in the development of diabetic retinopathy.⁶ Because angiotensin II induces increased VEGF, angiotensin II may potentiate VEGF induced changes in the diabetic retina.⁷⁻⁸ The results of the recent study show the importance of the renin-angiotensin system (RAS) in the pathogenesis of diabetic retinopathy.⁹ Losartan is a potent AT1 angiotensin II receptor antagonist that has beneficial effects on hypertension and diabetic nephropathy.¹⁰⁻¹²

In the present study, we evaluated quantitatively the effectiveness of losartan in the treatment of leucocyte entrapment in the retinal microcirculation of diabetic rats.

METHODS

Animals

Eighteen Brown-Norway male rats (weight approximately 250 g) were treated according to the Association for Research in Vision and Ophthalmology statement on the use of animals in vision and ophthalmic research. The rats were anaesthetised

for all procedures. Hyperglycaemia was induced with one intraperitoneal injection of streptozotocin (STZ) (60 mg/kg, Sigma Chemical Co, St Louis, MO, USA) in citrate buffered saline (pH 4.5) after an overnight fast. The plasma glucose concentration was measured 48 hours after the STZ injection. The animals were determined to be diabetic if their non-fasting plasma glucose concentrations were greater than 250 mg/dl. Non-fasting blood glucose concentration, body weight, and water intake were monitored weekly.

After the animals developed diabetes, they were divided into two subgroups. The first subgroup (n = 6) was left untreated; the second subgroup (n = 6) was given fresh drinking water supplemented with losartan potassium (Mikromol GmbH, Luckenwalde, Germany; concentration 50 mg/l). The rats ingested approximately 5 mg/kg/day of losartan for 4 weeks. The rats that were not injected with STZ or medications served as controls (n = 6).

Acridine orange digital fluorography

The leucocyte dynamics in the retina were observed by acridine orange digital fluorography using the modified methods of Nishiwaki *et al.*¹³⁻¹⁴ Leucocytes were labelled with a fluorescent nuclear dye of acridine orange (Wako Pure Chemical, Osaka, Japan) administered intravenously and then imaged with a confocal scanning laser ophthalmoscope (SLO) (Heidelberg Retinal Angiograph, Heidelberg Engineering, Carlsbad, CA, USA). The argon blue laser was the illumination source. A regular emission filter for fluorescein angiography was used, because the spectral properties of leucocytes stained with acridine orange are similar to those of sodium fluorescein.

Four weeks after intervention, the animals were anaesthetised with intramuscular ketamine hydrochloride and intraperitoneal pentobarbitone sodium, and the pupils were dilated. Each rat had a catheter placed into the right jugular vein. Acridine orange was dissolved in sterile saline at a concentration of 1.0 mg/ml, and the solution was injected through

Table 1 Animal characteristics

	Untreated diabetic rats (n=6)	Control rats (n=6)	Diabetic rats treated with losartan (n=6)
Body weight (g)	242.5 (16.0)*	303.3 (16.3)	230.0 (34.6)*
Blood glucose (mg/dl)	390.3 (65.4)*	85.5 (6.2)	363.5 (63.8)*

*p<0.05 compared with control rats.

the right jugular vein catheter at a rate of 1 ml/min. The fundus of the left eye was observed by SLO in the 10° field. Thirty minutes after injection, the fundus was observed to evaluate leucocyte entrapment in the retina. The digital images were saved on a hard drive in a confocal SLO. An observation area surrounding the optic disc was determined by drawing a polygon surrounded by the adjacent major retinal vessels. The area was measured in pixels on a monitor in a confocal SLO, and the density of trapped leucocytes was calculated by dividing the number of trapped leucocytes, seen as fluorescent dots, by the area of the observation region. The densities of the leucocytes were calculated generally in eight peripapillary observation areas. An average density of trapped leucocytes for each rat was obtained by averaging the eight density values. The numbers of trapped leucocytes were calculated by a masked observer. After the experiment, the rats were killed with an overdose of anaesthesia, and the eyes were enucleated to determine a calibration factor for converting values measured on a monitor (in pixels) in a confocal SLO into real values (in μm). The calibration factor is the ratio between the actual size of each optic disc measured by microscopy and the apparent value on a computer monitor in a confocal SLO. All data were converted into real values using the calibration factor.

Statistical analysis

All comparisons were performed using Fisher's PLSD after one way analysis of variance. All results are expressed as mean (SD). A p value of 0.05 was considered statistically significant.

RESULTS

Table 1 shows the body weights and blood glucose levels after 4 weeks of intervention in the three groups. Digitised images of the fundus obtained from an untreated diabetic rat (A), a control rat (B), and a diabetic rat treated with losartan (C) 30 minutes after dye administration are shown in Figure 1.

Figure 2 shows the number of leucocytes trapped in the retinal microcirculation in the three groups. In the untreated diabetic rats, the number of trapped leucocytes (6.1 (1.4) cells/mm²) increased significantly compared to the control rats (2.8 (1.2) cells/mm²; p = 0.005) and the diabetic rats treated with losartan (3.1 (0.9) cells/mm²; p = 0.0002).

Figure 2 Leucocytes trapped in the retinal microcirculation. Values are means (SD). *p<0.05 compared with control rats and diabetic rats treated with losartan.

DISCUSSION

In the present study, we demonstrated the inhibitory effects of losartan, an AT1 angiotensin II receptor antagonist, on leucocyte entrapment in the diabetic retina. Miyamoto *et al* reported that leucocyte entrapment increased in the diabetic retina and VEGF induces retinal leucocyte entrapment, in part, through intercellular adhesion molecule-1.⁴⁻¹⁵ Otani *et al* reported that AT II induced VEGF mRNA expression in retinal vascular pericytes under high glucose conditions and induced mRNA expression of kinase insert domain protein receptor, a VEGF receptor, in retinal vascular endothelial cells through the AT1 receptor.⁷⁻⁸ AT1 angiotensin II receptor antagonist may inhibit increased leucocyte entrapment in the diabetic retina mediated by RAS-VEGF interaction. Further mechanistic studies about this effect are needed.

Losartan normalised increased leucocyte entrapment in the diabetic retina. However, there were no differences in blood glucose levels between the treated and untreated diabetic groups. A few studies have reported a protective effect of losartan on a model of retinal angiogenesis.¹⁶⁻¹⁷ We also reported that losartan inhibited development of laser induced choroidal neovascularisation in rats.¹⁸ Losartan seemed to inhibit not only retinal and choroidal angiogenesis but also leucocyte

Figure 1 Leucocytes trapped in the retinal microcirculation were observed as fluorescent dots 30 minutes after acridine orange injection in an untreated diabetic rat (A), a control rat (B), and a diabetic rat treated with losartan (C).

entrapment in the diabetic retina mediated by RAS independent of blood glucose.

Several investigators reported that losartan is a valuable new drug in the clinical treatment of another diabetic complication, diabetic nephropathy.¹⁰⁻¹² Several investigators reported that increased leucocyte entrapment in the retina with early stage diabetes may be associated with vascular non-perfusion and vascular leakage and may initiate a series of events leading to diabetic retinopathy.³⁻⁵ Angiotensin converting enzyme inhibitor was associated with reduced proliferative diabetic retinopathy, providing a potential clinical role for suppression of RAS in preventing and treating retinal neovascularisation.⁹ AT1 angiotensin II receptor antagonist may be a valuable new drug in the clinical treatment of not only diabetic nephropathy but also retinopathy.

In the present study, we demonstrated that losartan may have therapeutic efficacy in preventing the development of diabetic retinopathy. To our knowledge, there have been no reports that AT1 angiotensin II receptor antagonist has therapeutic efficacy in preventing the development of diabetic retinopathy clinically. Further clinical studies of the effect of the angiotensin receptor antagonist on preventing the development of diabetic retinopathy are needed.

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The authors have no proprietary interest in any aspect of this technology.

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