

Optic nerve lesions in diabetic rats: blood flow to the optic nerve, permeability of micro blood vessels and histopathology

Jun-Ping Zhao¹, Zhi-Zhong Ma², Chen Song³, Xiang-Hong Li⁴, Yu-Zhen Li⁵, Yu-Ying Liu⁵

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¹ Institute of Medical Informatics, Chinese PLA General Hospital, Beijing 100853, China

² The Ophthalmologic Center of Beijing University, Beijing 100083, China

³ Department of Ophthalmology, Chinese PLA General Hospital, Beijing 100853, China

⁴ Department of Pathology, Chinese PLA General Hospital, Beijing 100853, China

⁵ The Research Center of Pathophysiology, Chinese PLA General Hospital, Beijing 100853, China

Correspondence to: Jun-Ping Zhao. Institute of Medical Informatics, Chinese PLA General Hospital, Beijing 100853, China. zhaojp@301hospital.com.cn

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Abstract

• **AIM:** To study optic nerve lesions, changes in blood flow to the optic nerve, and permeability of micro blood vessels and histopathology in diabetic rats.

• **METHODS:** Male Wistar rats ($n=20$) were randomly divided into control and diabetic groups. The diabetic model was prepared by a single injection of streptozotocin (50mg/kg) into the caudal vein. Three months later, laser Doppler perfusion imaging was used to observe the changes in blood flow to the optic nerve. Each rat was injected with 15g/L Evans blue (5 μ L/g). The permeability of microvessels in diabetic optic nerves was measured by spectrophotometry. Optic nerves were observed by light and transmission electron microscopy.

• **RESULTS:** Diabetic rats had atrophic optic nerve fibers with neurite swelling, loss of myelin, and a greater-than-normal proliferation of astrocytes, occurring within 3 months of induction of diabetes. Blood flow to the optic nerve was lower in diabetic rats than in controls. Microvessel permeability in diabetic rats increased 2.03-fold compared to controls.

• **CONCLUSION:** Diabetic rats develop significant pathological changes in the optic nerve, reduced blood flow to the optic nerve and increase microvessel permeability.

• **KEYWORDS:** blood flow; diabetes; optic nerve lesion; permeability of microvessels

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INTRODUCTION

Diabetic ophthalmopathy is one of the most common chronic complications of diabetes mellitus. Thanks to recent advances in comprehensive systemic therapies (laser photocoagulation, microsurgery, vitrectomy, cryogenesis, and lens implantation), great progress has been made in the treatment of various diabetic ophthalmopathies, *e.g.*, diabetic retinopathy complications and diabetic cataract. As the national general status and living standards improve, diabetic optic neuropathy, which was only rarely noticed in the past, has now become the focus of many investigational studies. Diabetic optic neuropathy research has traditionally focused primarily on descriptions of clinical signs and symptoms, associated physiological electrical activities, and experimental drug treatments [1-3]. However, the pathogenic mechanism(s) underlying diabetic optic neuropathy remains unclear. In this study, a 3-month diabetic rat model was used to observe pathological changes in the optic nerve, changes in microvessel permeability, blood flow to the optic nerve and histopathology, all with the purpose of discovering the underlying pathogenic mechanism(s). In addition, the study provides laboratory-based evidence that could be useful for development of new effective treatments for the complications associated with diabetic optic neuropathy.

MATERIALS AND METHODS

Materials Healthy male Wistar rats ($n=20$), with body weights of 190-200g (provided by the Animal Research Center of the General Hospital of the Chinese People's Liberation Army) were randomly assigned into diabetic ($n=10$) and control ($n=10$) groups. Before preparation of the

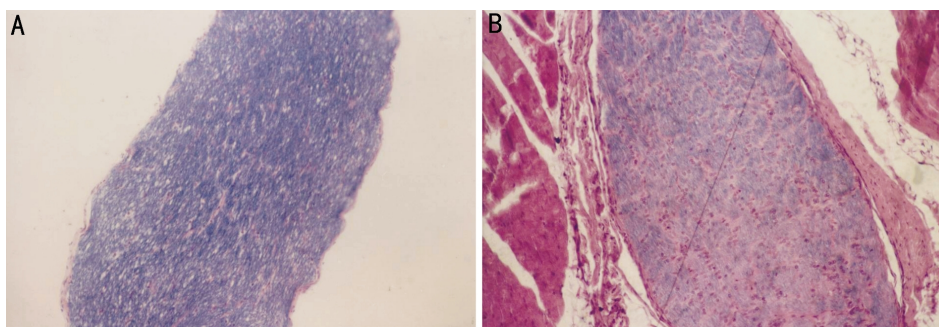


Figure 1 Optic nerve fiber structure in rats A: Control; B: Diabetic

diabetic model, all rats were restrained from eating for 12 hours. Diabetes was induced by injecting streptozotocin (50mg/kg) into the caudal vein. Rats in the control group were injected with an equivalent amount of acidified saline solution. After injection, all rats were placed in metabolic cages and fed routinely. Rats were observed daily. Food intake, excretion, and urine and blood glucose were measured and recorded periodically. The criteria for success of model preparation were 1) blood glucose ≥ 16.67 mmol/L; 2) 24-hour water intake >40 mL; 3) urine glucose $+++$, and 4) gradual decrease in body weight over the course of 3 months. After successful preparation of the model, rats were placed back into general larger cages.

Methods

Measurement of blood flow to the optic nerve At 3 months, diabetic rats were anesthetized by intraperitoneal injection of 200g/L urethane. Following surgery, their optic nerves were isolated, exposed, and viewed under a dissecting microscope. The probe of the laser Doppler's perfusion imaging instrument (PIMI 1.0, Retrac Co., Sweden) was aimed at the optic nerve and tested by dynamic single-point measurements. At each point, sampling was performed 4,096 times, and the average, maximum, and minimum values recorded.

Measurement of microvessel permeability Caudal injection of 15g/L Evans blue (5 μ L/g) was performed and the rat's head was severed one hour later. The optic nerve (with inner, intraocular, and intracranial segments) was obtained from both eyes. Each nerve was weighed and placed in 2mL formamide solution. Each was then placed in a water bath at a constant temperature of 54°C for 24 hours in order to allow the Evans blue in the tissues to be fully dissolved in the formamide solution. After filtering, the solution was tested spectrophotometrically at 620nm wavelength to obtain its absorbance value. Results are presented as the optic nerve's relative value of mg Evans blue/g.

Optic nerve pathology One eyeball, containing a small segment of optic nerve, was removed from each rat and

fixed in 100g/L formaldehyde before being routinely prepared for paraffin embedding and sectioning for light microscopy. Myelin was stained by Luxol fast blue and the optic nerve was viewed by light microscopy. The other eyeball of each rat was removed and the optic nerve near the screening plate was fixed immediately for 2 hours in 30g/L glutaraldehyde. It was then post-fixed in 10g/L osmium and dehydrated. Tissue was embedded in Epon 812 resin and sectioned on an ultramicrotome. Ultrathin sections were stained with acetate acid and lead citrate, and viewed on a transmission electron microscope.

Statistical Analysis All data are presented in the form of mean \pm SD. Intergroup comparisons were made using the paired *t*-test.

RESULTS

Pathology of optic nerve Light microscopy revealed that optic nerve fiber structures in control group rats were normal, with no proliferation of glial cells and no loss of myelin (Figure 1A). The diabetic group showed apparent myelin loss, accompanied by a greater-than-normal proliferation of astrocytes (Figure 1B). Electron microscopy revealed homogeneously intact neural myelin and no atrophic optic nerve fibers in the control group. No proliferation of astrocytes or fibrous tissue was seen. Blood vessels and pial capillaries were also normal in the control group (Figure 2A). In the diabetic group, there were formation of vacuoles and loss of optic nerve myelin. Atrophic neural fibers, and proliferation of astrocytes were seen. Additionally, there was a reduced number of organelles in the endothelial cells of optic nerve capillaries and the neurites were swollen. In pial capillaries, signs of aggregation of white blood cells and adhesion to endothelial cells were noted (Figure 2B).

Blood Flow to the optic nerve The average blood flow to the optic nerve of rats in the control group was 1.43 ± 0.58 v, while the average blood flow to the optic nerve in the diabetic group was 0.68 ± 0.05 v. There was a statistically significant difference between the two groups ($P < 0.01$).

Microvessel permeability The permeability of microvessels

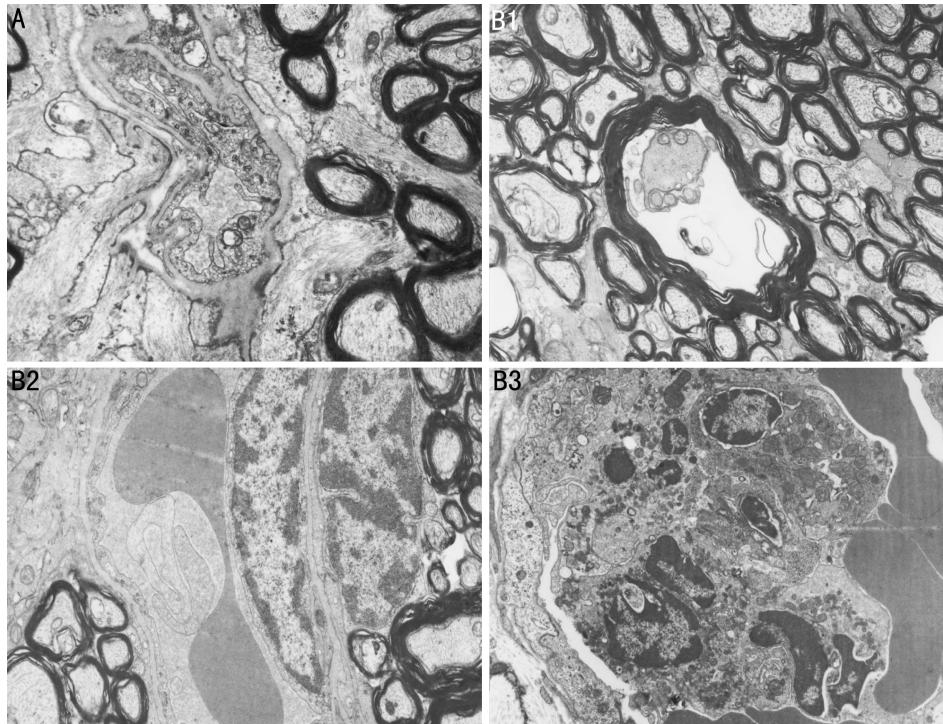


Figure 2 Optic nerve fiber ultrastructure in rats A: Control; B1, B2 and B3: Diabetic

in the optic nerve of control group rats was 6.34 ± 0.85 mg Evans blue/g optic nerve, while that of 3-month diabetic rats increased by 2.03 fold to 19.41 ± 6.18 mg Evans blue/g. There was a statistically significant difference between the two groups ($P < 0.01$).

DISCUSSION

Most researches have demonstrated that there is significant obstructive microcirculation in diabetes and that this circumstance can affect blood perfusion into tissues. Therefore, the measurement of blood perfusion into a tissue provides a comprehensive index of the tissue's microcirculatory status. There are numerous reports of blood flow in diabetic ophthalmopathy, specifically in diabetic retinopathy. Takagi *et al*^[4] demonstrated that blood flow to the retina in diabetic rats is significantly reduced and that normal automatic regulatory function is reduced as well. Bursell *et al*^[5] discovered that blood flow to the retina of insulin-dependent diabetic patients is also reduced. However, Grunwald *et al*^[6] discovered that blood flow to the retina increases 15% with high blood glucose in uncontrolled insulin-dependent diabetic patients.

In optic nerve studies, Ogasawara *et al*^[7] used laser Doppler to show that the rate of blood flow in optic papilla capillaries is significantly lower when there was diabetes without retinopathy. However, the question of regulation of changes in blood supply to the optic nerve during diabetes remains unanswered.

The present study used laser Doppler perfusion imaging to

measure the changes in blood flow to the optic nerve of 3-month diabetic rats, showing that blood flow is significantly reduced and that the optic nerve exhibits low blood perfusion at 3-month duration of diabetes. According to other reports, high perfusion status and increased blood flow are seen in tissues and organs, *e.g.*, kidneys, during the early stage of diabetes^[8]. In this study, the low perfusion phenomenon in the optic nerve suggests that the 3-month period of diabetes was not early-stage diabetes, since these rats already exhibited pathological tissues changes.

Behind the screen plate of the optic nerve, there are myelinated nerve fibers that are extremely sensitive to ischemia and hypoxia. Our histopathological findings in 3-month diabetic rats clearly demonstrated signs, *e.g.*, atrophy, vacuolization of myelin, segmentation loss, and proliferation of astrocytes in optic nerve fibers, that support the existence of pathological lesions in the optic nerve in diabetes. Induced diabetic lesions include termination of plasma axial flow in optic nerve fibers under ischemia and hypoxia, induced by low blood perfusion in the optic nerve. This also causes interruption in the supply of nutritional neural factors, another possible reason for primary injury to the optic nerve. At the same time, because the optic nerve is part of the axis of retinal ganglia, low blood perfusion causes increases in excitable toxins and free radicals, which, through intracellular signal transduction pathways, activate apoptotic genes that further cause both apoptosis in ganglion cells and secondary injury to the optic nerve. The optic

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nerve's nutritional metabolism is also dysfunctional, causing degeneration of nerve fibers and injury to glial cells. Small glial cells cannot regenerate and the damaged region is filled in primarily by astrocytes, which have stronger proliferative capabilities. Therefore, the proliferation of astrocytes can indirectly reflect the degree of severity of injury to optic nerve fibers.

Edema in optic nerve papilla is a neural pathological sign of diabetes; its incidence rate in patients with diabetes is about 0.4% . In this study, the permeability of optic nerve microvessels in diabetic rats was significantly increased. Permeability of microvessels represents a comprehensive index of the health status of the tight junctions of endothelial cells and capillary basal membranes. The histopathology findings of this study show that there was a reduction in the number of organelles in capillary endothelial cells of diabetic rats; many observed organelles were swollen. This result demonstrates that endothelial cells of optic nerve microvessels are damaged after a 3-month duration of diabetes, resulting in increased permeability of optic nerve microvessels and edema of the optic nerve papilla.

Overall, 3-month diabetic rats showed significant histopathological changes. The underlying causes of these changes (with the exception of high blood glucose), *i.e.*, reduced blood flow to the optic nerve and increased microvessel

permeability, perhaps contribute to the development and progression of diabetic optic neuropathy^{9,10}.

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