Finasteride Reduces Microvessel Density and Expression of Vascular Endothelial Growth Factor in Renal Tissue of Diabetic Rats

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Abstract: *Background:* Vascular endothelial growth factor (VEGF) plays a critical role in the pathogenesis of diabetic microvascular complications. Finasteride has been confirmed to decrease VEGF expression in prostate and prostatic suburethral tissue resulting in limiting hematuria from human benign prostatic hyperplasia. The purpose of this study was to evaluate the effects of finasteride on microvessel density (MVD), VEGF protein and mRNA expressions in the renal tissue of diabetic rats. *Methods:* Diabetic rats induced by streptozotocin were intragastrically given finasteride at 30 mg/kg body weight once a day for 4 weeks. Histomorphologic changes in kidney were observed under light microscope. Immunohistochemistry for CD34 and VEGF on kidney sections was performed to assess MVD and VEGF protein expression in glomeruli of rats, respectively. The VEGF mRNA expression in the renal tissue was examined using reverse transcription polymerase chain reaction analysis. *Results:* The glomerular tuft area, glomerular volume, MVD, VEGF protein expression in glomeruli and VEGF mRNA expression in the renal cortex tissue were significantly increased in diabetic rats and finasteride-treated rats when compared with controls ($P < 0.01$, $P < 0.05$). When compared with diabetic rats, the glomerular tuft area, glomerular volume, MVD, VEGF protein expression in glomeruli and VEGF mRNA expression in the renal cortex tissue of finasteride-treated rats were significantly decreased ($P < 0.05$, $P < 0.01$). *Conclusions:* Finasteride reduces the VEGF expression and decreases the MVD in the renal tissue of diabetic rats, suggesting the therapeutic potential of finasteride on diabetic microvascular complications.

Key Indexing Terms: Finasteride; Vascular endothelial growth factor; Microvessel density; Kidney; Diabetes mellitus. **[Am J Med Sci 2015;349(6):516–520.]**

Patients with diabetes with microvascular complications are suffering serious troubles on the quality of life. Pathologic angiogenesis plays an important role in the development and progression of diabetic microvascular complications. Vascular endothelial growth factor (VEGF), a member of the family of heparin-binding growth factors, is very potent in stimulating endothelial cell proliferation and angiogenesis under both physiologic and pathologic conditions.[1,2] Finasteride is the first 5α-reductase inhibitor that received clinical approval for the treatment of human benign prostatic hyperplasia (BPH).[3,4] Several studies have demonstrated that finasteride can inhibit angiogenesis and reduce prostate bleeding due to decreasing VEGF expression in human prostate and prostatic suburethral tissue.[5,6] An animal study has shown that finasteride is effective to prevent VEGF expression and microvascular development in the retina of early diabetic rats.[7] However, the effects of finasteride on VEGF expression in diabetic renal tissue remain unclear. In this study, the authors evaluated the effects of finasteride on microvessel density, protein and mRNA expressions of VEGF in renal tissue of diabetic rats induced by streptozotocin.

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MATERIALS AND METHODS

Reagents

Finasteride (Lot 20120801) was from Kailun Chemical and Advanced Material Co (Wuhan, China). Streptozotocin (Cat ALX-380–010-G001) was from Alexis Biochemical (San Diego, CA). Monoclonal mouse anti-CD34 antibody (Cat ZM-0046) and monoclonal rabbit anti-VEGF antibody (Cat ZA-0509) were from Zhongshan Golden Bridge Biotechnology Co (ZSGB Bio, Beijing, China). Trizol reagent (Cat SK1321) was from Sangon Biotech (Shanghai, China). Taq PCR reagent (Cat BS9296) was from Thermo Scientific (Shanghai, China).

Diabetic Model

Male Wistar rats weighting 230 to 250 g were supplied by Laboratory Animal Center of Hebei (China). All animals were housed in polypropylene cages under 12-hour light/dark cycle with free access to rodent chow and tap water. The rats were intraperitoneally given a single dose of STZ at 65 mg/kg body weight after fasting for 12 hours. STZ was freshly dissolved in cold citrate buffer (pH 4.4) for immediate use within 5 minutes. Rats in which fasting blood glucose level exceeded 17 mmol/L at 72 hours after STZ injection were considered diabetic. Control rats (n = 7) received an equivalent volume of citrate buffer alone. The diabetic rats were randomly stratified into diabetic model group and finasteride-treated group (n = 7 per group). The rats in finasteride-treated group were intragastrically given finasteride at 30 mg/kg body weight once a day for 4 weeks. Finasteride was prepared as a fine suspension in 0.5% sodium carboxymethyl cellulose solution in water. The rats in control group and diabetic model group were intragastrically given the same volume of sodium carboxymethyl cellulose solution for 4 weeks. Blood glucose level and body weight of all animals were weekly examined during the whole experiment period. All animals used in this study received humane care in compliance with institutional animal care guidelines. The study was approved by the Local Institutional Committee.

Tissue Preparation

Fasting blood glucose concentration and body weight of rats were measured after the last administration. All animals were intraperitoneally anesthetized with urethane at 1.5 g/kg

TABLE 1. Body weight and blood glucose level of rats

Groups	Body weight, g		Blood glucose, mmol/L	
	Day 3	Week 4	Day 3	Week 4
Control group	314.0 ± 14.4	354.0 ± 20.3	4.1 ± 0.6	4.9 ± 0.7
Diabetic model group	262.3 ± 28.4[a]	249.8 ± 38.3[a]	20.7 ± 3.9[a]	20.8 ± 3.1[a]
Finasteride-treated group	267.8 ± 23.1[a]	244.3 ± 32.5[a]	19.8 ± 2.2[a]	25.3 ± 3.6[a]

Each of the 3 groups contained 7 rats (n = 7).
[a] $P < 0.01$ compared with control group.

body weight, and the kidneys were dissected out. The right kidney was weighed and the wet weight index of kidney was calculated as follows: wet weight index = wet weight of kidney (in grams)/body weight (in grams) × 1,000. After the measurement, the right kidney was immediately fixed in 10% neutral formalin solution, routinely embedded in paraffin and consecutively cut into 4 μm sections for histomorphology and immunohistochemistry. The cortex of the left kidney was separated, cut into fine fragments and stored in −70°C refrigerator for reverse transcription polymerase chain reaction (RT-PCR) analysis.

Histomorphology

Sections from right kidney of rats were stained with hematoxylin and eosin. The histomorphologic changes were observed under light microscope (Olympus, Japan). In each animal, the glomerular tuft areas (G_a) were measured using an image analysis system (BI-2000; Taimeng Software Co, Ltd, Chengdu, China) at a magnification of ×400 as the average area of a total of 70 glomerular profiles. The glomerular volume (G_v) was determined from the glomerular tuft area and calculated as follows: $G_v = \beta/k \times (G_a)^{3/2}$, where $\beta = 1.38$, which is the shape coefficient for spheres (the idealized shape of glomeruli), and $k = 1.1$, which is a size distribution coefficient.[8] Each section was examined in a blinded fashion.

Immunohistochemistry

Immunohistochemistry Methods for CD34 and VEGF

Sections from right kidney of rats were floated onto polylysine coated slides for immunohistochemical staining using a standard streptavidin peroxidase method. The slides were dewaxed in xylene and dehydrated through a graded series of ethanols. Heat-induced epitope retrieval was performed by immersing the slides in citrate buffer (pH 6.0) and heating at 95°C for 20 minutes, before cooling and rinsing with phosphate-buffered saline (PBS). Endogenous peroxidase activity was quenched by incubation with 3% hydrogen peroxide at 37°C for 10 minutes. Nonspecific protein binding sites were blocked by incubating the sections with normal goat serum. The slides were then incubated with mouse anti-CD34 (1:100) or rabbit anti-VEGF (1:100) primary antibody overnight at 4°C in a humidified chamber. Negative control sections were incubated in PBS without primary antibody. After rinsing with PBS, the tissue-bound primary antibody was detected using biotinylated secondary antibody and horseradish peroxidase biotinylated streptavidin complex (Histostain Plus Kits; ZSGB Bio), with diaminobenzidine (ZSGB Bio) as the chromogen. Finally, the slides were counterstained with hematoxylin, dehydrated and mounted with DPX.

Microvessel Counting

Any endothelial cell or endothelial cell cluster positively stained for CD34 and clearly separated from the adjacent structures was considered a single, countable microvessel. Microvessels were counted in 10 nonrepeated glomeruli for each slide under light microscope at a magnification of 400×, and the mean of the microvessel counts was recorded as the microvessel density.

VEGF Immunostaining Score

To evaluate the expression of VEGF protein, we established a score corresponding to the staining intensity and the percentage of positively staining cells. The cell intensity of VEGF expression (I) was graded for absent or weak staining, moderate staining and strong staining of VEGF, and scores of 1, 2 and 3 were assigned, respectively. The percentage of cells with VEGF positive staining (P) was estimated in 10 nonrepeated glomeruli for each slide. Therefore, a VEGF index (I × P) was calculated for comparison of VEGF expression among 3 groups.[6] Each section was examined in a blinded manner.

Semiquantitative RT-PCR

Total RNA was extracted from cortex tissue of the left kidney using a Trizol reagent. Single strand cDNA was

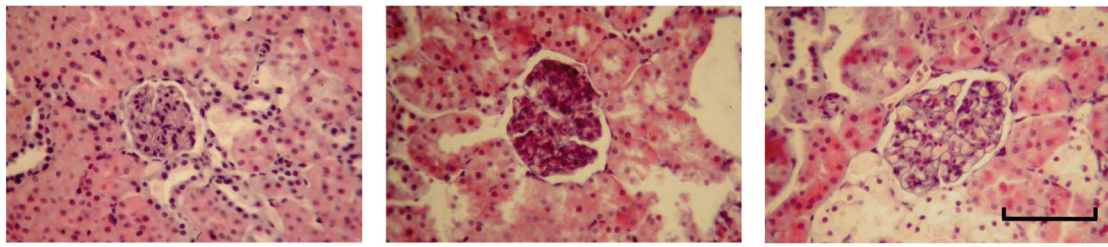

FIGURE 1. Histomorphologic changes in the renal tissue of rats (HE staining; magnification, 400×). Note the enlarged glomeruli in diabetic rats (B) and finasteride-treated rats (C) compared with control rats (A). Each of the 3 groups contained 7 rats (n = 7). Bar: 50 μm.