Effect of Curcumin on Vascular Endothelial Growth Factor Expression in Diabetic Mice Kidney Induced by Streptozotocin

Tarinee Sawatpanich MSc*, Hattaya Petpiboolthai MSc*,
Busaba Punyarachun PhD*, Vipavee Anupunpisit PhD*

** Department of Anatomy, Faculty of Medicine, Srinakharinwirot University, Bangkok, Thailand*

Objective: *To localize and demonstrate the effect of curcumin on vascular endothelial growth factor in diabetic mice kidney induced by streptozotocin*

Material and Method: *Diabetic mice were induced by streptozotocin (60 mg/kg BW). Male mice were divided into three groups, control mice, diabetic mice (DM) and diabetic mice treated with curcumin (DMC) (200 mg/kg BW). At 4 and 8 weeks, animals were sacrificed and kidneys were processed by immunohistochemistry technique.*

Results: *At the end of 4 and 8 week experiments, glomeruli were slightly enlarged and showed diffuse thickening of the glomerular capillary walls in diabetic mice. Administration with curcumin presented the better improvement and recovery of cells and tissues compared with diabetic mice. Immunohistochemical staining for vascular endothelial growth factor (VEGF) demonstrated that VEGF was mainly detected in the podocytes and renal tubules. There was an increase in VEGF expression in diabetic mice as compared to control. Treatment with curcumin significantly inhibited the expression of VEGF in the kidney tissue of diabetic mice in both 4 and 8 weeks. Comparing the diabetic mice between 4 and 8 week experiments, the expression of VEGF in the podocytes and renal tubules at 8 week were significantly stronger than at 4 week which represented time-dependent change. Nevertheless, the intensity of VEGF was not different in DMC mice when it was compared between 4 and 8 weeks.*

Conclusion: *VEGF immunoreactivity of the podocytes and the renal tubules at 4 and 8 weeks in DM mice showed strong intensity more than in control mice. However, the intensity of VEGF in DMC mice was less when it was compared with DM mice. Moreover, VEGF was a key modulator of angiogenesis and a potent mitogen for endothelial cells. These results demonstrated the potential use of antiangiogenic curcumin as a novel therapeutic agent in diabetic mellitus and maintain normal structure of the kidney.*

Keywords: *Curcumin, Vascular endothelial growth factor, Streptozotocin, Immunohistochemistry*

J Med Assoc Thai 2010; 93 (Suppl. 2): S1-8
Full text. e-Journal: *http://www.mat.or.th/journal*

Diabetic nephropathy is one of major microvascular complications and is the leading cause of end stage renal disease worldwide. Vascular endothelial growth factor (VEGF) is an important mediator in maintaining normal kidney function. It is a potent cytokine that is considered to be an important mediator in the pathogenesis of endothelial dysfunction in diabetes. In addition, several lines of evidence suggest that up-regulation of VEGF in glomeruli may be associated with or cause renal dysfunction such as diabetic nephropathy[1]. In the kidney, VEGF was detected pre-dominantly in glomerular podocytes and in renal tubular epithelial cells. VEGF is an endothelial specific growth factor that induces microvascular hyperpermeability and participates in interstitial matrix remodeling, promotes endothelial cell proliferation, differentiation and survival, mediates endothelial-depen-

Correspondence to: Anupunpisit V, Department of Anatomy, Faculty of Medicine, Srinakharinwirot University, Sukhumvit 23, Wattana, Bangkok 10110, Thailand. Phone: 0-2260-2234 ext. 4501, 4502, Fax: 0-2260-1532. E-mail: vipavee19@yahoo.com

dent vasodilatation[2]. VEGF is up-regulation in streptozotocin (STZ) induced diabetic animal and human with type I and type II diabetes. Therefore inhibition VEGF function has beneficial effect on diabetic nephropathy[3,4]. Treatment of monoclonal neutralizing anti VEGF antibodies for 6 weeks caused clearly dysfunction in DM type I, abolished the urinary albumin excretion (UAE), glomerular hypertrophy and glomerular hyper-filtration without an effect on metabolic control[5]. The glomerular VEGF expression was highest in the type I diabetic patients with mildest sclerotic changes and was particularly strong in viable podocyte[5], but was lowered or absent in sclerotic glomeruli[6].

Management of diabetes without any side effect is still a challenge to the medical system. There is an increasing demand by patient to use the natural products with antidiabetic activity[7]. Curcumin, the natural occurring compound in turmeric is known as a wonderful molecule with several proven medical effects, including anti-inflammatory, anti-diabetic, antioxidant properties and anti-renal lesion effect[8,9].

Nephropathy is one of the dangerous secondary complications induced by diabetes. In an animal model study, it was observed the dietary curcumin brought about significant inhibition in the progression of renal lesion. Curcumin fed at 0.5% level in the diet to STZ-induced diabetic rats lessened renal damage and preserved the integrity and function of the kidney[9].

Hyperglycemia causes release of tissue damaging reactive oxygen species (ROS) balance between radical production and antioxidant defense[11]. It has been proposed that STZ acts as a diabetogenic agent owing to its ability to destroy pancreatic beta cells[12]. Curcumin and its derivations, demethoxycurcumin and bis-demethoxycurcumin had the potential to control the diseases through its potent antioxidant activity[10,11]. Curcumin lowered the production of ROS *in vitro*[12], so it also maintained the activities of antioxidant enzymes like superoxide dismutase, glutathione peroxidase and catalase[13]. *In vitro*, curcumin could significantly inhibit ROS generation by activated macrophages, which play an important role in inflammation[12].

In this work, the cellular localizations and comparative expression of VEGF in diabetic mice's kidney were studied before and after administration of curcumin, which is a coloring principle of the commonly used spice turmeric (*Curcuma longa).* Curcumin was expected to have application as a novel natural material to control and attenuates diabetic mellitus renal damage.

Material and Method

Induction of diabetes and experimental protocols

Male mice (30-35 g) obtained from National Laboratory Animal Center of Mahidol University. Experimental diabetes were induced by triple intra peritoneal of streptozotocin (STZ) (Sigma, St, Louis, MO, USA) (60 mg/kg BW) dissolved in 0.9% normal saline. Control mice received injection with 0.9% normal saline alone. Mice with blood sugar level > 250 mg/dl were used as diabetic animals. Control and diabetic mice were randomly selected and divided in three groups (10 mice/group), control mice, diabetic mice (DM) and diabetic mice treated with curcumin (99.99% pure, Sigma) (200 mg/kg BW) (DMC). At the end of 4 and 8 weeks of experimental duration, the animals were sacrificed. Kidneys were quickly excised and processed for immunohistochemistry.

Immunohistochemistry

Five μm thick sections from Bouin's solution fixed paraffin-embedded kidney tissue were placed onto slides, rehydrated in xylene and graded alcohols. Tissues were immersed in 3% H_2O_2 in methanol for 1 hour to block endogenous peroxidase, followed by incubation in serum blocking solution. The primary antibody, monoclonal antibody VEGF (Santa Cruz Biotechnology Inc, USA) was added at a 1:25 dilution for 72 hours at 4°C. Negative control sections were stained under identical conditions with the buffer solution substituting for the primary antibody. After washing with phosphate buffered saline (PBS), sections were incubated with biotinylated secondary antibody IgG for 30 minutes, washed in PBS. Sections were next incu-bated with avidin-biotin complex (Zymed Laboratories, USA). Peroxidase conjugated were subsequently localized by diaminobenzidine tetrahydro chloride as a chromogen, then the positive reaction result was presented in brown color in cells and tissues. Slides were counterstained with hematoxyline, dehydrated through series of alcohol and xylene, covered with a glass cover slipped and observed by light microscope. Each slide was evaluated the intensity of VEGF by observers un-aware of the experiment details (50 slides/group). Comparative intensity of VEGF was revealed by using the following scales; 0, absent or very low intensity; +, low intensity; ++, mild intensity; +++, moderate inten-sity; ++++, high intensity[14].

Results

In general, immunohistochemical staining for VEGF in control mice demonstrated that VEGF was

fundamentally stained in the glomerular visceral epithelial cell or podocytes (P), evidenced by brown cytoplasmic staining. In renal tubule compartment, all positive stained cells showed a localized cytoplasmic pattern, which was focally increased in the collecting tubules (CT), distal convoluted tubules (DCT), thick (Tk) and thin (Tn) loops of Henle and to a lesser extent in proximal convoluted tubules (PCT), while it was abscence in mesangial (M) and glomerular capillary cells (Ca) in control mice (Fig. 1A-D).

The kidney at 4 and 8 weeks revealed glomerular and renal tubular hypertrophy, and glomerular capillary cells dilatation in all DM mice. After treatment by curcumin, kidney tissues in DMC mice dramatically reduced glomerular and renal tubular hypertrophy and glomerular capillary dilatation as compared with DM mice.

Concerning the result of each of 4 and 8 week experiments, VEGF immunoreactivity of the podocytes and renal tubules inducing collecting tubules, distal convoluted tubules and proximal convoluted tubules in DM (Fig. 2C-D, 3C-D) and DMC (Fig. 2E-F, 3E-F) revealed strong intensity more than in control group (Fig. 2A-B, 3A-B). However, the intensity of VEGF of the podocytes, collecting tubules, distal convoluted tubules and proximal convoluted tubules in DMC group (Fig. 2E-F, 3E-F) was less when it was compared with DM group (Fig. 2C-D, 3C-D). In comparison, the expression of VEGF in DM group between 4- and 8-week experiments, it was demonstrated that the VEGF intensity was strongly stained in 8 week group (Fig. 2C-D, 3C-D). Nevertheless, the expression of VEGF in DMC group was not significantly different between 4 and 8 week experiments (Fig. 2E-F, 3E-F). In summary, the comparison of VEGF intensity among control, DM and DMC mice of 4- and 8-week experiments was demonstrated in Table 1.

Discussion

STZ-injected mice showed significant increase in blood glucose, polyuria and a decrease in bodyweight compared with age- matched control mice[15]. We developed a model for DM type I by the injection of STZ into mice. It is a highly effective cyto-

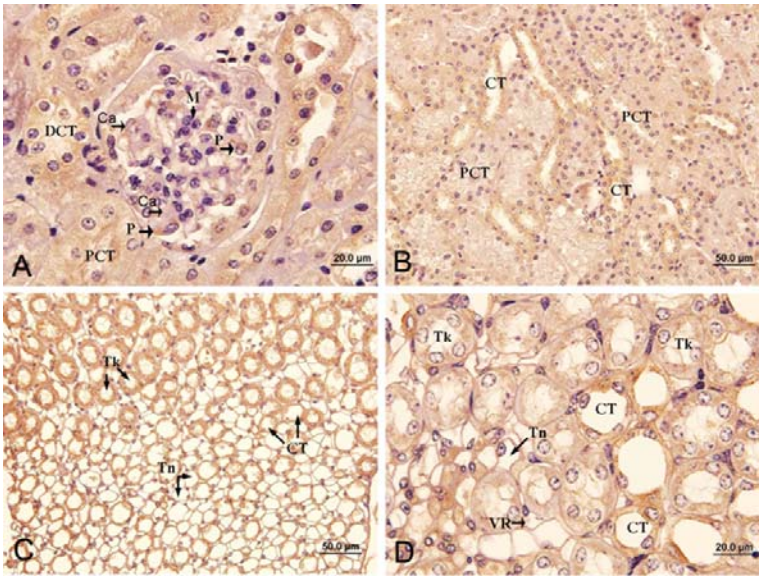

Fig. 1 Micrographs of immunoreactivity staining of mice kidney in the control group showed the distribution of VEGF the podocyte (P), collecting tubules (CT), distal convoluted tubules (DCT), loops of Henle (Tk = thick, Tn = thin), proximal convoluted tubules (PCT), whereas VEGF was not presented in mesangial cells (M), glomerular capillary cells (Ca) and vasa recta (VR). A = The glomerulus with positive staining for VEGF with labeling of podocyte (P) and renal tubules (DCT and PCT), B = Low magnification of the renal cortex showing the immunoreactivity staining of VEGF in the CT, DCT and PCT, C-D = Low (C) and high (D) magnifications of the renal medulla showing the immunoreactivity staining of VEGF in the CT, Tk and Tn

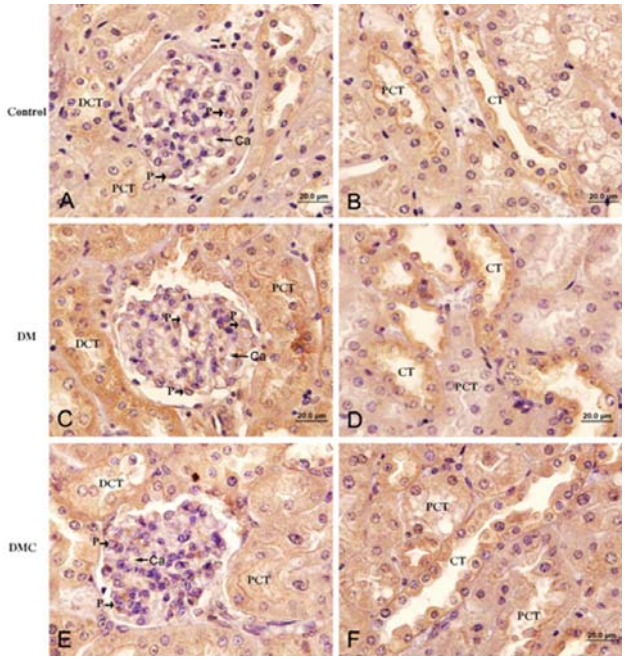

Fig. 2 Comparison of VEGF immunoreactivity in the podocytes (P), collecting tubules (CT), distal convoluted tubules (DCT) and proximal convoluted tubules (PCT) among three groups at 4 week, A-B = Control mice; C-D = Diabetic mice (DM); E-F = Diabetic mice treated with curcumin (DMC) Abbreviation; Ca : glomerular capillary cells

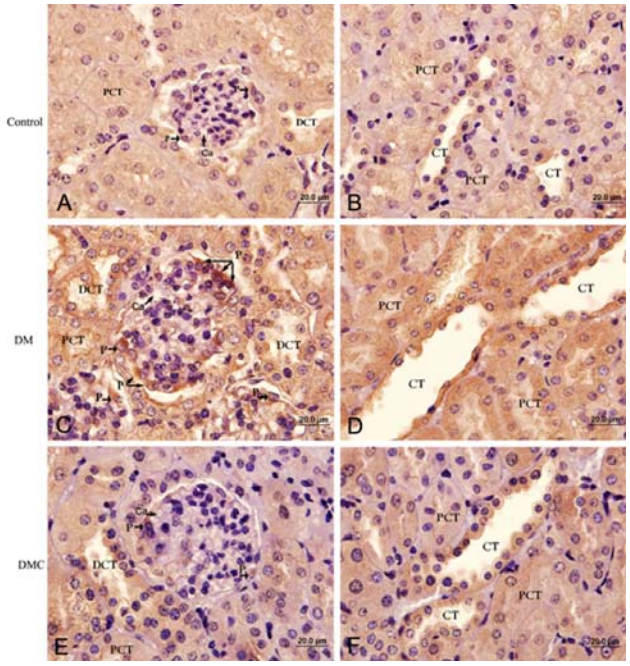

Fig. 3 Comparison of VEGF immunoreactivity in the podocytes (P), collecting tubules (CT), distal convoluted tubules (DCT) and proximal convoluted tubules (PCT) among three groups at 8 week, A-B = Control mice; C-D = Diabetic mice (DM); E-F = Diabetic mice treated with curcumin (DMC) Abbreviation; Ca: glomerular capillary cells

Table 1. VEGF intensity level of the podocytes and renal tubules among three groups at 4 and 8 weeks experiments

Week	Control	DM	DMC
4	+	+++	++
8	+	++++	++

toxic agent for pancreatic beta cells, and STZ treated animals have been used as a model for DM type I[16]. The kidney at 4 and 8 weeks revealed glomerular and renal tubular hypertrophy and glomerular capillary cells dilatation in all DM mice. The treatment of curcumin (200 mg/kg BW) in DMC mice dramatically reduced glomerular and renal tubular hypertrophy and glomerular capillary cells dilatation as compared with the DM mice. These results indicated that STZ-induced histological damages associated with glomerular and renal tubular hypertrophy and glomerular capillary cells dilatation were diminished after curcumin treatment.

Glomerular and renal tubular hypertrophy are well-recognized of diabetic mice. They are characterized by an increased number of glomerular capillary cells, which are due to the formation of new capillaries rather than lengthening or dilating of existing capillaries[17]. As an important mediators of physiological and pathological angiogenesis, glomerular hypertrophy is associated with glomerular hyperfiltration and hyperglycemia in type I diabetes[18].

In the present study, VEGF expression in kidney tissues increased significantly during the early period of diabetes. These findings agreed with those of previous reports that renal VEGF mRNA expression increased during the early period of diabetic nephropathy in type II diabetic rats[19-21] and pronounced VEGF up-regulation occurring in glomerular podocytes in the early stage of diabetic nephropathy[22].

VEGF is likely to play a role in the process by inducing the proliferation of glomerular capillary cells. In early diabetic nephropathy in STZ-induced diabetic mice, the increased glomerular volume and glomerular capillary cells were accompanied by enhance VEGF expression[23]. During the course of crescentic glomerular nephritis, loss of glomerular capillaries was associated with decreased VEGF immunoreactivity[24].

In this study, the immunoreactivity of VEGF in renal tissues was high in diabetic state and dietary curcumin showed a pronounced reversing trend on this at 4 and 8 weeks. Diabetic retinopathy is accompanied by increase in various angiogenic factors, including VEGF and hypoxia-induced growth factor (HIF), which are considered to play a pivotal role in the induced cellular permeability and angiogenesis[25]. We have demonstrated that curcumin administration suppresses VEGF levels in the diabetic kidney. In agreement, others have shown that curcumin can abolish IL-18, which induces the increasing of VEGF production in rheumatoid arthritis synovial fibroblastd via AP-1 dependent pathways[26].

The increase of VEGF in the diabetic kidney is consistent with *in vitro* studies suggesting that higher glucose level can induce VEGF expression. It has been reported that acute hyperglycemic stimulates VEGF gene expression in vascular smooth muscle cells, via protein kinase C dependent pathway[27]. Protein kinase C beta II inhibition was associated not only with reduced VEGF-induced retinal permea bility[28], but also with retardation of development of albuminuria in diabetic rats induced by STZ[29]. Elevated glucose level leaded to activate of the sorbital pathway, protein kinase C, hexosamine pathway, advanced glycation end product[30], reactive oxygen species, cytokines and growth factor production[31]. Direct high glucose-induced protein kinase C and mitogen activated protein kinase activation also cause increased production of oxidative stress in the diabetic kidney[32]. Curcumin inhibited phorbol-12 myristate-13 acetate, which induced reactive oxygen species generation, suggesting that curcumin has an inhibitory action on protein kinase C activity[33].

Conclusion

STZ-induced histological damages associated with glomerular, renal tubular hypertrophy and upregulated VEGF expression were diminished after curcumin treatment. VEGF may participate in the progression of the early stage of diabetic renal injury. Treatment with curcumin significantly inhibited expression of VEGF in kidney of diabetic group. These results demonstrated the potential use of antiangio-genic curcumin as a novel therapeutic agent in diabetic nephropathy.

Acknowledgements

This work is supported by Research Grant year 2007 and Graduate School, Srinakharinwirot University 2010

References

1. Liu E, Morimoto M, Kitajima S, Koike T, Yu Y, Shiiki H, et al. Increased expression of vascular

endothelial growth factor in kidney leads to progressive impairment of glomerular functions. J Am Soc Nephrol 2007; 18: 2094-104.
2. Schrijvers BF, Flyvbjerg A, De Vriese AS. The role of vascular endothelial growth factor (VEGF) in renal pathophysiology. Kidney Int 2004; 65: 2003-17.
3. De Vriese AS, Tilton RG, Elger M, Stephan CC, Kriz W, Lameire NH. Antibodies against vascular endothelial growth factor improve early renal dysfunction in experimental diabetes. J Am Soc Nephrol 2001; 12: 993-1000.
4. Flyvbjerg A, Dagnaes-Hansen F, De Vriese AS, Schrijvers BF, Tilton RG, Rasch R. Amelioration of long-term renal changes in obese type 2 diabetic mice by a neutralizing vascular endothelial growth factor antibody. Diabetes 2002; 51: 3090-4.
5. Grone HJ, Simon M, Grone EF. Expression of vascular endothelial growth factor in renal vascular disease and renal allografts. J Pathol 1995; 177: 259-67.
6. Shulman K, Rosen S, Tognazzi K, Manseau EJ, Brown LF. Expression of vascular permeability factor (VPF/VEGF) is altered in many glomerular diseases. J Am Soc Nephrol 1996; 7: 661-6.
7. Kameswara RB, Appa RB. Hypoglycemic and antihyper- glycemic activity of Syzygium alternifolium (Wt.) Walp. seed extracts in normal and diabetic rats. Phytomedicine 2001; 8: 88-93.
8. Arun N, Nalini N. Efficacy of turmeric on blood sugar and polyol pathway in diabetic albino rats. Plant Foods Hum Nutr 2002; 57: 41-52.
9. Suresh BP, Srinivasan K. Amelioration of renal lesions associated with diabetes by dietary curcumin in streptozotocin diabetic rats. Mol Cell Biochem 1998; 181: 87-96.
10. Chen H, Zhang ZS, Zhang YL, Zhou DY. Curcumin inhibits cell proliferation by interfering with the cell cycle and inducing apoptosis in colon carcinoma cells. Anticancer Res 1999; 19: 3675-80.
11. Korutla L, Kumar R. Inhibitory effect of curcumin on epidermal growth factor receptor kinase activity in A431 cells. Biochim Biophys Acta 1994; 1224: 597-600.
12. Folkman J. Can mosaic tumor vessels facilitate molecular diagnosis of cancer? Proc Natl Acad Sci U S A 2001; 98: 398-400.
13. Arbiser JL, Klauber N, Rohan R, van Leeuwen R, Huang MT, Fisher C, et al. Curcumin is an in vivo inhibitor of angiogenesis. Mol Med 1998; 4: 376-83.
14. Cha DR, Kang YS, Han SY, Jee YH, Han KH, Han JY, et al. Vascular endothelial growth factor is increased during early stage of diabetic nephropathy in type II diabetic rats. J Endocrinol 2004; 183: 183-94.
15. Sharma S, Kulkarni SK, Chopra K. Curcumin, the active principle of turmeric (Curcuma longa), ameliorates diabetic nephropathy in rats. Clin Exp Pharmacol Physiol 2006; 33: 940-5.
16. Okazaki M, Zhang H, Tsuji M, Morio Y, Oguchi K. Blood coagulability and fibrinolysis in streptozotocin-induced diabetic rats. J Atheroscler Thromb 1997; 4: 27-33.
17. Schrijvers BF, Flyvbjerg A, De Vriese AS. The role of vascular endothelial growth factor (VEGF) in renal pathophysiology. Kidney Int 2004; 65: 2003-17.
18. Osterby R, Gundersen HJ. Fast accumulation of basement membrane material and the rate of morphological changes in acute experimental diabetic glomerular hypertrophy. Diabetologia 1980; 18: 493-500.
19. Tsuchida K, Makita Z, Yamagishi S, Atsumi T, Miyoshi H, Obara S, et al. Suppression of transforming growth factor beta and vascular endothelial growth factor in diabetic nephropathy in rats by a novel advanced glycation end product inhibitor, OPB-9195. Diabetologia 1999; 42: 579-88.
20. Braun L, Kardon T, Reisz-Porszasz ZS, Banhegyi G, Mandl J. The regulation of the induction of vascular endothelial growth factor at the onset of diabetes in spontaneously diabetic rats. Life Sci 2001; 69: 2533-42.
21. Hoshi S, Shu Y, Yoshida F, Inagaki T, Sonoda J, Watanabe T, et al. Podocyte injury promotes progressive nephropathy in zucker diabetic fatty rats. Lab Invest 2002; 82: 25-35.
22. Cha DR, Kim NH, Yoon JW, Jo SK, Cho WY, Kim HK, et al. Role of vascular endothelial growth factor in diabetic nephropathy. Kidney Int Suppl 2000; 77: S104-S112.
23. Yamamoto Y, Maeshima Y, Kitayama H, Kitamura S, Takazawa Y, Sugiyama H, et al. Tumstatin peptide, an inhibitor of angiogenesis, prevents glomerular hypertrophy in the early stage of diabetic nephropathy. Diabetes 2004; 53: 1831-40.
24. Yuan HT, Tipping PG, Li XZ, Long DA, Woolf AS. Angiopoietin correlates with glomerular capillary loss in anti-glomerular basement membrane glomerulonephritis. Kidney Int 2002; 61: 2078-89.
25. Aiello LP. Angiogenic pathways in diabetic retin-

opathy. N Engl J Med 2005; 353: 839-41.
26. Cho ML, Jung YO, Moon YM, Min SY, Yoon CH, Lee SH, et al. Interleukin-18 induces the production of vascular endothelial growth factor (VEGF) in rheumatoid arthritis synovial fibroblasts via AP-1-dependent pathways. Immunol Lett 2006; 103: 159-66.
27. Williams B, Gallacher B, Patel H, Orme C. Glucose-induced protein kinase C activation regulates vascular permeability factor mRNA expression and peptide production by human vascular smooth muscle cells in vitro. Diabetes 1997; 46: 1497-503.
28. Aiello LP, Bursell SE, Clermont A, Duh E, Ishii H, Takagi C, et al. Vascular endothelial growth factor-induced retinal permeability is mediated by protein kinase C in vivo and suppressed by an orally effective beta-isoform-selective inhibitor. Diabetes 1997; 46: 1473-80.
29. Ishii H, Jirousek MR, Koya D, Takagi C, Xia P, Clermont A, et al. Amelioration of vascular dysfunctions in diabetic rats by an oral PKC beta inhibitor. Science 1996; 272: 728-31.
30. Yamagishi S, Inagaki Y, Okamoto T, Amano S, Koga K, Takeuchi M, et al. Advanced glycation end product-induced apoptosis and overexpres- sion of vascular endothelial growth factor and monocyte chemoattractant protein-1 in human-cultured mesangial cells. J Biol Chem 2002; 277: 20309-15.
31. Tilton RG, Kawamura T, Chang KC, Ido Y, Bjercke RJ, Stephan CC, et al. Vascular dysfunction induced by elevated glucose levels in rats is mediated by vascular endothelial growth factor. J Clin Invest 1997; 99: 2192-202.
32. Lehmann R, Schleicher ED. Molecular mechanism of diabetic nephropathy. Clin Chim Acta 2000; 297: 135-44.
33. Wilmer WA, Dixon CL, Hebert C. Chronic exposure of human mesangial cells to high glucose environments activates the p38 MAPK pathway. Kidney Int 2001; 60: 858-71.

ผลของ curcumin ต่อการแสดงออกของ vascular endothelial growth factor ในไตหนูถีบจักรที่เป็นเบาหวานจากการเหนี่ยวนำของ streptozotocin

ธาริณี สวัสดิ์พาณิชย์, หัทยา เพชรพิบูลย์ไทย, บุษบา ปันยารชุน,วิภาวี อนุพันธ์พิศิษฐ์

วัตถุประสงค์ : เพื่อหาตำแหน่งและการแสดงออกของ vascular endothelial growth factor (VEGF) เปรียบเทียบกับหนูเบาหวานที่ได้รับ curcumin ในหนูที่ถูกเหนี่ยวนำให้เป็นเบาหวานด้วยสาร streptozotocin (STZ)

วัสดุและวิธีการ : หนูถีบจักรถูกเหนี่ยวนำให้เป็นเบาหวานโดยสาร STZ (60 mg/kg BW) และได้แบ่งหนูออกเป็น 3 กลุ่ม ได้แก่ หนูกลุ่มควบคุม กลุ่มเบาหวานและกลุ่มเบาหวานที่ได้รับชมิ้นชัน (200 mg/kg BW) โดยทำการทดลองเป็นเวลา 4 และ 8 สัปดาห์ เมื่อครบกำหนดได้เก็บเนื้อเยื่อไตมาศึกษาดูการแสดงออกของ VEGF ด้วยวิธี immunohistochemistry (IHC)

ผลการศึกษา : . ในไตของหนูที่เป็นเบาหวานที่ 4 และ 8 สัปดาห์ จะมี glomeruli ขนาดใหญ่ขึ้น มีผนังของ glomerular capillary cells หนาตัวขึ้น เมื่อเปรียบเทียบกับกลุ่มควบคุม ส่วนในหนูเบาหวานที่ได้รับขมิ้นชันพบว่าลักษณะของเนื้อเยื่อไตโดยทั่วไปดีขึ้นเมื่อเปรียบเทียบกับกลุ่มเบาหวานที่ไม่ได้รับขมิ้นชัน เมื่อนำเนื้อเยื่อไตมาศึกษาโดยวิธี IHC พบว่าเซลล์ที่มีการแสดงออกของ VEGF ได้แก่เซลล์ podocyte และ renal tubular การแสดงออกของ VEGF จะชัดเจนมากในหนูกลุ่มที่เป็นเบาหวานเมื่อเปรียบเทียบกับกลุ่มควบคุม ในหนูเบาหวานที่ได้รับชมิ้นชันพบว่ามีการแสดงออกของ VEGF ลดลงเมื่อเปรียบเทียบกับกลุ่มเบาหวานที่ไม่ได้รับขมิ้นชัน และเมื่อนำหนูเบาหวานของระยะ 4 และ 8 สัปดาห์มาเปรียบเทียบกันพบว่า ในหนูกลุ่มเบาหวานที่ระยะ 8 สัปดาห์จะมีการแสดงออกของ VEGF ในเซลล์ podocytes และ renal tubules ที่ชัดเจนมากกว่าในหนูเบาหวานระยะ 4 สัปดาห์ แต่ในหนูเบาหวานที่ได้รับขมิ้นชันทั้งสองระยะมีการแสดงออกของ VEGF ไม่แตกต่างกัน

สรุป : ในหนูกลุ่มเบาหวานทั้ง 4 และ 8 สัปดาห์ มีการแสดงออกของ VEGF ที่ชัดเจนมากในเซลล์ podocytes และ renal tubules เมื่อเปรียบเทียบกับหนูกลุ่มควบคุมและลดลงในหนูเบาหวานที่ได้รับขมิ้นชัน เนื่องจาก VEGF เป็นสารที่มีบทบาทสำคัญที่เกี่ยวข้องกับการสร้างหลอดเลือด ผลการศึกษาในครั้งนี้แสดงว่า ขมิ้นชันมีคุณสมบัติในการยับยั้งการ สร้างหลอดเลือดและมีแนวโน้มในการช่วยรักษาและคงสภาพเนื้อเยื่อไตที่เสื่อมสภาพจากสภาวะเบาหวานได้