

The Role of Basic-Fibroblast Growth Factor (b-FGF) in Cyclosporine-induced Nephrotoxicity

A. EFTHIMIADOU¹, M. LAMBROPOULOU², O. PAGONOPOULOU¹,
I. VAKALOPOULOS¹, N. PAPADOPOULOS² and N. NIKOLETTOS¹

¹Department of Physiology and ²Department of Histology – Embryology, Democritus University of Thrace, Medical School, Alexandroupolis, Greece

Abstract. *Background: The effect of the b-fibroblast growth factor (b-FGF) on cyclosporine A (CsA)-induced nephrotoxicity in the rat kidney was investigated. Materials and Methods: The rats were divided into six groups: A (control), B (b-FGF-treated), C, D: (CsA-treated and sacrificed on days 14 or 21), E, F (CsA - and b-FGF- treated and sacrificed on days 14 or 21). The antibody mouse anti-rat CD31 was used to evaluate the kidney vessels present in histological preparations. Results: The kidney vessels in group B were increased in comparison with the control group ($p < 0.05$). Reduction of kidney vessels in groups C and D ($p < 0.05$) in comparison with the controls was observed, while in groups E and F they were increased when compared to group C ($p < 0.05$) and D ($p < 0.05$), respectively. Conclusion: The angiogenic role of b-FGF was confirmed in normal rats and a possible "protective" role of b-FGF was shown in rat kidney with CsA-induced nephrotoxicity.*

Angiogenesis, the formation of new blood vessels from pre-existing vascular beds, occurs normally during organ development and differentiation in embryogenesis, and is required for wound healing and reproductive functions in the adult. Angiogenesis is also involved in the pathogenesis of several disorders, including chronic inflammatory diseases and cancer.

Fibroblast growth factors (FGFs) are heparin-binding proteins involved in many biological processes and exert a pivotal role in angiogenesis under physiological and pathological conditions (1). The FGF family has at least 21 members (2), only some of which have been examined in the

kidney. Of those, a-FGF and b-FGF are present in both adult and embryonic kidneys and crude kidney extracts. FGF-2 specifically has mitogenic and angiogenic activity in the kidneys (3). An alternate transcript for a-FGF is specifically expressed in the kidney (4). b-FGF protein and mRNA have been localized in human kidneys (5). Also, a-FGF and b-FGF proteins are colocalized in glomeruli, S3 segments of proximal tubules, distal tubules and collecting ducts of adult rat kidneys (6).

Cyclosporine A (CsA) is a nephrotoxin that is still widely used in the treatment of autoimmune diseases and for immuno-suppression following organ transplantation (7,8). CsA-induced nephrotoxicity has been associated with an increase in the renal production of prostaglandins and thromboxane (9). CsA also stimulates the release of endothelin-1 (ET-1) from endothelial, tubular and mesangial cells in rats and has been associated with the increased expression of angiotensin II (ANG II) type I receptors in the kidney (10, 11). CsA nephrotoxicity is dose-dependent and has been associated with significant morphological changes, in which the toxicity is manifested by a variety of lesions affecting tubules, vessels and the renal interstitium (12).

Since FGFs are present in the kidney and have a special angiogenic activity, the purpose of this study was to investigate whether the intramuscular administration of b-FGF would exhibit a "protective" role against CsA-induced nephrotoxicity in rat kidney.

Materials and Methods

Experimental design. Thirty male Sprague-Dawley rats, weighing 260-280 g, were used. They were housed in individual cages in a temperature- and light-controlled environment, received a free diet and were allowed free access to water. The rats were assigned to the following experimental groups (no.=5/group).

Group A (controls): rats received a daily subcutaneous (s.c.) injection of corn oil solution 1 ml/kg/day for 10 days and were sacrificed on the 14th day; Group B (b-FGF): rats received a daily intramuscular (i.m.) injection of 1 µg b-fibroblast growth factor (b-FGF) for 10 days and were sacrificed on the 14th day; Group C (Cs,14): rats received a daily s.c. injection of cyclosporine (CsA)

Correspondence to: Anna Efthimiadou, Department of Physiology, Medical School, Democritus University of Thrace, University Campus, Alexandroupolis, 68100, Greece. Tel: 25510-30513, Mob: 6974197997, Fax: 25510-30524, e-mail: aefthim@med.duth.gr

Key Words: b-Fibroblast growth factor, cyclosporine, angiogenesis, nephrotoxicity.

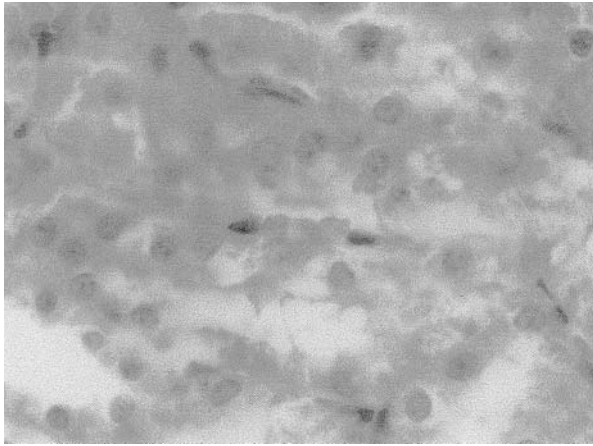


Figure 1. Hematoxylin and eosin (H&Ex200) staining of kidney tissue slides of Group D, showing cyclosporine A-induced tubular necrosis.

20 mg/kg for 10 days and were sacrificed on the 14th day; Group D (Cs,21): rats received a daily *s.c.* injection of CsA 20 mg/kg. for 10 days and were sacrificed on the 21st day; Group E: (Cs+FGF,14): rats received both a daily *s.c.* injection of CsA 20 mg/kg and an *i.m.* injection of 1µg b-FGF for 10 days and were sacrificed on the 14th day; Group F (Cs+FGF,21): rats received both a daily *s.c.* injection of CsA 20 mg/kg and an *i.m.* injection of 1 µg b-FGF for 10 days and were sacrificed on the 21st day .

On day 14 or 21, the rats were anesthetized with ether, the abdomen was opened through a midline incision and the kidneys were removed and processed for microscopy. A blood sample was collected from the heart. The animals were then euthanized by deep anesthesia with ether. The serum creatinine and blood urea nitrogen (BUN) concentrations were measured using a Hitachi biochemical analyzer.

Drugs. Cyclosporine was purchased from Novartis Pharma AG, Basle, Switzerland. Recombinant b-fibroblast growth factor (b-FGF) was purchased from Cytolab/peprotech Asia, Israel.

Histological examination. The slides were stained with conventional hematoxylin and eosin (H&E). Unstained slides were obtained for the detection of the CD31 monoclonal mouse anti-rat antibody (CD31, clone: PECAM-1) (Dako, Glostrup, Denmark), which recognizes the surface antigen CD31 of mouse endothelial cells.

Immunohistochemistry. Immunohistochemistry was performed with CD31 used on serial sections. Tissue specimens were fixed in formalin and embedded in paraffin according to standard procedures. Four-micron sections (4 µm) of representative blocks from each case were deparaffinized, rehydrated and treated with 0.3% H₂O₂ for 5 min in methanol to prevent endogenous peroxidase activity. The slides were then incubated for 75 min with the CD31 monoclonal mouse anti-rat antibody at a 1:50 dilution. Control slides were incubated for the same period with normal serum (negative control). A positive control was always run in the assay. The "Envision Kit" (dextran-free biotin one-step, Dako) was used according to the manufacturer's instructions. Finally, bound antibody complexes were stained for 10 min with 0.05%

Table I. Comparison of the vessel number per optical field (p.o.f.) in the various experimental groups.

Experimental group	Treatment	Number of vessels p.o.f (mean ± SE)
Group A	Controls	19.60 ± 1.33
Group B	FGF	28.30 ± 3.23 *
Group C	Cs, 14	14.42 ± 1.07*
Group D	Cs, 21	13.18 ± 0.9*
Group E	Cs+FGF,14	17.03 ± 0.51**
Group F	Cs+FGF, 21	20.25 ± 0.91 ⁺

**p* < 0.05 compared to controls.

***p* < 0.05 compared to Group C.

⁺*p* < 0.05 compared to Group D.

diaminobenzidine. The sections were then briefly counterstained with Mayer's haematoxylin, mounted and examined under an Olympus BX40 light microscope. High vascularization fields (hot-spots) were selected and the measurement of the blood vessels was achieved under X200 magnification in three selected fields with the highest vascular density. The medium average of the measurements of three fields with increased vascularization was considered as the final microvessel density.

Statistics. The number of vessels per optical field were analyzed in every specimen and are presented as mean ± S.E.M. Descriptive statistics were calculated for all five groups. The comparisons among the groups were performed using one way ANOVA followed by Tykey's HDS *post hoc* test (SPSS, v. 12).

Results

Histological changes. CsA treatment for 14 days produced a marked nephrotoxicity associated with dilation, interstitial fibrosis with tubular atrophy, sloughing of tubular epithelial cells, thickening of tubular basement membranes and peritubular capillary congestion (Figure 1). More lesions were observed at 21 days. The results of these experiments are presented in Table I. Vehicle-treated rats comprised the control groups.

The number of kidney vessels in group B (b-FGF treatment) was increased significantly in comparison with the controls (*p* < 0.05) (Figure 2A, B).

A significant reduction in the number of kidney vessels from both group C (CsA, 14) and D (CsA,21), (*p* < 0.05) in comparison with controls, was observed indicating that CsA damaged the kidney vessels (Table I). Greater differences were observed 21 days after interruption of the treatment (Figure 2 C, D).

The concurrent administration of b-FGF with CsA statistically significantly increased the number of kidney vessels in group E (CsA + b-FGF,14) when compared to the CsA,14 - only treated group (*p* < 0.05) (Table I) (Figure 2 E, C). However, the mean number of vessels was still

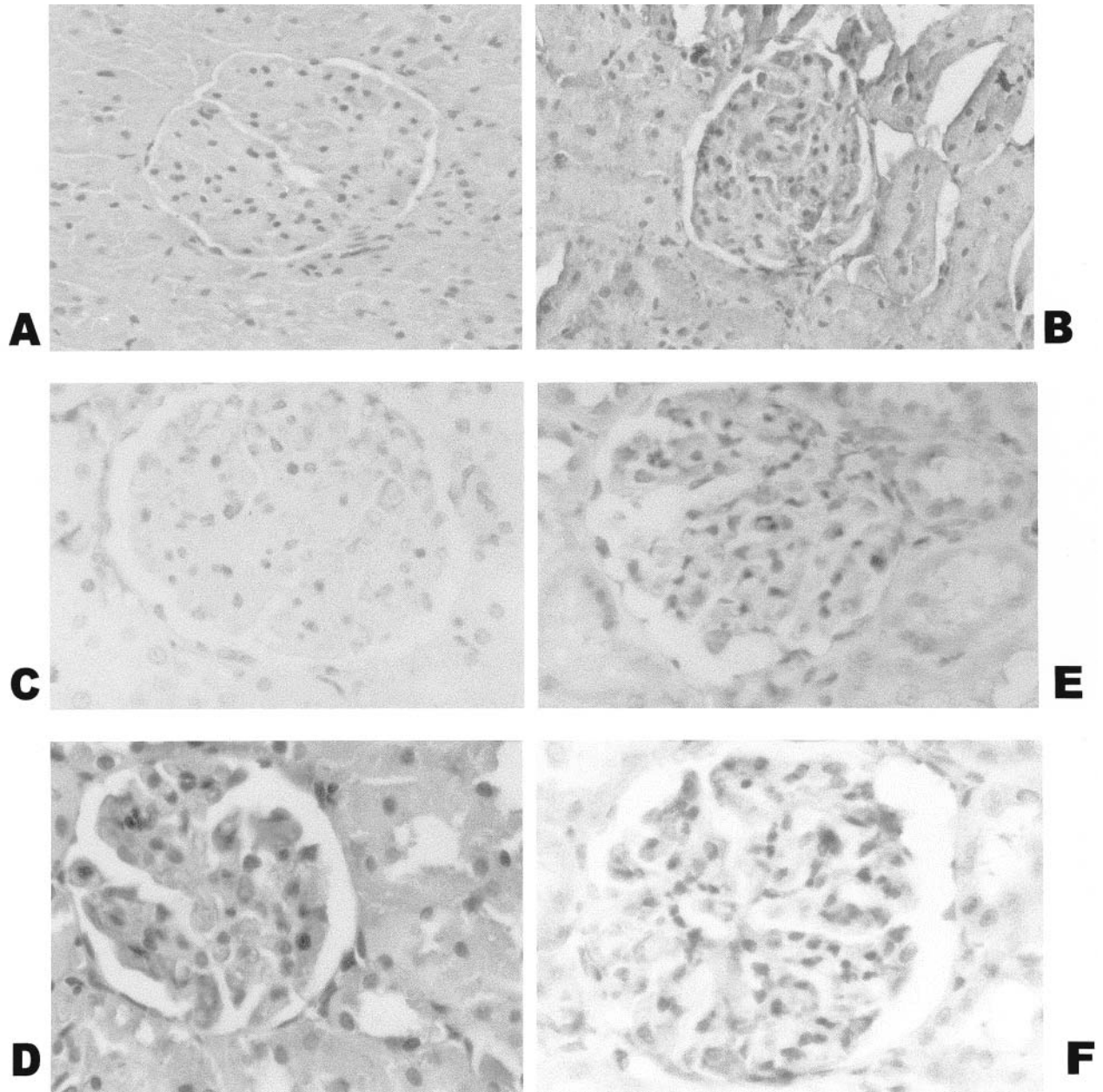


Figure 2. (A) Kidney tissue of controls. Vessels were detected by the use of a monoclonal mouse anti-rat antibody (CD31, clone: PEKAM-1). (B) Group B, b-FGF treatment. Immunohistochemical staining of the kidney tissue with significant increase of kidney vasculature compared to controls, using monoclonal antibody CD31. (C) Group C, CsA-14. Immunohistochemical staining of the reduction in blood vessels of the kidney. (D) Group D, CsA-21. Immunohistochemical staining of the kidney tissue with significantly decreased kidney vasculature compared to controls. (E) Group E, combined CsA and b-FGF-14. Immunohistochemical staining of the kidney tissue with significant increase of blood vessels. (F):Group F, combined CsA and b-FGF-21. Immunohistochemical staining of the kidney tissue with the maximum increase of blood vessels.

significantly reduced in comparison with the controls.

There was also a statistically significant increase in the number of kidney vessels in group F (CsA + b-FGF,21) ($p < 0.05$) when compared to group D (CsA,21) ($p < 0.05$) (Table I). Interestingly, the mean values of kidney vessels in group F (CsA + b-FGF,21) were higher, but not statistically

significantly, than those of the group E (CsA + b-FGF,14) and were almost equal to the controls.

Biochemical changes. Table II summarizes the values for blood urea nitrogen (BUN) and serum creatinine in the experimental groups. There was not a significant difference

Table II. Comparison of the biochemical parameters in the various experimental groups.

Experimental group	Treatment	Blood urea nitrogen (BUN) mg/dL	Serum creatinine mg/dL
Group A	Controls	28.8±3.11	0.63±0.02
Group B	b-FGF	29.1±2.88	0.60±0.03
Group C	CsA, 14	37.2±1.65*	1.03±0.05*
Group D	CsA, 21	43.6±5.36*	1.2±0.05*
Group E	CsA + b-FGF, 14	38.6±3.04*	0.95±0.05*
Group F	CsA + b-FGF, 21	30.2±4.32+	0.82±0.07+

* $p < 0.05$ compared to controls.+ $p < 0.05$ compared to Group D.

in serum creatinine levels and BUN between group B (b-FGF) and the controls. On the contrary, the serum creatinine levels and BUN were increased significantly at the end of CsA-treatment in groups C (CsA, 14) and D (CsA, 21) ($p < 0.05$) in comparison with the controls. In group E (CsA + b-FGF, 14) the serum creatinine levels and BUN were increased significantly ($p < 0.05$) in comparison with the controls. Despite that, in group F (CsA + b-FGF, 21) there was an improvement in serum creatinine and BUN in comparison to the controls but it was significantly lower than in group D (CsA, 21).

It seems that the concurrent administration of b-FGF with CsA improved the biochemical parameters of the kidney, indicating a possible "protective" role of b-FGF.

Discussion

This study demonstrated the specific role of b-FGF in rats with CsA-induced nephrotoxicity. Previous data on the action of FGFs in the rat kidney are limited (14). The localization of a-FGF receptors (FGFR-1) overlapped with the expression and localization of b-FGF (FGFR-2) receptors in the human kidney (5). Also FGFR-2 protein has been found in distal straight tubules and tubule segments adjacent to glomeruli and blood vessels, suggesting paracrine interactions with the ligands produced by Bowman's capsule cells, such as a-FGF and b-FGF in the rat kidney (6).

Other investigators, using immunohistochemistry, revealed specific immunostaining for all FGFRs within glomeruli indicating expression of a-FGF and b-FGF and their receptors in the normal rat. The differential expression pattern of FGFR isoforms between the glomeruli and whole cortex, and the mutually exclusive nature of the expression of IIIc but not IIIb isoforms within the glomeruli, indicate that FGFR expression and, thereby, FGF activity, is tightly regulated in the glomeruli (15).

We have previously reported the angiogenic effect of vascular endothelial growth factor (VEGF) in rat heart (16). In this study, significant angiogenesis was found in the kidney vessels of rats receiving b-FGF *i.m.* in comparison to the controls, confirming the angiogenic role of b-FGF in normal adult rat kidney.

In a rat model of CsA nephrotoxicity, Schrijvers *et al.* (17) reported the expression of the VEGF system to be increased. VEGF-blockade aggravated and VEGF-administration ameliorated the induced injury, suggesting a role for VEGF in the repair process induced by CsA nephrotoxicity.

Other investigators showed that CsA inhibits the migration of the primary endothelial cells and the angiogenesis induced by VEGF, this effect being mediated through the inhibition of cyclooxygenase-2, the transcription of which is activated by VEGF in primary endothelial cells (18). In a recent report, investigators showed that increased VEGF expression in chronic CsA nephrotoxicity seemed to be related to up-regulation of angiotensin II. In addition, VEGF probably exerted its effect *via* the KDR/Flk-1 receptor. The actions of VEGF in this model remain speculative, but may be related to its effect on macrophage infiltration or matrix deposition (19).

The role of FGFs in renal diseases has also been reported. b-FGF secreted by tubular epithelial cells damaged by gentamycin, FGFR1 expressed in damaged renal tubules and interstitial fibroblast b-FGF can not only promote the proliferation of tubular epithelia, but can also stimulate interstitial fibroblasts to proliferate. A correlation exists between renal tubular injury and interstitial lesions (20). CsA or FK506 (a potent immunosuppressant that blocks calcineurin-mediated T cell activation by binding to immunophilin and is used to prevent allograft rejection in solid organ transplantation) treatment delays recovery from tubular necrosis, and this may be associated with decreased epidermal growth factor (EGF) expression CsA or FK506 (21).

Other investigators have pointed to a selective bFGF-mediated enhancement of distinct pro-apoptotic pathways induced by TNF-alpha in glomerular endothelial cells (22). In experimental mesangioproliferative glomerulonephritis, it was suggested that the release of constitutively expressed FGF-2 after immune-mediated cell injury, contributed to glomerular cell damage and, thus, b-FGF was identified as a novel mediator of cytotoxicity (23). Strutz *et al.* showed that FGF-2 makes an important contribution to the mechanisms of epithelial-mesenchymal transformation (EMT) by stimulating microenvironmental proteases essential for the disaggregation of organ-based epithelial units (24).

Here, we showed, for the first time, that the concurrent administration of b-FGF to animals with CsA-induced nephrotoxicity could minimize the nephrotoxic effects of Cs

by increasing the number of vessels in those animals. Our results also showed that the factor is still active 21 days after the onset of treatment (11 days after cessation of CsA and b-FGF treatment) (Groups E, F). Since the nephrotoxic action of CsA seemed to be stable during the same period of time (Groups C, D), the continuing angiogenic effect of b-FGF seems to help the nephrons reach their pretoxicity levels (Group A), indicating a protective or even reparative mechanism of action supported by biochemical changes.

Thus, this study confirmed the angiogenic role of b-FGF in normal adult rats and showed a possible protective role of b-FGF in rat kidney with CsA-induced nephrotoxicity. The mechanism by which b-FGF exerts such a protective role is the subject of further investigation.

References

- Goldfarb M: Functions of fibroblast growth factors in vertebrate development. *Cytokine Growth Factor Rev* 7: 311-325, 1996.
- Nishimura T, Nakatake Y, Konishi M and Itoh N: Identification of a novel FGF, FGF-21, preferentially expressed in the liver. *Biochim Biophys Acta* 1492: 203-206, 2000.
- Baird A and Bohlen P: Fibroblast growth factors. In: *Peptide Growth Factors and Their Receptors*. Handbook of Experimental Pharmacology Sporn MB, Roberts AB (eds.), Berlin, Springer-Verlag, pp. 369-418, 1990.
- Myers RL, Payson RA, Chotani MA, Deaven LL and Chiu IM: Gene structure and differential expression of acidic fibroblast growth factor mRNA: identification and distribution of four different transcripts. *Oncogene* 8: 341-349, 1993.
- Floege J, Hudkins KL, Eitner F, Cui Y, Morrison RS, Schelling MA and Alpers CE: Localization of fibroblast growth factor-2 (basic FGF) and FGF receptor-1 in adult human kidney. *Kidney Int* 56: 883-897, 1999.
- Cauchi J, Alcorn D, Cancilla B, Key B, Berka JL, Nurcombe V, Ryan GB and Bertram JF: Light-microscopic immunolocalization of fibroblast growth factor-1 and -2 in adult rat kidney. *Cell Tissue Res* 285: 179-187, 1996.
- Kahan BD: Cyclosporine: the agent and its actions. *Transplant Proc* 17: 5-18, 1985.
- Sullivan BA, Hak LJ and Finn WF: Cyclosporine nephrotoxicity: studies in laboratory animals. *Transplant Proc* 17: 145-154, 1985.
- Coffman TM, Carr DR, Yarger WE and Klotman PE: Evidence that renal prostaglandin and thromboxane production is stimulated in chronic cyclosporine nephrotoxicity. *Transplantation* 43: 282-285, 1987.
- Moutabarrid A, Ishibashi M, Fukunaga M, Kameoka H, Takano Y, Kokado Y *et al*: FK506 mechanism of nephrotoxicity: stimulatory effect on endothelin secretion by cultured kidney cells and tubular cell toxicity *in vitro*. *Transplant Proc* 23: 3133-3136, 1991.
- Nakahama H: Stimulatory effect of cyclosporine A on endothelin secretion by a cultured renal epithelial cell line, LLC-PKI cells. *Eur J Pharmacol* 180: 191-192, 1990.
- Mihatsch MJ, Thiel G and Ryffel B: Morphologic diagnosis of cyclosporine nephrotoxicity. *Semin Diagn Pathol* 5: 104-121, 1988.
- Shibab FS, Bennett WM, Yi H, Choi SO and Anhoh TF: Sirolimus increases transforming growth factor-beta1 expression and potentiates chronic cyclosporine nephrotoxicity. *Kidney Int* 65(4): 1262-1271, 2004.
- Cancilla B, Davis A, Cauchy JA, Risbridger P and Bertam JF: FGF receptors and their ligands in the adult rat kidney. *Kidney Int* 60(1): 147-155, 2001.
- Ford MD, Cauchi J, Greferath U and Bertram JF: Expression of fibroblast growth factors and their receptors in rat glomeruli. *Kidney Int* 51(6): 1729-1738, 1997.
- Efthimiadou A, Asimakopoulos B, Nikolettos N, Giatromanolaki A, Sivridis E, Lialiaris TS, Papachristou D and Kontoleon E: The angiogenetic effect of intramuscular administration of VEGF on muscle. The influence of exercise on angiogenesis. *In Vivo* 18: 825-830, 2004.
- Schrijvers BF, Flyvbjerg A and De Zeeuw AS: The role of vascular endothelial growth factor (VEGF) in renal pathophysiology. *Kidney Int*, 65, pp. 2003-2017, 2004.
- Hernandez GL, Volperb OV, Iniguez MA, Lorenzo E, Martinez-Martinez S, Graua R, Fresno M and Redondo JM: Selective inhibition of VEGF-mediated angiogenesis by cyclosporin A: roles of the nuclear factor of activated T cells and cyclooxygenase 2. *J Exp Med* 193(5): 607-620, 2001.
- Shihab FS, Bennett WM, Isaac J, Yi H and Andoh TF: Angiotensin II regulation of vascular endothelial growth factor and receptors Flt-1 and KDR/Flk-1 in cyclosporine nephrotoxicity. *Kidney Int* 62(2): 422-433, 2002.
- Du A and Zou W: The role of b-FGF in renal tubular injury and the interstitial lesion. *Zhonghua Bing Li Xue Za Zhi* 29(3): 208-211, 2000.
- Yang CW, Lee SH, Lim SW, Jung JY, Kim HW, Choi BS, Cha JH, Kim J and Bang BK: Cyclosporine or FK506 decrease mature epidermal growth factor protein expression and renal tubular regeneration in rat kidney with ischemia/reperfusion injury. *Nephron* 92(4): 914-921, 2002.
- Messmer UK, Briner VA and Pfeilschifter J: Basic fibroblast growth factor selectively enhances TNF-alpha-induced apoptotic cell death in glomerular endothelial cells: effects on apoptotic signaling pathways. *J Am Soc Nephrol* 11(12): 2199-2211, 2000.
- Floege J, Burg M, Hugo C, Gordon KL, Van Goor H, Reidy M, Couser WG, Koch KM and Johnson RJ: Endogenous fibroblast growth factor-2 mediates cytotoxicity in experimental mesangioproliferative glomerulonephritis. *J Am Soc Nephrol* 9(5): 792-801, 1998.
- Strutz F, Zeisberg M, Ziyadeh FN, Yang CQ, Kalluri R, Muller GA and Neilson EG: Role of basic fibroblast growth factor-2 in epithelial-mesenchymal transformation. *Kidney Int* 61(5): 1714-1728, 2002.

Received October 4, 2005
Revised December 14, 2005
Accepted December 27, 2005