

Effect of Pentoxifylline on Renal Ischemic Reperfusion

¹Doustar Yousef, ¹Mohajeri Daryoush, ¹Hossein Hashenzadeh,
²Ali Rezaei and ²Mehrdad Neshat Gharamaleki

¹Department of Pathobiology, ²Department of Clinical Sciences,
Tabriz Branch, Islamic Azad University, Tabriz, Iran

Abstract: Ischemic/reperfusion induced cell death and inflammatory reaction (acute renal failure) is a common clinical problem associated with acute renal failure and renal transplantation. In this study, the effects of pentoxifylline on the attenuation of an ischemia reperfusion injury and inflammatory reaction were examined. Twelve adult mixed breed dogs of both sexes, weighing 10-20 kg were chosen and then the dogs were assigned randomly into control and treatment groups (n = 6). Celiotomy was performed by ventral midline incision. The left kidney was isolated and then both the renal artery and vein were clamped. After 60 min of warm ischemia, the vessels were unclamped and followed by 72 h of reperfusion while the right kidney was removed. Blood samples were collected before ischemia and at 24, 48 and 72 h after ischemia for determination of serum creatinine and BUN level. After 72 h of ischemia-reperfusion tissue samples from the left kidney were taken for histopathology examination. The treatment group showed lower creatinine, BUN (p<0.001 each), cell death (p<0.05 each) and of renal inflammatory reactions than did the control group.

Key words: Pentoxifylline, renal, ischemic reperfusion, apoptosis, necrosis, Iran

INTRODUCTION

Several studies have examined the role of leukocytes and their surface adhesion molecules and apoptosis in the pathogenesis of post ischemic renal damage. Leukocyte adhesion molecules seem to facilitate polymorphonuclear neutrophils recruitment during reperfusion, being implicated as mediators of renal IRI (Rabb *et al.*, 1997; Lauriat and Linas, 1998). Renal injury as a result of Ischemia Reperfusion (I/R) is a major clinical problem and is the most common cause of acute renal failure after renal transplantation, shock, sepsis and renal artery stenosis. For patients in shock, acute tubular necrosis is associated with a mortality rate of approximately 50% and little has changed in the past 40 years (Liano and Pascual, 1996; Cursio *et al.*, 2002; Fish and Molitoris, 1994). Treatment of I/R injury is still only supportive. Therefore, development of therapeutic interventions to prevent or reduce renal tissue injury after I/R remains the focus of research. Tumor necrosis factor- α (TNF- α) is an important cytokine participating in damage and inflammation in both animal and human renal diseases (Edelstein *et al.*, 1997; Thadhani *et al.*, 1996; Cuppage *et al.*, 1972; French-Constant *et al.*, 1989). TNF- α affects differentiation of macrophages into inflammatory cells and primes neutrophils to increase secretory responses and generation of reactive oxygen radicals and NOS. TNF- α also triggers cell death by apoptosis and necrosis. These effects all promote tissue injury in the target organ. Gomez-Chiarri (Edelstein *et al.*, 1997; Bush *et al.*, 2000)

have shown that in ADR nephropathy Platelet-Activating Factor (PAF) and TNF- α production precede proteinuria. Furthermore, treatment with BN52021 (a PAF antagonist) resulted in both a striking decrease in proteinuria and diminution in glomerular TNF- α and PAF production. Significant correlations between urinary TNF- α levels and both the proteinuria and the clinical activity scores in patients with active renal disease and cellular proliferation have been demonstrated (Thadhani *et al.*, 1996; Breuss *et al.*, 1995; Counts *et al.*, 1995). Pentoxifylline (PTX) is a methylxanthine derivative and phosphodiesterase inhibitor which inhibits the generation of mRNA for TNF- α *in vitro* and decreases the *in vivo* production of TNF- α in humans and in experimental animals (Bonventre, 1993; Boogaard *et al.*, 1990). PTX has also been shown to inhibit programmed cell death in short-term cell cultures as well as iNOS enzyme activity in macrophage cell culture (Rabb *et al.*, 1997; Lauriat and Linas, 1998; Abbate and Remuzzi, 1996; Basile *et al.*, 1998). Thus, we hypothesized that PTX would be interesting to study in the ADR-induced nephropathy model. We have studied the effects of PTX on TNF- α , proteinuria and apoptosis in rats with ADR nephropathy. Ischemic/reperfusion induced cell death and inflammatory reaction (acute renal failure) is a common clinical problem associated with acute renal failure and renal transplantation. In this study, the effects of pentoxifylline on the attenuation of an ischemia-reperfusion injury and inflammatory reaction were examined.

MATERIALS AND METHODS

Twelve adult mixed breed dogs of both sexes, weighing 10-20 kg were chosen and then the dogs were assigned randomly into control and treatment groups (n = 6). Celiotomy was performed by ventral midline incision. The left kidney was isolated and then both the renal artery and vein were clamped. After 60 min of warm ischemia, the vessels were unclamped and followed by 72 h of reperfusion while the right kidney was removed. Blood samples were collected before ischemia and at 24, 48 and 72 h after ischemia for determination of serum creatinine and BUN level. After 72 h of ischemia-reperfusion tissue samples from the left kidney were taken for histopathology examination.

RESULTS AND DISCUSSION

Ischemia/reperfusion Injury (IRI) is an important cause of acute renal failure in native kidneys and allograft (Liano and Pascual, 1996). Cellular and molecular responses of the kidney to acute ischemic injury are complex and not fully understood (Edelstein *et al.*, 1997; Thadhani *et al.*, 1996; Bonventre, 1993). The treatment group showed lower creatinine, BUN ($p < 0.001$ each), cell

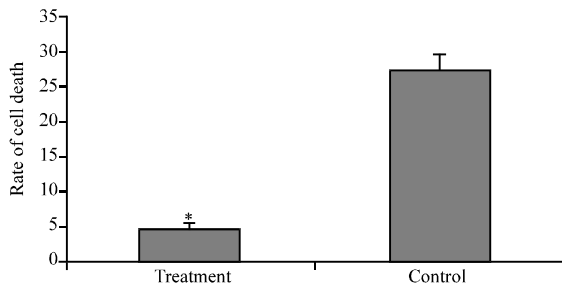


Fig. 1: Ordinary means of cell death in renal tissue of treatment and control groups. Data present with form of mean±SEM and * $p < 0.005$ compression between groups

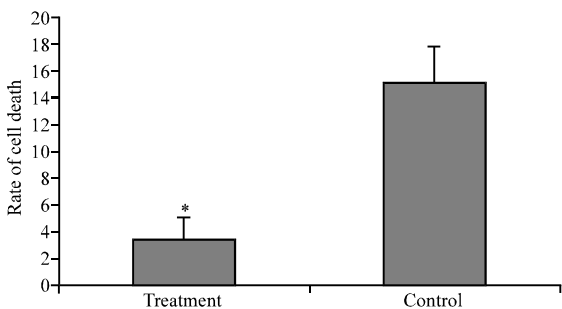


Fig. 2: Ordinary means of inflammatory reaction in renal tissue of treatment and control groups. Data present with form of mean±SEM and * $p < 0.005$ compression between groups

death ($p < 0.05$ each) and of renal inflammatory reactions than did the control group. All results of present study is shown in Fig. 1-7.

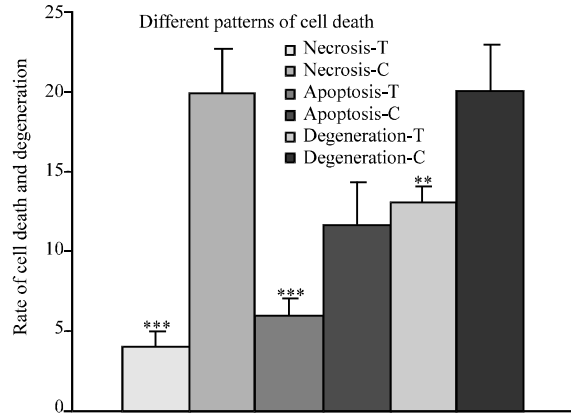


Fig. 3: Compression of necrosis and apoptosis changes in treatment and control groups (n = 6). Data present with form of mean±SEM. *** $p < 0.005$ and ** $p < 0.01$ on compression between groups

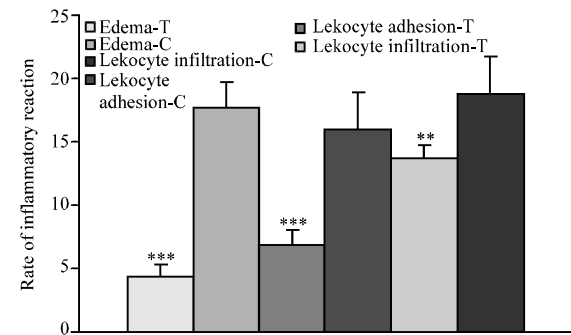


Fig. 4: Compression inflammatory reactions in treatment and control groups (n = 6). Data present with form of mean±SEM. *** $p < 0.005$ and ** $p < 0.01$ on compression between groups

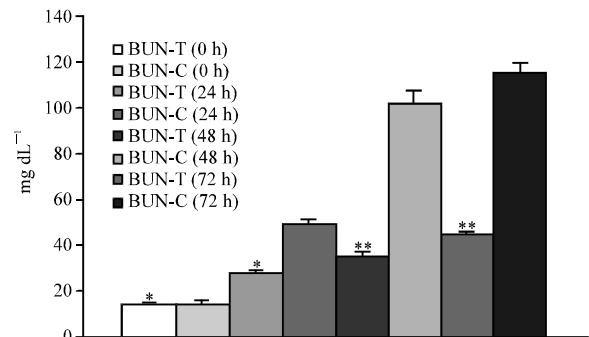


Fig. 5: Compression of BUN changes in treatment and control groups (n = 6). Data present with form of mean±SEM. *** $p < 0.005$, ** $p < 0.01$ and * $p > 0.05$ in compression between groups

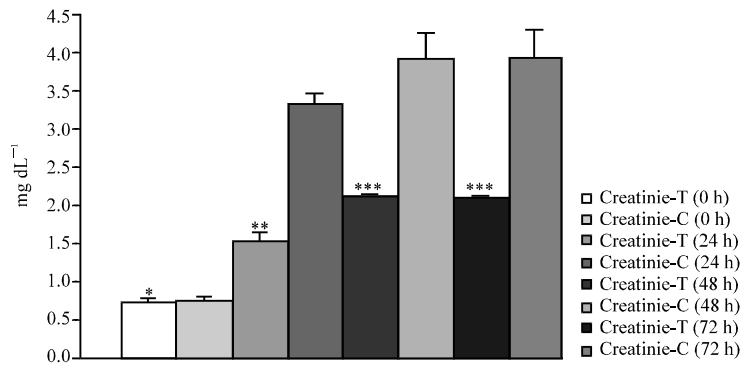


Fig. 6: Comparison of creatinine changes in treatment and control groups (n = 6). Data present with form of mean±SEM. ***p<0.005, **p<0.01 and *p>0.05 in comparison between groups

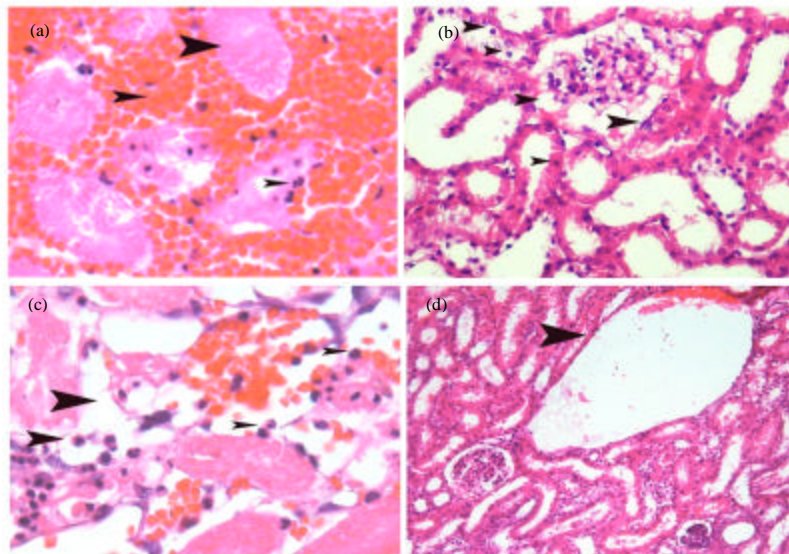


Fig. 7: a) Photomicrograph of renal tubular, hemorrhage and leukocyte infiltration in control group, H and E (×100); b) photomicrograph of renal tubular in treatment group with a few degeneration H and E (×100); c) photomicrograph of renal tubular in control group with leukocyte infiltration and tubular cells necrosis H and E (×100); d) photomicrograph of renal tubular in treatment group without of leukocyte infiltration and tubular cells necrosis H and E (×100)

CONCLUSION

The result of this study indicates that pentoxifylline alone might play a role in attenuation ischemia-reperfusion cell injury and reduced inflammatory reaction that may contribute to the ischemic renal damage.

REFERENCES

Abbate, M. and G. Remuzzi, 1996. Acceleration of recovery in acute renal failure: From cellular mechanisms of tubular repair to innovative targeted therapies. *Renal Fail.*, 18: 377-388.

Basile, D.P., D.R. Martin and M.R. Hammerman, 1998. Extracellular matrix-related genes in kidney after ischemic injury: Potential role of TCF-β in repair. *Am. J. Physiol. Renal Physiol.*, 275: F894-F903.

Bonventre, J.V., 1993. Mechanisms of ischemic acute renal failure. *Kidney Int.*, 43: 1160-1178.

Boogaard, P.J., J.F. Nagelkerke and G.J. Mulder, 1990. Renal proximal tubular cells in suspension or in primary culture as in vitro models to study nephrotoxicity. *Chem. Biol. Interact.*, 76: 251-292.

- Breuss, J.M., J. Gallo, H.M. DeLisser, I.V. Klimanskaya and H.G. Folkesson *et al.*, 1995. Expression of the beta 6 integrin subunit in development, neoplasia and tissue repair suggests a role in epithelial remodeling. *J. Cell Sci.*, 108: 2241-2251.
- Bush, K.T., S.H. Keller and S.K. Nigam, 2000. Genesis and reversal of the ischemic phenotype in epithelial cells. *J. Clin. Investig.*, 106: 621-626.
- Counts, R.S., G. Nowak, R.D. Wyatt and R.G. Schnellmann, 1995. Nephrotoxicant inhibition of renal proximal tubule cell regeneration. *Am. J. Physiol. Renal Physiol.*, 269: F274-F281.
- Cuppige, F.E., M. Chiga and A. Tate, 1972. Cell cycle studies in the regenerating rat nephron following injury with mercuric chloride. *Lab Investig.*, 26: 122-126.
- Cursio, R., B. Mari, K. Louis, P. Rostagno and M.C. Saint-Paul *et al.*, 2002. Rat liver injury after normothermic ischemia is prevented by a phosphinic matrix metalloproteinase inhibitor. *FASEB J.*, 16: 93-95.
- Edelstein, C.L., H. Ling and R.W. Schrier, 1997. The nature of renal cell injury. *Kidney Int.*, 51: 1341-1351.
- Ffrench-Constant, C., L. Van de Water, H.F. Dvorak and R.O. Hynes, 1989. Reappearance of an embryonic pattern of fibronectin splicing during wound healing in the adult rat. *J. Cell. Biol.*, 109: 903-914.
- Fish, E.M. and B.A. Molitoris, 1994. Extracellular acidosis minimizes actin cytoskeletal alterations during ATP depletion. *Am. J. Physiol.*, 267: F566-F572.
- Fujikawa, L.S., C.S. Foster, T.J. Harrist, J.M. Lanigan and R.B. Colvin, 1981. Fibronectin in healing rabbit corneal wounds. *Lab Investig.*, 45: 120-129.
- Lauriat, S. and S.L. Linas, 1998. The role of neutrophils in acute renal failure. *Semin Nephrol.*, 18: 498-504.
- Liano, F. and J. Pascual, 1996. Epidemiology of acute renal failure: A prospective multicenter community based study. The madrid acute renal failure group. *Kidney Int.*, 50: 811-818.
- Rabb, H., Y.M. O'Meara, P. Maderna, P. Coleman and H.R. Brady, 1997. Leukocytes, cell adhesion molecules and ischemic acute renal failure. *Kidney Int.*, 51: 1463-1468.
- Thadhani, R., M. Pascual and J.V. Bonventre, 1996. Acute renal failure. *N. Engl. J. Med.*, 334: 1448-1460.