RENOPROTECTIVE EFFECT OF LEFLUNOMIDE AGAINST ISCHEMIA-REPERFUSION INJURY IN RATS

By

Amany A. Shalaby¹, Amany A. Atwa², Heba K. Morsy²

From

Clinical Pharmacology dept.¹, and Medical Biochemistry dept.²

Faculty of Medicine, Mansoura University

ABSTRACT

Renal ischemia is of great clinical interest because of its role in renal failure and renal graft rejection. The purpose of this study was to investigate the possible protective effect of leflunomide against ischemia/reperfusion (I/R) injury in the rat. Methods: Three groups of Sprague-Dawley rats (10 rats each), the control group, I/R group and the leflunomide - treated I/R group. A renal I/R injury was induced by a left renal pedicle occlusion to induce ischemia for 45 min, followed by 60 mins of reperfusion with contralateral nephrectomy in rats. The rats in Leflunomide treated I/R group were pretreated intragastrically with a leflunomide suspension (10 mg/kg) 60 min before the ischemia induction. Thiobarbituric acid reactive substances (TBARS),

nitric oxide (NO), tumor necrosis factor alpha (TNF- α), catalase (CAT) superoxide dismutase (SOD) activities were determined in renal tissue, while, creatinine, blood urea nitrogen (BUN) were measured in blood. Results: Our results indicate that TBARS, NO, TNF- α , BUN and creatinine levels, were significantly higher in the I/R group than those in the control group. Leflunomide administration significantly decreased these parameters. SOD and CAT activities significantly decreased after I/R injury when compared to the control group. Leflunomide treatment significantly increased activities of these enzymes when compared to the I/R group.

Conclusions : These results demonstrated that reactive oxygen species (ROS) and TNF- α play causal role in I/R induced renal injury and

leflunomide exerted renoprotective effects by anti-inflammatory effect with radical scavenging and antioxidant activities.

Key words : Ischemia-reperfusion, Oxidative stress, Leflunomide, TNF- α .

INTRODUCTION

Renal failure as a consequence of ischemia /reperfusion is of particular relevance to transplantation⁽¹⁾, coronary bypass surgery⁽²⁾, aortic cross clamping⁽³⁾, and sepsis⁽⁴⁾ and remains a major cause of morbidity and mortality among patients in intensive care units.

I/R reperfusion injury leads to production of excessive amounts of reactive oxygen species (ROS) and reactive nitrogen species (RNS), causing oxidative stress which results in alterations in mitochondrial oxidative phosphorylation, depletion of ATP, an increase in intracellular calcium and activation of protein kinases, phosphatases, proteases, lipases and nucleases leading to loss of cellular function/integrity.⁽⁵⁾

Many studies showed that an inflammatory response induced by is-

chemia followed by reperfusion is largely responsible for tissue damage (6, 7). The acute inflammatory response initiated by I/R is characterized among others by the induction of a proinflammatory cytokine cascade (8), expression of adhesion molecules (9) and cellular infiltration (10). Peripheral monocytes infiltrating the kidney have been considered the primary source of TNF- α .

TNF- α is capable of upregulating its own expression as well as the expression of other genes pivotal to the inflammatory response (11) ultimately leading to a reduction in the glomerular filtration rate (GFR). TNF- α also stimulates the production of reactive oxygen species from mesangial cells as well as the production of other inflammatory mediators (12).

The expression of proinflammatory cytokines during I/R injury results in the upregulation of inducible nitric oxide synthase (iNOS) thereby producing large amounts of nitric oxide (NO) for longer periods of time. Under oxidative stress conditions, NO reacts with superoxide to generate peroxynitrite, which is capable of nitrating tyrosine residues of proteins and enzymes leading to tissue injury (13-15).

Identification of drugs that down regulate the production of TNF- α and NO should provide the opportunity for therapeutic intervention against I/R injury

Leflunomide, an isoxazole derivative, is a unique immunomodulatory agent capable of treating rheumatoid arthritis, allograft and xenograft rejection, systemic lupus erythematosus, prostate carcinoma, and neuronal glial tumours (16-27). Recent study demonstrated the protective effect of leflunomide against hepatic ischemia/reperfusion injury in rats owing to its antioxidant and anti-inflammatory effects (28).

Thus based on this study and on the immunological dysfunction in renal ischemia/reperfusion and leflunomide's immunomodulatory feature with high efficacy and low toxicity, we assumed that leflunomide might have protective effect on renal ischemia reperfusion injury. In this study therefore we aimed to clarify the possible protective effect of leflunomide on renal ischemia/reperfusion injury in rat.

MATERIALS AND METHODS

Drugs :

Leflunomide (Arthfree tab. EVA

pharma.Co.Egypt) is a prodrug that is rapidly converted in the gastrointestinal tract and plasma to its active metabolites A 77 1726. The A 77 1726 was highly bound to plasma protein (> 99%) and had a half life of between 15 and 18 days .The total plasma clearance was 0.3ml/Kg/hr.The majority of A 77 1726 (60-70%) is metabolized in liver and excreted in urine.

Chemicals :

Chemicals for TBARs, Catalase and superoxide dismutase were purchased from Sigma Chemical Co. (St Louis, MO). TNF- α detection kit was purchased from Genzyme, (Cambridge, MA, USA), NO detection chemicals from R&D system Inc. (U.S.A). other chemicals were from analytical grades.

Animals and experimental protocols :

Sprague-Dawley rats weighing 200-250 gm were used. The rats were fed with a standard rat chow and allowed to freely drink water. The rats were anaesthetized with thiopental (50mg/kg intraperitoneally) and the body temperature was kept at 36-38°C by placing the rats under light source. The abdominal region was

shaved with a safety razor and sterilized with povidine iodine solution. A midline incision was made and a right nephrectomy is done after ligating the pedicle. A non-traumatic vascular clamp was applied to the left renal pedicle. The control group (n=10) underwent identical surgical treatment, including isolation of the left renal pedicle but without pedicle occlusion. Ischemia was applied for 45 min to I/R group (n=10) followed by 60 min of reperfusion. The rats in I/R + leflunomide (I/R + lefl) group (n=10) were pretreated intragastric with leflunomide suspension [10 mg/kg] (28) in physiological saline 60 min before the ischemia induction. The control group and the I/R group received a comparable volume of vehicle physiological saline. At the end of each experimental procedure, the left kidneys were removed and kept frozen at 20°C until analyses. Blood samples were collected and the serum sample was stored at 20°C until detection of blood urea nitrogen (BUN) and serum creatinine as indices of renal injury

Tissue homogenization and Biochemical assay :

The left kidney of each rat was ho-

mogenized for all assays. The homogenization was performed in 1:10 (w:v) 0.1 M potassium phosphate buffer (pH 7.4) with tissue tearor homogenizer (cole parmer Chicago IL, 60698). Renal homogenates were centrifuged at 5000 rpm, and +4°C for 10 min, the supernatant was separated for analysis of the followings.

- Thiobarbituric acid reactive substances (TBARs) level was measured by the modified method of Ohkawa et al (29).
- Nitric oxide level was measured according to the method of Green et al (30).
- Catalase (CAT) activity was measured by Beutler's methods (31).
- Superoxide dismutase (SOD) activity was determined by the method of Winterbourn et al (32).
- TNF- α level was determined using a commercially available ELISA kit.

The result was expressed as nmol/mg tissue for TBARs and NO levels, as U/mg tissue for SOD and CAT activities and as pmol/mg tissue for TNF- α level. Serum samples were analyzed for creatinine level by the method of Jaffe (33) and blood urea nitrogen by the method of Patton and Crouch (34).

STATISTICAL ANALYSIS

Results are expressed as means $\pm$ standard deviation of mean. For statistical analysis, the non-parametrical Mann-Whitney U test was used. A p-value of less than 0.05 was considered statistically significant.

RESULTS

BUN and creatinine levels in the I/R group were significantly higher than in control rats ($p < 0.001$) table (1). When leflunomide was administered before I/R, BUN levels were still significantly higher than control group, but the elevation in both BUN and creatinine were significantly lower in comparison to I/R group alone ($p < 0.001$).

TBARS levels and TNF- α levels were significantly higher in the I/R group than those of the control group [Table 2, Fig 1]. Pretreatment with leflunomide in the I/R + leflunomide group, significantly lower than these levels in comparison to I/R group alone, ($p < 0.001$, $p < 0.001$) respectively. Nitric oxide level was significantly increased in I/R group in comparison to control group. Pretreatment with leflunomide decreased the level of NO in comparison to I/R group ($p < 0.01$) [Table 2]. CAT and SOD activities significantly decrease in I/R group when compared to the control group [Table 3, Fig 2,3]. Leflunomide treatment increased the CAT and SOD enzyme activities in comparison to the I/R group ($p < 0.001$, $p < 0.05$) respectively.

Table 1: Serum creatinine and blood urea nitrogen(BUN) levels in control and studied groups .(Mean $\pm$ SD).

Groups	Creatinine (mg/dl)	BUN (mg/dl)
Control	0.85 $\pm$ 0.03	14.27 $\pm$ 0.24
I/R group	1.61 $\pm$ 0.03	27.60 $\pm$ 0.62
P ₁	<0.001	<0.001
I/R +leflunomide	0.93 $\pm$ 0.15	18.05 $\pm$ 0.91
P ₂	<0.05	<0.01
P ₃	<0.001	<0.001

P₁: statistical significance between control group and saline treated ischemia-reperfusion(I/R) group.

P₂: statistical significance between control group and Leflunomide- treated ischemia-reperfusion(I/R) group.

P₃: statistical significance between saline treated ischemia- reperfusion(I/R) group and Leflunomide- treated ischemia- reperfusion(I/R) group.

Table 2: Thiobarbituric acid reactive substance(TBARs), Tumour necrosis factor alpha(TNF- α) and Nitric oxide (NOx) levels in control and studied groups .(Mean $\pm$ SD).

Groups	TBARs (nmol/mg tissue)	TNF- α (Pmol/mg tissue)	NOx (nmol/mg tissue)
Control	0.459 $\pm$ 0.111	39 $\pm$ 13.88	2.53 $\pm$ 0.928
I/R group	0.647 $\pm$ 0.117	53.8 $\pm$ 6.03	4.49 $\pm$ 0.989
P ₁	<0.001	<0.001	<0.001
I/R +leflunomide	0.490 $\pm$ 0.120	17.5 $\pm$ 6.04	3.53 $\pm$ 0.948
P ₂	<0.05	<0.001	<0.001
P ₃	<0.001	<0.001	<0.01

P₁: statistical significance between control group and saline treated ischemia-reperfusion(I/R) group.

P₂: statistical significance between control group and Leflunomide- treated ischemia- reperfusion(I/R) group.

P₃: statistical significance between saline treated ischemia- reperfusion(I/R) group and Leflunomide- treated ischemia- reperfusion(I/R) group.

Table 3: *Catalase and superoxide dismutase(SOD) levels in control and studied groups .(Mean $\pm$ SD).*

Groups	Catalase (U/mg tissue)	SOD (U/mg tissue)
Control	2276 $\pm$ 286.9	8.84 $\pm$ 0.736
I/R group	1800 $\pm$ 150.92	7.14 $\pm$ 0.59
P ₁	<0.001	<0.05
I/R +leflunomide	2190 $\pm$ 96.69	8.82 $\pm$ 0.749
P ₂	NS	NS
P ₃	<0.001	<0.01

P₁: statistical significance between control group and saline treated ischemia-reperfusion(I/R) group.

P₂: statistical significance between control group and Leflunomide- treated ischemia-reperfusion(I/R) group.

P₃: statistical significance between saline treated ischemia- reperfusion(I/R) group and Leflunomide- treated ischemia- reperfusion(I/R) group.

TBARS

Fig. 1: Renal tissue TBARS levels in control rats, in rats after 45 min ischemia and 60 min reperfusion (I/R) and in I/R rats pretreated with leflunomide (10 mg/kg) (I/R+ leflunomide)

* P < 0.001 vs. control group

** P < 0.001 vs. I/R group.

CAT

Fig. 2: Renal tissue CAT activities in control rats, in rats after 45 min ischemia and 60 min reperfusion (I/R) and in I/R rats pretreated with leflunomide (10 mg/kg) (I/R+ leflunomide)

* $P < 0.001$ vs. control group
** $P < 0.001$ vs. I/R group.

SOD

Fig. 3: Renal tissue SOD activities in control rats, in rats after 45 min ischemia and 60 min reperfusion (I/R) and in I/R rats pretreated with leflunomide (10 mg/kg) (I/R+ leflunomide)

* $P < 0.05$ vs. control group
** $P < 0.01$ vs. I/R group.

DISCUSSION

Lipid peroxidation, as a free radical generating system, may be closely related to I/R induced tissue damage, and TBARs is a good indicator of the degree of lipid peroxidation⁽³⁵⁾. In the present study, the levels of TBARs were significantly increased by I/R. This observation is in agreement with previous studies, in which levels of lipid peroxidation products were increased from 40 to 100% from baseline⁽³⁶⁻³⁷⁾. Our results show that leflunomide causes significant inhibition of TBARs production probably in part by scavenging the very reactive hydroxyl(OH $\cdot$) and superoxide anion (ROO $\cdot$) radicals indicating a reduction in lipid peroxidation and cellular injury.

Superoxide radicals formed by I/R injury are converted into H₂O₂, either spontaneously (in pH 4.8) or by dismutation with the SOD enzyme (especially, in neutral and alkaline pH). H₂O₂ is then converted to H₂O by either CAT or glutathione peroxidase. It has been reported that SOD activity was reduced after I/R injury^(38, 39). Dobashi et al⁽⁴⁰⁾ also demonstrated mRNA levels of CAT significantly decreased after I/R. In our study SOD and CAT activities were found to be

significantly decreased in the I/R group when compared to the control group. The decrease in renal SOD and CAT activities is probably the result of the inactivation by ROS produced by I/R. leflunomide treatment increased levels of these enzymes in comparison with the I/R group. The increase in the SOD and CAT activities is possibly due to the scavenging of ROS, i.e. $\cdot$ O₂ and $\cdot$ OH by leflunomide.

Induction of iNOS under inflammatory conditions leads to the production of large amounts of NO for longer periods of time. The toxic effects of NO may be attributed to peroxynitrite (ONOO $\cdot$) which is a reaction product of NO with $\cdot$ O₂. NO has also been seen to inactivate the antioxidant enzymes glutathione peroxidase⁽⁴¹⁾ and catalase⁽⁴²⁾. Alterations in NO synthesis have been implicated in pathophysiological changes of ischemia/reperfusion injury in several key organs^(43, 44). For example, nephrotoxicity^(45, 46) or neurotoxicity⁽⁴⁷⁾. An animal model of kidney ischemia and brain focal ischemia is mediated at least in part by NO, since this toxicity is blocked by antisense to iNOS and inhibitors of NOS respectively. Several groups have reported that

iNOS is the injurious isoform involved in ischemic and endotoxin-induced acute renal failure, based on in vivo studies in both rats and mice (48, 49). In a recent study, kidneys of iNOS knockout mice have been protected against ischemic acute renal failure (50), where treatment with the iNOS inhibitor has improved renal function and has decreased apparent OONO- formation. OONO- is believed to be responsible for the shedding of proximal tubule cells seen in ischemic sections (51).

Previous studies have shown that proinflammatory cytokines play a key role in ischemia/reperfusion injury. Ischemia/reperfusion-induced renal TNF- α expression may result in renal cell injury by at least two distinct mechanisms: (i) direct cytotoxicity (induction of dysfunction and/or apoptosis) (52) and (ii) neutrophil mediated tissue injury (53). In a recent study, ischemia/reperfusion-induced renal TNF- α production has been found to be associated with impaired renal function and anti- TNF- α treatment results in diminished histologically evident damage and improved renal function (54).

Our data demonstrated that leflunomide

plays an important role in the attenuation of I/R-induced renal injury by decreasing TBARs, TNF- α levels and NO activities and by increasing SOD and CAT activities. These results are in agreement with others (28) who demonstrated the protective effects of leflunomide in hepatic I/R injury by decreasing levels of TBARs, nitric oxide and TNF- α and by increasing activity of catalase and SOD respectively. Also Jankovic reported that A771726, leflunomide's active metabolite, also had inhibitory effect on NO production and iNOS mRNA expression in interferon gamma (IFN- γ) + lipopolysaccharide (LPS)-activated murine and rat primary fibroblast (55-56). Furthermore, it was found that leflunomide lower significantly the TBARs content as well as the generation of NO and TNF- α in liver homogenate in mice (57). Also others (58) demonstrated that leflunomide within the therapeutic range causes a dose-dependent reduction of production of IL-1, TNF- α and NO in synovial tissue culture media.

The mechanism of the immunomodulatory and anti-inflammatory actions of Leflunomide, is mainly due to inhibition of the activity of dihydroorotate dehydrogenase (DHODH) in-

volved in de novo pyrimidine biosynthesis. Also, at a higher concentration, it mainly inhibited protein tyrosine kinases initiating signaling (59-62), and therefore could reduce the cell response to mitogen and cytokine. Other investigators found that leflunomide exerts its action via inhibition of nuclear factor kappa (NF-KB) activation in T cell and other cells by suppression of the MAPK activated by TNF- α (63-64).

Finally, these results indicate that the renoprotective effects of leflunomide in the renal injury induced by I/R could be related to its antioxidant properties, which reduce the lipid peroxidation and increase SOD activity, CAT activity, and anti-inflammatory properties by reducing level of TNF- α . Therefore, leflunomide can have a role as renoprotective against ischemia-reperfusion injury.

REFERENCES

1. Almond PS, Matas AJ, Gillingham K, Dunn DL, Payne WD, Gores P, Gruessner R, Najarian JS. (1993) : Predictors of chronic rejection in renal transplant recipients. *Transplant Proc.*; 25: 936.
2. Mangos GJ, Brown MA, Chan WY, Horton D, Trew P, Whithworth JA. (1995) : Acute renal failure following cardiac surgery: incidence, outcomes and risk factors. *Aust NZJ Med.*; 25 : 284-9.
3. Myers BD, Miller DC, Mehigan JT, Olcott CO, Golbetz H, Robertson CR, Derby G, Spencer R, Friedman S. (1984) : Nature of renal injury following total renal ischemia in man. *J Clin Invest.*; 73:329-41.
4. Groeneveld AB. (1994) : Pathogenesis of acute renal failure during sepsis. *Nephrol Dial Transplant.*; 9 (suppl 4):47-51.
5. Charanpal SS, Bipanjeet KS, Inderjit S, John KO, Avtar KS. (2003) : Attenuation of renal ischemia/reperfusion injury by a triple drug combination therapy. *J. nephrol.*; 16(!)63-74.
6. Takada M, Nadeau KC, Shaw GD, Marquette KA, Tilney NL.

- (1997) : The cytokine-adhesion molecule cascade in ischemia/reperfusion injury of the rat kidney. Inhibition by a soluble P-selectin ligand. *J Clin Invest*; 99: 2682-90.
7. Vedder NB, Winn RK, Rice CL, Chi EY, Arfos K. (1990) : Inhibition of leukocyte adherence by anti-CD18 monoclonal antibody attenuates reperfusion injury in the rabbit ear. *Proc Natl Acad Sci U S A.*; 87: 2643-6.
8. Herskowitz A, Choi S, Ansari AA, Wesselingh S. (1995) : Cytokine mRNA expression in postischemic/reperfused myocardium. *Am J Pathol.*; 146: 419-28.
9. Kelly KJ, Williams WW Jr, Colvin RB, Meehan SM, Springer TA, Gutierrez-Ramos JC, Bonventre JV. (1996) : Intercellular adhesion molecule-1-deficient mice are protected against ischemic renal injury. *J Clin Invest.*; 97: 1056-63.
10. Linas SL, Whittenburg D, Parsons PE, Repine JE. (1995) : Ischemia increases neutrophil retention and worsens acute renal failure: role of oxygen metabolites and ICAM-1. *Kidney Int.*; 48: 1584-91.
11. Donnahoo KK, Shames BD, Harken AH, Meldrum DR. (1999) : Review article: the role of tumor necrosis factor in renal ischemia-reperfusion injury. *J Urol.*; 162: 196-203.
12. Akbulut G, Nuridilek O, Kahraman A, Koken T, Serteser M. (2005) : The correlation between renal tissue oxidative stress and TNF- α levels in an experimental model of ischemia-reperfusion injury in mice. *Turkish J of trauma and surgery.* ; 11(1):11-16.
13. Walker LM, Walker PD, Imam SZ, Ali SF, Mayeux PR. (2000) : Evidence for peroxynitrite formation in renal ischemia-reperfusion injury: studies with the inducible nitric oxide synthase inhibitor L-N (6)-(1-Iminoethyl) ly-

- sine. *J Pharmacol Exp Ther.*; 295: 417-22.
14. Yu L, Gengaro PE, Niederberger M, Burke TJ, Schrier RW. (1994) : Nitric oxide: a mediator in rat tubular hypoxia/reoxygenation injury. *Proc Natl Acad Sci U S A*; 91: 1691-5.
15. Noiri E, Peresieni T, Miller F, Goligorsky MS. (1996) : In vivo targeting of inducible NO synthase with oligodeoxynucleotides protects rat kidney against ischemia. *J Clin Invest.*; 97: 2377-83.
16. Sanders S, Harisdangkul V. (2002) : Leflunomide for the treatment of rheumatoid arthritis and autoimmunity. *Am J Med Sci*; 323: 190 - 193.
17. Wendling D. (2002) : Leflunomide in the treatment of rheumatoid arthritis. *Ann Med Interne.*; 153: 21-24.
18. Alldred A, Emery P. (2001) : Leflunomide: a novel DMARD for the treatment of rheumatoid arthritis. *Expert Opin Pharmacother.*; 2: 125-137.
19. Jakes-Ocampo J, Richaud-Patin Y, Simon JA, Llorente L. (2002) : Weekly dose of leflunomide for the treatment of refractory rheumatoid arthritis: an open pilot comparative study. *Joint Bone Spine.*; 69: 307-311.
20. Williams JW, Mital D, Chong A, Kottayil A, Millis M, Longstreth J, Huang W, Brady L, Jensik S, Anita C, Anita K, Michael M, James L, Wanyun H, Lynda B, Stephen J. (2002) : Experiences with leflunomide in solid organ transplantation. *Transplantation.*; 73: 358-366.
21. Jin MB, Nakayama M, Ogata T, Fujita M, Mino K, Taniguchi M, Suzuki T, Shimamura T, Furukawa H, Todo S. (2002) : A novel leflunomide derivative, FK778, for immunosuppression after kidney transplantation in dogs. *Surgery.*; 132: 72-79.

22. Barthel HR. (2001) : Leflunomide for the treatment of systemic lupus erythematosus: comment on the article by McMurray. *Arthritis Rheum.*; 45: 472.
23. Kessel A, Toubi E. (2002) : Leflunomide in systemic lupus erythematosus. *Harefuah.*; 141: 355-357.
24. Remer CF, Weisman MH, Wallace DJ. (2001) : Benefits of leflunomide in systemic lupus erythematosus: a pilot observational study. *Lupus.*; 10: 480-483.
25. Shawver LK, Schwartz DP, Mann E, Chen H, Tsai J, Chu L, Taylorson L, Longhi M, Meredith S, Germain L, Jacobs JS, Tang C, Ullrich A, Berens ME, Hersch E, McMahon G, Hirth KP, Powell TJ. (1997) : Inhibition of platelet-derived growth factor-mediated signal transduction and tumor growth by [4-(trifluoromethyl)-phenyl] 5-methylisoxazole-4-carboxamide. *Clin Cancer Res.*; 3: 1167-1177.
26. Xu X, Shen J, Mall JW, Myers JA, Huang W, Blinder L, Saclarides TJ, Williams JW, Chong ASF. (1999) : In vitro and in vivo antitumor activity of a novel immunomodulatory drug, leflunomide: mechanisms of action. *Biochem Pharmacol.*; 58: 1405-1413.
27. Huang M, Wang Y, Collins M, Mitchell BS, Graves LM. (2002) : A77 1726 induces differentiation of human myeloid leukemia K562 cells by depletion of intracellular CTP pools. *Mol Pharmacol.*; 62: 463-472.
28. karaman A, Fadilloğlu E, Turkmen E, Tas E, Yilmaz Z (2006) : protective effects of leflunomide against ischemia-reperfusion injury in rat liver. *Pediatr.Surg Int.*; 24: 44-50.
29. Ohkawa H, Ohishi N, Yagi K. (1979) : Assay for lipid peroxidase in animal tissues by

- thiobarbituric acid reaction.
Anal Biochem.; 95: 351-8.
30. Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS, Tannenbaum SR. (1982) : analysis of nitrate, nitrite and (15N) nitrate in biological fluids. Anal. Biochem.; 126:131-138.
31. Beutler E, ed. (1973) : Red cell metabolism, a manual of biochemical methods. New York: Grune & Stratton, Inc; 76-85.
32. Winterbourn CC, Hawkins RE, Brian M, Correll RW. (1957) : The estimation of red cell superoxide dismutase activity. J Lab Clin Med.; 49: 84-95.
33. Jaffe MZ. (1886) : Phys Chem.: 10; 391.
34. Patton CJ and Crouch SR. (1977) : Anal.Chem.: 49; 464-469.
35. Granger DN, Korthuis RJ. (1995) : Physiological mechanisms of postischemic tissue injury. Annu Rev Physiol.; 57: 311-32.
36. Eschwege P, Paradis V, Conti M, Holstege A, Richet F, Deteve J, Menager P, Legendre A, Jardin A, Bedossa P, Benot G. (1999) : In situ detection of lipid peroxidation by-products as markers of renal ischemia injuries in rat kidneys. J Urol.; 162: 553-7.
37. Omar R, Nomikos I, Piccorrelli G, Savino J, Agarwal N. (1989) : Prevention of post-ischemic lipid peroxidation and liver cell injury by iron chelation. Gut.; 30: 510-4.
38. Sumimoto K, Oku J, Dohi K, Kawasaki T. (1990) : Lipid peroxidation in transplanted rat liver. Transplant Proc.; 22: 2023-4.
39. Yuceyar S, Gumus-tas K, Erturk S, Hamzaog-brevelu IH, Uygun N, Ayaz M, Cengiz A, Kafadar Y. (1998) : The role of oxygen free radicals in acute renal failure compli-

- cating obstructive jaundice: an experimental study. *HPB Surg.*; 10: 378-93.
40. Dobashi K, Ghosh B, Orak JK, Singh I, Singh AK. (2000) : Kidney ischemia-reperfusion: Modulation of antioxidant defenses. *Mol Cell Biochem.*; 205: 1-11.
41. Asahi M, Fujii J, Suzuki K, Seo HG, Kuzuya T, Hori M, Tada M, Fujii S, Taniguchi N. (1995) : Inactivation of glutathione peroxidase by nitric oxide. Implication for cytotoxicity. *J Biol Chem.*; 270: 21035-9.
42. Brown GC. (1995) : Nitric oxide regulates mitochondrial respiration and cell functions by inhibiting cytochrome oxidase. *FEBS Lett.*; 369: 136-9.
43. Giraldez RR, Panda A, Xia Y, Sanders SP, Zweier JL. (1997) : Decreased nitric-oxide synthase activity causes impaired endothelium-dependent relaxation in the postischemic heart. *J Biol Chem.*; 272: 21420-6.
44. Huang PL, Huang Z, Mashimo H, Bloch KD, Moskowitz MA, Bevan JA, Fishman MC. (1995) : Hypertension in mice lacking the gene for endothelial nitric oxide synthase. *Nature.*; 377: 239-42.
45. Wang L, Kubodera S, Ueno A, Takeda M. (2001) : Effects of nitric oxide synthesis inhibition on FK506-induced nephrotoxicity in rats. *Ren Fail.* 2001; 23: 11-9.
46. Bobadilla NA, Gamba G, Tapia E, Garcia-Torres R, Bolio A, Lopez-Zetina P, Herrera-Acosta J. (1998) : Role of NO in cyclosporin nephrotoxicity: effects of chronic NO inhibition and NO synthases gene expression. *Am J Physiol.*; 274 : F791-8.
47. Nowicki JP, Duval D, Poignet H, Scatton B. (1991) : Nitric oxide mediates neuronal death after focal cerebral ischemia in the mouse. *Eur J Neurosci.*; 3: 112-20.

- J Pharmacol.; 204: 339-40.
48. Chiao H, Kohda Y, McLeroy P, Craig L, Housini I, Star RA. (1997) : Alpha-melanocyte-stimulating hormone protects against renal injury after ischemia in mice and rats. *J Clin Invest.*; 99: 1165-72.
49. Schwartz D, Mendonca M, Schwartz I, Xia Y, Satriano J, Wilson CB, Blantz RC. (1997) : Inhibition of constitutive nitric oxide synthase (NOS) by nitric oxide generated by inducible NOS after lipopolysaccharide administration provokes renal dysfunction in rats. *J Clin Invest.*; 100: 439-48.
50. Ling H, Edelstein C, Gengaro P, Meng X, Lucia S, Knotek M, Wangsiripaisan A, Shi Y, Schrier R. (1999) : Attenuation of renal ischemia-reperfusion injury in inducible nitric oxide synthase knockout mice. *Am J Physiol.*; 277: F383-90.
51. Wangsiripaisan A, Gengaro PE, Nemenoff RA, Ling H, Edelstein CL, Schrier RW. (1999) : Effect of nitric oxide donors on renal tubular epithelial cell-matrix adhesion. *Kidney Int.*; 55: 2281-8.
52. Lieberthal W, Koh JS, Levine JS. (1998) : Necrosis and apoptosis in acute renal failure. *Semin Nephrol.*; 18: 505-18.
53. Rabb H, O'Meara YM, Maderna P, Coleman P, Brady HR. (1997) : Leukocytes, cell adhesion molecules and ischemic acute renal failure. *Kidney Int.*; 51:1463-8.
54. Manna SK, Mukhopadhyay A, Aggarwal BB. (2000) : Leflunomide Suppresses TNF-Induced Cellular Responses: Effects on NF-kB, Activator Protein-1, c-Jun N-Terminal Protein Kinase, and Apoptosis. *J Immunol.*; 165: 5962-5969.
55. Jankovic V, Samardzic T, Stosic-Grujicic S, Popadic D,

- Trajkovic V. (2000)** : Cell-specific inhibition of inducible nitric oxide synthase activation by leflunomide. *Cell Immunol.*; 199: 73-80.
- 56. Donnahoo KK, Meng X, Ayala A, Cain MP, Harken AH, Meldrum DR. (2000)** : Early kidney TNF-alpha expression mediates neutrophil infiltration and injury after renal Breedveld FC, Dayer JM. Leflunomide: mode of action in the treatment of rheumatoid arthritis. *Ann Rheum Dis.*; 59: 841-849.
- 57. Yao HW, Li J, Jin Y, Zhang YF, Chang YL, Shu YX. (2003)** : Effect of leflunomide on immunological liver injury in mice. *World J Gastroenterol.*; 15:9(2): 320-323.
- 58. Dimitrijevic M, Barlett RR. (1996)** : Leflunomide, a novel immunomodulating drug, inhibits homotypic adhesion of peripheral blood and synovial fluid mononuclear cells in rheumatoid arthritis. *Inflamm. Res.*; 45: 550-6.
- 59. Sanders S, Harisdangkul V. (2002)** : Leflunomide for the treatment of rheumatoid arthritis and autoimmunity. *Am J Med Sci.*; 323: 190-193.
- 60. Herrmann ML, Schleyerbach R, Kirschbaum BJ. (2000)** : Leflunomide: an immunomodulatory drug for the treatment of rheumatoid arthritis and other autoimmune diseases. *Immunopharmacology*; 47: 273-289.
- 61. Breedveld FC, Dayer JM. (2000)** : Leflunomide: mode of action in the treatment of rheumatoid arthritis. *Ann Rheum Dis.*; 59: 841-849.
- 62. Xu X, Gong H, Blinder L, Shen J, Williams JW, Chong AS. (1997)** : Control of lymphoproliferative and autoimmune disease in MRL-lpr/lpr mice by brequinar sodium:

- mechanisms of action. *J Pharmacol Exp Ther.*; 283: 869-875.
63. Elder RT, Xu X, Williams JW, Gong H, Finnegan A, Chong AS. (1997) : The immunosuppressive metabolite of leflunomide, A771726, affects murine T cells through two biochemical mechanisms. *J Immunol.*; 159: 22-27.
64. Sunil K, Manna B, Aggarwal L. (1999) : Immunosuppressive leflunomide metabolite (A77 1726) blocks TNF-dependent nuclear factor B activation and gene expression. *J Immunol.*; 162:2095-2102.

التأثير الوقائي لعقار الليفلوناميد على القصور المؤقت في الدورة الدموية الكلوية للفئران

د. أماني عبد الرحمن شلبي^١ ، د. أماني عبد المجيد عطوه^٢

د. هبه كمال مرسى^٢

قسم الفارماكولوجى الاكلينيكي^١ - قسم الكيمياء الحيوية الطبية^٢

كلية الطب - جامعة المنصورة

قصور الدورة الدموية الكلوية المؤقت له أهمية إكلينيكية لدوره في أحداث الفشل الكلوى ورفض أنسجة الكلى ولذلك هدفت هذه الدراسة إلى بحث التأثير الوقائي لعقار الليفلوناميد على حماية الأنسجة ضد قصور الدورة الدموية المؤقت بكلية الفئران. وقد أجريت هذه الدراسة على ثلاث مجموعات من الفئران، المجموعة الأولى، المجموعة الضابطة، والثانية مجموعة قصور الدورة الدموية المؤقت والأخيرة مجموعة قصور الدورة الدموية + الليفلوناميد. أحدث قصور الدورة الدموية المؤقت بواسطة غلق العنق الكلوى الأيسر لمدة ٤٥ دقيقة ثم فكه لمدة ٦٠ دقيقة مع استئصال الكلية اليمنى. تم إعطاء فئران المجموعة الثالثة عقار الليفلوناميد ٥٠ مجم/كجم فى المعدة بواسطة أنبوبة معدية ٦٠ دقيقة قبل إحداث القصور المؤقت. تم قياس كل من معامل نخر الورم ألفا، الشقوق الحرة، أكسيد النيتريك، إنزيم الكاتاليز وإنزيم السوبر أوكسيد الدسميوتيز فى أنسجة الكلى وكذلك نسبة الكرياتينين واليوريا فى الدم. وقد أظهرت النتائج زيادة ذات دلالة إحصائية فى مستوى معامل نخر الورم ألفا، الشقوق الحرة وأكسيد النيتريك وكذلك نسبة الكرياتينين واليوريا فى الدم فى مجموعة قصور الدورة الدموية عن المجموعة الضابطة وإعطاء عقار الليفلوناميد يؤدى إلى إنخفاض مستوى هذه العناصر.

انخفض نشاط إنزيم الكاتاليز وإنزيم السوبر أوكسيد الدسميوتيز بعد إحداث القصور المؤقت عن المجموعة الضابطة. العلاج بعقار الليفلوناميد أدى إلى زيادة نشاط هذه الإنزيمات. ولذلك فإن العلاج بعقار الليفلوناميد ذو فاعلية فى حماية الكلى من قصور الدورة الدموية المؤقت .