Zagazig J. Pharm. Sci. Dec., 2014 ISSN 1110-5089
Vol. 23, Issue. 2, pp, 16-25

The protective effect of low dose methotrexate on renal ischemia-reperfusion injury

Abd-elaal M, El-fayomi HM, Elshazly SM, Elbatrik MH*
Department of Pharmacology and Toxicology-Faculty of Pharmacy-Zagazig University-Zagazig Egypt.
***Corresponding Author E-mail: elbatreek_m@yahoo.com**

ABSTRACT

Methotrexate was developed as a cytostatic agent, but at low doses, it has shown potent anti-inflammatory activity. Previous studies have demonstrated that the anti-inflammatory effects of methotrexate are primarily mediated by the release of adenosine. This study was designed to investigate the effects of low-dose methotrexate in I/R-induced renal injury in rats.

Forty male albino rats were divided equally into five groups: (I) control; (II) sham operated (only unilateral nephrectomy); (III) I/R; (IV) methotrexate (0.5 mg/kg prior to experiment) plus I/R; and (V) levamisole (12.5 mg/kg prior to experiment) plus group IV. In groups III, IV and V after unilateral nephrectomy, the rats were subjected to 45 min of left renal pedicle occlusion, followed by 6 h of reperfusion. At the end of the reperfusion period, rats were killed and kidneys and blood were removed. Myeloperoxidase and superoxide dismutase activities, and interleukin 10, malondialdehyde, monocyte chemoattractant protein 1, reduced glutathione and tumor necrosis factor alpha contents were determined in renal tissue. Serum creatinine and blood urea nitrogen were measured for the evaluation of renal function.

After I/R, increases were found in serum levels of creatinine and urea, MPO activity, tissue contents of MDA, MCP-1 and TNF-α. In contrast, SOD activity, GSH and IL-10 contents were decreased. After the administration of a low-dose of methotrexate, decreases were observed in serum levels of creatinine and urea, MPO activity, tissue contents of MDA, MCP-1 and TNF-α. In contrast, SOD activity, GSH and IL-10 contents were increased. These effects diminished in presence of levamisole. Low-dose methotrexate exhibits meaningful renoprotective activity following ischemia–reperfusion injury of the kidney.

Key Words: methotrexate- renal ischemia reperfusion- adenosine- immunosuppressants

INTRODUCTION

Acute kidney injury (AKI) caused by ischemia-reperfusion (I/R) injury is a common and important problem in both native kidneys and transplanted kidneys. The mortality during native kidney AKI is close to 50% in the intensive care unit, and AKI in early transplants leads to more rejections and worse long-term outcomes (Li et al., 2012). Ischemia, can occur for a number of reasons, for example, with the use of vasoconstrictive drugs or radiocontrast agents; hypotension linked to sepsis or blood loss after surgery and trauma (Meinel et al., 2014).

In ischemic acute kidney injury, hypoxic and anoxic cell injuries occur early during the ischemic phase, followed by inflammatory responses in the reperfusion phase. Renal ischemia reperfusion induces renal synthesis or activation of pro-inflammatory cytokines and chemokines, and recruits leukocytes into the post-ischemic kidneys (Jang & Rabb, 2009). A number of processes have been implicated in the pathogenesis of ischemic kidney injury. These include generation of reactive oxygen species (ROS), depletion of adenosine triphosphate (ATP), neutrophil infiltration, phospholipase activation and accumulation of intracellular calcium (Takasaki et al.,

1998; Bonventre, 2010; and Abdelkader et al., 2014).

Methotrexate (MTX) is a folate analogue that was introduced in the clinical practice more than 50 years ago. It is currently one of the most widely used disease-modifying antirheumatic drugs (DMARDs) (Romano Danesi et al., 1999). When used in high dose, MTX competitively inhibits the enzyme dihydrofolate reductase and prevents the formation of tetrahydrofolate which is necessary for purine and pyrimidine synthesis, so it has been used to treat oncological diseases (Sweetman, 2009). Low dose MTX has both immunosuppressive and anti-inflammatory properties resulting in inhibition of proliferation of lymphocytes, monocytes-macrophages and neutrophils. Also it enhances release of adenosine (Romano Danesi et al., 1999; and Montesinos et al., 2007).

In fact, previous studies have concluded that both adenosine and immunosuppression protect the kidney from I/R injury (Okusa et al., 2001; Okusa, 2002; and Karaman et al., 2006). Methotrexate has also been reported to limit infarct size and has shown a potent cardioprotective effect against I/R injury of the heart (Asanuma et al., 2004). Moreover, MTX has been reported to protect spinal cord from I/R injury (Kertmen et al., 2013). There are no previous studies examining the protective effect of MTX in renal I/R injury. Based on these results, the purpose of this study was to evaluate whether MTX administration could protect the kidney from I/R injury in rats.

MATERIAL and METHODS

Animals

Forty male albino rats, weighing 180–220 g، were obtained from the Faculty of Veterinary Medicine, Zagazig University, Egypt, and were randomly divided into four groups, with 8 rats in each group. The control group (group I, control) animals received normal saline without I/R procedure. The sham group (group II, sham) underwent right nephrectomy, but the left renal pedicle was not occluded. The animals in the I/R control group (group III, I/R) underwent right nephrectomy and, after 10 min of stabilization, 45 min of left renal ischemia followed by 6 h reperfusion. Methotrexate-treated ischemic group (group IV, MTX + I/R) animals were treated with MTX 15 min prior to ischemia and the rest of the protocol was the same as in group III. Levamisole-treated group (group V, LEV+MTX+I/R) animals were treated with LEV 30 min prior to ischemia and the rest of the protocol was the same as in group IV. Rats were housed in controlled light/ dark cycles of 12 h and allowed free access to water and rat chow.

Experimental procedure

On the experiment day, rats were fasted overnight. Rats were anesthetized with thiopental sodium (EPICO, 10th of Ramadan city. Egypt) (40 mg/kg) administered i.p. before the operation.Under anesthesia, a midline laparotomy was made and, using minimal dissection, bilateral renal blood vessels were isolated. Right nephrectomy was performed on all rats except group I. After 10 min of stabilization, the left renal pedicle was occluded for 45 min to induce ischemia and then subjected for 6 h of reperfusion (I/R groups). Methotrexate (Sanofi-Aventis, Compiegne, France) (0.5 mg/kg) (MTX group & LEV/MTX group) or saline (control &I/R group) were administered i.v 15 min prior to the experiment, while levamisole (Kahira Pharmaceutical Co. Egypt) (12.5 mg/kg) (LEV/MTX group) was administered p.o 30 min prior to experiment. Sham-operated animals only underwent right nephrectomy. At the end of the reperfusion period، the animals were decapitated. Trunk blood samples were collected and the serum

samples were stored at −80 C، until determination of renal function. The renal tissue samples were immediately stored at −80 C for subsequent biochemical analyses. On the day of analysis, the tissues were homogenized in physiologic saline and then centrifuged. All protocols were approved by Ethical Committee for Animal Handling, Faculty of Pharmacy, Zagazig University.

Serum creatinine and urea nitrogen analyses

Serum creatinine and blood urea nitrogen (BUN) levels was quantitatively determined by colorimetric method using the kit provided by Diamond diagnostic, Egypt according to the manufacturer's protocol.

Determination of malondialdhyde (MDA) content

MDA content in the kidney was determined as an indicator of lipid peroxidation using the kit provided by Diamond diagnostic, Egypt following the protocol described by (Ohkawa *et al.*, 1979).

Determination of reduced glutathione (GSH) content

GSH content was measured in kidney tissue using the kit provided by Biodiagnostic, Egypt following the protocol described by (Beutler *et al.*, 1963). The method is based on the reduction of 5,5' dithiobis (2-nitrobenzoic acid) (DTNB) with (GSH) to produce a yellow compound. The reduced chromogen is directly proportional to GSH concentration and its absorbance can be measured at 405 nm.

Determination of superoxide dismutase (SOD) activity

SOD activity was measured in renal tissue using the kit provided by Biodiagnostic, Egypt following the protocol described by (Nishikimi *et al.*, 1972**)**. This assay relies on the ability of the enzyme to inhibit the phenazine methosulphate-mediated reduction of nitro blue tetrazolium dye.

Determination of tumor necrosis factor (TNF-α), monocyte chemoattractant protein (MCP-1), interleukin-10 (IL-10) contents and myeloperoxidase (MPO) activity

TNF-α, MCP-1, IL-10 contents and MPO activity were measured using enzyme-linked immunosorbent assay (ELISA) methods according to (Yan-Lian *et al.*, 1994).

Statistical analyses

Data were analyzed using computer based fitting program (Prism, Graphpad 5.). The results expressed as mean values ± SE were compared between groups using one-way analysis of variance (ANOVA). Differences were considered to be statistically significant when $P < 0.05$.

RESULTS

Serum creatinine and urea nitrogen analyses

Serum creatinine and urea levels were significantly elevated following I/R injury when compared to both sham operated and control groups ($P < 0.05$). Treatment with MTX significantly reduced both serum creatinine and urea levels when compared to I/R group and LEV/MTX group ($P < 0.05$) (fig. 1, fig. 2).

Malondialdhyde analyses

Renal MDA content was significantly elevated following I/R injury when compared to both sham operated and control groups ($P < 0.05$). Treatment with MTX significantly reduced renal MDA content when compared to I/R group and LEV/MTX group ($P < 0.05$) (fig. 3).

Antioxidant parameters analyses

Renal GSH content and SOD activity were significantly reduced following I/R injury when compared to both sham operated

and control groups (P < 0.05). Treatment with MTX showed a significant increase in renal GSH content and SOD activity when compared to I/R group and LEV/MTX group (P < 0.05) (fig. 4, fig. 5).

MPO was significantly higher in the kidney tissue of I/R group than in the kidney tissue of both sham operated and control groups (P < 0.05). The rats in MTX group had significantly lower MPO activity than the rats in I/R group and LEV/MTX group (P < 0.05) (table 1).

Cytokines and chemokines analyses

A significant increase in TNF -α and MCP-1 contents, but a significant decrease in IL-10 content following I/R injury were observed when compared to both sham operated and control groups (P < 0.05). Treatment with MTX showed a significant decrease in TNF-α and MCP-1 contents, but a significant increase in IL-10 content when compared to I/R group and LEV/MTX group (P < 0.05) (table 1).

Myeloperoxidase activity analyses

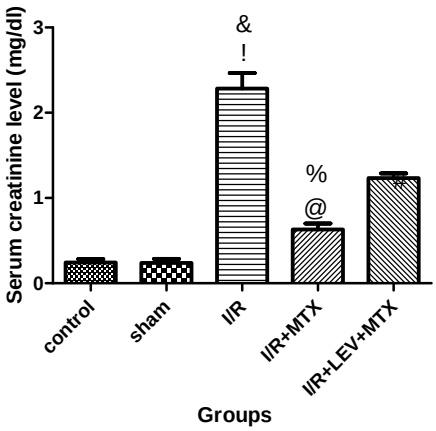

Fig. 1 Effect of MTX on serum creatinine level

! Significantly different from control group.

&Significantly different from sham operated group.

@ Significantly different from I/R group.

% Significantly different from LEV/MTX group.

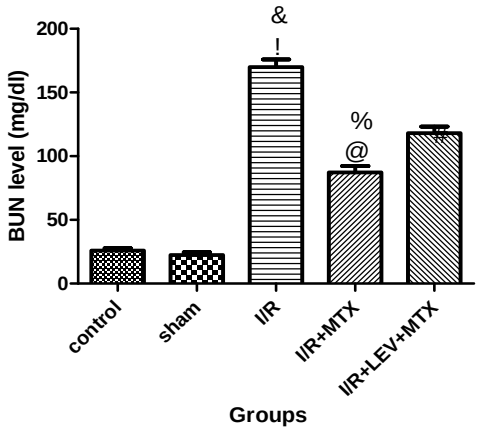

Fig. 2 Effect of MTX on serum urea level

! Significantly different from control group.

&Significantly different from sham operated group.

@ Significantly different from I/R group.

% Significantly different from LEV/MTX group.

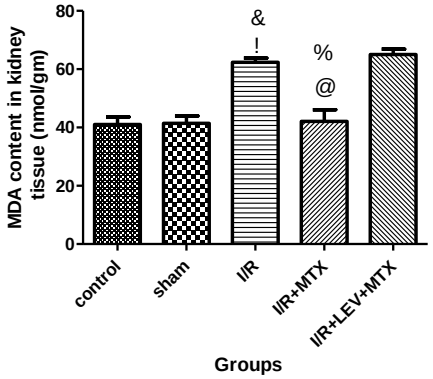

Fig. 3 Effect of MTX on renal MDA content
! Significantly different from control group.
&Significantly different from sham operated group.
@ Significantly different from I/R group.
% Significantly different from LEV/MTX group.

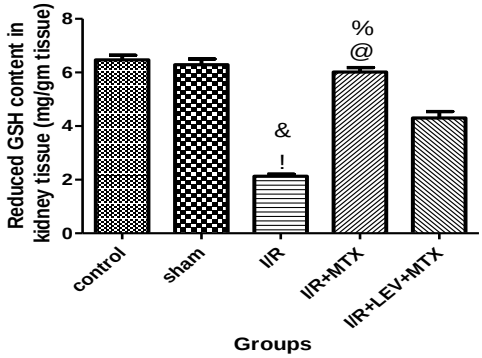

Fig. 4 Effect of MTX on renal GSH content
! Significantly different from control group.
&Significantly different from sham operated group.
@ Significantly different from I/R group.
% Significantly different from LEV/MTX group.

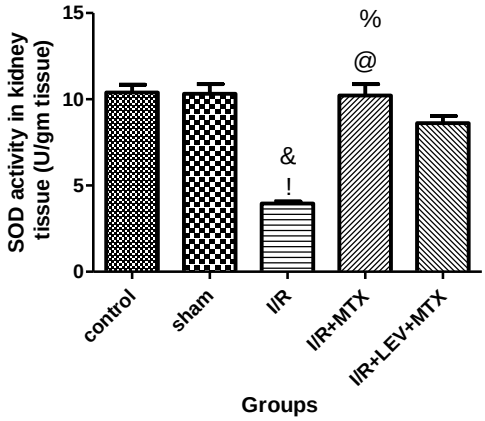

Fig. 5 Effect of MTX on renal sod activity
! Significantly different from control group.
&Significantly different from sham operated group.
@ Significantly different from I/R group.
% Significantly different from LEV/MTX group.

Table 1: Activity of myeloperoxidase (MPO) and contents of tumor necrosis factor TNF-α, monocyte chemoattractant protein (MCP-1) and interleukin-10 (IL-10) in renal tissue of control, sham, ischemia-reperfusion (I/R) and methotrexate (MTX) groups

Variables	Control	Sham	I/R	MTX	LEV/MTX
MPO (ng/ml)	12.78 ± 0.049	12.92 ± 0.14	$21.05^{!\&}$ ± 0.77	$13.75^{@\%}$ ± 0.46	16.76 ± 0.88
TNF-α (pg/ml)	30.2 ± 0.77	30.84 ± 0.80	$123.3^{!\&}$ ± 1.03	$63.55^{@\%}$ ± 3.97	103 ± 6.59
MCP-1 (pg/ml)	1.18 ± 0.04	1.25 ± 0.037	$7.41^{!\&}$ ± 0.45	$4.69^{@\%}$ ± 0.58	7.6 ± 0.45
IL-10 (pg/ml)	120.9 ± 4.11	114.8 ± 3.68	$49.28^{!\&}$ ± 3.62	$106.9^{@\%}$ ± 5.54	73.69 ± 1.86

All data are expressed as mean ± SE

! Significantly different from control group, **&** Significantly different from sham operated group, **@** Significantly different from I/R group, **%** Significantly different from LEV/MTX group.

DISCUSSION

Acute renal failure (ARF) is a common clinical problem with increasing incidence, serious consequences, unsatisfactory therapeutic options, and an enormous financial burden to society (Devarajan, 2006). One of the most common causes of ARF is ischemia reperfusion injury which occurs when blood supply is interrupted in clinical situations such as kidney transplantation, partial nephrectomy and renal artery angioplasty (Chatauret *et al.*, 2014). The pathophysiology of renal ischemia-reperfusion injury (RIRI) can be summarized by a primary energy deficit during ischemia and a secondary phase of oxidative stress and inflammation (Denecke & Tullius, 2014). In the current study, animals subjected to renal I/R demonstrated a significant increase in blood urea nitrogen and creatinine levels, confirming the occurrence of marked renal dysfunction.

Lipid peroxidation that occurs in cell membranes is one of the main pathophysiological mechanisms involved in I/R damage (Ivanov *et al.*, 2014). MDA content increase after renal I/R, demonstrating the involvement of lipid peroxidation, thus supporting the presence of reperfusion injury (Sancaktutar *et al.*, 2014). This is in accordance with Grekas *et al.* (1996); and Rhoden *et al.* (2001) who reported that MDA content was significantly increased following I/R. Reactive oxygen species (ROS) play a key role in mediating the injury induced by reperfusion. Neutrophil activation causes the generation of ROS and thus results in a considerable amount of damage to the tissue. The endogenous antioxidants as SOD and GSH have the capacity to scavenge ROS (Scaduto, Jr. *et al.*, 1988; and Cong *et al.*, 2013). The results of the current study confirm that there is significant decrease in renal SOD activity and GSH content in ischemic groups and this is in accordance with previous reports (Bayrak *et al.*, 2008; and Ozturk *et al.*, 2014). Pretreatment with methotrexate (MTX) prevented the renal I/R-induced lipid peroxidation and protected the kidneys from severe attenuation of antioxidant activity in rats exposed to the renal I/R. Furthermore, the renal functional damage was significantly improved by MTX.

Several methods have been used to define the role of neutrophils in reperfusion tissue injury. In neutrophils, MPO is stored in azurophilic granules and released during phagocytosis. Hypochlorous acid is produced largely from stimulated neutrophils by MPO

activity. Hypochlorous acid causes oxidation of other molecules such as proteins, amino acids, carbohydrates, nucleic acids and lipids, expanding kidney tissue damage (Yagmurca *et al.*, 2004; and Kabasakal *et al.*, 2004). Results of the current study showed that renal I/R induced a significant increase in MPO activity. These results are in harmony with that of (Karaman *et al.*, 2006; and Punuru *et al.*, 2014).

Pro-inflammatory cytokines as tumor necrosis factor alpha (TNF-α) and chemokines as monocyte chemoattractant protein- 1 (MCP-1) play a pivotal role in renal I/R injury (Huen & Cantley, 2014). TNF-α has been shown to be secreted at early stages of ischemia-reperfusion injuries and mediate the induction of other chemokines such as MCP-1 to attract leukocytes migrating to the inflammatory site which finally results in the inflammation (Tomasoni *et al.*, 2000). The compensatory anti-inflammatory response is a secondary immune response that is characterized by the production of anti-inflammatory cytokines, aimed at offsetting pro-inflammatory responses. Interleukin-10 (IL-10) is the most potent anti-inflammatory cytokine, and its release inhibits the production of TNF-α and IL-1β (Opal & Huber, 2000). The results of this work demonstrated that TNF-α and MCP-1 contents were significantly increased, but IL-10 content was significantly decreased after renal I/R. These data are in agreement with the studies of Dong *et al.* (2007); and Collino *et al.* (2013).

The current study revealed that MTX significantly reduced MPO activity in renal tissue. Thus, the observed decrease in MPO activity in response to MTX indicates a reduction in the number of neutrophils at the site of injury. This supports the existence of anti-inflammatory activity for MTX. These findings are in harmony with Kertmen et al. (2013) who indicated that MTX was effective in reducing MPO activity in I/R injury of rabbits spinal cord. Results of the present study illustrated that MTX induced a significant decrease in TNF-α and MCP-1 contents and also induced a significant increase in IL-10 content indicating anti-inflammatory activity. These observations were in accordance with the findings of Neurath *et al.* (1999); Rudwaleit *et al.* (2000); and Riksen *et al.* (2006).

The anti-inflammatory action of MTX may be explained by its immunosuppressive effects and this was confirmed by prior administration of LEV that decreased the anti-inflammatory actions of MTX. In several studies, MTX has exerted a wide range of anti-inflammatory activities that are primarily mediated via the release of adenosine. Once adenosine is released in the extracellular environment, it binds to different types of adenosine receptors (i.e. adenosine A(1), A(2A), A(2B) and A(3) receptors) expressed on various innate immune cells [Neutrophils, macrophages, mast cells, dendritic cells and natural killer cells] (Kumar & Sharma, 2009). It is commonly accepted that the anti-inflammatory effects of adenosine are predominantly due to A2A-receptor stimulation. Adenosine A2A-receptor activation has been shown to have protective anti-inflammatory effects against renal I/R injury in many previous studies (Okusa *et al.*, 2001; and Day *et al.*, 2006). Concurrently, MTX administration has been shown to increase adenosine concentrations and activate adenosine A2A –receptors (Montesinos *et al.*, 2007). Therefore, it is hypothesized that MTX may have a renoprotective effect in renal I/R injury.

In conclusion, this study may suggest that acute administration of methotrexate would be helpful in clinical practice, for example, in reconstructive renal surgery and transplantation. Furthermore, new studies are needed to improve roles of methotrexate on ARF in both other animal models and in

vitro human cell lines, before clinical applications.

REFERENCES

1. Abdelkader A.; Ho J.; Ow C. P., Eppel G. A., Rajapakse N. W., Schlaich M. P. and Evans R. G. (2014): Renal oxygenation in acute renal ischemia-reperfusion injury. *Am J Physiol Renal Physiol.* 306(9): 1026-1038.
2. Asanuma H., Sanada S., Ogai A., Minamino T., Takashima S., Asakura M., Ogita H., Shinozaki Y., Mori H., Node K., Tomoike H., Hori M. and Kitakaze M. (2004): Methotrexate and MX-68, a new derivative of methotrexate, limit infarct size via adenosine-dependent mechanisms in canine hearts. *J Cardiovasc Pharmacol*., 43: 574-579
3. Bayrak O., Bavbek N., Karatas O. F., Bayrak R., Catal F., Cimentepe E., Akbas A., Yildirim E., Unal D. and Akcay A. (2008): Nigella sativa protects against ischaemia/reperfusion injury in rat kidneys. *Nephrol Dial Transplant*., 23: 2206-2212.
4. Beutler E., Duron O. and Kelly B. M. (1963): Improved method for the determination of blood glutathione. *J Lab Clin Med*., 61: 882-888.
5. Bonventre J. (2010): Mechanisms of Acute Kidney Injury and Repair.In Management of acute kidney problems. ed. Jarres,A., Ronco,C. & Kellum,J.A. pp. 13-20. Springer Berlin Heidelberg. Chatauret N., Badet L., Barrou B. and Hauet T. (2014): Ischemia-reperfusion: From cell biology to acute kidney injury. Prog Urol., 24S1: S4-S12
6. Collino M., Rogazzo M., Pini A., Benetti E., Rosa A. C., Chiazza F., Fantozzi R., Bani D. and Masini E. (2013): Acute treatment with relaxin protects the kidney against ischaemia/reperfusion injury. *J Cell Mol Med*., 17: 1494-1505.
7. Cong G., Cui L., Zang M. and Hao L. (2013): Attenuation of renal ischemia/reperfusion injury by a polysaccharide from the roots of Dipsacus asperoides. *Int J Biol Macromol*., 56: 14-19.
8. Danesi R., Bocci G., Di Paolo A. and Del Tacca M. (1999): Cytotoxic drugs.In Principles of immunopharmacology. ed. Nijkamp,F.P. & Parnham,M.J. pp. 465-479. Birkh_luser Verlag.
9. Day Y. J., Huang L., Ye H., Li L., Linden J. and Okusa M. D. (2006): Renal ischemia-reperfusion injury and adenosine 2A receptor-mediated tissue protection: the role of CD4+ T cells and IFN-gamma. *J Immunol*., 176: 3108-3114..
10. Denecke C. and Tullius S. G. (2014): Innate and adaptive immune responses subsequent to ischemia-reperfusion injury in the kidney. *Prog Urol*., 24S1: S13-S19..
11. Devarajan P. (2006 :(Devarajan P. (2006): Update on Mechanisms of Ischemic Acute Kidney Injury. *Journal of the American Society of Nephrology*., 17: 1503-1520..
12. Dong X., Swaminathan S., Bachman L. A., Croatt A. J., Nath K. A. and Griffin M. D. (2007): Resident dendritic cells are the predominant TNF-secreting cell in early renal ischemia-reperfusion injury. *Kidney Int*., 71: 619-628..
13. Grekas D., Dioudis C., Papageorgiou G., Iliadis S., Zilidis C., Alivanis P., Dimitriadou A. and Tourkantonis A. (1996): Lipid peroxidation after acute renal ischemia and reperfusion in rats: the effect of trimetazidine. *Ren Fail*., 18: 545-552..
14. Huen S. C. and Cantley L. G. (2015): Macrophage-mediated injury and repair after ischemic kidney injury. *Pediatr Nephrol*. 30 (2):199-209
15. Ivanov M., Mihailovic-Stanojevic N., Grujic M. J., Jovovic D., Markovic-Lipkovski J., Cirovic S. and Miloradovic Z. (2014): Losartan improved antioxidant defense, renal function and structure of postischemic hypertensive kidney. *PLoS One*., 9: e96353.
16. Jang H. R. and Rabb H. (2009): The innate immune response in ischemic acute kidney injury. *Clin Immunol*., 130: 41-50.
17. Kabasakal L., Sehirli A. O., Cetinel S., Cikler E., Gedik N. and Sener G. (2004): Mesna (2-mercaptoethane sulfonate) prevents ischemia/reperfusion induced renal oxidative damage in rats. *Life Sci*., 75: 2329-2340.
18. Karman A. B. D. U., Turkemen E. M. I. N., GURSUL C. E. B. R., TAS E. R. K. A. and Fadilluglo E. R. S. I. (2006): Prevention of

renal ischemia/reperfusion-induced injury in rats by leflunomide. *International Journal of Urology*., 13: 1434-1441.
19. Kertmen H., Gurer B., Yilmaz E. R., Sanli A. M., Sorar M., Arikok A. T., Sargon M. F., Kanat M. A., Erguder B. I. and Sekerci Z. (2013): The protective effect of low-dose methotrexate on ischemia-reperfusion injury of the rabbit spinal cord. *Eur J Pharmacol*., 714: 148-156.
20. Kumar V. and Sharma A. (2009): Adenosine: an endogenous modulator of innate immune system with therapeutic potential. *Eur J Pharmacol*., 616: 7-15.
21. Li X., Liu M., Bedja D., Thoburn C., Gabrielson K., Racusen L. and Rabb H. (2012): Acute renal venous obstruction is more detrimental to the kidney than arterial occlusion: implication for murine models of acute kidney injury. *Am J Physiol Renal Physiol*., 302: F519-F525.
22. Meinel F. G., De Cecco C. N., Schoepf U. J. and Katzberg R. (2014): Contrast-Induced Acute Kidney Injury: Definition, Epidemiology, and Outcome. *Biomed Res Int*., 2014: 859328.
23. Montesinos M. C., Takedachi M., Thompson L. F., Wilder T. F., Fernandez P. and Cronstein B. N. (2007): The antiinflammatory mechanism of methotrexate depends on extracellular conversion of adenine nucleotides to adenosine by ecto-5'-nucleotidase: findings in a study of ecto-5'-nucleotidase gene-deficient mice. *Arthritis Rheum*., 56: 1440-1445.
24. Neurath M. F., Hildner K., Becker C., Schlaak J. F., Barbulescu K., Germann T., Schmitt E., Schirmacher P., Haralambous S., Pasparakis M., Meyer Zum Buschenfelde K. H., Kollias G. and Marker-Hermann E. (1999): Methotrexate specifically modulates cytokine production by T cells and macrophages in murine collagen-induced arthritis (CIA): a mechanism for methotrexate-mediated immunosuppression. *Clin Exp Immunol*., 115: 42-55.
25. Nishikimi M., Appaji Rao N. and Yagi K. (1972): The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. *Biochemical and Biophysical Research Communications*., 46: 849-854.
26. Ohkawa H., Ohishi N. and Yagi K. (1979): Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical Biochemistry*., 95: 351-358.
27. Okusa M. D. (2002): A(2A) adenosine receptor: a novel therapeutic target in renal disease. *Am J Physiol Renal Physiol*., 282: F10-F18.
28. Okusa M. D., Linden J., Huang L., Rosin D. L., Smith D. F. and Sullivan G. (2001): Enhanced protection from renal ischemia: Reperfusion injury with A2A-adenosine receptor activation and PDE 4 inhibition. *Kidney Int*., 59: 2114-2125.
29. Opal S. M. and Huber C. E. (2000): The role of interleukin-10 in critical illness. *Curr Opin Infect Dis*., 13: 221-226.
30. Ozturk H., Ozturk H., Terzi E. H., Ozgen U., Duran A. and Uygun I. (2014): Protective effects of rosmarinic acid against renal ischaemia/reperfusion injury in rats. *J Pak Med Assoc*., 64: 260-265.
31. Punuru P., Sujatha D., Kumari B. P. and Charisma V. V. (2014): Evaluation of aqueous extract of Murraya koenigii in unilateral renal ischemia reperfusion injury in rats. *Indian J Pharmacol*., 46: 171-175.
32. Rhoden E. L., Lucas M. L., Pereira-Lima L., Rhoden C. R. and Souto C. A. (2001): Effects of L-arginine on the kidney levels of malondialdehyde in rats submitted to renal ischaemia-reperfusion. *BJU Int*., 88: 273-277.
33. Riksen N. P., Smits P. Rongen G. A. (2006): The nonspecific anti-inflammatory therapy with methotrexate for patients with chronic heart failure. *Am Heart J*., 151: e5-e7.
34. Rudwaleit M., Yin Z., Siegert S., Grolms M., Radbruch A., Braun J. and Sieper J. (2000): Response to methotrexate in early rheumatoid arthritis is associated with a decrease of T cell derived tumour necrosis factor alpha, increase of interleukin 10, and predicted by the initial concentration of interleukin 4. *Ann Rheum Dis*., 59: 311-314.
35. Sancaktutar A. A., Bodakci M. N., Hatipoglu N. K., Soylemez H., Basarili K. and Turkcu G. (2014): The protective effects of pomegranate extracts against renal ischemia-

reperfusion injury in male rats. *Urol Ann*., 6: 46-50.
36. Scaduto R. C., Jr., Gattone V. H., Grotyohann L. W., Wertz J. and Martin L. F. (1988): Effect of an altered glutathione content on renal ischemic injury. *Am J Physiol*., 255: F911-F921.
37. Sweetman S. C. (2009): Antineoplastics.In Martindale: The Complete Drug Reference. ed. Sean C Sweetman & Paul S Blake. pp. 635-790. Pharmaceutical Press.
38. Takasaki J., Kawauchi Y., Urasaki T., Tanaka H., Usuda S. and Masuho Y. (1998): Antibodies against type II phospholipase A2 prevent renal injury due to ischemia and reperfusion in rats. *FEBS Lett*., 440: 377-381.
39. Tomasoni S., Azzollini N., Casiraghi F., Capogrossi M. C., Remuzzi G. and Benigni A. (2000): CTLA4Ig gene transfer prolongs survival and induces donor-specific tolerance in a rat renal allograft. *J Am Soc Nephrol*., 11: 747-752.
40. Yagmurca M., Erdogan H., Iraz M., Songur A., Ucar M. and Fadillioglu E. (2004): Caffeic acid phenethyl ester as a protective agent against doxorubicin nephrotoxicity in rats. *Clin Chim Acta*., 348: 27-34.
41. Yan-Lian C., Le Vraux V., Giroud J. P. and Chauvelot-Moachon L. (1994): Anti-tumor necrosis factor properties of non-peptide drugs in acute-phase responses. *European Journal of Pharmacology*., 271: 319-327.

التأثير الوقائى لجرعة صغيرة من عقار ميثوتريكسات على حبس الدموية الكلوية ثم اعادة التروية

محمد عبدالعال محمد، حسن محمود الفيومى، شيماء مصطفى الشاذلي، محمود حسن البطريق
قسم الفارماكولوجى والسموم- كلية الصيدلة-جامعة الزقازيق

تتألف الكلية من العديد من الخلايا التى تعمل معا لتنفيذ عدد من العمليات المعقدة .إصابة الكلى الحاد هو حدث سريرى شائع ويعطل هذا التوازن، مما يؤدى إلى ارتفاع معدلات المرض والوفيات بشكل غير مقبول .ولاتزال عملية حبس الدموية الكلوية ثم اعادة التروية هى السبب الرئيسى لحدوث الفشل الكلوى الحاد .وقد لوحظ أن عملية حبس الدموية الكلوية ثم اعادة التروية تحدث فى مجموعة متنوعة من الحالات السريرية مثل زرع الكلى.

فى هذا النوع من الفشل الكلوى، تحدث اصابات للخلايا فى وقت مبكر نتيجة نقص الأكسجين فى مرحلة حبس الدموية و تليها حدوث الالتهابات فى مرحلة اعادة التروية. ميثوتريكسات هو عقار تم تصميمه لعلاج السرطان ولكن فى الجرعات المنخفضة له تأثير مضاد للالتهاب وقد أظهرت دراسات سابقة أن هذا التأثير يرجع الى تحفيز الأدينوسين. وقد تم تصميم هذه الدراسة للتحقيق في الآثار المترتبة على جرعة منخفضة من الميثوتريكسيت فى الضرر الناتج عن اصابة الكلى فى الجرذان.

وقد قسمت الجرذان عشوائيا الى خمس مجموعات بكل منها ثمانية جرذان. المجموعة الأولى لم يتم عمل تدخل جراحى فيها، المجموعة الثانية تم تعريضها لجميع العمليات الجراحية عدا استئصال الكلية ومنع الدموية الكلوية، المجموعة الثالثة تم استئصال احدى الكلى واحداث حبس الدموية عن الكلية الأخرى لمدة خمسة وأربعين دقيقة ثم اعادة التروية لمدة ست ساعات، المجموعة الرابعة تم حقنها بعقار ميثوتريكسات ٠,٥ مجم/ كجم وتعرضت لنفس الجراحة بالمجموعة الثالثة، المجموعة الخامسة تم حقنها بعقار ليفاميسول ١٢,٥ مجم/ كجم وتعرضت لنفس ظروف المجموعة الرابعة. وقد تم قياس مستوى كلا من الكرياتينين و اليوريا فى مصل الدم ومحتوى مالوندای ألدهيد، الجلوتاثيون المختزل، عامل النخر الورمى الفا، البروتين الجاذب الكيميائى-١و انترليوكين ١٠ بالاضافة الى نشاط كلا من انزيم سوبر أكسيد ديسميوتيز و ميلوبيروكسيداز فى انسجة الكلى.

وقد تم التوصل الى النتائج التالية: احدث حبس الدموية ومايتبعه من اعادة التروية فى الكلى زيادة جوهرية فى كلا من مستوى الكرياتينين واليوريا فى مصل الدم .كما أدى أيضا الى زيادة جوهرية فى محتويات مالوندای ألدهيد، عامل النخر الورمى الفا و البروتين الجاذب الكيميائى1 - ونشاط انزيم ميلوبيروكسيداز فى الكلى .بينما ترتب على هذه العملية انخفاض جوهرى فى محتويات الجلوتاثيون المختزل والانترليوكين 10ونشاط انزيم سوبر أكسيد ديسميوتيز فى الكلى. ولكن أدت الوقاية باستخدام عقار ميثوتريكسات الى انخفاض جوهرى فى مستوى كلا من الكرياتينين واليوريا فى مصل الدم .كما أدى أيضا الى انخفاض جوهرى فى محتويات مالوندای ألدهيد، عامل النخر الورمى الفا و البروتين الجاذب الكيميائى 1 -ونشاط انزيم ميلوبيروكسيداز فى الكلى .بالاضافة الى زيادة جوهرية فى محتويات الجلوتاثيون المختزل والانترليوكين -١٠ ونشاط انزيم سوبر أكسيد ديسميوتيز فى الكلى. كما أدى عقار ليفاميسول الى تقليل الآثار المفيدة من عقار ميثوتريكسات.

ومن ثم فان هذه الدراسة توصى بأن ميثوتريكسات يمكن استخدامها للوقاية من التلف الناجم عن حبس الدموية ومايتبعه من اعادة التروية فى الكلى ولكن هناك حاجة لدراسات جديدة في كل من النماذج الحيوانية الأخرى وعلى الخلايا البشرية قبل التطبيقات السريرية.