Renal Failure

ISSN: 0886-022X (Print) 1525-6049 (Online) Journal homepage: https://www.tandfonline.com/loi/irnf20

Effects of melatonin on the serum levels of pro-inflammatory cytokines and tissue injury after renal ischemia reperfusion in rats

Elif Oguz, Zehra Yilmaz, Hatice Ozbilge, Fusun Baba, Suzan Tabur, Mukerrem Betul Yerer & Askin Hekimoglu

To cite this article: Elif Oguz, Zehra Yilmaz, Hatice Ozbilge, Fusun Baba, Suzan Tabur, Mukerrem Betul Yerer & Askin Hekimoglu (2015) Effects of melatonin on the serum levels of pro-inflammatory cytokines and tissue injury after renal ischemia reperfusion in rats, Renal Failure, 37:2, 318-322, DOI: 10.3109/0886022X.2014.991263

To link to this article: https://doi.org/10.3109/0886022X.2014.991263

Published online: 18 Dec 2014.

Submit your article to this journal

Article views: 581

View related articles

View Crossmark data

Citing articles: 7 View citing articles

Full Terms & Conditions of access and use can be found at
https://www.tandfonline.com/action/journalInformation?journalCode=irnf20

RENAL FAILURE

http://informahealthcare.com/rnf
ISSN: 0886-022X (print), 1525-6049 (electronic)

Ren Fail, 2015; 37(2): 318–322
© 2014 Informa Healthcare USA, Inc. DOI: 10.3109/0886022X.2014.991263

informa
healthcare

LABORATORY STUDIES

Effects of melatonin on the serum levels of pro-inflammatory cytokines and tissue injury after renal ischemia reperfusion in rats

Elif Oguz[1], Zehra Yilmaz[1], Hatice Ozbilge[2], Fusun Baba[3], Suzan Tabur[4], Mukerrem Betul Yerer[5], and Askin Hekimoglu[6]

[1]Department of Pharmacology, Faculty of Medicine, Harran University, Sanliurfa, Turkey, [2]Department of Microbiology, Faculty of Pharmacy, Erciyes University, Kayseri, Turkey, [3]Department of Pathology, Faculty of Medicine, Selcuk University, Konya, Turkey, [4]Department of Endocrinology, Faculty of Medicine, Gaziantep University, Gaziantep, Turkey, [5]Department of Pharmacology, Faculty of Pharmacy, Erciyes University, Kayseri, Turkey, and [6]Department of Pharmacology, Faculty of Medicine, Dicle University, Diyarbakir, Turkey

Abstract

We investigated the changes in the serum levels of tumor necrosis factor (TNF)-α and interleukin (IL)-6, the pro-inflammatory cytokines, and the possible effect of melatonin on the modulation of these inflammatory molecules after renal ischemia reperfusion (IR). The study was carried out in the laboratory of Department of Pharmacology. Forty-six male Wistar albino rats were divided into five groups as control ($n = 6$), positive control ($n = 4$), sham ($n = 12$), renal IR ($n = 12$), and renal IR melatonin ($n = 12$). After 1 h renal pedicle occlusion, the blood samples were taken for the measurement of cytokine levels at second hour of the reperfusion. The rats were sacrificed after 24 h of reperfusion for histopathological evaluation. Melatonin or vehicle was administrated to IR rats. Lipopolysaccharide (LPS) was administered to the positive control group and the blood was taken at fourth hour. Serum TNF-α levels increased significantly in renal IR and LPS groups. Serum IL-6 levels were not different from control except the LPS group. There was no significant correlation between the serum TNF-α levels and the histopathological score after renal IR. Melatonin treatment reversed the increase of serum TNF-α levels and histopathological injury in renal tissue after renal IR. Melatonin may have a protective effect by reducing the serum level of TNF-α in renal IR.

Keywords

Cytokines, ischemia, kidney, melatonin, reperfusion

History

Received 9 July 2014
Revised 9 October 2014
Accepted 18 November 2014
Published online 18 December 2014

Introduction

Renal ischemia, the most common cause of acute kidney injury, is appeared in many clinical situations such as renal transplantation, partial nephrectomy, renal artery angioplasty, aortic aneurysm surgery, cardio-pulmonary bypass, and elective urological operations[1] and is the main cause of high mortality rates in the intensive care units.[2]

Ischemic renal injury leads to a rise in antibody production, which may be harmful to renal allografts, possibly explaining a mechanism underlying the link between delayed graft function and long-term allograft failure.[3]

Pro-inflammatory cytokines, such as tumor necrosis factor (TNF)-α and interleukin (IL)-1β, are increased after ischemia/reperfusion (IR) in some model systems.[4,5] Renal tubular epithelial cells, simultaneously targets, and mediators of IR injury express several pro-inflammatory and chemotactic cytokines such as monocyte chemoattractant protein 1 (MCP-1), IL-1β, IL-6, IL-8, TNF-α, and transforming growth factor beta (TGF-β) are expressed in acute kidney injury (AKI), a severe clinical problem induced by IR injury.[6] TNF-α has a major role in IR injury, occurred after liver, kidney, intestine, heart, lung, and pancreas transplantation.[7,8] The plasma concentrations of TNF-α-and IL-1 are increased after renal ischemia.[9] Unilateral renal IR induces bilateral TNF-α production and neutrophil infiltration through a TNF-α-dependent mechanism.[10] Zager et al.[11] have also shown that IR failed to increase TNF-α levels in serum, ipsilateral, and contralateral kidney after reperfusion. More studies are required to identify the role of pro-inflammatory cytokines on renal IR injury.

Melatonin, a pineal secretory product, reduces the IR-induced brain,[12] heart,[13] kidney,[14,15] and liver[16] damages in rats. These effects of melatonin are related with scavenging of a variety of toxic oxygen and nitrogen-based reactants and stimulation of antioxidative enzymes.[17,18] It has been shown that postoperative elevated values of TNF-α, IL-6, and IL-8 reduced by melatonin in newborns.[19] Lipopolysaccharide (LPS)-induced TNF-alpha production reduced in human monocytes by melatonin.[20] Rodriguez-Reynosa et al.[16] showed that melatonin reduces TNF-alpha production in plasma in IR-exposed liver of rats. These studies suggest that melatonin may have protective effect through the modulation of cytokine levels.

Address correspondence to Elif Oguz, Ph.D., Department of Pharmacology, Faculty of Medicine, Harran University, Sanliurfa, Turkey. Tel: + 90 535 4200852. E-mail: elifoguz73@harran.edu.tr

In this study, we aimed to investigate the possible changes in the serum levels of TNF-α and IL-6 in and to reveal the relationship between histopathological injury and the serum levels of these pro-inflammatory cytokines. We also determined whether the protective effect of melatonin is related to the modulation of these inflammatory molecules in serum after renal IR.

Materials and methods

Animals

Male Wistar albino rats (180–300 g) were placed in a temperature (21 ± 2 °C) and humidity (60 ± 5%) controlled room in with a 12 h light:12 h dark cycle. All experiments in this study were performed in accordance with the ''Principles of Laboratory Animal Care'' (NIH publication No. 86-23, revised 1984) and were approved by the Committee on Animal Research at Harran University, Sanliurfa (Approval number: 101.5/60).

Surgery and experimental protocol

Forty-six male Wistar albino rats were divided into five groups as control ($n = 6$), positive control ($n = 4$), sham ($n = 12$), renal IR ($n = 12$), and renal IR + melatonin ($n = 12$).

All surgical procedures were performed under anesthesia (75 mg/kg ketamine hydrochloride and 8 mg/kg xylazine, intraperitoneally). In the renal IR group, after 30 min of the right nephrectomy, the left renal pedicle (artery and vein) was occluded for 1 h to induce ischemia and then subjected to reperfusion for 2 and 24 h ($n = 6$ for each group). The sham group received only nephrectomy without occlusion, while no surgery was performed on the control group.[21] Melatonin (Sigma Chemical Co., St. Louis, MO) was dissolved in ethanol and then diluted in saline (0.09% NaCl wt/vol) to give a final alcohol concentration of 1% ethanol. Melatonin injected to the rats 10 mg/kg intraperitoneally prior to ischemia.

LPS (*Escherichia coli* LPS 0111:B4; Sigma, St. Louis, MO) was administrated to the positive control group with 10 mg/kg intraperitoneally ($n = 4$).

Measurement of cytokine levels

The serum levels were collected at second hour from the renal IR group[10,22] and at fourth hour from the LPS-administrated group.[23] TNF-α and IL-6 levels of serum samples were quantified according to the instructions of the manufacturer and guidelines using enzyme-linked immunosorbent assay (ELISA) kit specific for the previously mentioned rat cytokines (Biosource International, Nivelles, Belgium).

Histological analysis

The rats were sacrificed at 24 h of reperfusion for histopathological evaluation. For light microscopic evaluation, kidneys fixed in 10% neutral buffered formalin, processed routinely by automatic tissue processor and embedded in paraffin wax. Four micrometer sections were stained with hematoxylin–eosin (H–E) before investigation under light microscopy (Olympus BX 51 TF, Tokyo, Japan).

Histological findings in renal cortical tubuli were evaluated using semi-quantitative scores developed by Kang et al.[24] Tubular injury was defined as tubular dilatation, tubular atrophy, tubular cast formation, vacuolization, degeneration, and sloughing of tubular epithelial cells or loss of the brush border and thickening of the tubular basement membrane. The tubules were evaluated according to the following scoring system: 0 = no tubular injury; 1 ≤ 10% of tubules injured; 2 = 10–25% of tubules injured; 3 = 26–50% of tubules injured; 4 = 51–75% of tubules injured; 5 ≥ 75% of tubules injured.

Statistics

All data were expressed as the arithmetic mean ± SEM. $p < 0.05$ was considered to be statistically significant. Distribution of the samples in the groups was analyzed with one sample of the Kolmogorov–Smirnov test. For analysis of data, analysis of variance was used to assess the differences between multiple groups. If statistically significance was determined, the mean values obtained from each group were then compared by LSD multiple comparisons' test. The histological results were statistically analyzed by the Kruskal–Wallis H test. The differences between the groups were evaluated by the Mann–Whitney U test. Spearman correlation test was used for the correlation between serum TNF-α levels and histopathological score.

Results

Serum level of TNF-α showed a significant increase in renal ischemia and the LPS group (Figure 1). Melatonin decreased the TNF-α levels in the renal IR group but did not reversed to the sham group (Figure 1). Serum IL-6 level was not different in the renal ischemia group compared with control whereas was significantly higher in the LPS group (Table 1).

The evaluations of ischemic injury in renal cortical tubuli were evaluated according to Kang et al.'s[24] method (Table 1). Extensive necrosis and apoptosis were noted in proximal tubule epithelium in the renal IR group. Regressive cellular changes such as nuclear chromatin condensation, nuclear hyperchromasia, and cytoplasmic shrinkage were noted in non-necrotic tubule epithelium (Figure 2A). Melatonin treatment significantly reduced the tubular damage and extensive reactive cellular changes such as nucleomegaly and prominent nucleoli were observed (Figure 2B). Sham (Figure 2C) groups showed only focal tubular epithelial sloughing. There was no histopathologic finding stated in the control group. There was no significant correlation between the serum TNF-α levels and the histopathologic score after renal IR ($R = 0.175$; $p = 0.244$).

Discussion

To reveal the roles of pro-inflammatory cytokines in the renal ischemia, we will give insights regarding the pathophysiology of these processes and we will aid to the development of therapies to rescue the kidney from the adverse effects of IR.

Renal ischemia, the process resulted in after partial or complete blocking renal blood flow, causes endothelial disfunction, inflammation, and tubular injury.[7] Several pro-inflammatory and chemotactic cytokines regulated by NF-kB, including IL-1β, IL-6, IL-8, and TNF-α are expressed from

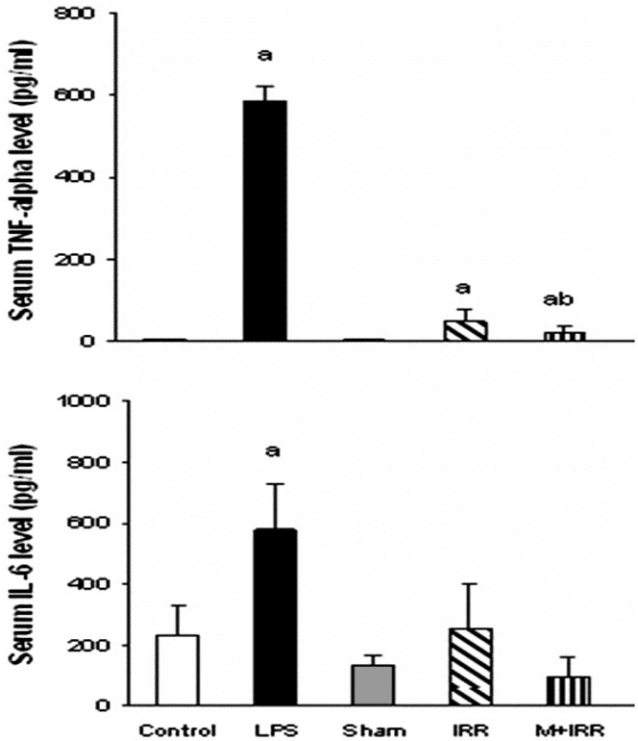

Figure 1. Serum tumor necrosis-alpha (TNF-α) levels and interleukin (IL)-6 levels of control, lipopolysaccharide (LPS), sham (only nephrectomy), renal ischemia reperfusion (IRR), melatonin + renal ischemia reperfusion (M+IRR). Notes: [a]$p < 0.05$, compared with the values in the control group. [b]$p < 0.05$, compared with the values in the IRR group.

Table 1. Semi-quantitative estimates of renal tubular injury at 24h of reperfusion*.

Groups	Renal tubular injury
Control	0.0 ± 0.0^{b}
Sham	$0.2 \pm 0.16^{a,b}$
IR	4.8 ± 0.18^{a}
M+IR	$2.8 \pm 0.16^{a,b}$

Notes: Data were expressed as mean ± SEM.
[a]$p < 0.05$ versus the control group.
[b]$p < 0.05$ versus the IR group.
*The tubules were evaluated according to the following scoring system: 0 = no tubular injury; 1 ≤ 10% of tubules injured; 2 = 10–25% of tubules injured; 3 = 26–50% of tubules injured; 4 = 51–75% of tubules injured; 5 ≥ 75% of tubules injured.

renal tubular epithelial cells, the targets, and mediators of IR.[25]

TNF-α is capable of up-regulating its own expression as well as the expression of other genes pivotal to the inflammatory response.[26] It has been suggested that IR-induced TNF-α expression may result in cell injury via distinct mechanisms such as direct cytotoxicity like induction of dysfunction and/or apoptosis[27] and neutrophil-mediated tissue injury.[11] Furthermore IR-induced renal TNF-α production was shown to be associated with impaired renal function.[28] Other pro-inflammatory cytokines, IL-1β, and IL-6 increase renal inflammation by recruiting more neutrophils into the injured kidney.[9,29,30] After short periods of renal ischemia, significant renal dysfunction and cellular degradation occur.[30] Meldrum et al.[10] determined that a significant TNF protein level occurred at 1 and 2 h of reperfusion in both the injured ipsilateral and the non-ischemic contralateral kidney. Donnahoo et al.[22] also reported that renal TNF protein expression and bioactivity peaked following 1 h of ischemia and 2 h of reperfusion.

It has been shown that serum[31] and renal[29] IL-6 levels were increased at 4 h of reperfusion. In the present study, serum TNF-α levels increased significantly in the renal IR group at 2 h of reperfusion. There was no difference in serum IL-6 levels except LPS group. Serum TNF-α levels of LPS, the positive control group, were higher 10 times than the renal IR group. The increase in the renal IR group was not markedly like the LPS group. There was no significant correlation between the serum TNF-α levels and histopathologic score after renal IR. Supporting these data, Donnahoo et al.[32] reported that TNF-binding protein attenuates reversible (30 min ischemia/48 h reperfusion), but not irreversible (60 min ischemia/48 h reperfusion) injury. However, Zager et al.[11] have shown that IR failed to increase TNF-α levels in serum, ipsilateral, and contralateral kidney after 30 min, 1 h, or 18 h of reperfusion while increased markedly blockage by LPS.

Acute kidney injury (AKI) may lead to systemic inflammatory events that contribute to remote organ injury and distant organ injuries, the main reason of morbidity and mortality.[33] The data of this study show that serum TNF-α levels increase after renal IR, indicating that renal IR may cause a systemic inflammation. TNF-α is involved in the production of nitric oxide and induction of vasodilation and hypotension, which may induce renal ischemia and hypoxia, and finally acute tubular necrosis and renal failure.[22] It is shown that cytokines may have a role in small intestine and liver injury after renal ischemia.[34] Bilateral kidney IR injury leads to an increase in both systemic and cardiac TNF-α.[35] A recent study identified that ischemic AKI induces lung endothelial apoptosis via TNFR1 and limited data suggest a role for systemic elevation of inflammatory cytokines and apoptosis; however, the mechanism was not expressed clearly.[36]

Melatonin is an effective regulator of the immune system. Melatonin may act as an anti-inflammatory agent, inhibiting immune responses in some cases[37] and has been shown to be protective in septic shock,[38] an animal model of ulcerative colitis,[39] and experimental pancreatitis.[40] Sezgin et al. reveal that 1,25-dihydroxyvitamin D3 with the combination of melatonin may help to prevent I/R injury via increasing renal SOD activity and GSH levels and decreasing NO*x* levels. It is showed that erythropoietin and melatonin combination treatment exerted more nephroprotective effects than erythropoietin treatment[41] and, in another study, it is reported that melatonin protects against inflammation and apoptosis better than erythropoietin in renal IR injury pretreatment.[42]

Melatonin is likely to be well absorbed in humans,[43] crosses all physiological barriers easily, for example, blood–brain barrier, membranes of cells, and organelles, additionally, has no reproducible adverse effects in humans or animals.[44] Exogenous melatonin has a short half-life (20–60 min) in human. The apparent elimination half-life of melatonin following an intravenous dose of 3 mgkg^{-1} (5 mgkg^{-1} in rats)

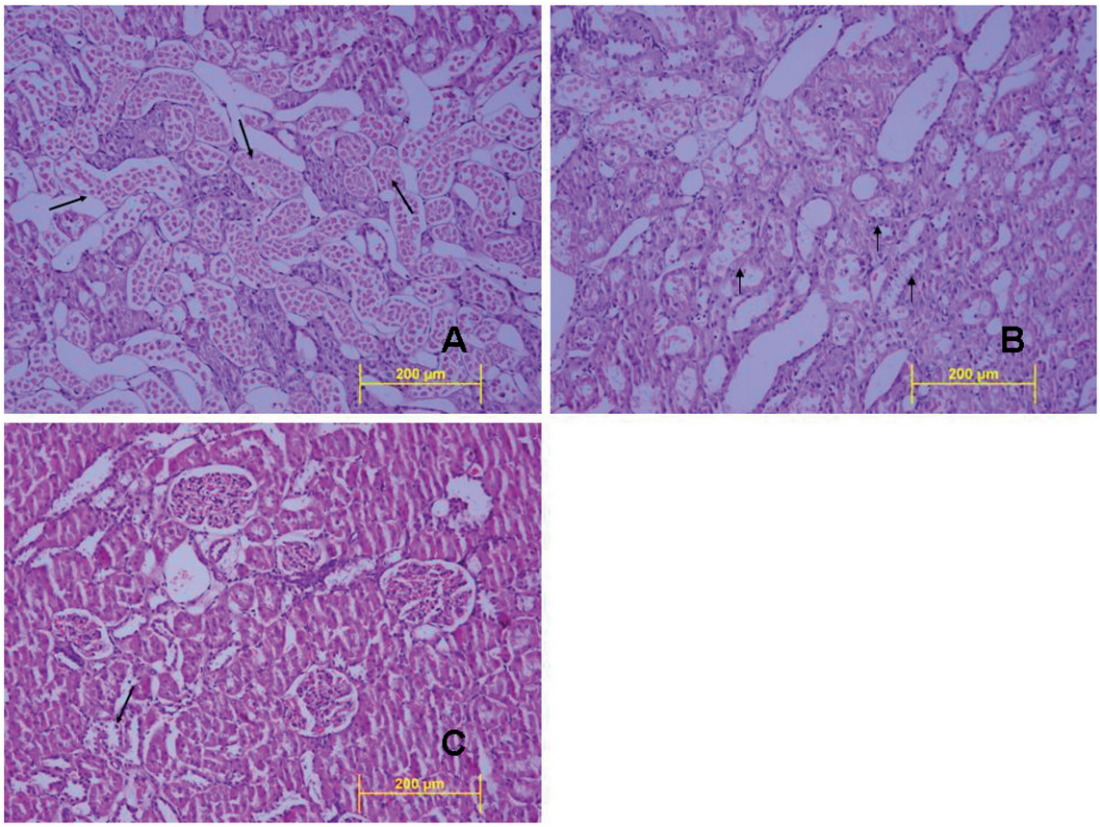

Figure 2. (A) Extensive necrosis of renal proximal tubule epithelium (arrows) in IR group (HE, 100×). (B) Regenerative changes in tubule epithelium (arrows) in the melatonin-treated IR group (HE, 100×). (C) A few sloughed tubule epithelium in lumen (arrows) in the sham group (HE, 100×).

is reported as 19.8 min in rats. Bioavailability of melatonin following a 10 mgkg $^{-1}$ intraperitoneal administration in rats is 74.0%, suggesting the lack of substantial first-pass hepatic extraction of melatonin in rats.[43] It was demonstrated that immediately administration melatonin before reperfusion had a protective effect on I/R injury.[45] In this study, we used melatonin at a dose of 10 mgkg^{-1} intraperitoneally prior to ischemia similar to other studies, used melatonin for protecting from renal IR injury.[42,46] However, Souza et al.[47] reported that a high dose (50 mgkg^{-1}) of melatonin attenuated renal injury without transient hyperglycemia, but was unable to prevent acute tubular necrosis in rats with hyperglycemia.

In the current study, we showed that melatonin at a dose of 10 mgkg^{-1} intraperitoneally prior to ischemia reversed the increase of serum TNF-α levels in the renal IR group. Similarly the studies reported that melatonin ameliorates hemorrhagic shock-induced organ damage[48] and protects liver after intestinal IR[49] via decreasing serum TNF-α levels.

In conclusion, this study reveals first that the serum level of TNF-α increases after renal ischemia without a relationship to renal histopathological score and melatonin treatment reduces the serum level of this pro-inflammatory cytokine. We think that some other studies must be performed to reveal the role of cytokines in renal IR and also the effect of melatonin on TNF-α level in renal IR.

Declaration of interest

Authors disclose no funding sources and no potential conflicts of interest.

References

1. Paller MS. Acute renal failure: Controversies, clinical trials, and future directions. *Semin Nephrol.* 1998;18:482–489.
2. Rodriguez F, Bonacasa B, Fenoy FJ, Salom MG. Reactive oxygen and nitrogen species in the renal ischemia/reperfusion injury. *Curr Pharm Des.* 2013;19: 2776–2794.
3. Fuquay R, Renner B, Kulik L, et al. Renal ischemia-reperfusion injury amplifies the humoral immune response. *J Am Soc Nephrol.* 2013;24(7):1063–1072.
4. Mitsui Y, Okamoto K, Martin DP, Schmelzer JD, Low PA. The expression of proinflammatory cytokine mRNA in the sciatic-tibial nerve of ischemia-reperfusion injury. *Brain Res.* 1999;844: 192–195.
5. Konturek PCh, Duda A, Brzozowski T, et al. Activation of genes for superoxide dismutase, interleukin-1b, tumor necrosis factor-a, and intercellular adhesion molecule-1 during healing of ischemia-reperfusion-induced gastric injury. *Scand J Gastroenterol.* 2000;35: 452–463.
6. Kusch A, Hoff U, Bubalo G, et al. Novel signalling mechanisms and targets in renal ischemia and reperfusion injury. *Acta Physiol [Oxf].* 2013;208:25–40.
7. Pascher A, Klupp J. Biologics in the treatment of transplant rejection and ischemia/reperfusion injury: New applications for TNF alpha inhibitors? *Bio Drugs.* 2005;19:211–231.
8. Di Paola R, Genovese T, Impellizzeri D, Ahmad A, Cuzzocrea S, Esposito E. The renal injury and inflammation caused by ischemia-reperfusion are reduced by genetic inhibition of TNF-αR1: A comparison with infliximab treatment. *Eur J Pharmacol.* 2013;700: 134–146.
9. Kelly KJ. Distant effects of experimental renal ischemia/reperfusion injury. *J Am Soc Nephrol.* 2003;14:1549–1558.
10. Meldrum KK, Meldrum DR, Meng X, Ao L, Harken AH. TNF-α-dependent bilateral renal injury is induced by unilateral renal ischemia-reperfusion. *Am J Physiol Heart Circ Physiol.* 2002;282: H540–H546.

11. Zager RA, Johnson ACM, Hanson SY, Lund S. Ischemic proximal tubular injury primes mice to endotoxin-induced TNF-α generation and systemic release. *Am J Physiol Renal Physiol.* 2005;289: F289–F297.
12. Kilic E, Ozdemir YG, Bolay H, Kelestimur H, Dalkara T. Pinealectomy aggravates and melatonin administration attenuates brain damage in focal ischemia. *J Cereb Blood Flow Metab.* 1999; 19:511–516.
13. Sahna E, Acet A, Ozer MK, Olmez E. Myocardial ischemia-reperfusion in rats: Reduction of infarct size by either supplemental physiological or pharmacological doses of melatonin. *J Pineal Res.* 2002;33:234–238.
14. Aktoz T, Aydogdu N, Alagol B, Yalcin O, Huseyinova G, Atakan IH. The protective effects of melatonin and vitamine E against renal ischemia-reperfusion injury in rats. *Ren Fail.* 2007;29:535–542.
15. Li Z, Nickkholgh A, Yi X, et al. Melatonin protects kidnet grafts from ischemia/reperfusion injury through inhibition of NFkB and apoptosis after experimental kidney transplantation. *Pineal Res.* 2009;46:365–372.
16. Rodriguez-Reynoso S, Leal C, Portilla-de-Buen E, Olivares N. Effects of exogenous melatonin on hepatic energetic status during ischemia-reperfusion: Possible role of TNF-α and nitric oxide. *J Surg Res.* 2001;110:141–149.
17. Rodriguez C, May JC, Sainz RM, et al. Regulation of antioxidant enzymes: A significant role for melatonin. *J Pineal Res.* 2004;36: 1–9.
18. Tomas-Zapico C, Coto-Montes A. A proposed mechanism to explain the stimulating effect of melatonin on antioxidative enzymes. *J Pineal Res.* 2005;39:99–104.
19. Gitto E, Romeo C, Reiter RJ, et al. Melatonin reduces oxidative stress in surgical neonates. *J Pediatr Surg.* 2004;39:184–189.
20. Perianayagam MC, Oxenkrug GF, Jaber BL. Immune-modulating effects of melatonin, N-acetyl serotonin, and N-acetyl dopamine. *Ann N Y Acad Sci.* 2005;1053:386–393.
21. Karaman A, Turkmen E, Gursul C, Tas E, Fadillioglu E. Prevention of renal ischemia/reperfusion-induced injury in rats by leflunomide. *Int J Urol.* 2006;13:1434–1441.
22. Eren Z, Coban J, Ekinci ID, Kaspar C, Kantarci G. Evaluation of the effects of a high dose of erythropoietin-beta on early endotoxemia using a rat model. *Adv Clin Exp Med.* 2012;21: 321–329.
23. Donnahoo KK1, Meldrum DR, Shenka R, Chung CS, Abraham E, Harken AH. Early renal ischemia, with or without reperfusion, activates NFkappaB and increases TNF-alpha bioactivity in the kidney. *J Urol.* 2000;163:1328–1332.
24. Kang DH, Kim YG, Andoh TF, et al. Post-cyclosporine-mediated hypertension and nephropathy: Amelioration by vascular endothelial growth factor. *Am J Physiol Renal Physiol.* 2001;280: F727–F736.
25. Bonventre JV, Yang L. Cellular pathophysiology of ischemic acute kidney injury. *J Clin Invest.* 2011;121:4210–4221.
26. Dinarello CA, Cannon JG, Wolff SM, et al. Tumor necrosis factor (cachectin) is an endogenous pyrogen and induces production of IL-1. *J Exp Med.* 1986;163:1433–1450.
27. Lieberthal W, Koh JS, Levine JS. Necrosis and apoptosis in acute renal failure. *Semin Nephrol.* 1998;18:505–518.
28. Donnahoo KK, Meng X, Ayala A, Cain MP, Harken AH, Medrum DR. Early kidney TNF-α expression mediates neutrophil infiltration and injury after renal ischemia-reperfusion. *Am J Physiol Regul Integr Comp Physiol.* 1999;277:R922–R929.
29. Kielar ML, John R, Bennett M, et al. Maladaptive role of IL-6 in ischemic acute renal failure. *J Am Soc Nephrol.* 2005;16: 3315–3325.
30. Haq M, Norman J, Saba SR, Ramirez G, Rabb H. Role of IL-1 in renal ischemic reperfusion injury. *J Am Soc Nephrol.* 1998;9: 614–619.
31. Jo SK, Sung SA, Cho WY, Go KJ, Kim HK. Macrophages contribute to the initiation of ischemic acute renal failure in rats. *Nephrol Dial Transplant* 2006;21:1231–1239.
32. Donnahoo KK, Meldrum DR, Shenkar R, Chung CS, Abraham E, Harken AH. Early renal ischemia, with or without reperfusion, activates NfkappaB and increases TNF-alpha bioactivity in the kidney. *J Urol.* 2000;163:1328–1332.
33. McCullough JW, Renner B, Thurman JM. The role of the complement system in acute kidney injury. *Semin Nephrol.* 2013; 33:543–556.
34. Park SW, Chen S, Kim M, et al. Cytokines induce small intestine and liver injury after renal ischemia or nephrectomy. *Lab Invest.* 2011;91:63–84.
35. Kelly KJ. Distant effects of experimental renal ischemia/reperfusion injury. *J Am Soc Nephrol.* 2003;14:1549–1558.
36. Hassoun HT, Lie ML, Grigoryev DN, Liu M, Tuder RM, Rabb H. Kidney ischemia-reperfusion injury induces caspase-dependent pulmonary apoptosis. *Am J Physiol Renal Physiol.* 2009;297: F125–F137.
37. Szczepanik M. Melatonin and its influence on immune system. *J Physiol Pharmacol.* 2007;58:115–124.
38. Escames G, Acuña-Castroviejo D, López LC, et al. Pharmacological utility of melatonin in the treatment of septic shock: Experimental and clinical evidence. *J Pharm Pharmacol.* 2006;58:1153–1165.
39. Nosálová V, Zeman M, Cerná S, Navarová J, Zakálová M. Protective effect of melatonin in acetic acid induced colitis in rats. *J Pineal Res.* 2007;42:364–370.
40. Leja-Szpak A, Jaworek J, Tomaszewska R, et al. Melatonin precursor; L-tryptophan protects the pancreas from development of acute pancreatitis through the central site of action. *J Physiol Pharmacol.* 2004;55:239–254.
41. Ahmadiasl N, Banaei S, Alihemati A, Baradaran B, Azimian E. Effect of a combined treatment with erythropoietin and melatonin on renal ischemia reperfusion injury in male rats. *Clin Exp Nephrol*. 2014. [Epub ahead of print]. DOI: 10.1007/s10157-014-0937-6.
42. Ahmadiasl N, Banaei S, Alihemmati A, Baradaran B, Azimian E. The anti-inflammatory effect of erythropoietin and melatonin on renal ischemia reperfusion injury in male rats. *Adv Pharm Bull.* 2014;4(1):49–54.
43. Yeleswaram K, McLaughlin LG, Knipe JO, Schabdach D. Pharmacokinetics and oral bioavailability of exogenous melatonin in preclinical animal models and clinical implications. *J Pineal Res.* 1997;22:45–51.
44. García JJ1, López-Pingarrón L, Almeida-Souza P, et al. Protective effects of melatonin in reducing oxidative stress and in preserving the fluidity of biological membranes: A review. *J Pineal Res.* 2014; 56:225–237.
45. Sahna E, Parlakpinar H, Ozturk F, Cigremis Y, Acet A. The protective effects of physiological and pharmacological concentrations of melatonin on renal ischemia-reperfusion injury in rats. *Urol Res.* 2003;31:188–193.
46. Sezgin G, Oztürk G, Güney S, Sinanoğlu O, Tunçdemir M. Protective effect of melatonin and 1,25-dihydroxyvitamin D3 on renal ischemia-reperfusion injury in rats. *Ren Fail.* 2013;35: 374–379.
47. de Souza AV1, Golim MA2, Deffune E2, et al. Evaluation of renal protection from high doses of melatonin in an experimental model of renal ischemia and reperfusion in hyperglycemic rats. *Transplant Proc.* 2014;46(5):1591–1593.
48. Yang FL, Subeq YM, Lee CJ, Lee RP, Peng TC, Hsu BG. Melatonin ameliorates hemorrhagic shock-induced organ damage in rats. *J Surg Res.* 2011;167:e315–e321.
49. Li JY1, Yin HZ, Gu X, Zhou Y, Zhang WH, Qin YM. Melatonin protects liver from intestine ischemia reperfusion injury in rats. *World J Gastroenterol.* 2008;14:7392–7396.