Original
ISSN 2413-0516

Erythropoietin Protects Against Renal Ischemia/Reperfusion Injury in Rats Via Inhibition of Oxidative Stress, Inflammation and Apoptosis

Elaf R. Alaasam[1], Ali M. Janabi[2*]

[1]Alsadar Medical City, Directorate of Health, Ministry of Health, Najaf, Iraq.
[2]Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Kufa, Najaf, Iraq.
[*]Correspondence to: Ali M. Janabi, (E-mail: alim.hashim@uokufa.edu.iq)
(Submitted: 12 May 2023 – Revised version received: 09 June 2023 – Accepted: 01 July 2023 – Published Online: 26 August 2023)

Abstract

Objectives: Study investigates erythropoietin's nephroprotective effect in adult male rats with renal ischemia and reperfusion injury, examining biochemical parameters and renal tissue alterations.

Methods: Twenty-eight male rats of Sprague Dawley randomized into four groups: Sham, Iscemia/Reperfusion, NS, and Erythropoietin. Sham group treated kidneys identically without clamping pedicles. Ischemia/Reperfusion group underwent midline laparotomy, 30 minutes ischemia, 2 hours reperfusion, Rats received NS vehicle for Erythropoietin 30 minutes before ischemia, Erythropoietin dose administered 30 minutes before ischemia/reperfusion. Operation performed under maintained anesthesia using ketamine and xylazine injections.

Results: Renal ischemia /reperfusion increased serum Cr, BUN, IL-1β, NF-kB, Caspase-3, while SOD, GSH, Bcl-2 decreased in induced rats. Erythropoietin treatment reduced Cr and BUN levels, Reduced inflammation, and inflammatory markers. Renal tissue antioxidant markers increased; apoptotic markers decreased. Significant increase in Bcl-2 antiapoptotic marker. Erythropoietin-treated group had significantly better renal histological score compared to induced group.

Conclusion: Erythropoietin protects against Ischemia/Reperfusion-mediated renal injury, possessing antioxidant, anti-inflammatory, and anti-apoptotic properties.

Keywords: Ischemia, reperfusion, renal, erythropoietin, antioxidants

Introduction

Ischemia/reperfusion injury (IRI) is defined as a decrease in blood flow to an organ followed by reoxygenation and restoration of blood flow. Injuries are predictable after an sepsis, infarction, or organ transplantation, and this phenomenon exacerbates tissue damage by triggering an inflammatory cascade of reactive oxygen species (ROS), chemokines, cytokines, and leukocyte activation.[1,2] Clinical manifestations of I/R include myocardial hibernation/stun, reperfusion arrhythmias, systemic inflammatory response syndrome, impaired cerebral function, gastrointestinal barrier breakdown, and, most tragically, multiorgan dysfunction syndrome.[3] Several physiological mechanisms, including atherosclerosis and acute myocardial infarction, promote ischemia and lead to hypoxia and hypoperfusion.[4] One of the leading causes of renal dysfunction and acute kidney injury is renal ischemia/reperfusion injury (RIRI), which is also common in renal transplantation and is linked to increased morbidity, mortality, and length of hospital stay. Unfortunately, there are no preventative or therapeutic drugs currently available for RIRI.[5,6] Direct injury to the kidneys, including the tubules, glomeruli, renal blood vessels, or interstitium, is what defines RIRI.[7] Acute kidney injury (AKI) is a syndrome characterized by rapid kidney dysfunction and high mortality, both of which are exacerbated by IRI in the kidney.[8] AKI, also known as acute kidney failure, occurs in the renal system as a result of a decrease in blood flow (renal ischemia), which results in a decreased oxygen supply to the organ (renal hypoxia). It causes a decrease in renal output and glomerular filtration rate, increasing morbidity and mortality. The kidney is one of the organs sensitive to IRI because of its unique tissue function and structure, as well as its high oxygen demand.[9,10] Some pathological pathways, such as neutrophil activation, the release of reactive oxygen species, and inflammatory mediators such as adhesion molecules and a variety of cytokines, are involved in the development of renal I/R.[11] A depletion of ATP, a lack of glycogen and oxygen, DNA damage, an activated immune system, leaking blood vessels, activated endothelial cells, and adhering leukocytes are the hallmarks of RIRI.[12] Consequently, Vascular leakage and interstitial edema are exacerbated by the damage done to renal tubular epithelial cells and endothelial cells, as well as the activation of tissue-resident leukocytes.[13]

Red blood cell (RBC) production requires the glycoprotein hormone erythropoietin (EPO). In adults, peritubular cells in the kidney are responsible for synthesizing EPO and secreting it into the bloodstream. Red blood cell (RBC) production is stimulated by EPO when it binds to the EPO receptor expressed on erythroid progenitor cells in the bone marrow.[14] When bound to red series progenitor cell receptors, EPO promotes their proliferation and differentiation. Because of this, it causes a dose-dependent rise in hematocrit by stimulating the release of reticulocytes from the bone marrow into the peripheral blood, where they can develop into erythrocytes.[15] With the aid of revolutionary recombinant DNA technology, an erythropoiesis stimulating agent such as rhEOP has been created. Epoetin alfa was the first rhEPO to be approved by regulatory authorities.[16] EPOR is not restricted to erythroid cells in terms of tissue expression. Non-hematopoietic tissues, such as the brain, heart, and skeletal muscle, all show EPO response in animal models, indicating EPOR-mediated EPO activity.[17]

We measured biochemical parameters, renal function parameters (urea, creatinine), anti-inflammatory markers (IL-1, NF-kB), antioxidant markers (SOD, GSH), anti-apoptotic marker (Bcl-2), and apoptotic marker(caspase-3), and histopathological alterations in renal tissue to evaluate the potential nephroprotective effect of erythropoietin in adult male rats with renal ischemia and reperfusion injury.

Methods

Animals preparation

Overall, the University of Kufa/Faculty of Science provided 28 male Sprague Dawley rats weighing 150-250g and aged 15-20 weeks. The rats were housed in the animal facility at the University of Kufa/Faculty of Pharmacy. The animals were kept in a separate chamber, in a group-caging system, with temperature and humidity regulated to 24±2 C° and 60-65 percent, respectively, and a 12/12-hour light/dark cycle. The rats were fed a standard diet consisting of food and water. After submitting the necessary applications, the Institutional Animal Care and Use Committee (IACUC) at the University of Kufa approved all experimental protocols.

Study Design

A total of twenty- eight adult male of Sprague Dawley rats were randomized into four groups ($n = 7$ in each group): Sham group, I/R group, NS group and Erythropoietin group. In the Sham group, the kidneys were treated identically with I/R group, without clamping the renal pedicles, in the ischemic/reperfusion injury (I/R) group, the midline laparotomy was performed and both renal pedicles were subjected to 30 minutes of ischemia followed by 2 hr reperfusion, in NS group rats received NS vehicle for Erythropoietin by intraperitoneal injection at 30 minutes before ischemia.,[18] in the erythropoietin group, erythropoietin was administered at a dose of 1000 IU/kg intraperitonially 30 min prior to suffering I/R.[19,20] In summary, the operation was performed under fully maintained anesthesia with intraperitoneal injections of 100 mg/kg ketamine and 10 mg/kg xylazine.

Preparation of the drug

Erythropoietin was given in 0.9% N/S (200 µl in 800 µl a stock solution), as standard vehicle prepared immediately before use. The dose was administered intraperitoneally according to body weight.[18, 19]

Collection of Samples

Blood sample collection

At the conclusion of the procedure, while the rats were still sedated, approximately 2–4 milliliters of blood were extracted directly from the heart. The blood sample was placed in a gel tube without anticoagulant, and it will be centrifuged at 6000 rpm for 10 minutes to obtain serum that will be used to determine urea, creatinine, NF-kB, and IL-1β using commercially available ELISA Kits.

Tissue sample preparation

Following the collection of blood samples, samples of left kidney tissue were obtained immediately. One portion of the tissue was fixed in 10% formaldehyde for histopathological analysis, while the other was frozen at -80°C. After that, it will be homogenized in a 1:10 W/V phosphate-buffered saline solution containing 1% Triton X- 100 and a protease inhibitor cocktail using a high-intensity ultrasonic liquid processor.[21] The homogenate was centrifuged at 5000 rpm at 4 for 10 minutes, and the supernatants were analyzed with commercially available ELISA Kits for GSH, SOD, Caspase-3, and Bcl-2.

Tissue Sampling for Histopathology

Section of kidney tissue that was fixed in 10% formaldehyde, dehydrated in an alcohol series, cleared in xylene, and then embedded in paraffin. Kidney tissues were embedded in paraffin and then cut into 5-m thick sections. The tissue was then stained with hematoxylin and eosin.[22]

Measurement of study parameters

Measurement of urea and creatinine

Using urea Elisa kit obtained from SunLong Biotech Co., Ltd, China and creatinine Elisa kit obtained from Bioassay Technology Laboratory, China, the levels of urea and creatinine were measured in accordance with the manufacturer's instructions.

Measurement of NF-kB, and IL-1β

NF-kB and IL-1β levels were measured using NF-kB and IL-1β Elisa kits purchased from Bioassay Technology Laboratory, China, in accordance with the manufacturer's instructions.

Measurement of GSH and SOD

GSH and SOD levels were measured using GSH and SOD Elisa kits purchased from Bioassay Technology Laboratory, China, in accordance with the manufacturer's instructions.

Measurement-of Caspase -3, and Bcl-2

Caspase -3 and Bcl-2 levels were determined using Caspase -3 and Bcl-2 Elisa kits purchased from Bioassay Technology Laboratory, China, in accordance with the manufacturer's instructions.

Statistical-Analysis

SPSS version 27 was used for the analyses. Data were expressed as mean ± standard error mean. For the numerous comparisons between all groups, one-way analysis of variance (ANOVA) was used, followed by LSD post-hoc testing. Additionally, Kruskal-Wallis one-way ANOVA and Fisher's exact test were used to compare changes in histopathology between groups. In every test, the level of statistical significance was set at $P < 0.05$.

Results

Serum Cr, BUN, IL-1β, NF-kB, and Caspase-3 were all significantly increased in the induced group compared to the sham group after renal I/R, while SOD, GSH, and Bcl-2 were all significantly decreased. Treatment with erythropoietin significantly decreased serum Cr and BUN levels (Figure 1, Figure 2), as well as the levels of inflammatory markers

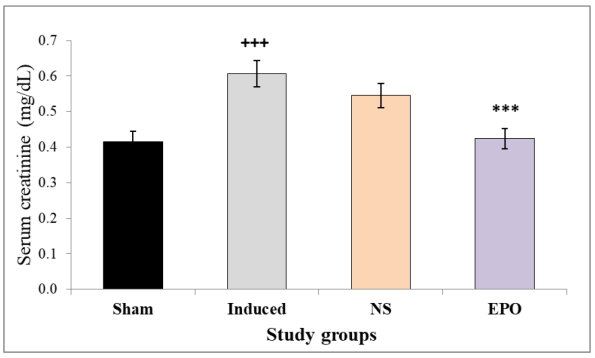

Fig. 1 **Serum creatinine level of study groups. Rats were subjected to ischemia for 30 min and reperfusion for 2 hr. Rats were pretreated with either vehicle NS, erythropoietin (1000 IU\kg) or left untreated (sham, and induced group) 30 min before ischemia. Creatinine concentration was determined using creatinine ELISA kit. One-way ANOVA followed by LSD multiple comparison test was used for analysis. Data are presented as mean ± SEM. $^{+++}P <$ 0.001 vs sham group, ***$P <$ 0.001 vs induced group.**

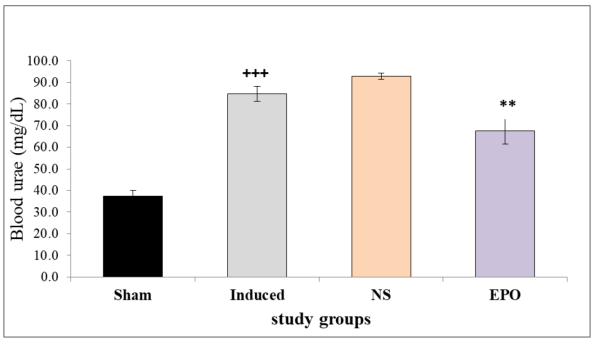

Fig. 2 **Blood urea level of study groups. Ischemia for 30 min and reperfusion for 2 hr rats were exposed to. They were pretreated 30 min before ischemia with either vehicle NS, erythropoietin (1000 IU\kg) or left untreated (sham, and induced group). BUN concentration was determined using BUN ELISA kit. One-way ANOVA followed by LSD multiple comparison test was used for analysis. Documents are presented as mean ± SEM. $^{+++}P <$ 0.001 vs sham group, **$P <$ 0.01 vs induced group.**

(IL-1β, NF-kB)also reduced (Figure 3, Figure 4), Renal tissue antioxidant markers (SOD, GSH) increased significantly (Figure 5, Figure 6), Significantly decreased levels of the apoptotic marker (Caspase-3) in renal tissue (Figure 7), whereas antiapoptotic marker (Bcl-2) increased significantly (Figure 8), compared to the induced group. In addition, the renal histological score of the erythropoietin-treated group was significantly better than that of the induced group (Figure 9, Figure 10).

Discussion

In this study, erythropoietin significantly lowers the levels of urea and creatinine as compared with that of induced group (Figure 1, Figure 2), representing maintenance of the renal function. This result is agreement with those reported by Kwak and colleagues indicated that the pretreatment of rats with erythropoietin appreciably reduced the level of BUN, serum creatinine and restored normal renal function.[23]

Inflammation appears to link the various cell types and play a significant role in the pathophysiology of kidney IRI

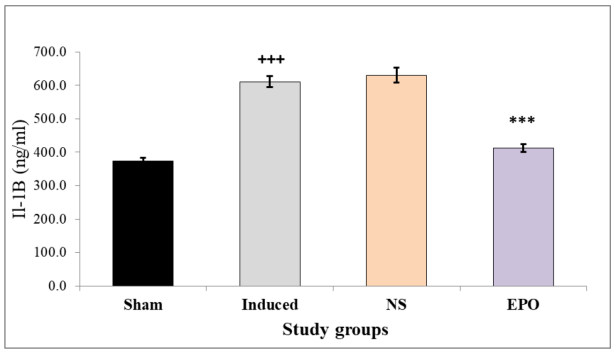

Fig. 3 **IL-1β level among study groups. Animals were exposed to ischemia for 30 min and reperfusion for 2 hr. Approximately 30 minutes prior to ischemia, rats were pretreated with either vehicle NS, erythropoietin (1000 IUkg), or left untreated (sham, and induced group). IL-1β concentration was determined using IL-1β ELISA kit. One-way ANOVA followed by LSD multiple comparison test was used for analysis. Data are presented as mean ± SEM. $^{+++}P <$ 0.001 vs sham group, ***$P <$ 0.001 vs induced group.**

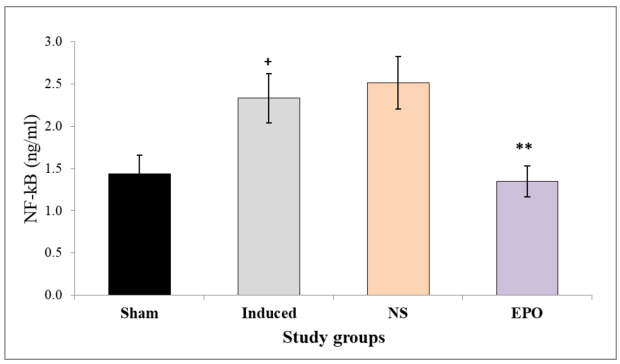

Fig. 4 **NF-kB level of study groups. Ischemia lasted for 30 minutes and reperfusion lasted for 2 hours in these rats. Approximately 30 minutes prior to ischemia, rats were pretreated with either vehicle NS, erythropoietin (1000 IUkg), or left untreated (sham, and induced group). The NF-KB ELISA kit was used to measure the levels of NF-KB in the sample. The data was analyzed with one-way ANOVA and then the LSD multiple comparison test. Data are presented as mean ± SEM. + $P <$ 0.05 vs sham group, **$P <$ 0.01 vs induced group.**

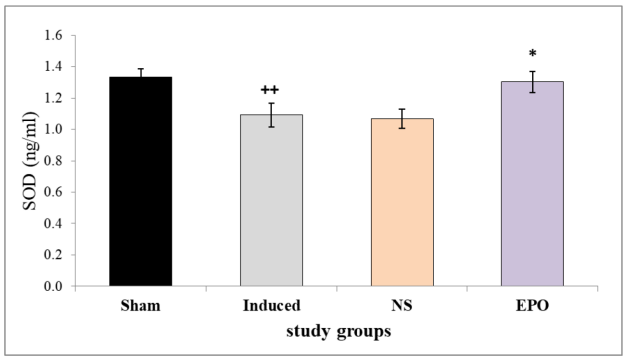

Fig. 5 **SOD level of study groups. Ischemia lasted for 30 minutes, and then the rats were given 2 hours of reperfusion. Thirty minutes before ischemia, rats were given either vehicle NS, erythropoietin (1000 IUkg), or were left untreated (sham, and induced group). Concentration of SOD was determined using SOD ELISA kit. One-way ANOVA followed by LSD multiple comparison test was used for analysis. Data are presented as mean ± SEM. $^{++}P <$ 0.01 vs sham group, *$P <$ 0.05 vs induced group.**

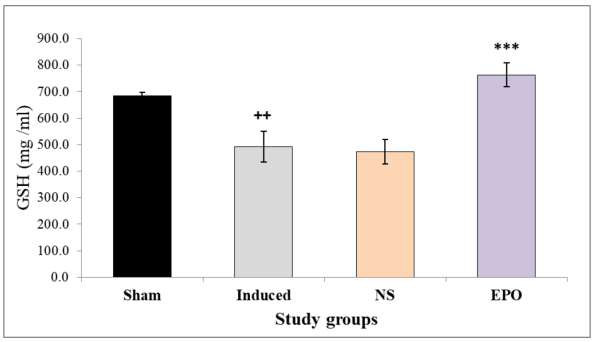

Fig. 6 **GSH level of study groups. Rats were imperiled to ischemia for 30 min and reperfusion for 2 hours. Pretreatment with either vehicle NS, erythropoietin (1000 IU\kg) or left untreated (sham, and induced group) 30 min before ischemia. Using GSH ELISA kit for the determination of GSH concentration. One-way ANOVA followed by LSD multiple comparison test was used for analysis. Data are presented as mean ± SEM. $^{++}P < 0.01$ vs sham group, $^{***}P < 0.001$ vs induced group.**

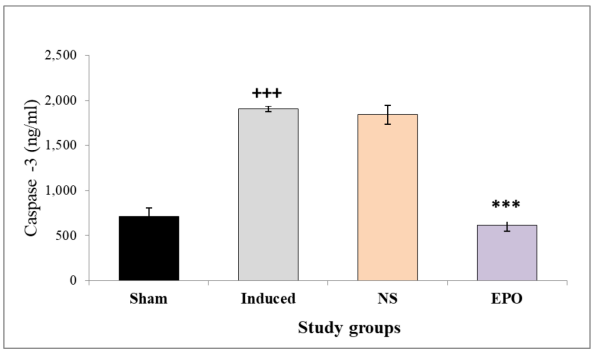

Fig. 7 **Caspase-3 level of study groups. The rats were put through 30 minutes of ischemia followed by 2 hours of reperfusion. Thirty minutes prior to ischemia, rats were given either vehicle NS, erythropoietin (1000 IUkg), or were left untreated (sham, and induced group). Caspase-3 concentration was determined using caspase-3 ELISA kit. Analysis was performed using one-way ANOVA and the LSD multiple comparison test for further interpretation. Data are presented as mean ± SEM. $^{+++}P < 0.001$ vs sham group, $^{***}P < 0.001$ vs induced group.**

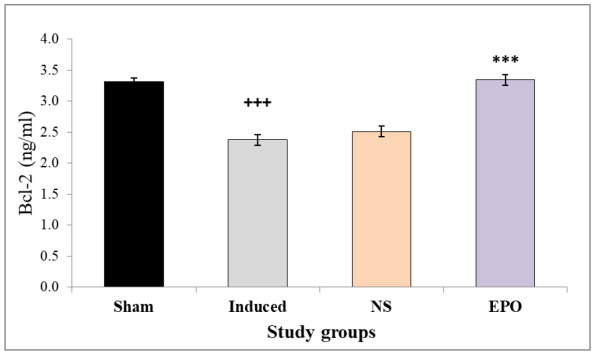

Fig. 8 **Bcl-2 level of study groups. Ischemia lasted for 30 minutes and reperfusion lasted for 2 hours in these rats. Approximately 30 minutes prior to ischemia, rats were pretreated with either vehicle NS, erythropoietin (1000 IUkg), or left untreated (sham, and induced group). Bcl-2 ELISA kit was used to determine Bcl-2 concentration. One-way ANOVA followed by LSD multiple comparison test was used for analysis. As mean ± SEM. $^{+++}P < 0.001$ vs sham group, $^{***}P < 0.001$ vs induced group data are presented.**

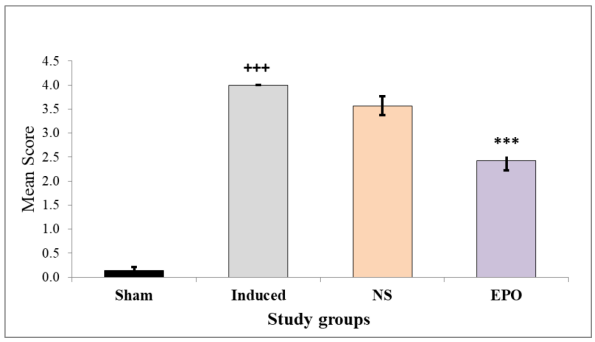

Fig. 9 **Mean score of histopathological renal tubular damage. $^{+++}P < 0.001$ vs sham group, $^{***}P < 0.001$ vs induced group.**

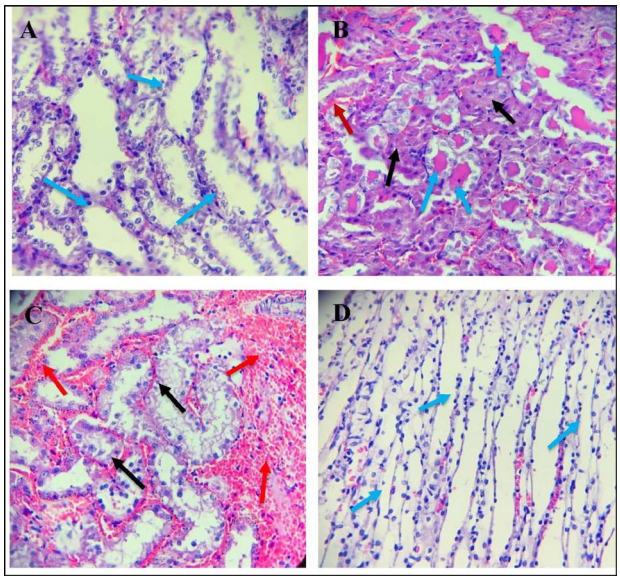

Fig. 10 **(A) sham group, normal histology of renal tubules (blue arrows). H&E x400. (B) Control group, renal tubules with score 4 damage. Cellular swelling increased cytoplasmic eosinophilia (black arrows), eosinophilic cast (blue arrows) & hemorrhage (red arrow). H&E. x400. (C) Vehicle group, renal tubules with score 4 damage. Cellular swelling increased cytoplasmic eosinophilia (black arrows) and hemorrhage (red arrows). H&E x400. (D) Erythropoietin group, renal tubules with score 2 damage. involving 30% of the examined tubules, normal tubules (blue arrows). H&E x400.**

as a common abnormality. Because RIRI initiates an inflammatory cascade that contributes to renal tissue damage, inhibiting inflammatory responses is a therapeutic approach for protecting renal tissue.[24] Pro-inflammatory factors such as IL-1 β, IL-6, and TNF α are found at injury and inflammation sites and can stimulate the production of a number of other pro-inflammatory cytokines and inflammatory mediators. Patients with chronic renal failure, as well as AKI rats induced by cisplatin and paraquat, have significantly elevated levels of IL-1β, IL-6, and TNF α[25]. NF-kB is a key signal transduction mediator that is involved in the effector phase of inflammation after being triggered by a number of major inflammatory cytokines such as tumor necrosis factor alpha and interleukin-1.[26] A positive feedback mechanism of NF-kB regulation occurs because of the interaction between I/R and TNFα, which increases TNFα production in an NF-kB-dependent manner, and

TNF α, which binds to its receptor and activates NF-kB. This feedback loop plays a critical role in the development of RIRI pathology.[27] The current study discovered a significant decrease in the level of (IL-1β and NF-kB) for erythropoietin pretreated group in comparison to induced group (Figure 3, Figure 4). Our findings showed that pretreatment with erythropoietin could significantly reduce pro-inflammatory cytokine secretion following renal I/R injury. In agreement with a study conducted on a rat model that underwent 30 minutes of ischemia and 24 hours of reperfusion, our findings showed that pretreatment with EPO significantly reduced the level of IL-1β and NF-kB in the serum as compared with that of induced group[28]. Another study confirmed anti-inflammatory effect of erythropoietin through lowering IL-1β, IL-6, TNF-a, and NF-kB when compared to induced group by subjecting rats to bilateral renal ischemia 45 min followed by reperfusion 24hr which indicate that erythropoietin has nephroprotective effect through suppressing inflammatory response.[29]

Oxidative stress was also important in renal I/R injury. Renal tissue injury is brought on by free radical formation, which in turn peroxidizes membrane lipids and causes oxidative damage to proteins and DNA, ultimately leading to apoptosis and cell death. Catalase, glutathione peroxidase and superoxide dismutase, are all antioxidant enzyme systems that may play a role in the pathophysiology of ischemia-reperfusion injury through their downregulation. Thus, protecting tissue during IRI entails inhibiting this pathway or preventing free radical production.[30] ROS are important in the development of hypertension, acute and chronic kidney injury, and diabetes-induced nephropathy [31]. SOD and GSH levels in ischemic renal tissues were found to be significantly higher in the erythropoietin-pretreated group compared to the induced group in this study (Figure 5, Figure 6). Based on these findings, it can be concluded that erythropoietin exhibits antioxidant activity in kidney tissues that have been subjected to I/R. These results are consistent with research into the effects of oxidative stress on renal function using an adiabatic rat model of ischemia/reperfusion. Oxidative stress has been shown to increase cellular damage and death via peroxidation of membrane lipids, protein oxidation, and DNA damage. It was found in this study that the activity of SOD and GSH level was decreased in the renal tissue in diabetic RIRI, indicating an oxidative injury, when pretreated with erythropoietin SOD and GSH levels were both significantly increased, in comparison to the I/R group. The results indicate the nephroprotective effect of erythropoietin via antioxidant activity.[32]

The death of cells is a key factor in the development of many diseases. IRI causes activation of cell death pathways, with necrosis and apoptosis being suggested as the primary contributors to IRI pathology.[33] During hypoxic stress in ischemic injury and during the production of reactive oxygen species (ROS) in reperfusion injury, cells undergo a programmed death process known as apoptosis.[34] Caspases' activation is a crucial biochemical aspect of apoptosis in cells. Procedures for apoptosis in mammals are initiated and carried out with the help of the caspase family of cysteine proteases. Caspase-3 is generated from a zymogen through the death ligand and mitochondrial pathways. This zymogen is a caspase effector that triggers cell death during the terminal stages of apoptosis. Bcl-2, an anti-apoptotic protein, inhibits not only the production of free radicals and lipid peroxides, but also the release of endoplasmic reticulum Ca2+. By decreasing Bcl-2, IRI induced renal cell apoptosis.[35,36] The results of the study demonstrated significant elevated level of Bcl2 and decrease level of caspase-3 in erythropoietin pretreated group as compared with induced and vehicle groups (Figure 7, Figure 8), these findings suggest that erythropoietin has antiapoptotic effect after RIRI. These results are compatible with the study in which rat was subjected to bilateral renal ischemia for 45 min followed by reperfusion 24or 48 hr, which demonstrated that pretreatment with erythropoietin prior to I/R injury resulted in an elevated Bcl-2 protein Level and caspase-3 activity decreased significantly compared with that in the I/R group. erythropoietin exerts its antiapoptotic effect by inducing Bcl-2 protein in kidneys.[37]

Rats pretreated with erythropoietin before ischemia induction enhanced renal damage significantly when compared to induce group and average group severity scores indicated mild renal injury (Figure 9, Figure 10). This means that erythropoietin pretreatment, which was given before renal I/R injury, prevented renal injury over histopathological parameters. Other Studies have proven the protective effect of erythropoietin in preserving normal morphology of renal tissue by exposing rat models to renal ischemia 45min/24hr reperfusion. Following IRI, there were significant tubular changes, including dilation of renal tubules, loss of brush border, and degeneration and necrosis of renal tubular epithelial cells. EPO treatment, on the other hand, significantly improved tubular lesions.[29,32]

Conclusion

Erythropoietin was found to have a nephroprotective effect against I/R-mediated renal injury. Inhibiting inflammation, modulating oxidative stress, and apoptotic markers were all ways in which erythropoietin showed its antioxidant, anti-inflammatory, and anti-apoptotic properties following renal I/R injury.

Conflict of Interest

None.

References

1. Jang, H.R. and H. Rabb, The innate immune response in ischemic acute kidney injury. Clinical Immunology, 2009. 130(1): p. 41–50.
2. Sharfuddin, A.A. and B.A. Molitoris, Pathophysiology of ischemic acute kidney injury. Nature Reviews Nephrology, 2011. 7(4): p. 189–200.
3. Collard, C.D. and S. Gelman, Pathophysiology, clinical manifestations, and prevention of ischemia-reperfusion injury. The Journal of the American Society of Anesthesiologists, 2001. 94(6): p. 1133–1138.
4. Wu, M.-Y., et al., New insights into the role of inflammation in the pathogenesis of atherosclerosis. International Journal of Molecular Sciences, 2017. 18(10): p. 2034.
5. Ponticelli, C., Erratum: Ischaemia-reperfusion injury: A major protagonist in kidney transplantation (Nephrology Dialysis Transplantation (2014) 29: 6 (1134-1140. Nephrology Dialysis Transplantation, 2014. 29(8): p. 1612.

6. Zhong, D., et al., Ganoderma lucidum polysaccharide peptide prevents renal ischemia reperfusion injury via counteracting oxidative stress. Scientific Reports, 2015. 5(1): p. 1–14.
7. Beker, B.M., et al., Novel acute kidney injury biomarkers: Their characteristics, utility and concerns. International Urology and Nephrology, 2018. 50: p. 705–713.
8. Ja, K., Unruh Ml and Murugan r: Acute kidney injury. BMJ Clin Evid, 2011. 2001: p. 2011.
9. Rojas-Morales, P., et al., Fasting reduces oxidative stress, mitochondrial dysfunction and fibrosis induced by renal ischemia-reperfusion injury. Free Radical Biology and Medicine, 2019. 135: p. 60–67.
10. Van Avondt, K., E. Nur, and S. Zeerleder, Mechanisms of haemolysis-induced kidney injury. Nature Reviews Nephrology, 2019. 15(11): p. 671–692.
11. Dennis, J.M. and P.K. Witting, Protective role for antioxidants in acute kidney disease. Nutrients, 2017. 9(7): p. 718.
12. Kezić, A., N. Stajic, and F. Thaiss, Innate immune response in kidney ischemia/reperfusion injury: potential target for therapy. Journal of Immunology Research, 2017. 2017.
13. Park, J.S., et al., Small heterodimer partner attenuates hydrogen peroxide-induced expression of cyclooxygenase-2 and inducible nitric oxide synthase by suppression of activator protein-1 and nuclear factor-κB in renal proximal tubule epithelial cells. International Journal of Molecular Medicine, 2017. 39(3): p. 701–710.
14. Tsagalis, G., Renal anemia: A nephrologist's view. Hippokratia, 2011. 15 (Suppl 1): p. 39-43.
15. Priyadarshi, A. and J.I. Shapiro. Hematology: Issues in the dialysis patient: Erythropoietin resistance in the treatment of the anemia of chronic renal failure. In Seminars in dialysis. 2006. 19(4): p. 267–348
16. Kalantar-Zadeh, K., History of erythropoiesis-stimulating agents, the development of biosimilars, and the future of anemia treatment in nephrology. American Journal of Nephrology, 2017. 45(3): p. 235–247.
17. Suresh, S., et al., Erythropoietin-induced changes in bone and bone marrow in mouse models of diet-induced obesity. International Journal of Molecular Sciences, 2020. 21(5): p. 1657.
18. Sheldon, R.A., et al., Erythropoietin treatment exacerbates moderate injury after hypoxia-ischemia in neonatal superoxide dismutase transgenic mice. Developmental Neuroscience, 2017. 39(1–4): p. 228–237.
19. Tasar, P. and Y. Ozen, Effects of Recombinant Human Erythropoietin and 2-Mercaptoethane Sulfonate on Liver Ischemia-Reperfusion Injury in Rats. Experimental and Clinical Transplantation: Official Journal of the Middle East Society for Organ Transplantation, 2022.
20. Shawky, H.M., et al., Effect of recombinant erythropoietin on ischemia–reperfusion-induced apoptosis in rat liver. Journal of Physiology and Biochemistry, 2012. 68(1): p. 19–28.
21. Tiba, A.-T., H. Qassam, and N.R. Hadi, Semaglutide in renal ischemia-reperfusion injury in mice. Journal of Medicine and Life, 2023. 16(2): p. 317.
22. Erkılıç, E., et al., Does remifentanil attenuate renal ischemia–reperfusion injury better than dexmedetomidine in rat kidney? Drug Design, Development and Therapy, 2017.11: p. 677–683.
23. Kwak, J., et al., Erythropoietin ameliorates ischemia/reperfusion-induced acute kidney injury via inflammasome suppression in mice. International Journal of Molecular Sciences, 2020. 21(10): p. 3453.
24. Stroo, I., et al., Chemokine expression in renal ischemia/reperfusion injury is most profound during the reparative phase. International Immunology, 2010. 22(6): p. 433–442.
25. Li, Y., et al., Hydralazine protects against renal ischemia-reperfusion injury in rats. European Journal of Pharmacology, 2019. 843: p. 199–209.
26. Tak, P.P. and G.S. Firestein, NF-κB: A key role in inflammatory diseases. The Journal of Clinical Investigation, 2001. 107(1): p. 7–11.
27. Zhang, H. and S.-C. Sun, NF-κB in inflammation and renal diseases. Cell & bioscience, 2015. 5(1): p. 1–12.
28. Zhang, J., et al., Erythropoietin pretreatment ameliorates renal ischaemia-reperfusion injury by activating PI3K/Akt signalling. Nephrology, 2015. 20(4): p. 266–272.
29. Hu, L., et al., Erythropoietin ameliorates renal ischemia and reperfusion injury via inhibiting tubulointerstitial inflammation. Journal of Surgical Research, 2012. 176(1): p. 260–266.
30. Malek, M. and M. Nematbakhsh, Renal ischemia/reperfusion injury; from pathophysiology to treatment. Journal of Renal Injury Prevention, 2015. 4(2): p. 20–27.
31. Xu, N., et al., Reactive oxygen species in renal vascular function. Acta Physiologica, 2020. 229(4): p. e13477.
32. Tang, Y., et al. Recombinant human erythropoietin restrains oxidative stress in streptozotocin-induced diabetic rats exposed to renal ischemia reperfusion injury. Transplantation Proceedings. 2019. 51(6): p. 2076-2080.
33. Sun, Z., et al., Dexmedetomidine attenuates spinal cord ischemia–reperfusion injury through both anti-inflammation and anti-apoptosis mechanisms in rabbits. Journal of Translational Medicine, 2018. 16: p. 1–11.
34. Liu, H., et al., Overexpression of TIMP3 protects against cardiac ischemia/reperfusion injury by inhibiting myocardial apoptosis through ROS/Mapks pathway. Cellular Physiology and Biochemistry, 2017. 44(3): p. 1011–1023.
35. Hu, L., et al., HBx sensitizes cells to oxidative stress-induced apoptosis by accelerating the loss of Mcl-1 protein via caspase-3 cascade. Molecular cancer, 2011. 10: p. 1–16.
36. Renault, T.T. and J.E. Chipuk, Death upon a kiss: Mitochondrial outer membrane composition and organelle communication govern sensitivity to BAK/BAX-dependent apoptosis. Chemistry & Biology, 2014. 21(1): p. 114–123.
37. Yang, C.W., et al., Preconditioning with erythropoietin protects against subsequent ischemia-reperfusion injury in rat kidney. The FASEB Journal, 2003. 17(12): p. 1754–1755.