Study on Co-Administration of Erythropoietin and Nandrolone Decanoate against Injury Induced By Ischemia-Reperfusion in Rats

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Abstract: Erythropoietin which is a cytokine has been known for a long time as a hematopoietic hormone. Its effectiveness mechanism is to reduce apoptosis in cells making erythroblast in bone marrow. Its artificial form is available, too. The hormone is used to improve anemia in patients with renal failure. With regard to high rate of renal patients, high cost of renal treatment with erythropoietin, and unavailability of the drug versus easy availability of nandrolone even as an OTC combination in drugstores, we decided to study the coincident effects of both drugs for using nandrolone in place of erythropoietin in case of obtaining suitable results. For this purpose 24 Vistar male rats with weight of 200-250 gram were divided randomly in to four groups, each group consisted of 6 rats: the first group was Sham (6 Vistar male rats), the second one was control group in which vascular pedicles were blocked, the third group received 500 IU/kg peritoneally every week, two weeks before ischemia via injection, the fourth group received 3 mg/kg nandrolone decanoate intramuscularly and 500 IU/kg peritoneally every week, two weeks before ischemia. The data were reported as mean+SEM. ANOVA statistical method was used for data analysis and Tokay comparative tests was used to compare the difference among groups. The rate of $p<0.05$ has been considered as a meaningful level among groups. The obtained results demonstrated that erythropoietin had a positive effect on renal function by itself and administration of the drug without nandrolone could decrease and improve degeneration of tubule cells, decrease the hyaline cast and decrease the necrosis of tubule cells. Also, in functional phase all changes associate to serum keratinin level showed that administrating of erythropoietin by itself as well as coincidently with nandrolone had not a meaningful effect on serum keratinin level but it was observed that EPO singularly and coincident with nandrolone, the blood urea level decreased meaningfully. It is clear that the effect of EPO on the changes of blood urea is more meaningful compared its effect on serum keratinin level. The present study demonstrated that EPO singularly and without any other medical additions like nandrolone can be used effectively in ARF that ischemia is one of ARF cases also it promotes renal function and decrease the rate mortality among ARF patients.

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1. Introduction

Ischemic acute renal failure (ARF) is a clinical syndrome that can occur following the interruption or reduction of renal blood flow. Despite preventive and therapeutic measures, this disease is still associated with high mortality (Liano et al., 1998). Tissue damage begins from the ischemic phase. Reperfusion after ischemia in the early phase leads to a new damages in the organ, so, these actions are called Ischemic Reperfusion (Sheridan and Bonventre, 2001).

Erythropoietin (EPO) is a cytokine that has been known as a hematopoietic hormone. Its mechanism is reduction of apoptosis in Erythroblasts in the bone marrow. It is also available in synthetic form and is used for correction of anemia in patients with renal failure frequently (Juul, 2002). Studies have shown that EPO has anti-apoptotic effects in kidney, nervous system and heart damages. EPO in renal tubule cells inhibits apoptosis by increasing the activity of nuclear factor-kβ. This action appears to be due to an increase in anti-apoptotic factors such as heat shock proteins (HSP70), BCL-XL, XIAP, BCL-2 that inhibits apoptosis pathway by stabilization of inner membrane of mitochondria and inhibition of entrance of cytochrome c into the cytoplasm and prevention of Kaspaz's initiating apoptosis (Juul, 2002; Patel and Sharples, 2004). Anti-apoptotic effects of erythropoietin and its protective role against ischemic injury is one of the novel fields of research and many studies have been done in this field during recent years. Anabolic steroids, which are synthetic compounds with testosterone in their structure, are used in treatment of reproductive disorders, cancer and anemia widely (Lubna et al., 2010). Almost all tissues have androgen receptors so; these compounds can affect the entire body (Lubna et al., 2010). The hematopoietic effects of Nandrolone Decanoate seem

to be direct stimulation of kidneys to produce erythropoietin or by increasing the sensitivity of erythroid precursors of bone marrow to erythropoietin (Teruel et al., 1995). Researchers have shown that nandrolone may enhance the effects of erythropoietin in dialysis patients (Gonzalez et al., 1988). Considering the high renal disease occurrence and high cost of treatment by erythropoietin, Lack of easy access to the compound and easy access to the nandrolone as OTC in the pharmacies, in this study, we aimed to assay the protective role of nandrolone compared with erythropoietin in terms of reduction of inflammation and cell damage following the ischemia-reperfusion, and in order to obtain optimum results, we recommend it as an appropriate alternative for erythropoietin.

2. Materials and Methods

Twenty four wistar rats (200-250g) were divided into the 4 groups of 6. Group 1 considered as sham, group 2 considered as ischemia-reperfusion, group 3 considered as ischemia-reperfusion + treatment with erythropoietin and group 4 considered as ischemia-reperfusion + concomitant treatment with erythropoietin and nandrolone decanoate. Rats of group 3 received erithropoitn at the dose of 500 IU/kg intraperitoneally weekly and two weeks prior the induction the ischemia (Chevalier et al., 1996, Gonzalez et al., 1988, Kiris et al., 2008). After 2 weeks, animals were anesthetized using the ketamine and then pedicles of kidneys were obstructed and after 45 min were released (Gonzalez et al., 1988, Kiris et al., 2008). After 2 hours, left kidney nephrectomy was applied (Aggarwal et al., 2005). Rats of group 4 received 3 mg/kg nandrolone weekly intramuscularly two weeks prior the induction the ischemia (Chevalier et al., 1996) and erythropoietin at the dose of 500 IU/kg intraperitoneally (Gonzalez et al., 1988, Kiris et al., 2008). After 2 weeks, animals were anesthetized using the ketamine and then pedicles of kidneys were obstructed and after 45 min were released (Gonzalez et al., 1988, Kiris et al., 2008). After 2 hours, left kidney nephrectomy was applied (Aggarwal et al., 2005).

2.1. Blood Sampling

On days 0 (before administration of nandrolone decanoate) and at the end of the period, blood samples were collected and the values of serum urea and creatinine were measured.

2.2. Histopathological evaluations

After euthanization of animals, left kidneys were harvested and were fixed in the formalin 10% and were sent to histopathology laboratory for evaluation the pathological events. In the laboratory, slides were prepared and were stained by Hematoxylin Eosin method.

2.3. Statistical analysis

Data were presented as Mean±SEM. Data were analysed by SPSS software using the ANOVA following the post-tukey multiple test for assessment the difference between groups. $P<0.05$ considered as significant difference.

3. Results

Histopathological changes in groups are shown in the figures 1-4. Also, severity of changes is given in table 1. In control group, kidney tissues structure was normal and no certain pathological changes was observed. In group 2, degenerative changes of tubular cells, acute tubular necrosis, edema, hyperemia and interstitial bleeding were observed. Glomerular hyperemia and hemorrhage were obvious. In group 3, erythropoietin showed healing of pathological changes significantly so that; the severity of pathological changes was reduced. In group 4, slight healing in the pathological changes was observed. Pathological damages observed in this group include edema, moderate hyperemia and hemorrhage in renal glomeruli and interstitial tissue and mild degenerative changes with necrosis of tubular epithelial cells in the cortex and medulla.

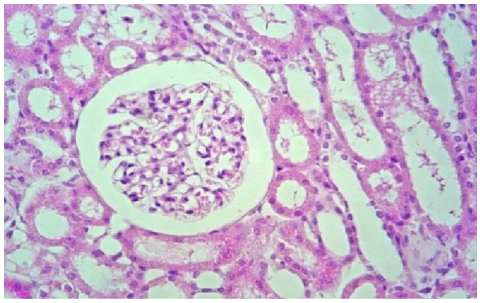


Fig 1: microscopic view of kidneys of rats of control group. Renal structure is normal. H&E, 40x.

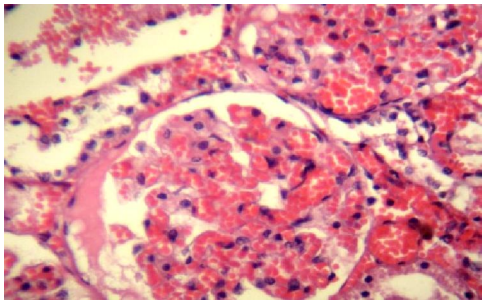
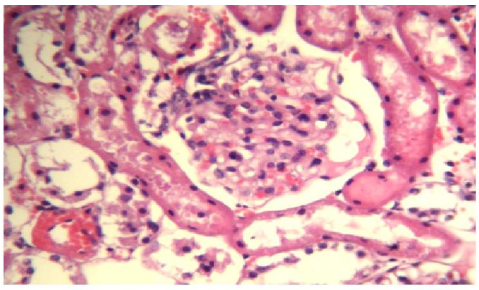


Fig 2: microscopic view of kidneys of rats of group 2. Interstitial and glomerular hemorrhage and tubular necrosis of tubules with protein deposition in the Bowman capsule is obvious. H&E, 40x.



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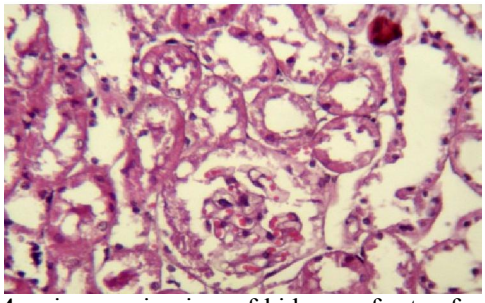

Fig 4: microscopic view of kidneys of rats of group 4. Degenerative changes and tubular and parietal membrane of Bowman's capsule necrosis with mild hyperemia and basal membrane thickness is obvious. H&E, 40x.

3.1. Results of biochemical parameters

Changes in biochemical parameters of serum in all groups are given in table 2.

Table 2: comparison of biochemical parameters changes in groups

Parameter / Group	Serum creatinine (mg/dl)	Urea (mg/dl)
SHAM	1.66±0.09	60.32±5.6
IR	4.34±0.21[a]	139.95±12.71[a]
EPO	1.96±0.08[c]	71.35±4.1[c]
NAN+EPO	2.71±0.11[b]	101.95±7.27[b]

a: P<0.001 in compared with control group;
b: P<0.05; c: P<0.01 in compared with group 2.

4. Discussion and Conclusion

The results of this study indicate a positive effect of erythropoietin (EPO) alone administration on renal function and it could prevent renal pathological damages. Also, in the functional phase, all changes in serum creatinine levels showed that administration of erythropoietin alone and in co-administration with nandrolone, significant effects on serum creatinine levels were not observed however, the level of BUN significantly decreased in both route of administration.

Table 1: comparison of pathological changes in groups

Groups	*Tubular Cell Necrosis*	*Tubular Cell Degeration*	*Podocyte Necrosis*	*Fibrin Depostion on Glomerulat Space*	*Haemorrahage*	*Protein Cast*
SHAM	0	0	0	0	0	0
IR	++++	++++	+++	++++	++++	++++
EPO	++	++	++	++	++	++
NAN+EPO	++++	+++	+++	+++	+++	+++

SCORE Information: Normal and without of pathologic changes(0), Minimal pathologic changes(+), mild pathologic changes(++), moderate pathologic changes (+++) and Sever pathologic changes(++++).

Obviously, the effect of EPO on changes in blood urea was more significantly than serum creatinine levels. However, serum creatinine level is more valuable than blood urea in then interpretation of renal disease and its performance because BUN levels beside of kidneys function are affected by the saliva and gut secretions and animal nutrition. However, in this research we can claim that EPO and its co-administration with nandrolone decreased BUN levels and didn't affect serum creatinine levels.

In this study also we found that EPO alone and in co-administrated with nandrolone can increase leucocytes particularly lymphocytes levels. But, more notably, EPO as alone increased lymphocytes more than EPO + nandrolone. Also, EPO + nandrolone increased neutrophils more than group IR but, this changes was not observed in the EPO alone administration. Perhaps, this is due to increased damage to kidneys in co-administration of EPO+ nandrolone. So, in terms of increased neutrophils, its infiltration into the renal interstitial also increased and may cause induction of necrosis in tubules and glomeruli. Fortunately, the results of this study are compatible with other studies that are referred to some of them.

In a research by Kiris et al., (2008) they found that aortic IR significantly increased the levels of MDA and superoxide dismutase (P<0.05 versus

control). Erythropoietin significantly decreased the levels of MDA, superoxide dismutase, and catalase ($P < 0.05$ versus aortic IR). Histological evaluation showed that aortic IR significantly increased ($P < 0.05$ versus control), whereas erythropoietin significantly decreased ($P < 0.05$ versus aortic IR) the focal glomerular necrosis, dilation of Bowman's capsule, degeneration of tubular epithelium, necrosis in tubular epithelium, interstitial inflammatory infiltration, and congestion of blood vessels. Their results indicate that erythropoietin has protective effects on renal injury induced by aortic IR in rats.

Yazihan et al., (2008) showed that renal TNF-alpha and caspase-3 levels were decreased in both glibenclamide and EPO-treated IR rats compared to untreated rats. The protection afforded by the pretreatment with EPO alone was greater than that of administering glibenclamide alone. Application of glibenclamide at the same time partly abolished the cytoprotective effect of EPO treatment. They concluded that K-ATP mediated cytoprotection is not the main mechanism of protective effect of EPO.

Nakazawa et al., (2010) demonstrated that Ischaemia-reperfusion injury of diabetic kidney resulted in significantly low protein expression levels of bcl-2, an anti-apoptotic molecule, and bone morphogenetic protein-7 (BMP-7), an anti-fibrotic and pro-regenerative factor, compared with non-diabetic kidneys. Diabetic kidney subsequently showed severe damage including increased tubular cell apoptosis, tubulointerstitial fibrosis and decreased tubular proliferation, compared with non-diabetic kidney. Treatment with asialoerythropoietin induced bcl-2 and BMP-7 expression in diabetic kidney and decreased tubular cell apoptosis, tubulointerstitial fibrosis and accelerated tubular proliferation. They concluded that reduced induction bcl-2 and BMP-7 may play a role in the acceleration of renal damage after ischemia-reperfusion injury in diabetic kidney. Also, they declared that the renoprotective effects of asialoerythropoietin on acute kidney injury may be mediated through the induction of bcl-2 and BMP-7.

Prokai et al., (2011) conducted a study and showed that EPO cause improve the renal function by increasing the HSP-72.

Credible mechanisms of the protective effects of EPO have been identified through the application of an ATP-dependent potassium channels. ATP-dependent potassium channels play an important role in the occurrence of injury and the induction of apoptotic death in kidney tissue. So, inhibit these channels enhance caspase-3 and TNF-a levels thus, destructive effects are began. While, EPO acts effecting on ATP-dependent potassium channels.

Another mechanism seems that EPO with their can apply its protective effects on the kidney tissue are induction the bcl-2 and Bone Morphogenic Protein- 7 that these are precursor of anti-apoptotic, anti-fibrotic and Pro-regenerative molecules respectively.

Another EPO protective direction is explained by Yang et al., (2011), especially in kidney transplant patients, they found that EPO can be done via caspase-3 and interleukin (IL-1b) and cause enhancement of apoptosis of inflammatory cells and reduces inflammation in the interstitial tissue of the kidney and renal Remodeling.

Ates et al., (2005) suggested that EPO is effective in attenuating renal ischemia/reperfusion injury, and this effect may be related to inhibition of tyrosine kinase activity by genistein.

However, the EPO is effective in the treatment of kidney disease and controlling of anemia resulted from the use of chemotherapy and other disorders affecting the kidneys that improve quality of life. EPO acts by reaction with itself receptors on non-hematopoietic tissues and performs its protective effects such as mitogenesis, angiogenesis and inhibition of apoptosis. Present research showed that EPO is useful in treatment of patients with renal disorders alone and without using the other adjuvants such as nandrolone.

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