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ORIGINAL ARTICLE

Effects of Erythropoietin on Cell Death Following of Renal Ischemic reperfusion in rats

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Mehrdad Neshatgharamaleki, Yousef Doustar, Ghafor Mousavi, Daryoush Mohajeri, Ali Rezaii, Mehrdad Nazeri: Effects of Erythropoietin on Cell Death Following of Renal Ischemic reperfusion in rats

ABSTRACT

I/R-induced acute renal failure (ARF) is a common clinical problem, which despite significant advances in critical care medicine are still associated with high morbidity and mortality.

Key words: Erythropoietin, Cell Death, Renal Ischemic reperfusion,

Introduction

I/R-induced acute renal failure (ARF) is a common clinical problem, which despite significant advances in critical care medicine is still associated with high morbidity and mortality. The mechanisms underlying renal I/R are complex, including ATP depletion, accumulation of intracellular Ca^{2+} and reactive oxygen species, mitochondrial dysfunction, multiple enzyme systems activation and pro-inflammatory cytokine production. Although reperfusion is essential for the survival of ischemic renal tissue, it causes additional cellular injury. Together, renal ischemia and reperfusion initiate a multiple and interrelated sequence of events, resulting in the injury and eventually the death of renal cells as a combination of both apoptosis and necrosis [5]. EPO is a cytokine that was originally identified as the major regulator of erythroid precursor cells. However, increasing evidence suggests that EPO has broader functions independent of its effects on erythropoiesis. Recent *in vitro* and *in vivo* studies have demonstrated that EPO attenuates cell damage. The favorable effects of the EPO-related changes are not fully known, although its anti-apoptotic, anti-oxidative and anti-inflammatory properties as well as its pro-angiogenic potential seem to be related to EPO-mediated protective effect. The biological effects of EPO are mediated by binding to its specific cell surface receptor (EPOR), and the presence of functional EPOR in renal tubular and mesansial cells has pointed to a potential autocrine or paracrine role for EPO in the kidney. Furthermore, in recent *in vivo* studies subjected to cisplatin or to I/R injury, EPO enhanced functional and morphologic tissue recovery, mainly through its anti-apoptotic action [5].

Multiple protective effects of erythropoietin (EPO), such as antiapoptotic, antioxidant, angiogenic and neuroprotective effects, against ischemia have been demonstrated in cell culture and animal models. The aims of the present study were to evaluate the effects of EPO on renal ischemia/reperfusion injury [4].

Materials and methods

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Animals:

Studies were performed on male Wistar rats weighing 200 to 250 g (n = 25). Rats received a standard diet and water ad libitum and were housed in a 12-h light/dark cycle. The animals were randomly allocated into three groups: [1] I/R-saline group, in which rats were subjected to renal ischemia for 45 min (n = 10); [2] I/R-EPO group, in which rats were administered EPO (500 U/kg, i.p.) 20 min prior to I/R (n = 10); and [3] sham-operated group, in which rats were subjected to identical surgical procedure without occlusion of both renal pedicles and maintained under anesthesia for the duration of the experiment (n = 5). Dose-dependent responses were not examined in our model. In previous studies, EPO was used in doses varying from 300–5000 IU/kg [8,11,12], and we choose a single rather low dose, which we expected to ensure the protective effect of EPO.

Animals:

Investigations using experimental animals were conducted in accordance with the internationally accepted principles for laboratory animal use and care as found in the United States guidelines (United States National Institutes for Health publication no. 85-23, revised in 1985), and our ethical committee on animal care approved the protocol. 15 male adult Sprague-Dawley rats, 250-300 gram were used. Rats were obtained from the central animal laboratory of Islamic Azad University-Tabriz Branch and were housed in colony rooms with 12/12 hr light/dark cycle at 21±2°C for 2 weeks before initiation of the study, fed with laboratory pellet chow and drinking water was given ad libitum. Animals were randomised in 3 groups (n=5), sham, control and experimental.

Surgical procedure:

Animals were anesthetized with Ketamine hydrochloride (Ketamine 10%, Alfasan, Woerden-Holland, 50 mg/kg) and Xylazine (Xylazin 2%, Alfasan, Worden-Holland, 5mg/kg) intraperitoneally. A midline incision was made in each rat and the left kidney became available. Ischemia-reperfusion injury was induced by applying a noncrushing microvascular clamp on the left renal artery for 45 minutes. After 45 minutes of ischemia, the clamp was removed and the tissue was closed in layers. The animals were then returned to their cages, reperfusion period was 24 hour after surgery. Sham-operated animals underwent the same operation but without clamping. Animals were sacrificialed after 24 hour postoperatively under general anesthesia, with an injection of over dosage of Thiopental sodium (60 mg/kg) and the left kidney was harvested.

Histopathology analyses:

kidneys sunk in 10% formalin buffer during seven days, for fixation; then. Each of those segments were included in paraffin blocks, all specimens were serially sectioned longitudinally at 5-µm intervals and stained with hematoxylin-eosin (H and E) method and used for light microscopic examination under a Nikon microscope (ECLIPSE E200, Japan).

Statistical analyses:

All values are expressed as mean±SEM. Statistical analysis was carried out using the SPSS 11.0 software. Biochemical parameters were evaluated by analysis of variance (ANOVA) followed by Bonferroni *post hoc* test. Student's *t*-test was performed for evaluation of scores of renal damage. *P*-values less than 0.05 were considered statistically significant.

Histological evaluation:

The kidneys were removed from the rats at the end of the experimental period and were cut in a sagittal section into two halves. Renal tissue was fixed in 10% buffered-formalin solution and embedded in paraffin. Paraffin kidney sections (5 µm) were prepared and stained with haematoxylin and eosin. Evaluation of renal injury was performed in a blinded manner by a pathologist and renal sections were scored with a semiquantitative scale designed to evaluate the degree of tubular necrosis. Injury was graded on a 5-point scale: 0 = normal kidney; 1 = minimal damage (<5% involvement of the cortex or outer medulla); 2 = mild damage (5–25% involvement of the cortex or outer medulla); 3 = moderate damage (25–75% involvement of the cortex or outer medulla); 4 = severe damage (>75% involvement of the cortex or outer medulla).

Results and discussion

Effects of EPO on the histological features of renal I/R:

The characteristic histopathological features of ischemic injury were readily evident at 24 h of reperfusion in kidneys obtained from I/R-treated rats compared with sham-operated rats. Specifically, the most severe and pronounced injuries were observed in the cortex and the outer stripe of outer medulla with a typical tubular necrosis pattern, which included widespread degeneration of tubular architecture detachment of epithelial cells from the basement membrane, tubular cell necrosis, luminal congestion and hemorrhage with loss of brush border and inflammatory cell infiltration. (Figure1A-

B).contrast, renal sections obtained from rats pretreated with EPO demonstrated marked reduction of the histological features of renal injury, consisting of more focal and mild characteristics of tubular necrosis (Figure1D). Semi-quantitative assessment of the histological lesions showed a significantly higher score in the I/R-treated rats compared to the EPO-treated rats at 24 h of reperfusion (P<0.05).

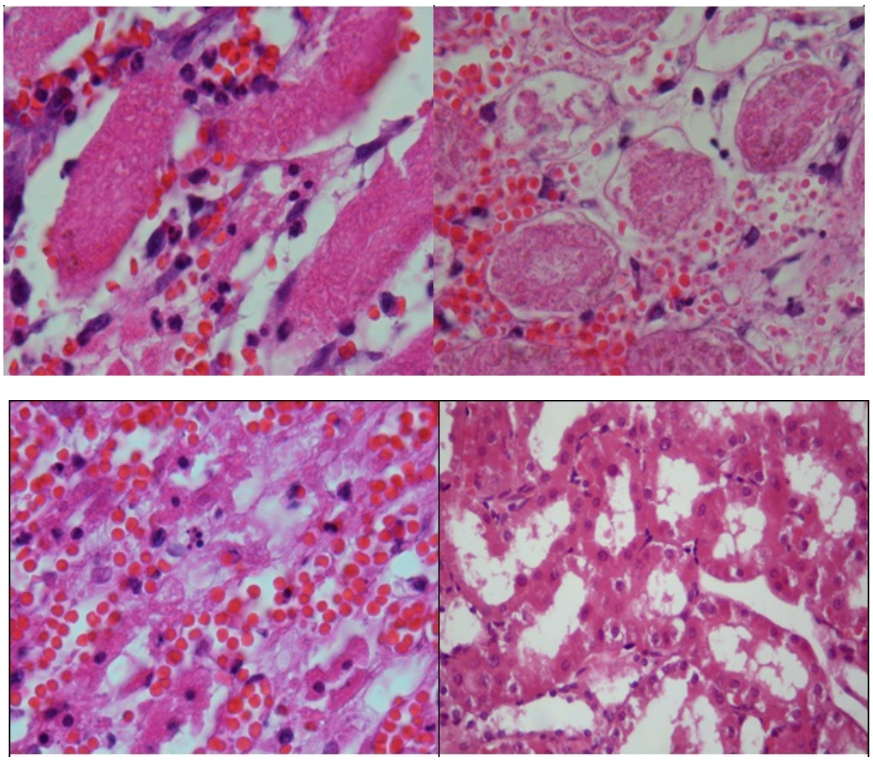

Fig. 1: Effect of EPO on I/R-induced renal injury. Representative haematoxylin-eosin stained renal sections from I/R (A, B, C) and EPO (D)-treated rats, demonstrating more severe lesions of tubular necrosis in I/R rats. EPO pretreatment significantly preserved renal tissue morphology. Magnification ×400. (D). Semi-quantitative assessment of the histological lesions based on tubular necrosis. Values represent scores±SEM. *P<0.01.

Discussion:

Erythropoietin by interfering in the cytokine complicated play important role in preventing from kidney ischemic reperfusion harm. Plasma concentration of this hormone is 1-26 mills units in the mills liter of blood (1-26mu/ml). It was clearly identify that erythropoietin and erythropoietin receptors have important role in prenatal growth and have vital role in stimulating red blood cells and again they act this vital role in product and development of vessel system. This erythropoietin variety impression originates from stimulating signals of different directions. Stimulating erythropoietin receptors cause variety impression inspiration for example erythropoietin have cyto-protective and erythropoietic effect in different doses [1]. In answering to the mammals ischemic cells a cycle from proteins by the names of erythropoietin and vessel endothelial growth factor (VEGE) were out braked but their accession were done by impressing a sensitive factor to hypoxia (HIF-1) [9]. Erythropoietin is a glycoprotein by the weight of 30.4 kd. Its first role is Erythropoiesis regulator. Erythropoietin production are adjusting by amount of the oxygen convey to the fiber about the 90 percent of the erythropoietin are product in the interstitial cell nearby the kidney's tubular. Decrease in the oxygen conveying to the kidney cause increase in stimulating erythropoietin production by kidneys and same stimulating the blood conveying. Erythropoietin is an accessible cytokine. Chat are used for treatment and uremic correction anemia, stimulating and production of red blood cells and increasing the work vigor of the sick people that suffering chronic renal failure in combination with iron for improvement of the heart working among sick people erythropoietin are used among children for treat the chronic renal kidney failure. Also In recent studies it has shown that erythropoietin keep the brain from the ischemic harms [6]. Wide observations have shown that erythropoietin cause decreasing in the negative effect of the hypoxia, oxidative stress, and hemorrhagic shock on the kidneys by decreasing the caspase

activity and restrain the apoptotic death in kidney's cells. Erythropoietin has a non- hematopoietic on the kidneys and has more effects on keeping of the cells and mitogenic. Oxidative stress is the most important cause of the kidney's ischemic harms. More over kidney's ischemic tubular – interstitial fibrosis, causes Kidney's vessel harms and nearby the tubular [10]. Erythropoietin is the most important tendency and propagation regulator of the erythroid cells precursor regulator with anti- apoptotic effects. Accession of the erythropoietin gene is under the control of the sensitive factor to hypoxia (HIF-1) or hypoxia inducible factor-1. This factor is under the unique HIF-1ß, HIF-1α and HIF-1 is the controlling cause of the cytokine multi accession like endothelial growth factor (VEGF) that inspirited in the answering to ischemia and interfere in the glucose metabolism. Peritubular fibroblasts are the most important place for erythropoietin production. From the other side erythropoietin's receptors in the proximal tubules of mesansial and glomerular cells are scattered [3]. In current study, role of the erythropoietin on the kidney's harm that originate from Ischemic reperfusion were surveyed. In this research it observed that erythropoietin can become a cause of reduction in the glomerular and tubular harms (histopathologic Parameter) that originate from 45minuts ischemic and 24 hours reperfusion. In this research erythropoietin were used at least one hour before ischemic in the peritoneal route. It was question that how erythropoietin was kept the kidneys and different kind of mechanisms were noticed by different kind of scholars. It seems that the increasing kidney's blood fluid by the erythropoietin that the cause of kidney protecting. By notice that erythropoietin can cause of the increasing in the urine [3], there for we can claim that erythropoietin by increasing perfusion in the cortical and Interglomerular part of kidney cause the increasing in the blood convey to this part of kidney and apply keeping effect. From the other side it can claimed the theory that erythropoietin has direct effect on the harmed proximal tubular epithelial cells from ischemic reperfusion. Erythropoietin apply their keeping effect directly on the epithelial proximal by restrain the oxidative harms and so by its receivers by applying the anti-apoptotic effect. [3] Erythropoietin with activation of JAK2 in proximal tubules causes activation of PI3K and phosphorylation of AKt. By activating AKt multipurpose like phosphorylation of Bad, Bax, caspase 9, GSK3β, potential constant mitochondrial membrane and ATP synthesis that all belonging to the anti – apoptotic effects starts to appear. Therefore erythropoietin by Juncture to its receptors cause keeping of proximal tubule cells by restrains apoptosis. XJAP is the most important mediator involved in keeping erythropoietin administration subsequent kidney [3]. For this, first it directly cause restrain in the 3,7,9 Caspase activity and second it act by regulating death receptor mean's FAS. From the other XJAR apply indirectly it's anti –apoptotic effect by stimulating P21CiP1 that cause stopping in the cell propagation cycle in the grade G1/S. erythropoietin by restrain a caspase fall both from the way of caspase 9 and with low severity by restrain the caspase 8's activity cause a decrease and restrain a caspase's activity production increasing TNF-α ischemic reperfusion subsequent and ultimately stimulating nuclear factor ß cause death inspiration of the kidney tubular cells, that erythropoietin can cause stopping in the nuclear factor's activity [3]. A probability for erythropoietin keeping activity and Ischemic kidney can originate from hemodynamic activity improvement. It seems that erythropoietin more over its anti-apoptotic effect, has anti inflammatory effects this theory were improve by influencing erythropoietin on the encephalomyelitis level Il-6. From the other side erythropoietin can decrease accession a effective gene in the inflammation process namely nuclear factor gene kappa B and decline the inflammation severity [2]. as effect of inflammatory process epithelial tubular cells were dig and tubular harm become accent. Pre inflammation cytokines like tumor necroses Factor-α, Interleukin 1 and interferon γ cause cellular matrix analysis namely BETA 1 (ß1) inter green and cells were draft into kidney's lumen [7]. From the other on an account of the inflammation is the most important cause of ischemic reperfusion administration in the kidney's harm [7]. Erythropoietin cause angiogenesis and mitogenic inspiration in the vessel by stimulating endothelial cells and it has an improvement role in oxygen convey to fiber. Erythropoietin support vessel reproduction and it's effective in garble relief. From the other side erythropoietin are the most important and power full motive for endothelial precursor cells and their exodus from marrow. That the attendance of precursor in the blood, cause improvement in the harmed endothelial regeneration power [13].

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