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Insulin Like Growth Factor -1(IGF-1) Promotes Angiogenesis and Reverses Ischemia Reperfusion Induced Acute Kidney Injury in Rats: Role of VEGF and TGF-β1

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Abstract

Previous studies highlighted the effect of administering insulin-like growth factor I (IGF-I) in a model of ischemic acute kidney injury (AKI) in rats. We studied the effect of IGF-1 treatment on ischemia reperfusion induced AKI in rats focusing on angiogenesis as a potential mechanism underlying the effect of IGF-1. AKI was induced by nephrectomy of the left kidney while the right one's pedicle was clamped for 45 min then the clamp was removed to start reperfusion. Compared to AKI rats, rats administered IGF-I (100 µg/day) for 7 days post occlusion had significantly lower serum creatinine, blood urea nitrogen (BUN) and Aspartate Aminotransferase (AST) levels. Serum creatinine and BUN were used as indicators of impaired renal function, while serum concentration of AST was measured as an indicator of renal injury. Examining the levels of vascular endothelial growth factor (VEGF) and transforming growth factor β1 (TGF- β1) in the renal tissue revealed higher levels in the I-R+IGF-1 group compared to I-R group. Histopathological examination of the renal tissue in the IGF-1treated group showed an obvious angiogenic reaction occurred as a result of IGF-1 treatment. Neovascularization by such angiogenic agents may explain the action of IGF-1 during AKI. Overall, the current results provide evidence that the effect of IGF-1 on ischemia-reperfusion AKI involves induction of angiogenesis.

Keywords: Insulin like growth factor -1, Ischemia reperfusion, angiogenesis, VEGF, TGF β1.

Abbreviations: **AKI**, acute kidney injury; **I-R**, Ischemia reperfusion; **IGF-1**, Insulin like growth factor -1; **VEGF**, vascular endothelial growth factor; **TGF-β1**, transforming growth factor- β1; **IGF-1**, Insulin-like

growth factor-1; **BUN**, blood urea nitrogen; **AST**, Aspartate Aminotransferase; **HIF-1α**, Hypoxia Inducible Factor-1α.

1. Introduction

Acute kidney injury (AKI) is one of the common reasons of morbidity and mortality allover the world [1]. The AKI is characterized by sudden decline in the kidney function that leads to the accumulation of wastes in blood and inability to maintain body fluid and electrolyte balance. [2]. Ischemia-reperfusion injury (I-R) is one of the common reasons for renal dysfunction observed in various clinical conditions involving kidney transplantation, renal artery angioplasty, partial nephrectomy, aortic aneurysm, and ureteral obstruction [3].

Angiogenesis is a process involving the formation of new blood vessels from the pre-existing one that occurs in many physiological and pathological situations [4]. Therapeutic angiogenesis is considered a new strategy that has been used to treat tissue ischemia by enhancing the proliferation of collateral vessels [5]. It holds new promise for the treatment of a variety of ischemic diseases including the kidney [6].

Angiogenesis process is regulated by the interplay of numerous cytokines and growth factors which include several angiogenic molecules such as vascular endothelial growth factor (VEGF), and transforming growth factor-beta 1 (TGF-β1) as well as angiostatic factors such as angiostatin and endostatin [7]. Under homeostatic conditions, the microvasculature is maintained by a delicate balance between these pro- and anti-angiogenic factors. In response to ischemia the balance tips in favor of pro-angiogenic factors to drive repair, as the hypoxia is generally regarded as a potent stimulus for angiogenesis [8].

Insulin-like growth factor-1 (IGF-1) is a peptide hormone structurally related to insulin, synthesized by the liver and also in many other tissues. In the kidney, it's found especially in the glomerulus and collecting ducts, with specific receptors that have been identified in glomeruli and proximal tubules [9].Previous studies established the benefits of IGF-1 on ischemic heart disease [10], cerebrovascular ischemia [11] and renal ischemia [12]. IGF-1was reported to improve the kidney function by several mechanisms such as its direct hemodynamic effects, as it increases renal plasma flow and glomerular filtration rate [13], another evidence suggests that IGF-1 may accelerate healing from acute ischemic nephron injury by its mitogenic actions [14]. Based on the previous demonstration of the effect of IGF-1 on renal ischemia the present study investigated one putative mechanism that might contribute to this beneficial effect focusing on the angiogenic role, this study was designed to examine the proangiogenic effect of IGF-1 in renal I-R rats. Parameters chosen to assess acute kidney injury and effects of IGF-1 administration included serum creatinine, BUN and AST, VEGF and TGF-β1 levels in the kidney tissue and histopathological examination of the kidney.

2. Materials and Methods

2. 1 Animals

This study was conducted on 32 adult Wistar albino male rats, 6-8 weeks old, weighing between 200 and 220 g. Animals were maintained on a standard diet with free access to food and water throughout the study. Rats were housed at room temperature, they have housed in metal cages; four rats in each cage. An adaptation period of one week was allowed before experimental protocol. The present study was carried out in accordance with the guidelines set by the Research Ethics Committee of Benha Faculty of Medicine.

2. 2 Experimental design

The rats were randomly assigned into four groups (eight rats in each group): sham group, sham +IGF-1 group, Ischemia-reperfusion (I-R) group and I-R +IGF-1 group. IGF-1 was given subcutaneously 100 µg daily started 30 min post-reperfusion for 7 days. This dose was chosen because it was shown that such dose enhances kidney function in rats without causing

hypoglycemia in rats [15]. Sham group and I-R group rats received equal volumes of vehicle injections (i.e., normal saline) instead of IGF-1.

2. 3 Surgical procedures

The ischemia-reperfusion was induced by using unilateral nephrectomy with ischemia-reperfusion of the other kidney. The rats were anesthetized by Thiopental Na (dose 40mg/kg, i.p) and injected intramuscularly with antibiotics (penicillin G procaine 40,000 U/kg). The anesthetized rats were placed on surgical platform in dorsal position, the dorsal surface was shaved and then cleaned with ethanol. Both kidneys were exposed through flank incisions, left one was subjected for nephrectomy while the right one's pedicle was clamped for 45 min then the clamp were then removed to start reperfusion. Occlusion was confirmed visually by a change in the color of the kidneys to a paler shade. Reperfusion was initiated with the removal of the artery clips and was confirmed visually by noting a blush. The surgical site was sealed by continuous sutures in two layers [16]. In the sham group, the animals were exposed to similar surgical procedure except for the nephrectomy and the occlusion of renal pedicle.

24 hours after the last treatment, the rats were anesthetized with ether, and blood samples were collected via cardiac puncture for serum separation and estimation of serum creatinine, blood urea nitrogen (BUN) and Aspartate Aminotransferase (AST). Rats were then sacrificed by decapitation and the kidney was rapidly isolated for histopathological examination and VEGF and TGF-β1 measurement in the kidney tissues.

2. 4 Biochemical analysis

The blood samples were centrifuged at 4500 rpm at 4°C for 10 minutes to separate out the serum that was stored at -20°C for biochemical analysis. Levels of creatinine, BUN and AST were measured using diagnostic kits (Audit Diagnostic, Co Cork, Ireland). Serum creatinine and BUN were used as indicators of impaired renal function, while serum concentration of AST an enzyme that occurs in the proximal tubule cells, was measured as an indicator of renal parenchymal cell injury [17].

Portions of the kidney tissue were homogenized in ice-cold phosphate buffered saline PBS buffer (0.1 M, pH 7.4) using a homogenizer and centrifuged (3000 rpm for 10 minutes). The supernatant of the homogenate was stored at -80 °C [18] and used to measure vascular endothelial growth factor (VEGF) and transforming growth factor-beta 1 (TGF-β1) protein levels using commercially available enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer's protocols (VEGF; USCN Life, China) and (TGF-β1 BioVendor, Czech Republic).

2. 5 Histopathology analysis

The kidneys were dissected out and it was preserved in 10% formalin. Paraffin blocks were prepared from the kidneys samples and thin sections (5 µm) were prepared and stained with haematoxylin-eosin (H&E) for light microscopy examination. Morphological analyses were undertaken by an experienced pathologist blinded to the tissue source for signs of cell injury and angiogenesis.

2. 6 Chemicals used

Recombinant human Insulin-like growth factor 1 (IGF-1): It was purchased from Peprotech, Inc., Rocky Hill, NJ, USA. It was supplied as a powder and dissolved in saline for injection.

2. 7 Statistical analysis

All analyses were performed using the program "Statistical Package for Social Sciences (SPSS) version 16" (SPSS Inc, Chicago, IL, USA). The data are presented as the mean ± standard deviation (SD). Comparisons between two groups, in all studied parameters, were analyzed by using unpaired Student "t" test. The probability of chance (P-value) < 0.05 was considered statistically significant.

3. Results

Table 1. Changes in serum creatinine, blood urea nitrogen (BUN) and AST in different experimental groups (mean ± SD), *n=8.*

	Sham group	Sham +IGF-I group	I-R group	I-R+ IGF-I group
Creatinine(mg/dl)	0. 46 ±0. 03	0. 46±0. 03	2. 89±0. 25*****	0. 96±0. 06**
BUN(mg/dl)	18. 27±1. 05	17. 70±0. 64	145. 27±10. 44*	31. 33±3. 50**
AST (U/ml)	72. 3 ± 9. 4	73. 1 ± 12. 4	784. 7 ± 12. 8 *	119. 2 ± 11. 3**

BUN, blood urea nitrogen; AST, Aspartate Aminotransferase.
** Significant difference (p<0. 001) compared with Sham group.*
*** Significant difference (p<0. 001) compared with I-R group.*

Table 2. Changes in renal angiogenic growth factors in different experimental groups (mean ± SD), n=8.

	Sham group	Sham+IGF-I group	I-R group	I-R + IGF-I group
VEGF (ng/mg protein)	1. 81±0. 06	2. 54±0. 14*	1. 85±0. 08	5. 46±0. 42**
TGFß1 (µg/gm tissue)	0. 15±0. 01	0. 22±0. 02*	0. 16±0. 01	0. 33±0. 16**

VEGF, vascular endothelial growth factor; TGF ß1, transforming growth factor ß1.
** Significant difference (p<0. 001) compared with Sham group.*
*** Significant difference (p<0. 001) compared with I-R group.*

3.1 Effect of IGF-1 administration on biochemical changes that associate ischemia-reperfusion induced acute kidney injury in rats (Table 1)

I-R-induced AKI was accompanied by a significant elevation (p<0.001) in the serum levels of creatinine and BUN which were used as indicators of kidney function and in the serum level of AST that was used as an indicator of renal parenchymal cell injury compared with sham group. Treatment with IGF-1 after I-R significantly (p<0.001) reduced the creatinine, BUN and AST to near normal values compared with an I-R group.

3.2 Effect of I-R and IGF-1 administration on renal angiogenic growth factors (Table 2)

As regard, the angiogenic growth factors, VEGF and TGF- ß1 increased significantly in sham +IGF-1 group (p<0.001) compared with sham rats and these factors also showed a significant increase (p<0.001) in an I-R+IGF-1 group compared with an I-R group.

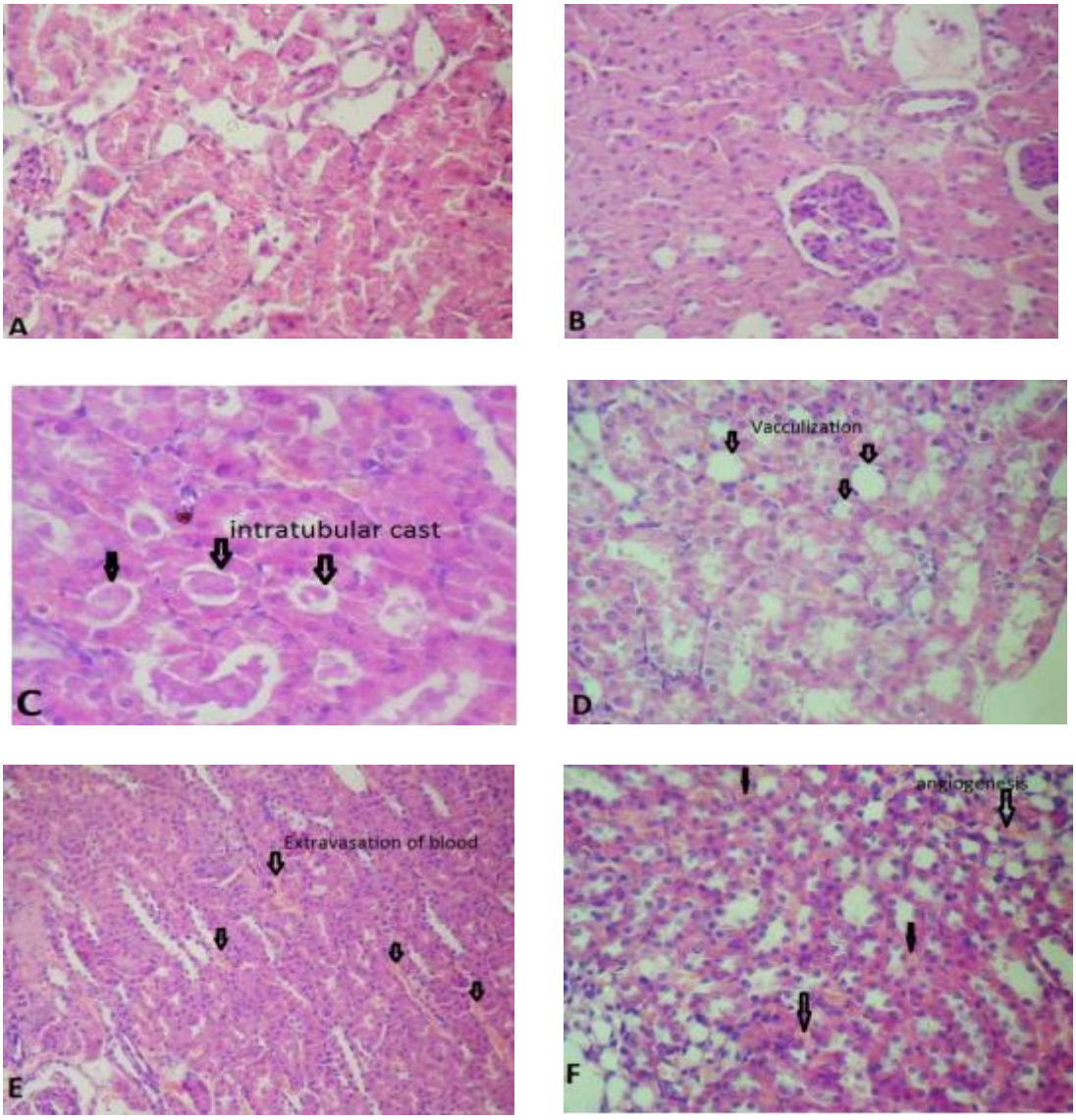

Figure 1. (A) kidney section of sham rats showing normal glomeruli and normal tubules; (B) kidney section of Sham + IGF-1 group shows normal histology; (C) ,(D) and (E) kidney section of renal I-R rat shows tubular cell swelling, cellular vacuolization, congestion, intratubular casts, extravasation of blood and cellular necrosis; (F) kidney section of I-R +IGF-1 rats showing angiogenesis and normal morphology. H & E staining, magnification × 400.

3.3 Histopathological examination of the renal tissue (Figure 1)

The histopathological examination of the Sham and Sham + IGF-1 groups did not show any morphologic changes (Fig. 1A and B, respectively). In contrast, the section of kidneys obtained from animals that underwent renal I-R demonstrated the recognized features of severe acute tubular damage (Fig. 1C, D and E). These features included cellular congestion, vacuolization, extravasation of blood, intratubular casts, cell exfoliations and cell necrosis. Treatment of I-R with IGF-1 preserved the normal morphology of the kidney demonstrating capillary sprouting and local angiogenic reaction (Fig. 1F).

4. Discussion

Several studies had shown interventions taken before I-R injury. However, the effects of intervention after I-R injury would be more valuable and of much more significance to the clinical treatment of AKI especially in patients

with unpredictable AKI caused by a severe injury such as hemorrhage or shock. Insufficient angiogenesis or loss of peritubular and glomerular capillaries have been observed in acute ischemic renal failure suggesting a role for angiogenesis in modulating renal injury [19]. To the best of our knowledge, the current study was the first to investigate the pro-angiogenic effect of IGF-1 in experimentally induced acute kidney injury following ischemia-reperfusion in rats.

In the current study, AKI in I-R group was confirmed by a significant elevation in the serum creatinine, BUN, AST and histologic features. These findings are in consonance with earlier reports on I-R-induced kidney injury where increased serum levels of creatinine, BUN and AST were observed [20,21,22]. Several factors such as alterations in morphology, microvasculature, tubule cell metabolism and structure, tubule dynamics, and inflammatory response all results from ischemia and believed to exert many deleterious effects on renal tissue and cause AKI [23]. Treatment with IGF-1 (100µg/day) started 30 min post-reperfusion for seven days decreased the serum level of creatinine, BUN and AST indicating that the IGF-1 reduced the extent of AKI.

In this study, it was found that renal levels of angiogenic factors VEGF and TGF-β1 didn't increase after I-R although it's known that the ischemia results in the stabilization of the transcription factor Hypoxia Inducible Factor-1α (HIF-1α) which induce the transcription of genes that encode proteins responsible for angiogenesis such as VEGF and TGF- β1[24]. This was in accordance with Basile et al., [25] who showed that although the expression of VEGF has been shown to increase in renal diseases and during hypoxia, experimental works did not find any increase in VEGF expression in a rat model of renal I-R. As a possible explanation for the lack of vascular repair, it is hypothesized that renal I-R results in a net shift of expressed factors in favor of anti-angiogenesis vs. angiogenesis/vascular stabilization. [26].

In the present study, significant increases in levels of VEGF and TGF- β1 in the renal tissue were observed after IGF-1 treatment, which is accompanied by improvement of the ischemia-induced AKI. Several studies showed that IGF-1 had proangiogenic effects and it was postulated to induce angiogenesis through interaction with factors such as VEGF which plays essential role in the angiogenesis and act as a key mediator of ischemia-driven angiogenesis [27,28] as it contributes to increased migration and mitosis of endothelial cells, creation of blood vessel lumen and fenestrations and stimulating vascular splitting and sprouting and acts as a 'branching factor' by directly affecting endothelial and/or smooth muscle cells [29]. Previous studies found that VEGF acts through receptors in the endothelium to increase the production of nitric oxide, and thus, the activity of the two growth factors may be associated [30].

There is a controversy regarding the role of TGF-β1 in angiogenesis it has been reported that TGF- β1 has a pronounced inhibitory effect on endothelial cell proliferation and migration in vitro with respect to cellular proliferation and DNA synthesis when tested on endothelial cells from a large number of sources including bovine aorta and pulmonary artery, adrenal cortex and retina [31]. Similarly, overexpression of TGF- β1 in a tissue-specific manner does not necessarily induce angiogenesis in vivo [32]. In contrast to the previous findings, TGF- β1 is a potent inducer of angiogenesis when administered subcutaneously to newborn, adult, mice or adult rats, when tested in the rabbit cornea and when applied to full of thickness wounds in a rabbit ear dermal ulcer model [33]. These results are in agreement with previous studies that shown that TGF-β1 play an essential role in angiogenesis through the establishment and maintenance of vessel wall integrity, stimulating extracellular matrix production, and enhancing endothelial cells that promote the pericyte differentiation and expansion which lead to vessel stabilization and, consequently, vessel maturation [34]. Stabilization and maturation of newly formed blood vessels is being essential as the newly generated vessels may present a disorganized architecture, functional abnormalities or increased permeability, therefore, leading to deficient regulation and delivery of blood flow [35].

Overall, histopathological comparison of kidneys from rats in I-R group and I-R+ IGF-1treated group showed an obvious angiogenic reaction occurred as a result of IGF treatment. Neovascularization by such angiogenic agents may explain the action of IGF-1 during AKI, the mechanism of which has yet to be fully elucidated.

Overall, the present study provided evidence that the ameliorating effect of IGF-1 on ischemia-induced AKI in rats involves induction of angiogenesis through stimulation of VEGF and TGF- β1. Further studies are still needed to examine the effect of IGF-1 on restoring blood flow in experimentally ischemic kidneys; this will represent a good follow-on experiments that would confirm the present hypothesis.

5. Conclusion

In conclusion, the present study revealed that IGF-1 treatment could improve the outcome of ischemia-reperfusion induced acute kidney injury in rats. This therapeutic effect could be explained on the basis of its angiogenic effects.

Limitation of the study

The duration of the study (one week) is not enough to detect any side effects of IGF-1. So I recommend doing further studies with more prolonged durations.

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