Erythropoietin Administration Promotes Expression of VEGF in Renal Ischemic–Reperfusion Injury in Rat Model

Supranee Kongkham PhD*,
Siriwan Sriwong BSc**, Adis Tasanarong MD***

** Division of Biochemistry, Department of Preclinical Sciences, Faculty of Medicine, Thammasat University (Rangsit Campus), Pathumthani, Thailand*
*** Laboratory Animal Centre, Thammasat University (Rangsit Campus), Pathumthani, Thailand*
**** Nephrology Unit, Department of Medicine, Faculty of Medicine, Thammasat University (Rangsit Campus), Pathumthani, Thailand*

Background: *Acute ischemia-reperfusion (I/R) injury is the most common causes of acute renal failure in daily clinical practice. It has been recognized that endothelial cell dysfunction and microvascular injury as the pathophysiological changes during I/R injury. Protective effects of erythropoietin (EPO) have been demonstrated in various experimental models of I/R induced injury. Therefore, the aim of the present study was to investigate whether EPO administration has renoprotective effect against acute renal failure I/R injury in rats by promotion of endothelial progenitor cells (EPCs) mobilization and neovascularization.*

Material and Method: *Male Sprague-Dawley rats were pretreated with EPO (1,000 IU/kg/day, ip); or the placebo for 3 days before the induction of I/R procedure. On day 4, the bilateral renal occlusion for 30 min operations to produce renal I/R injury or treatment with EPO 30 min before the initiation of I/R were done. At the end of the reperfusion period at day 1 day 2 and day 4, blood and renal tissues were collected to investigate renal function and pathohistological examination. The expression levels of CAV-1 and CD34 were determined for circulating of EPCs in blood, while CD34, CAV-1 and VEGFR-2 were investigated for mobilized EPCs in kidney, using real time PCR. The expression level of VEGF was also examined to indicate the angiogenesis in kidney using real time PCR and western blotting.*

Results: *In the I/R group, the significantly increased values of serum urea and creatinine were found on Day 1 after ischemia, as compared to sham group. The development of tubular epithelial cell necrosis, peritubular capillary congestion and mild interstitial infiltration has been observed in this group. Administration of EPO in I/R rat was significantly improved renal function and significantly less the tubular damage. The treatment with EPO significantly increased in expression levels of CD34 and CAV-1 in blood, and also CAV-1, VEGFR-2 and VEGF in kidney tissue in this group, as compared to the I/R group.*

Conclusion: *These results suggest that treatment with EPO protects the kidney from ischemic acute renal injury via increasing the mobilization and recruitment of EPCs, resulting in the induction of expression of VEGF that might play an important role in the repair response.*

Keywords: *Erythropoietin (EPO), Ischemia/reperfusion injury (I/R injury), Endothelial progenitor cells (EPCs), Angiogenesis*

J Med Assoc Thai 2016; 99 (Suppl. 4): S246-S255
Full text. e-Journal: *http://www.jmatonline.com*

Acute ischemia-reperfusion (I/R) injury is the most common causes of acute renal failure (ARF) in daily clinical practices[1,2]. The lack of effective therapy leads to a high mortality and morbidity rate[3]. The structure and tubular endothelial cell dysfunction and microvascular injury during I/R injury is a key importance for the pathophysiological consequence in various renal disease, therefore, immediate and adequate repair of endothelial injury is essential to preserve the renal function[4]. The loss of endothelial cell function may represent an important therapeutic target in which endothelial regeneration by progenitor cells may show promising alternative in the treatment of the acute or chronic effects of ischemic ARF[5-7]. Several studies demonstrated that circulating bone marrow-derived endothelial progenitor cells (EPCs) are mobilized and recruited in the ischemic area and that also they induce tissue repair via secretion of pro-

Correspondence to:
Kongkham S, Division of Biochemistry, Department of Preclinical Sciences, Faculty of Medicine, Thammasat University (Rangsit Campus), Pathumthani 12120, Thailand.
Phone: +66-2-9269710, Fax: +66-2-9269710
E-mail: sukongkham@yahoo.com

angiogenic growth factors including vascular endothelial growth factor (VEGF)[8-11] and repair injured microvasculature by formation of new blood vessels that demonstrated in both animal and human model of ischemic injury[12].

Erythropoietin (EPO) is a cytokine secreted by the kidneys in response to hypoxia induced condition and required for erythropoiesis by the proliferation of erythroid progenitor cells in the bone marrow. It has also many pleiotropic therapeutic properties by exerting anti-apoptotic and anti-inflammatory effects in many models of ischemia-reperfusion injury, including myocardial infarction[13] and acute kidney injury[14]. Also, EPO acts as a stimulator of EPCs mobilization in response to hypoxia in order to promote angiogenesis and also are able to secrete paracrine proteins, which include anti-inflammatory cytokines and growth factors including vascular endothelial growth factor (VEGF). Recent data demonstrated that EPO was used for therapeutic approach in experimental model such as ischemic heart disease[15,16] and ischemic spinal cord[17]. However, the effect of pretreatment with EPO before I/R induction on renal function and EPCs mobilization remains unclear. Thus, the present study was designed to investigate whether daily pre-treatment with EPO for a period of 3 days can modulate EPCs recruitment and promote angiogenesis, thereby contribute to attenuate the renal injury and its dysfunction induced by ischemic reperfusion injury.

Material and Method

Animals and surgical procedures

In the present study, 54 adult male Sprague-Dawley rats obtained from National Animal Center were used. All experiments were approved by the animal Ethics Committee of faculty of Medicine, Thammasat University. All rats were housed in temperature controlled room (24±1°C) and were given with standard food pellets and tape water until they weighed 250-300 g prior to surgery.

At the beginning of the experiment, rats were randomly assigned to sham control (n = 12, n = 4/group), IR (n = 15, n = 5/group), IR+EPO (n = 15, n = 5/group), and EPO (n = 12, n = 4/group). In pretreatment protocol, rats were administered subcutaneously with saline (8 ml/kg/day) or EPO (1,000 IU/kg/day) for 3 days prior to I/R. and also with intra-peritoneal injection before 30 min induction ischemia was occurred. All rats were anesthetized with sodium pentobarbital at dose of 40-60 mg/kg by intra-peritoneal injections. The abdominal region was shaved, and the animals were placed on a heating table to maintain them at constant body temperature at 37±1°C while under anesthesia. After midline laparotomy was performed, I/R group and IR+EPO group were subjected to bilateral renal ischemia for 30 min which the renal arteries and veins were occluded by placing a microvascular clamps. After the renal clamps were removed, the kidney were observed for further 5 min to ensure reflow and then the incision site was closed. Sham operated rats underwent identical surgical procedures but microaneurysm clamps were not applied.

Sample preparations

Animals from each group were sacrificed under inhalation ether anesthesia at day 1, day 2 and day 4. Kidneys were dissected from rats and sliced from the corona. These sections were fixed in 10% formalin and processed for histology using standard techniques. A small section of the kidney was frozen in liquid nitrogen stored at -70°C for protein studies using Western blot analysis, while another section was fixed in RNA later Stabilization Solution (Ambion, Inc.) for Realtime-PCR gene expression studies. Blood samples were collected from rats via cardiac puncture. The samples were centrifuged at 2,000 xg for 10 min. Plasma were collected to measure the levels of BUN and creatinine using automated analysis methods. Also buffy coated sample were obtained and collected in RNA later Stabilization Solution to investigate the expression of EPCs in bloods using Realtime PCR.

Histopathology Examination of Renal Tissues

The renal tissues were fixed with formalin followed by paraffin embedding. Five-micrometer paraffin sections were stained with H&E using standard procedure and examined under a light microscope for morphologic analysis by a pathologist functioning in a blind manner. Tubular necrosis and proteinaceous casts were graded according to a previous methodology[18], as follows: 0 = no damage; 1 = mild (unicellular, patchy isolated damage); 2 = moderate (damage <25%); 3 = severe (damage between 25 and 50%) and 4 = very severe (>50% damage). The degree of medullary congestion was defined as: 0 = no congestion; 1 = mild (vascular congestion with identification of erythrocytes); 2 = moderate (vascular congestion with identification of erythrocytes); 3 = severe (vascular congestion with identification of erythrocytes) and 4 = very severe (vascular congestion with identification of erythrocytes). Moreover, interstitial edema was graded

as follows: 0 = no damage; 1 = mild (unicellular, patchy isolated damage); 2 = moderate (damage <25%); 3 = severe (damage between 25 and 50%) and 4 = very severe (>50% damage).

Protein extraction and western blot analysis

Total proteins were extracted from fresh frozen kidney tissue. 100 mg of kidney (wet weight) was homogenized in 500 µl of RIPA buffer. Homogenates were treated with protease inhibitors and centrifuged in a pre-chilled rotor at 15,000 xg for 15 min. Supernatants were stored at -70°C. Protein content was measured using a BCA™ Protein Assay Kit (PIERCE, IL, USA).

Protein samples were separated by 10% SDS-PAGE and transferred onto nitrocellulose membranes. Membranes were treated with Odyssey@ blocking buffer and followed by an overnight incubation at 4°C with primary antibody (rabbit anti-VEGF and rabbit anti-actin, dilution 1:2,000). Blots were then incubated with goat anti-rabbit LI-COR IRDye 680 antibody (dilution 1:5,000). Densitometric analysis of bands was performed using LI-COR Odyssey@.

Quantitative RT-PCR analysis

Total RNA was extracted from Buffy coat or kidney tissue using Trizol reagent (Invitrogen Carlsbad, CA, USA). Total RNA concentration was measured at 260 nm (OD_{260}). Pure RNA possessed an OD_{260}/OD_{280} ratio of 1.6-1.9. Total RNA (0.25 ug) was reverse-transcribed to cDNA using random primer and High-capacity cDNA reverse transcription kits (Applied Biosystems, Foster City, California, USA) according to the manufacturer's instructions. Transcript expression was analyzed using quantitative PCR analysis with a Step One Plus™ (Applied Biosystems) using Tagman EXPRESS qPCR SuperMix Universal (Invitrogen). Values for each gene were normalized to the relative quantity of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA in each sample. The primers (Applied Biosystems) used for the PCR reaction are as follows: Assay ID; CAV-1; Rn00755834_m1, Assay ID; VEGFR-2 (Kdr); Rn00564986_m1, Assay ID; CD34; Rn03416140_m1, Assay ID; GAPDH; Rn99999916_s1 and Assay ID; VEGF; Rn01511601_m1.. The relative concentration of products was calculated using the comparative C_T ($_2^{-\Delta\Delta CT}$) method, according to the instructions supplied by Applied Biosystems.

Statistical analyses

Data were expressed as mean ± SD. The means of the different groups were evaluated by one-way analysis of variance (ANOVA) or unpaired t-test. The level of statistical significance was defined as $p<0.05$.

Results

Effect of EPO on renal dysfunction

All rats in the I/R injury group exhibited a significant increase in BUN and Cr concentrations when compared to sham, and also the increase in BUN and Cr in this group was higher than that in the other groups at day 1 and day 2, as shown in (Fig. 1A and 1B). However, significantly lower levels of BUN and Cr were detected in treatment groups, especially I/R + EPO treated rats, compared to that of I/R group at day 2 as shown in (Fig. 1A and 1B).

Effect of EPO on renal injury

Histological examination of the kidneys showed that there was extensive tubular damage in the I/R group at day 1 and day 2, consisting of tubular necrosis and proteinaceous cast. Damage was observed in the proximal tubular epithelial cells (TEC) in the outer stripe of the outer medulla and cortex (Table 1 and Fig. 2). The marked structural damage,

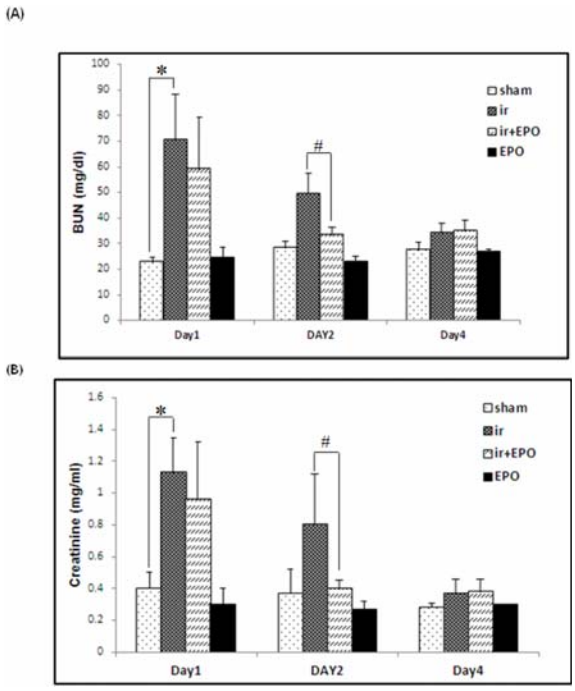

Fig. 1 EPO decreased levels of blood nitrogen urea (A) and serum creatinine (B) in I/R induced rats. Each histogram represents mean ± SE. * $p<0.05$ versus sham group (day 1), # $p<0.05$ versus IR group (day 2).

Table 1. Histological analysis of tubular necrosis, PTC congression and interstitial edema of the study groups

	Tubular epithelial cell necrosis	PTC congestion	Interstitial edema and infiltration
Day 1			
Sham	0.3±0.48	0.2±0.42	0.1±0.31
IR	3.4±0.52 *	2.9±0.56 *	2.2±0.42 *
IR + EPO	2.6±0.52 [a]	2.2±0.42 [a]	2.1±0.31
EPO	0.2±0.42	0.1±0.31	0.1±0.31
Day 2			
Sham	0.2±0.32	0.1±0.31	0.1±0.31
IR	3.3±0.48 [#]	3.2±0.78 [#]	2.2±0.42[#]
IR + EPO	2.5±0.52 [b]	2.1±0.31 [b]	1.6±0.51
EPO	0.1±0.31	0.1±0.31	0.1±0.31
Day 4			
Sham	0.1±0.31	0.1±0.31	0.1±0.31
IR	2.0±0.47 **	1.6±0.51 **	1.1±0.31 **
IR + EPO	1.3±0.48	0.7±0.48	0.7±0.48
EPO	0.1±0.31	0.1±0.31	0.1±0.31

All data are expressed as mean ± SE
* $p<0.05$ versus sham group (Day 1), [#] $p<0.05$ versus sham group (Day 2), ** $p<0.05$ versus sham group (Day 4), [a] $p<0.05$ versus IR group (Day 1), [b] $p<0.05$, versus IR group (Day 2)

included the widespread loss of brush border, a denudation of tubular cells, tubule dilatation and intratubular obstruction by granular casts, was presented. Moreover, the mean score of PTC congestion and interstitial edema in the outer medulla and cortex were also presented. These lesions were markedly different from those in the control kidney. In contrast, renal sections obtained from rats pretreated with EPO demonstrated a significantly decreased in tubular injury when compared with the I/R group at day 1 and day 2 ($p<0.05$).

Effect of EPO on circulating EPCs

Circulating EPCs measured from blood samples using real time PCR were performed to investigate whether the EPO pretreatment group was associated with increase in the number of circulating EPCs. RNA levels of EPCs marker genes, including CD34 and CAV-1, specific genes of EPCs representing for an attractive cell candidate for inducing angiogenesis, were investigated[19]. The expression of CD34 mRNA was markedly reduced in I/R group at day 1 and day 2, compared with sham group. In contrast, the expression of CD34 in pretreatment EPO group (I/R+EPO) at day2 was significantly increased, compared with I/R group ($p<0.05$), as shown in Fig. 3. The expression of CAV-1 levels in pretreatment EPO group (I/R+EPO) were higher than those I/R group, especially at day 2 where a significantly increased expression of CAV-1 gene was observed ($p<0.05$). These results suggested that, EPO increased the immobilization of EPCs into peripheral blood in EPO pretreatment group after reperfusion.

Effect of EPO on mobilization of EPCs in the kidney

EPCs measured in kidney samples were performed to investigate the mobilization of EPCs in kidney. RNA levels of EPCs marker genes, including CD34, Cav-1 and VEGFR-2, were analyzed. Lower levels of all of the EPCs marker gene expressions in I/R group and EPO pretreated group at day 1 were observed, compared with sham group. In contrast, higher levels of those gene expressions at day 2 were observed. Interestingly, VEGFR-2 and CAV-1 mRNA expression were significantly higher in EPO pretreated group compared with I/R group ($p<0.05$), as shown in Fig. 4. These results suggested that the accumulated EPCs in kidney are mediated by EPO.

Effect of EPO on expression of angiogenic factor in the kidney

Expression of VEGF on mRNA and protein levels were analyzed using realtime PCR and western blots, respectively. The VEGF expression levels in the

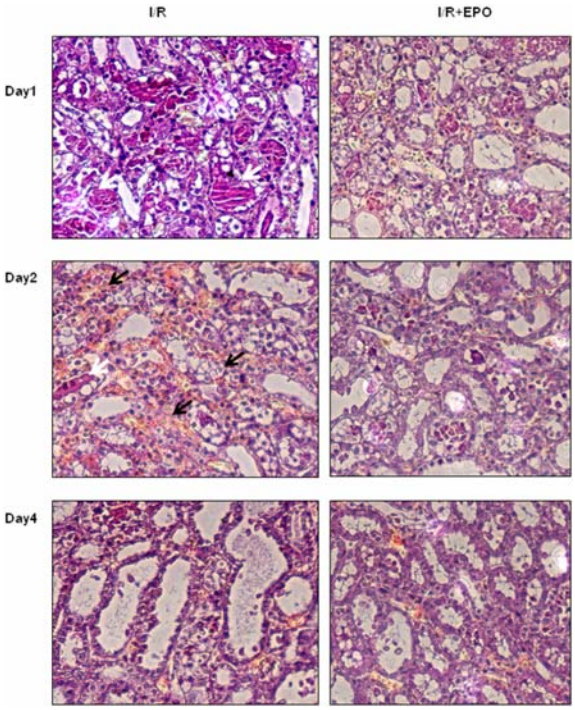

Fig. 2 Histopathological with H&E staining of rat kidney. Representative renal sections from I/R and I/R+EPO groups at Day 1, Day 2 and Day 4. Severe TEC necrosis, moderate PTC congestion (black arrows) and proteinaceous casts (white arrows) in the I/R group. EPO ameliorated renal histological damage. H&E staining, original magnification x400. PTC = peritubular capillaries, TEC = tubular epithelial cells.

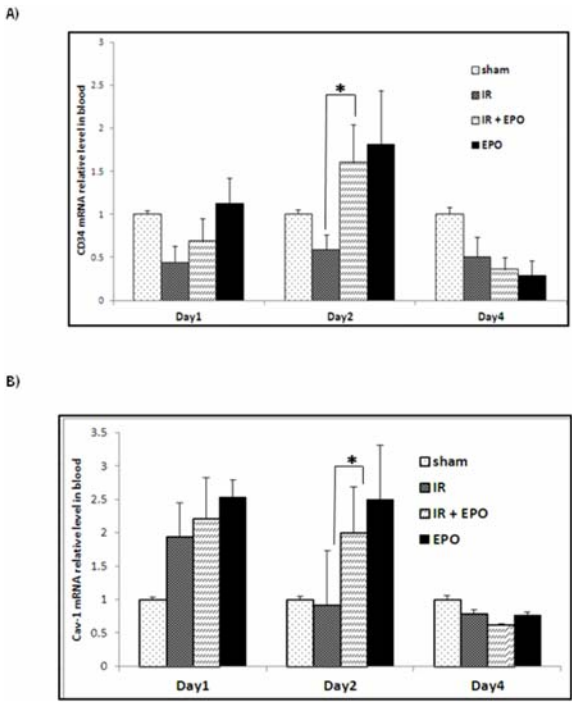

Fig. 3 Relative expression of circulation of EPCs; CD34 (A) and CAV-1 (B) mRNA. * $p<0.05$ versus IR (Day 2)

kidneys of I/R+EPO group were markedly increased compared with I/R group, especially at day 2, as shown in Fig. 5, 6. These result indicated that EPO induced increased the levels of paracrine factors in I/R rats pretreated with EPO.

Discussion

In the present study, we demonstrated that 3 daily pretreated with EPO prior to I/R induction can promote circulating EPCs and mobilization of EPCs in the kidney to stimulate angiogenesis in rats. We also evaluated for the first time the angiogenic potential of EPCs in renal I/R injury in rats by quantitating the expression of EPC-specific molecules, including CD34, CAV-1 VEGFR-2 using realtime PCR, in their peripheral blood and kidney, instead of using flow cytometer assay. These data demonstrate that EPCs recruitment and increasing the VEGF expression into I/R induced kidney can be modulated by daily pre-treatment with EPO for a prior period of 3 days before I/R induction in rats. The potential benefits of pretreatment with EPO may be not only dependent on increasing EPCs mobilization and paracrine effect by up regulating VEGF expression, which mediated in endothelial cell repair and the subsequent promotion of the process of angiogenesis[20], but also exert important anti-apoptosis actions[14,21] and anti-inflammation actions[22,23] in renoprotection in nephropathy.

AKI affects the structure and tubular endothelial cell dysfunction and microvascular injury during I/R injury which is a key importance for pathophysiological consequences of renal disease[24]. The injured epithelial cells lead to impairment of microcirculation and persistent endothelial damage is responsible for a delay functional recovery of the post-ischemic renal reperfusion and thereby prolongs kidney dysfunction[25-27]. These data suggested that the post-ischemic renal endothelial cell dysfunction is the important implication for the development of new therapeutic targets in the management of AKI. Several studies have been reported that EPCs may improve vascular regeneration in different ischemic organs, including atherosclerotic vascular diseas[28] and

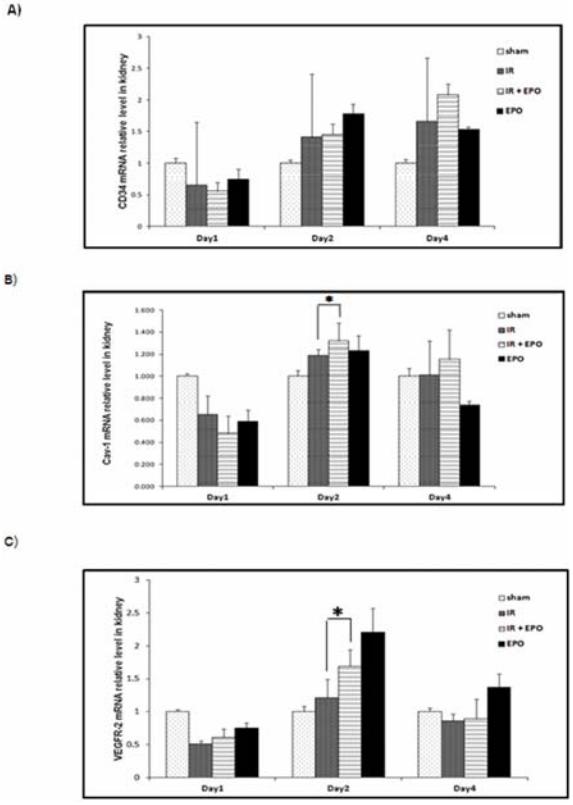

Fig. 4 Relative expression of mobilization of EPCs in the kidney; CD34 (A), CAV-1 (B), and VEGR-2 (C) mRNA. * p<0.05 versus IR (Day 2)

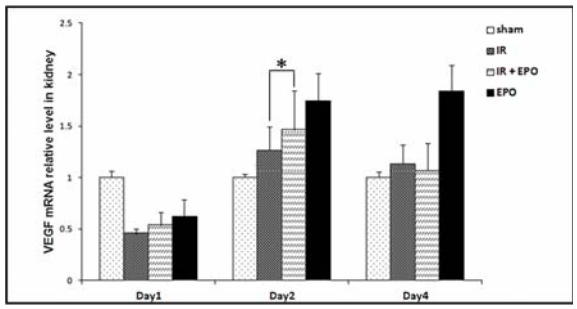

Fig. 5 Relative expression of angiogenesis marker in the kidney, VEGF mRNA. * p<0.05 versus IR (Day 2)

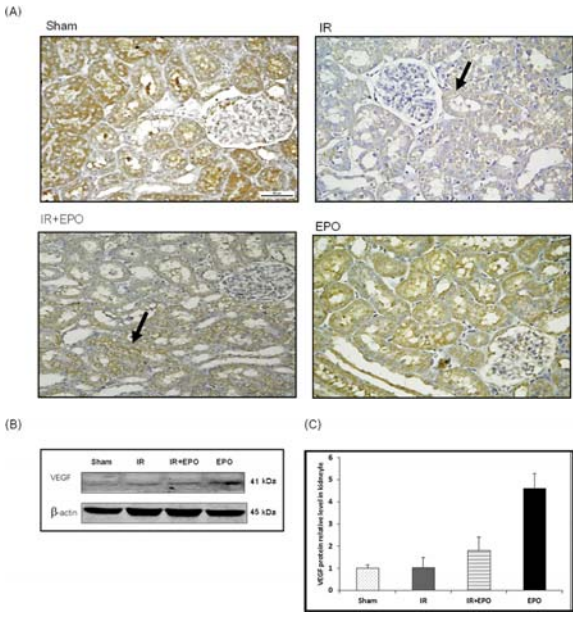

Fig. 6 Relative expression of angiogenesis in kidney; VEGF protein. (A) Immunohistochemical staining for VEGF, the tubular VEGF staining increased in IR+EPO group (black arrow) compared to IR group (black arrow). (B and C) Protein expression was assessed by Western blot analysis using B-actin as a sample loading control. VEGE level was higher in IR+EPO group compared with that in the IR or sham group (Day 2).

myocardial infraction[29]. Therefore, endothelial regeneration by EPCs playing a fundamental role in cell regeneration and vascular repair has been extensively studied in different model of renal disease. These data imply that a rapid EPCs mobilization after acute renal ischemia and an increased EPCs accumulation in ischemic kidney areas are able to maintain and repair of renal endothelium in acute ischemia and to promote angiogenesis and to secrete paracrine protein[5-7,11]. The bone marrow is alternative cellular source of progenitor cells to facilitate the form of circulating EPCs. Circulating EPCs are characterized by the expression of early hematopoitic stem cell markers CD34, CD133, and the vascular endothelial cell growth factor receptor-2 (VEGFR-2). These cells can differentiate into endothelial cells and are resident progenitors in vessel wall which can contribute to neovascularization and re-endothelialization. Several evidence have been reported that the potential of bone marrow derived EPCs to restore the renal microcirculation in animal model for glomerulonephritis[30].

A successful EPC-based therapeutic practice for acute ischemic renal injury with time point of administration or of activation of endogenous EPCs mobilization has been intensively investigated, especially in order to increase the number of circulating EPCs and improve the repair process. Several studies have investigated pharmacologic agents which

stimulate/modify endothelial progenitor cell number and/or function[31]. EPO, a well-known stimulator of EPC activation for therapeutic intervention, has been demonstrated the ability of EPO to mobilize EPCs in animal models[16,32]. These animal experiments have confirmed that endothelial cell proliferation of a number of cells markedly enhanced by using EPO application in a single dose of EPO (1,000 IU/kg) at time point reperfusion. However, Patel et al reported that daily pre-treatment with EPO (1,000 IU/kg/day, subcutaneously for 3 days) afford greater renoprotection in a mouse model of renal ischemia-reperfusion injury than a single dose (1,000 IU/kg subcutaneously) delivered at the time of reperfusion[33]. Therefore, in present study, a daily pre-treatment prior to the induction of renal ischemic reperfusion injury in rats. EPO 1,000 IU/kg/day for 3 days and on day 4, a single dose delivered in 30 min before I/R induction were designed in a protocol. The 3-day pretreatment period EPO has been used as a therapeutic strategy in a pre-clinical model of hemorrhagic shock, a state of global ischemia, which is able to attenuate the tissue and organ injury and dysfunction[34].

In present study, after EPO application, EPO treatment markedly increased the circulating EPCs and the accumulation of EPCs in injured kidney, as investigated using the expression level of EPCs genetic markers in peripheral blood and renal tissue, respectively. EPCs are classically described as cells expressing a combination of an endothelial marker (VEGFR-2) and a hematopoietic progenitor marker (CD34/CD133)[19,35]. However, it has been reported that co-expression of these markers may not fully specific for EPCs[36-38]. Indeed, several studies have demonstrated the sub-population of EPCs as called late EPCs which exhibit commitment to the endothelial lineage. These cells with characteristics of endothelial phenotype revealed high expression of VE-cadherin, claudin 11, caveolin 1 (CAV-1) and caveolin 2 (CAV-2) and directly incorporated them into the resident vasculature. Therefore, late EPCs have been informed that they might be an attractive cell candidate for inducing thereutic angiogenesis[39-41]. In the present study, this is the first time this precisely molecular fingerprint of EPCs has been used to demonstrate that the paracrine and angiogenesis manner of EPCs by investigating 3 cellular genetic markers of EPCs including CD34, CAV-1 and VEGFR2. The present study provides evidence that late EPCs seem to prefer to accumulate to the ischemic kidney. In addition to increasing of accumulation of EPCs into the kidney, pre-treatment with EPO also resulted in increased the levels of VEGF mRNA and protein expression in kidney compared with I/R group. Therefore, these results indicate that the effect of EPO therapy, including enhancing of EPCs accumulation in the ischemic kidney and upregulating local VEGF levels by the paracrine effect from localized EPCs[20], might provide angiogenesis and subsequent improvement in renal function.

Conclusion

This result suggests that treatment with EPO protects kidneys from ischemic acute renal injury via increasing the mobilization and recruitment of EPCs, resulting in the induction of expression of protein mediating vascular growth, VEGF, may play important roles to promote angiogenesis as well as to repair injured microvasculature. Therefore, the protective effect of EPO via EPCs accumulation in hypoxia kidney may find therapeutic application in the management of AKI.

What is already known on this topic?

EPO has also many therapeutic properties, for example it effects in many models of ischemia-reperfusion injury such as ischemic heart disease and ischemic spinal cord. EPO also acts as a stimulator of EPCs mobilization in response to hypoxia in order to promote angiogenesis.

What this study adds?

Pre-treatment with EPO for a period of 3 days before kidney ischemic reperfusion injury can modulate EPCs recruitment and promote paracrine effects for angiogenesis, which contribute to attenuating renal injury and its dysfunction.

Acknowledgements

The present study was supported by Thammasat University Research Fund.

Potential conflicts of interest

None.

References

1. Mehta RL, Pascual MT, Soroko S, Savage BR, Himmelfarb J, Ikizler TA, et al. Spectrum of acute renal failure in the intensive care unit: the PICARD experience. Kidney Int 2004; 66: 1613-21.
2. Hoste EA, Kellum JA, Katz NM, Rosner MH, Haase M, Ronco C. Epidemiology of acute kidney

injury. Contrib Nephrol 2010; 165: 1-8.
3. Xue JL, Daniels F, Star RA, Kimmel PL, Eggers PW, Molitoris BA, et al. Incidence and mortality of acute renal failure in Medicare beneficiaries, 1992 to 2001. J Am Soc Nephrol 2006; 17: 1135-42.
4. Kang DH, Kanellis J, Hugo C, Truong L, Anderson S, Kerjaschki D, et al. Role of the microvascular endothelium in progressive renal disease. J Am Soc Nephrol 2002; 13: 806-16.
5. Benigni A, Morigi M, Remuzzi G. Kidney regeneration. Lancet 2010; 375: 1310-7.
6. Herrera MB, Bussolati B, Bruno S, Fonsato V, Romanazzi GM, Camussi G. Mesenchymal stem cells contribute to the renal repair of acute tubular epithelial injury. Int J Mol Med 2004; 14: 1035-41.
7. Bussolati B, Bruno S, Grange C, Buttiglieri S, Deregibus MC, Cantino D, et al. Isolation of renal progenitor cells from adult human kidney. Am J Pathol 2005; 166: 545-55.
8. Urbich C, Aicher A, Heeschen C, Dernbach E, Hofmann WK, Zeiher AM, et al. Soluble factors released by endothelial progenitor cells promote migration of endothelial cells and cardiac resident progenitor cells. J Mol Cell Cardiol 2005; 39: 733-42.
9. Kwon O, Miller S, Li N, Khan A, Kadry Z, Uemura T. Bone marrow-derived endothelial progenitor cells and endothelial cells may contribute to endothelial repair in the kidney immediately after ischemia-reperfusion. J Histochem Cytochem 2010; 58: 687-94.
10. Patschan D, Plotkin M, Goligorsky MS. Therapeutic use of stem and endothelial progenitor cells in acute renal injury: ca ira. Curr Opin Pharmacol 2006; 6: 176-83.
11. Li B, Cohen A, Hudson TE, Motlagh D, Amrani DL, Duffield JS. Mobilized human hematopoietic stem/progenitor cells promote kidney repair after ischemia/reperfusion injury. Circulation 2010; 121: 2211-20.
12. Chade AR, Zhu X, Lavi R, Krier JD, Pislaru S, Simari RD, et al. Endothelial progenitor cells restore renal function in chronic experimental renovascular disease. Circulation 2009; 119: 547-57.
13. Brunner S, Winogradow J, Huber BC, Zaruba MM, Fischer R, David R, et al. Erythropoietin administration after myocardial infarction in mice attenuates ischemic cardiomyopathy associated with enhanced homing of bone marrow-derived progenitor cells via the CXCR-4/SDF-1 axis. FASEB J 2009; 23: 351-61.
14. Sharples EJ, Patel N, Brown P, Stewart K, Mota-Philipe H, Sheaff M, et al. Erythropoietin protects the kidney against the injury and dysfunction caused by ischemia-reperfusion. J Am Soc Nephrol 2004; 15: 2115-24.
15. Bahlmann FH, De Groot K, Spandau JM, Landry AL, Hertel B, Duckert T, et al. Erythropoietin regulates endothelial progenitor cells. Blood 2004; 103: 921-6.
16. Heeschen C, Aicher A, Lehmann R, Fichtlscherer S, Vasa M, Urbich C, et al. Erythropoietin is a potent physiologic stimulus for endothelial progenitor cell mobilization. Blood 2003; 102: 1340-6.
17. Hirano K, Wagner K, Mark P, Pittermann E, G□bel R, Furlani D, et al. Erythropoietin attenuates the sequels of ischaemic spinal cord injury with enhanced recruitment of CD34+ cells in mice. J Cell Mol Med 2012; 16: 1792-802.
18. Toprak O, Cirit M, Tanrisev M, Yazici C, Canoz O, Sipahioglu M, et al. Preventive effect of nebivolol on contrast-induced nephropathy in rats. Nephrol Dial Transplant 2008; 23: 853-9.
19. Asahara T, Murohara T, Sullivan A, Silver M, van der Zee R, Li T, et al. Isolation of putative progenitor endothelial cells for angiogenesis. Science 1997; 275: 964-7.
20. Chung AS, Lee J, Ferrara N. Targeting the tumour vasculature: insights from physiological angiogenesis. Nat Rev Cancer 2010; 10: 505-14.
21. Yang CW, Li C, Jung JY, Shin SJ, Choi BS, Lim SW, et al. Preconditioning with erythropoietin protects against subsequent ischemia-reperfusion injury in rat kidney. FASEB J 2003; 17: 1754-5.
22. Lee SH, Li C, Lim SW, Ahn KO, Choi BS, Kim YS, et al. Attenuation of interstitial inflammation and fibrosis by recombinant human erythropoietin in chronic cyclosporine nephropathy. Am J Nephrol 2005; 25: 64-76.
23. Kiris I, Kapan S, Kilbas A, Yilmaz N, Altunta□ I, Karahan N, et al. The protective effect of erythropoietin on renal injury induced by abdominal aortic-ischemia-reperfusion in rats. J Surg Res 2008; 149: 206-13.
24. Molitoris BA, Nelson WJ. Alterations in the establishment and maintenance of epithelial cell polarity as a basis for disease processes. J Clin Invest 1990; 85: 3-9.
25. Devarajan P. Update on mechanisms of ischemic acute kidney injury. J Am Soc Nephrol 2006; 17: 1503-20.
26. Bonventre JV, Weinberg JM. Recent advances in

the pathophysiology of ischemic acute renal failure. J Am Soc Nephrol 2003; 14: 2199-210.
27. Basile DP. The endothelial cell in ischemic acute kidney injury: implications for acute and chronic function. Kidney Int 2007; 72: 151-6.
28. Werner N, Kosiol S, Schiegl T, Ahlers P, Walenta K, Link A, et al. Circulating endothelial progenitor cells and cardiovascular outcomes. N Engl J Med 2005; 353: 999-1007.
29. Dai W, Hale SL, Martin BJ, Kuang JQ, Dow JS, Wold LE, et al. Allogeneic mesenchymal stem cell transplantation in postinfarcted rat myocardium: short- and long-term effects. Circulation 2005; 112: 214-23.
30. Li J, Deane JA, Campanale NV, Bertram JF, Ricardo SD. The contribution of bone marrow-derived cells to the development of renal interstitial fibrosis. Stem Cells 2007; 25: 697-706.
31. Bahlmann FH, Speer T, Fliser D. Endothelial progenitor cells in chronic kidney disease. Nephrol Dial Transplant 2010; 25: 341-6.
32. Bahlmann FH, DeGroot K, Duckert T, Niemczyk E, Bahlmann E, Boehm SM, et al. Endothelial progenitor cell proliferation and differentiation is regulated by erythropoietin. Kidney Int 2003; 64: 1648-52.
33. Patel NS, Sharples EJ, Cuzzocrea S, Chatterjee PK, Britti D, Yaqoob MM, et al. Pretreatment with EPO reduces the injury and dysfunction caused by ischemia/reperfusion in the mouse kidney in vivo. Kidney Int 2004; 66: 983-9.
34. Nandra KK, Collino M, Rogazzo M, Fantozzi R, Patel NS, Thiemermann C. Pharmacological preconditioning with erythropoietin attenuates the organ injury and dysfunction induced in a rat model of hemorrhagic shock. Dis Model Mech 2013; 6: 701-9.
35. Robb AO, Mills NL, Newby DE, Denison FC. Endothelial progenitor cells in pregnancy. Reproduction 2007; 133: 1-9.
36. Timmermans F, Plum J, Yoder MC, Ingram DA, Vandekerckhove B, Case J. Endothelial progenitor cells: identity defined? J Cell Mol Med 2009; 13: 87-102.
37. Barber CL, Iruela-Arispe ML. The ever-elusive endothelial progenitor cell: identities, functions and clinical implications. Pediatr Res 2006; 59: 26R-32R.
38. Case J, Mead LE, Bessler WK, Prater D, White HA, Saadatzadeh MR, et al. Human CD34+AC133+VEGFR-2+ cells are not endothelial progenitor cells but distinct, primitive hematopoietic progenitors. Exp Hematol 2007; 35: 1109-18.
39. Sieveking DP, Buckle A, Celermajer DS, Ng MK. Strikingly different angiogenic properties of endothelial progenitor cell subpopulations: insights from a novel human angiogenesis assay. J Am Coll Cardiol 2008; 51: 660-8.
40. Mukai N, Akahori T, Komaki M, Li Q, Kanayasu-Toyoda T, Ishii-Watabe A, et al. A comparison of the tube forming potentials of early and late endothelial progenitor cells. Exp Cell Res 2008; 314: 430-40.
41. Medina RJ, O'Neill CL, Sweeney M, Guduric-Fuchs J, Gardiner TA, Simpson DA, et al. Molecular analysis of endothelial progenitor cell (EPC) subtypes reveals two distinct cell populations with different identities. BMC Med Genomics 2010; 3: 18.

การได้รับอีรีโทรโพไออีตินส่งเสริมให้มีการสร้างสาร VEGF ในหนูที่เกิดภาวะไตถูกทำลายจากการขาดเลือด

สุปรานี กองคำ, สิริวรรณ ศรีวงค์, อดิศว์ ทัศณรงค์

ภูมิหลัง: ภาวะไตถูกทำลายจากการขาดเลือดเป็นสาเหตุหลักของภาวะไตวายเฉียบพลันในเวชปฏิบัติพบว่าการเปลี่ยนแปลงในระหว่างการเกิดภาวะการขาดเลือดจะส่งผลให้เซลล์เยื่อบุท่อไตและเซลล์เอนโดธีเลียลหลอดเลือดไตถูกทำลายและเกิดการบาดเจ็บขึ้น จากการศึกษาในสัตว์ทดลองพบว่าอีรีโทรโพไออีติน (อีพีโอ) สามารถใช้ป้องกันการเกิดพยาธิสภาพที่ไตเมื่อไตถูกเหนี่ยวนำให้เกิดการขาดเลือดและได้รับเลือดกลับฟื้นมาหล่อเลี้ยง

วัตถุประสงค์: เพื่อประเมินประสิทธิภาพของอีพีโอในกระตุ้นการเคลื่อนย้ายของเซลล์ต้นกำเนิดเอนโดธีเลียล และช่วยกระตุ้นการสร้างเลือดใหม่เพื่อการลดหรือป้องกันจากการถูกทำลายและบาดเจ็บของท่อไตในหนูที่ถูกเหนี่ยวนำภาวะไตวายเฉียบพลันจากการขาดเลือด

วัสดุและวิธีการ: หนู Sprague-Dawley ได้รับอีพีโอ (1,000 IU/กิโลกรัม/วัน) เป็นเวลา 3 วันและ 30 นาที ก่อนการเหนี่ยวนำให้เกิดภาวะไตถูกทำลายจากการขาดเลือด จัดเก็บเลือดและเนื้อไตหนู ณ วันที่ 1, 2 และ 4 เพื่อวิเคราะห์การหน้าที่ของไต พยาธิสภาพที่เกิดขึ้นที่เนื้อไต การเปลี่ยนแปลงของจีน CD34, VEGFR-2, CAV-1, VEGF และโปรตีน โดยใช้วิธี realtime PCR และ western blotting

ผลการศึกษา: จากการศึกษาพบว่าหนูกลุ่มที่เกิดภาวะไตถูกทำลายจากการขาดเลือดมีระดับยูเรียและครีเอตรีนินสูงอย่างมีนัยสำคัญทางสถิติ เมื่อเปรียบเทียบกับกลุ่ม sham และการตรวจพยาธิสภาพที่เนื้อไตพบว่าเซลล์ท่อไตมีการถูกทำลาย หลอดเลือดแดงเล็กที่อยู่รอบ ๆ ท่อไตมีการขยายตัวและมีเม็ดเลือดแดงคั่งอยู่เป็นจำนวนมาก พบการบวมและเซลล์อักเสบเพิ่มขึ้นในบริเวณ interstitium ส่วนหนูกลุ่มที่ได้รับอีพีโอก่อนการเหนี่ยวนำให้เกิดภาวะไตถูกทำลายจากขาดเลือด พบว่า ณ วันที่ 2 ผลตรวจการทำหน้าที่ไตให้ผลดีขึ้นอย่างมีนัยสำคัญและพยาธิสภาพที่เนื้อไต แสดงถึงการถูกทำลายและบาดเจ็บลดลงอย่างมีนัยสำคัญร่วมกับการแสดงออกของจีน CD34 และ CAV-1 ในเลือด รวมกับจีน CD34, VEGFR-2, CAV-1 ในไตและโปรตีน VEGF ในเนื้อเยื่อของไตที่เพิ่มขึ้นในหนูกลุ่มนี้ เมื่อเทียบกับหนูที่ได้รับการบาดเจ็บอย่างเดียว

สรุป: การให้อีพีโอก่อนการเหนี่ยวนำให้เกิดไตวายเฉียบพลันจากการขาดเลือดช่วยเพิ่มระดับ EPCs ในเลือดเลือดและในไตที่เกิดบาดเจ็บจากการขาดเลือดและมีส่วนเพิ่มการหลั่งสารปัจจัยซึ่งช่วยส่งเสริมการสร้างหลอดเลือดใหม่ เช่น VEGF ส่งผลให้สามารถช่วยป้องกันการถูกทำลาย และลดการสูญเสียหน้าที่ของไตจากภาวะขาดเลือดได้