SEMINARS IN
NEPHROLOGY

Erythropoietin and Acute Renal Failure

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The hemopoietic growth factor erythropoietin (EPO) has been recognized to be a multifunctional cytokine that plays a key role in ischemic preconditioning in the brain and heart. The EPO receptor is expressed widely in the kidney, and we review the important findings from the use of EPO in experimental models of acute renal failure that show that EPO reduces tubular cell death and hence the dysfunction induced by ischemia reperfusion injury, and we explore how these observations may be translated into the clinical arena.

KEYWORDS acute renal failure

Acute renal failure (ARF), characterized by a sudden deterioration of renal function over a period of hours or days, resulting in the failure of the kidney to excrete nitrogenous waste products and to maintain both electrolyte and fluid homeostasis, complicates approximately 7% of all hospital admissions in patients older than 40 years.[1]

The frequency of ARF varies greatly depending on the clinical setting, the age of the patient, and, particularly, the definition of renal insufficiency used. In certain scenarios, such as cardiac surgery requiring cardiopulmonary bypass, the frequency of ARF may be as high as 15%.[2] Hou et al[3] showed an incidence of ARF of 5% (109 of 2,216 medical and surgical patients), associated with decreased renal perfusion (42%), major surgery (18%), radiocontrast exposure (12%), and aminoglycoside administration (7%). Predictors of poor prognosis included oliguria and relatively modest degrees of renal dysfunction.

The classification and assessment of severity of ARF can be difficult to determine, and this hinders the design and recruitment to randomized clinical trials using novel agents identified in animal studies. These issues of classification also are hampered by the lack of accurate sensitive biomarkers to allow early detection of alterations in glomerular filtration rate (GFR). The recognition that serum creatinine is a relatively poor marker of acute changes in GFR has led to interest in novel biomarkers, including cystatin c and neutrophil gelatinase-associated lipocalin (NGAL).[4,5]

The severity of renal dysfunction may determine the natural history and patient outcome in ARF. Few studies have examined the association between small changes in serum creatinine level and patient outcome. Epidemiologic studies can show only an association between ARF and mortality, especially in (intensive therapy unit) ITU studies, because ARF often is part of the spectrum of multi-organ failure. However, published studies have shown a consistently increased relative risk associated with ARF despite adjustment for comorbid conditions and severity of illness. Levy et al[6] compared 183 patients with radiocontrast-associated ARF and 174 age-matched patients who received similar radiocontrast loads without developing ARF. The mortality rate was 34% in patients with ARF versus 7% in those patients without ARF. Adjusting for differences in comorbidity, the odds of death were increased 5.5-fold in the ARF group. This high relative risk has been shown for a more heterogenous cohort of patients. Chertow et al[7] recently showed that, in a study of 19,201 admissions to a large urban hospital, even a 50% increase in serum creatinine level was associated with an increased mortality adjusted odds ratio of 5.8 (confidence limits 4.6-7.5), with similar increases in mortality observed with only a 0.3 mg/dL increase in serum creatinine level.

Improvements in supportive therapy, and increased access to hemofiltration and ITU support, have not led to a reduction in the mortality associated with the development of ARF.[8] Currently therapy is limited to supportive and, in certain situations such as radiocontrast nephropathy, preventative strategies. There still is, therefore, an urgent need to discover new agents to alter the natural history of ARF. Despite a large number of agents that have proved successful in animal models of renal ischemia, the results of the clinical trials of these agents, such as insulin-like growth factor-1, have been disappointing.[9,10]

Improved understanding of the pathologic mechanisms of

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experimental acute kidney injury, and how the kidney responds and undergoes repair after an insult, is required to determine which new therapies may be effective when translated into clinical trials. In light of this knowledge, we review the recent literature investigating the potential use of erythropoietin (EPO) as a therapy in ARF.

A Model of Pathophysiology of Ischemic ARF

Prolonged renal ischemia initiates epithelial and vascular cell injury, resulting in a rapid decrease in GFR, correlating with the clinical initiation phase of ARF. The extent of cellular injury depends on the severity and duration of the ischemic insult. Sublethal insults disrupt the ability of vascular endothelial cells and tubular epithelial cells to maintain normal homeostatic processes, and initiate an inflammatory milieu that contributes to the extent of injury. In the commonly studied standard small rodent models of renal artery clamping, the S3 segment of the proximal tubule that traverses the outer-medullary segment, and the medullary thick ascending limb are extremely susceptible to ischemic injury. The sensitivity of the outer medulla derives from a combination of its microvascular architecture and a relatively low glycolytic capacity to generate adenosine triphosphate in the setting of rapid adenosine triphosphate (ATP) depletion resulting from impaired oxidative phosphorylation. The medullary thick ascending limb of the loop of Henle, although situated in the same region, does not undergo the same extent of cell death because there is a greater glycolytic capacity to generate adenosine triphosphate under ischemic conditions. The initiation phase is followed immediately by a phase that recently has been termed the *extension* phase.[11] During the extension phase, persistent hypoxia and hypoperfusion lead to worsening of epithelial and endothelial cell injury and cell death, primarily in the corticomedullary region of the kidney. The maintenance phase represents a phase of stabilization of injury, and subsequent correcting events leading to cellular repair, division, and redifferentiation. This sets the stage for improved epithelial and endothelial cell function and recovery of GFR during the recovery phase.

EPO

EPO is a glycoprotein hormone with a molecular weight of 30.4 kd. The gene for EPO, situated on chromosome 7q11 to 22, consists of 5 exons and 4 introns, and encodes a protein precursor of 193 amino acids. During posttranslational modification, which consists of cleavage of a 27 amino acid sequence, glycosylation of 3 *N*-linked (at Asn-24, Asn-38, and Asn-83) and 1 *O*-linked (ser-126) amino acids, the removal of arginine residue (Arg-166) from the C-terminal end yields the final circulating EPO molecule comprising 165 amino acids.[12] The tertiary structure of erythropoietin is defined by 4 antiparallel α-helices. EPO was purified successfully by Miyake et al[13] from the urine of a patient with aplastic anemia.[14] From tryptic fragments of this urinary EPO, DNA probes were synthesized for the isolation and cloning of the human EPO gene. Clinical trials of the use of recombinant human EPO in patients with anemia associated with end-stage renal failure commenced shortly after,[15,16] leading rapidly to universal uptake of EPO as standard therapy.

In adults, EPO secretion is primarily from the kidney, regulated in response to hypoxia, to maintain an appropriate red cell mass to manage normal tissue oxygen demand. Renal production is restricted to a population of cells in the interstitium of the cortex and outer medulla. Immunohistochemical characterization of EPO-producing cells by light and electron microscopy shows that they are fibroblast-like type I interstitial cells.[17] The basal level of EPO secretion in the picomolar range maintains a plasma concentration equivalent to 15 to 25 IU/ L. The effects of erythropoietin on the erythroid components of bone marrow are mediated by binding to specific receptors on erythroid precursors (intermediate-stage erythroid burst-forming units and the erythroid colony-forming units), which already have differentiated from pluripotent stem cells. Differentiation to this stage is not dependent on EPO because EPO receptor knock-out mice are incapable of erythropoiesis but have committed erythroid burst-forming units and erythroid colony-forming units in fetal liver tissue.[18] In the absence of EPO, the erythroid progenitors undergo apoptotic cell death.[19] EPO can support efficiently the proliferation of murine erythroid progenitor cells ex vivo, and induce entry into the cell cycle in dormant cells.[20]

EPO gene expression is under the control of the oxygen-sensitive transcription factor hypoxia-inducible factor (HIF-1), which consists of the regulatory subunit HIF-1α and the constitutively expressed subunit HIF-1β. Both subunits are members of multiprotein families and belong to the extended family of basic helix-loop-helix Per-Arnt-Sim homology (PAS) domain transcription factors. Low oxygen tension adverts enzymatic prolyl-residue hydroxylation by prolyl-4-hydroxylase, which, in normoxia, serves as a signal for von Hippel-Lindau–dependent polyubiquitination and proteosomal degradation, thereby preventing HIF degradation, leading to nuclear accumulation of HIF-1. The von Hippel-Lindau gene product is the recognition component of a multiprotein E3 ubiquitin-ligase complex that captures HIF 1α chains that have undergone enzymatic prolyl hydroxylation.[21]

HIF activation has a diverse range of effects on the expression of several cytokines that mediate the adaptive response to stress and ischemia, including pro-angiogenesis hormone vascular endothelial growth factor, glucose transporters (GLUT1) and glycolytic enzymes, iron metabolism (transferrin), and a variety of genes involved in cellular proliferation, differentiation, and viability.[22] The prolyl-4-hydroxylase requires iron as a cofactor, and administration of cobalt mimics the effect of hypoxia with upregulation of HIF-1α–dependent genes, including EPO.

There has been interest in the role of the HIF/EPO axis in ischemic preconditioning. Evidence supporting the essential role of EPO in delayed ischemic preconditioning in the heart comes from work in transgenic HIF−/+ mice, which express

only small amounts of constitutively expressed HIF-1, and are resistant to the beneficial effects of an ischemic preconditioning protocol in a model of myocardial ischemia.[23] Although there is debate about whether the kidney benefits from this type of preconditioning, cobalt administration to rats diminished the degree of renal injury caused by ischemia-reperfusion,[24] suggesting that the HIF-dependent production of adaptive mediators, particularly EPO, may play an important role in renal protection by ischemic preconditioning.

Erythropoietin and Ischemic Injury in the Brain and Heart

Both EPO and the EPO receptor are expressed functionally in the nervous system of rodents, primates, and human beings. In the mouse, EPO is present in the hippocampus, capsula interna, cortex, and midbrain areas.[25] In human beings, EPO and the EPO receptor are expressed in both astrocytes and neurones, although the level of expression varies according to gestation age, with reduced production after birth.[26]

Oxygen deficiency results in the induction of EPO in brain tissues. Accumulation of EPO messenger RNA and the EPO protein in response to hypoxia has been observed in cultured astrocytes. EPO and the EPO receptor also are inducible in the hippocampal neurones, although in vivo models show a broader upregulation during hypoxia.[27] Initial experiments therefore have examined the potential role of EPO in the nervous system during cerebral ischemia. Infusion of EPO into the lateral ventricle of gerbils subjected to the occlusion of the common carotid arteries prevented ischemia-induced learning disability and rescued hippocampal neurones from degeneration.[28] Several studies have confirmed the beneficial effects of EPO administration in the course of ischemic brain injury in vivo, using systemic administration to overcome the impracticalities of ventricular delivery systems.[29,30]

Ischemia-related investigations examining the potential of EPO to prevent neuronal injury also have been extended to the spinal cord, peripheral nervous system, and visual system. Systemic administration of EPO before or immediately after retinal insult protects retinal ganglion cells from apoptosis and promotes the recovery of retinal function in mice.[31] These findings led to the experimental use of EPO in models of myocardial infarction. Several studies have shown that EPO reduces infarct size, and reduces the degree of apoptosis, with beneficial effects on left ventricular function and remodeling at later time points.[32,33]

The discovery by Westenfelder et al[34] in 1999 that the EPO receptor is expressed throughout the kidney, including tubular epithelium, the glomerulus, and in mesangial cells, and that, in culture, proximal tubule epithelial cells proliferated in response to EPO, led a number of groups to examine the effects of EPO on the natural history of ischemia-reperfusion injury to the kidney.

In Vivo Evidence for the Protective Role of EPO in Renal Ischemic Injury

Several studies of the adverse effect of anemia on the course of ARF and the response to EPO were undertaken in the early 1990s. Nemato et al[35] administered a low- and high-dose EPO protocol (500/kg to 3,000 u/kg) to rats subjected to unilateral nephrectomy and ischemia-reperfusion injury. EPO administration was associated with an increased hematocrit level, and there was a trend to increased survival in the high-dose EPO-treated animals. In comparison with latter studies, the rats used in these experiments only weighed 200 g, in a relatively severe injury model with a high animal mortality rate, which may have caused the relative lack of observed effect of EPO on renal dysfunction.

Yang et al[36] administered high-dose EPO (3,000 U/kg) intraperitoneally as a single dose 24 hours before the onset of ischemia to simulate preconditioning before acute renal ischemia-reperfusion injury. EPO preconditioning significantly attenuated the degree of renal dysfunction (serum creatinine) observed by 24 hours, which was associated with a reduced proximal tubular epithelial cell death, measured by terminal deoxynucleotidyl-mediated deoxyuridine triphosphate nick end labeling (TUNEL) staining. EPO administration caused an increase in the expression of the antiapoptotic modulators $Bcl\text{-}_2$ and heat shock protein-70 in both sham-operated and animals subjected to ischemia reperfusion, with a concurrent reduction in the activation of stress-activated mitogen activation stress kinase c-jun N-terminal kinases (JNK) after renal ischemia.

Vesey et al[37] showed that EPO was effective when administered only 30 minutes before the initiation of ischemia, in a model in which Sprague-Dawley rats underwent nephrectomy and unilateral ischemia (30 min). A single high-dose EPO regimen (5,000 U/kg intraperitoneally) attenuated the increase in creatinine level observed at 24 hours, and the difference between treatment groups was maintained at 72 hours. EPO significantly reduced apoptotic cell death in the outer medullary thick ascending limb and S_3 segment of the proximal tubule. The decrease in cell death was associated with evidence of an effect on tubular proliferation, with increased proximal tubule epithelial cell mitosis seen at 24 hours. This enhanced tubular regeneration associated with EPO was confirmed by increased tubular staining for proliferating cell nuclear antigen. This apparent effect of a single preischemia administration on tubular recovery is an important potential mechanism by which EPO may act in the recovery phase of ARF, even when solely administered as a bolus pretreatment.

We have studied the effects of several different EPO protocols on the course of ARF in an established short-term rat model of bilateral ischemia-reperfusion injury.[38] Low-dose EPO was administered as a single intravenous bolus (300 U/kg) to 3 treatment groups: as a 30-minute pretreatment, at the time of reperfusion, or 30 minutes after the onset of reperfusion, to determine which protocol offered the best

organ protection. A single dose was studied to determine the efficacy of the timing of treatments. All 3 EPO-treated groups showed significant reduction in the degree of renal dysfunction observed at 6 hours, although administration 30 minutes after reperfusion was associated with lesser, but significant, functional and histologic protection. The improvement in renal function and preservation of normal tubular architecture on histologic examination was associated with a significant reduction in tissue caspase-3 activity, using immunohistochemistry for the active fragment of caspase-3 and quantitative analysis of apoptotic cell death in proximal tubule segments.[39] We also have observed that EPO therapy given as late as 2 hours into the reperfusion period still is associated with significant functional protection at 24 hours (Sharples EJ, unpublished data). A beneficial response to late initiation of EPO treatment has been observed in models of myocardial infarction and stroke, although there may be an important effect of dose on these late effects because Moon et al[40] showed only a beneficial effect 12 hours after reperfusion in those animals treated with very high EPO dosages.

These findings are encouraging, however, because even administration late in the clinical presentation may influence the subsequent natural history of acute ischemic injury, and allow those patients who present late to benefit from therapy.

Does EPO Solely Act on the Proximal Tubular Epithelium?

Although these studies have concentrated on the effects of EPO administration on tubular cell injury, the endothelium plays an important role in the development and maintenance of ischemic renal injury.

A decrease in renal blood flow is of critical importance in initiating and extending the pathophysiology of ischemic ARF. Under physiologic conditions, the oxygen tension of the kidney decreases from the outer cortex to the inner medulla.[41] The blood flow to the outer medullary or corticomedullary junction remains approximately 10% of normal during early reperfusion, leading to congestion caused by interstitial edema, red blood cell trapping, leukocyte adherence, and extravasation.[42] Permanent damage to the peritubular capillaries occurs in rats subjected to prolonged renal ischemia, and this may be associated with the development of tubulointerstitial fibrosis and poor urine concentrating ability in the postischemic kidney.[43]

Interventions designed to reverse endothelial dysfunction have been shown to minimize subsequent renal injury. Transplantation and engraftment of functionally mature endothelial cells into the circulation of postischemic rats protected the kidney from ischemic injury. In the same study, a reduction in renal dysfunction also was observed after transplantation of human embryonic kidney cells (HEK293) stably transfected with human endothelial nitric oxide synthase (eNOS), showing the importance of eNOS-derived NO in the maintenance of endothelial integrity.[44]

Capillary endothelial cells express the EPO receptor in their intraluminal surfaces. EPO antagonizes apoptosis of endothelial cells subjected to hypoxic stress in vitro,[45] and therefore might play a role in maintaining the integrity of the microvasculature. EPO also upregulates the expression of eNOS in endothelial cells, and might rapidly increase eNOS activity via protein kinase B (AKT)-dependent eNOS phosphorylation.[46] Through this mechanism, EPO may maintain normal vascular autoregulation, thereby preventing amplification of tubular hypoxia. The effects of EPO on endothelial cell survival and function are likely to be a major component of the mechanism of organ protection. These beneficial effects of EPO on the endothelium may be dose dependent because higher doses of EPO reduce the activity of dimethylarginine dimethylaminohydrolase, the enzyme that degrades asymmetric dimethylarginine, which is an endogenous inhibitor of eNOS and accumulation of asymmetric dimethylarginine is associated with oxidative stress and endothelial dysfunction.[47]

EPO also has been shown to stimulate endothelial cell mitogenesis and angiogenesis, which improve tissue oxygenation. EPO stimulates revascularization and healing in animal models of ischemic skin flaps.[48] Studies in mice and human beings have shown that EPO is a potent stimulator of endothelial progenitor cell mobilization from the bone marrow, increasing the number of circulating endothelial progenitor cells, which may play a role in regeneration of damaged endothelium.[49,50] A 3-day subcutaneous preconditioning EPO regimen (1,000 U/kg EPO per day), based on the regimen known to stimulate production of endothelial progenitor cells, was compared with a single subcutaneous administration of EPO (1,000 U/kg) at the time of reperfusion in a murine model of ischemia-reperfusion injury.[51] Male C57BL/6J mice weighing 25 to 30 g underwent 30-minute bilateral renal clamping followed by 24 hours of reperfusion. Both protocols conferred significant protection from ischemia-reperfusion injury, although a greater reduction in injury marker intensity was observed in the preconditioned group. Given the half-life of EPO, this is unlikely to be merely a dose effect, and suggests that genomic pathways induced by EPO are crucial to its effects. That mobilization of endothelial progenitor cells and either maintenance or repair of endothelial integrity are involved in the protective effect of EPO in this model is an attractive hypothesis. Further work is required to determine the contribution of endothelial progenitor cell mobilization and preservation of capillary architecture to the minimization of renal dysfunction after ischemia.

Does EPO Have Similar Effects in Other Models of Experimental Renal Failure?

Vaziri et al[52] previously had used EPO in a model of cisplatin nephrotoxicity (7 mg/kg intraperitoneally) in Sprague-Dawley rats, and then administered EPO 100 U/kg/d for 9 days. EPO significantly increased the calculated creatinine clearance at day 9 when compared with vehicle-treated animals. The early improvement in creatinine clearance in EPO-treated animals was maintained over the course of 6 weeks, at

which point full functional recovery occurred in the vehicle-treated animals. The enhanced functional recovery was accompanied by increased [^{3}H]thymidine incorporation as a marker of increased tubular regeneration.

The long-term benefit of reducing the severity of initial kidney injury also has been shown to reduce progressive dysfunction in a rat remnant kidney model. Sprague-Dawley rats underwent a single-stage 5/6th nephrectomy procedure, and were followed-up for 6 weeks. Darbopoetin (0.1 μg/kg) subcutaneously administered once weekly significantly reduced apoptotic cell death between days 4 and 14 after surgery, associated with persistent AKT phosphorylation, and this reduction in apoptosis led to partial preservation of renal function at 6 weeks when compared with vehicle-treated animals.[53]

The angiogenic effects of EPO and the synergistic interaction with vascular endothelial growth factor on vessel generation observed in vivo also may play an important role in remodeling peritubular capillary networks after ischemic injury.[54] Rarefaction of the networks can be seen in the remnant model, but also occurs after ischemic injury, and contributes to persistent tissue hypoxia and promotion of fibrosis.[43] Bahlmann et al[53] showed that EPO maintained peritubular capillary density in the remnant kidney by immunohistochemical staining for CD31.

Mechanisms of Cellular Protection by EPO

Much of the pathways activated by EPO binding to its membrane-bound homodimeric receptor have been established in hematopoietic cells.[12]

Fishbane et al[55] exposed porcine kidney epithelial cells (LLC-PK$_1$) to several insults with and without darbopoetin (50 ng/mL). Darbopoetin significantly reduced apoptotic cell death in response to 16 hours of hypoxia (1% oxygen). Similar experiments performed with an inactive recombinant EPO molecule did not offer protection. Vesey et al[37] showed that high doses of EPO reduced hypoxia-induced cell death in primary proximal tubular epithelial cells. These studies did not address the mechanism by which EPO exerts these effects.

We have examined the effects of EPO on immortalized human proximal tubular epithelial cells (HK-2) in a variety of experimental settings. EPO caused a dose-dependent (10-100 U/mL) increase in cell viability in serum-deprived cells, associated with a reduction in DNA fragmentation observed at 24 and 48 hours. EPO increased the expression of several antiapoptotic proteins, including Bcl-X$_L$ and XIAP, and prevented the activation of caspase-3.[39] There is evidence that the phosphatidylinositol 3-kinase/AKT pathway is involved in EPO-dependent cell survival with the demonstration that the phosphatidylinositol 3-kinase inhibitor LY294002 inhibits the protective effects of EPO in these models. AKT phosphorylates multiple targets that influence apoptotic signaling. These preliminary investigations allow us to develop a paradigm for the multiple effects of EPO that may contribute to the reduction in ischemic injury (Fig 1).

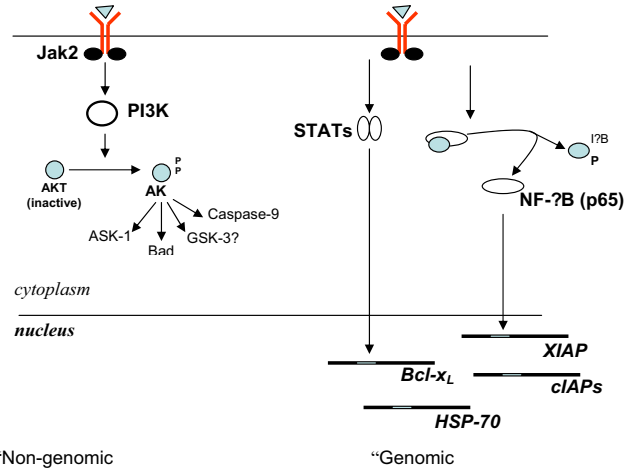

Figure 1 Potential mechanism of antiapoptotic effects of erythropoietin. EPO and the EPO receptor prevent apoptosis and cellular inflammation through a series of pathways that originate with autophosphorylation and activation of Janus kinase-2 (JAK2) after binding of EPO to its receptor. JAK2 phosphorylates several tyrosine residues on the intracellular portion of the receptor, facilitating binding of proteins containing src-homology (SH-2) domains. The p85 subunit of phosphatidylinositol-3 kinase (PI3K) interacts with the receptor, possibly via scaffolding proteins, and this leads to phosphorylation of protein kinase B (AKT). AKT has multiple effects on cell survival by maintaining mitochondrial integrity directly and through the inhibition of several pro-apoptotic mediators including Bad, caspase-9, and GSK-3, and inhibits the activation of c-jun N-terminal kinases by stabilization of ASK-1. Via JAK2, EPO activates members of the signal transducer and activator of transcription (STAT) family of transcription factors that enhance cell proliferation and survival. The STAT member activated appears to be cell-type specific. Activation of the transcription factor nuclear factor-κB is dependent on JAK2 activity, and might require phosphorylation of inhibitor of kappa β-alpha (IκB) kinase by AKT. NFκB induces expression of endogenous inhibitors of apoptosis, including X-linked inhibitor of apoptosis, which inhibits caspase-3, caspase-7, and caspase-9. EPO maintains mitochondrial membrane integrity and prevents apoptosis by enhancing expression of Bcl-X$_L$, which interacts with the pro-apoptotic bcl-2 homology region 3 (BH3) protein Bax. This interaction prevents the release of cytochrome C and activation of caspase-9 and caspase-3. (Color version of figure is available online.)

Can These Experimental Findings Be Translated to Clinical Practice?

There now is experimental evidence from these rodent models of ARF that EPO can reduce the severity of acute kidney injury induced by ischemia reperfusion, systemic shock, and nephrotoxic insults, and also may contribute to the process of tubular regeneration through direct effects on tubular epithelial cells. It has been established that EPO exerts direct effects on both endothelial and tubular epithelial cells that contrib-

ute to the reduction in organ damage, although the relative importance of the site of action remains to be elucidated.

It is still to be determined which of the different treatment strategies used in these models may translate best to human beings, and particularly the timing of intervention and how late in the natural history of ARF can EPO still achieve a significant response are of great interest. The doses used in several early trials (3-5,000 U/kg) would equate to massive doses in human beings, with particular concerns on the adverse effects on polycythemia, endothelial dysfunction, and thrombogenicity, although it seems as though significantly lower doses, such as those used by Vaziri et al[52] and Bahlmann et al,[53] would have similar therapeutic potential. The successful outcome of a proof-of-concept trial in stroke published in 2002 should encourage clinical trials in ARF.[56] The introduction of new derivatives of EPO that do not significantly affect hemopoiesis may make these agents attractive for use in clinical trials, but standard EPO formulations have the advantage of long-term patient safety data, familiarity, and availability.[57,58]

EPO therapy does suffer from potential side effects. Recently, the observation that a large number of tumors and tumor cell lines express a functional EPO receptor,[59,60] and the early stoppage of a trial of EPO in patients with head and neck cancers because of increased mortality rates,[61] have led to a great deal of debate over the potential use of EPO in new clinical arenas. As yet, the scientific studies have not come to a conclusion, with some evidence suggesting a synergistic effect of EPO and conventional chemotherapy in some tumors.[62]

The management of the patient with ARF remains a major challenge, with significant morbidity and mortality. The experimental evidence suggests that EPO may open a new direction in the treatment of these patients.

References

1. Nash K, Hafeez A, Hou S: Hospital-acquired renal insufficiency. Am J Kidney Dis 39:930-936, 2002
2. Zanardo G, Michielon P, Paccagnella A, et al: Acute renal failure in the patient undergoing cardiac operation. Prevalence, mortality rate, and main risk factors. J Thorac Cardiovasc Surg 107:1489-1495, 1994
3. Hou SH, Bushinsky DA, Wish JB, et al: Hospital-acquired renal insufficiency: A prospective study. Am J Med 74:243-248, 1983
4. Herget-Rosenthal S, Marggraf G, Husing J, et al: Early detection of acute renal failure by serum cystatin C. Kidney Int 66:1115-1122, 2004
5. Mishra J, Dent C, Tarabishi R, et al: Neutrophil gelatinase-associated lipocalin (NGAL) as a biomarker for acute renal injury after cardiac surgery. Lancet 365:1231-1238, 2005
6. Levy EM, Viscoli CM, Horwitz RI: Effect of acute renal failure on mortality. A cohort analysis. JAMA 275:1489-1494, 1996
7. Chertow GM, Burdick E, Honour M, et al: Acute kidney injury, mortality, length of stay, and costs in hospitalized patients. J Am Soc Nephrol 16:3365-3370, 2005
8. Liangos O, Wald R, O'Bell JW, et al: Epidemiology and outcomes of acute renal failure in hospitalised patients: a national survey. Clin J Am Soc Nephrol 1:43-51, 2006
9. Hladunewich MA, Corrigan G, Derby GC, et al: A randomized, placebo-controlled trial of IGF-1 for delayed graft function: a human model to study postischemic ARF. Kidney Int 64:593-602, 2003
10. Hirschberg R, Kopple J, Lipsett P, et al: Multicenter clinical trial of recombinant human insulin-like growth factor I in patients with acute renal failure. Kidney Int 55:2423-2432, 1999
11. Sutton TA, Fisher CJ, Molitoris BA: Microvascular endothelial injury and dysfunction during ischemic acute renal failure. Kidney Int 62: 1539-1549, 2002
12. Lacombe C, Mayeux P: Biology of erythropoietin. Haematologica 83: 724-732, 1998
13. Miyake T, Kung CK, Goldwasser E: Purification of human erythropoietin. J Biol Chem 252:5558-5564, 1977
14. Lin FK, Suggs S, Lin CH, et al: Cloning and expression of the human erythropoietin gene. Proc Natl Acad Sci U S A 82:7580-7584, 1985
15. Eschbach JW, Egrie JC, Downing MR, et al: Correction of the anemia of end-stage renal disease with recombinant human erythropoietin. Results of a combined phase I and II clinical trial. N Engl J Med 316:73-78, 1987
16. Winearls CG, Oliver DO, Pippard MJ, et al: Effect of human erythropoietin derived from recombinant DNA on the anaemia of patients maintained by chronic haemodialysis. Lancet 2:1175-1178, 1986
17. Maxwell PH, Osmond MK, Pugh CW, et al: Identification of the renal erythropoietin-producing cells using transgenic mice. Kidney Int 44: 1149-1162, 1993
18. Wu H, Liu X, Jaenisch R, et al: Generation of committed erythroid BFU-E and CFU-E progenitors does not require erythropoietin or the erythropoietin receptor. Cell 83:59-67, 1995
19. Witthuhn BA, Quelle FW, Silvennoinen O, et al: JAK2 associates with the erythropoietin receptor and is tyrosine phosphorylated and activated following stimulation with erythropoietin. Cell 74:227-236, 1993
20. Spivak JL, Pham T, Isaacs M, et al: Erythropoietin is both a mitogen and a survival factor. Blood 77:1228-1233, 1991
21. Maxwell P: HIF-1: An oxygen response system with special relevance to the kidney. J Am Soc Nephrol 14:2712-2722, 2003
22. Iyer NV, Kotch LE, Agani F, et al: Cellular and developmental control of O2 homeostasis by hypoxia-inducible factor 1 alpha. Genes Dev 12: 149-162, 1998
23. Cai Z, Manalo DJ, Wei G, et al: Hearts from rodents exposed to intermittent hypoxia or erythropoietin are protected against ischemia-reperfusion injury. Circulation 108:79-85, 2003
24. Matsumoto M, Makino Y, Tanaka T, et al: Induction of renoprotective gene expression by cobalt ameliorates ischemic injury of the kidney in rats. J Am Soc Nephrol 14:1825-1832, 2003
25. Digicaylioglu M, Bichet S, Marti HH, et al: Localization of specific erythropoietin binding sites in defined areas of the mouse brain. Proc Natl Acad Sci U S A 92:3717-3720, 1995
26. Juul SE, Yachnis AT, Christensen RD: Tissue distribution of erythropoietin and erythropoietin receptor in the developing human fetus. Early Hum Dev 3:235-249, 1998
27. Sakanaka M, Wen TC, Matsuda S, et al In vivo evidence that erythropoietin protects neurons from ischemic damage. Proc Natl Acad Sci U S A 95:4635-4640, 1998
28. Bernaudin M, Marti HH, Roussel S, et al: A potential role for erythropoietin in focal permanent cerebral ischemia in mice. J Cereb Blood Flow Metab 19:643-651, 1999
29. Digicaylioglu M, Lipton SA: Erythropoietin-mediated neuroprotection involves cross-talk between Jak2 and NF-kappaB signalling cascades. Nature 412:641-647, 2001
30. Brines ML, Ghezzi P, Keenan S, et al: Erythropoietin crosses the blood-brain barrier to protect against experimental brain injury. Proc Natl Acad Sci U S A 97:10526-10531, 2000
31. Junk AK, Mammis A, Savitz SI, et al: Erythropoietin administration protects retinal neurons from acute ischemia-reperfusion injury. Proc Natl Acad Sci U S A 99:10659-10664, 2002
32. Parsa CJ, Matsumoto A, Kim J, et al: A novel protective effect of erythropoietin in the infarcted heart. J Clin Invest 112:999-1007, 2003
33. Calvillo L, Latini R, Kajstura J, et al: Recombinant human erythropoietin protects the myocardium from ischemia-reperfusion injury and promotes beneficial remodelling. Proc Natl Acad Sci U S A 100:4802-4806, 2003
34. Westenfelder C, Biddle DL, Baranowski RL: Human, rat, and mouse kidney cells express functional erythropoietin receptors. Kidney Int 55:808-820, 1999

35. Nemato T, Yokota N, Keane WF, et al: Recombinant erythropoietin rapidly treats anemia in ischemic acute renal failure. Kidney Int 59: 246-251, 2001
36. Yang CW, Li C, Jung JY, et al: Preconditioning with erythropoietin protects against subsequent ischemia-reperfusion injury in rat kidney. FASEB J 17:1754-1755, 2003
37. Vesey DA, Cheung C, Pat B, et al: Erythropoietin protects against ischaemic acute renal injury. Nephrol Dial Transplant 19:348-355, 2004
38. Chatterjee PK, Thiemermann C: An in vivo model of ischemia/reperfusion and inflammation of the kidneys of the rat. Methods Mol Biol 225:223-237, 2003
39. Sharples EJ, Patel N, Brown P, et al: Erythropoietin protects the kidney against the injury and dysfunction caused by ischemia-reperfusion. J Am Soc Nephrol 15:2115-2124, 2004
40. Moon C, Krawczyk M, Paik D, et al: Cardioprotection by recombinant human erythropoietin following acute experimental myocardial infarction: Dose response and therapeutic window. Cardiovasc Drugs Ther 19:243-250, 2005
41. Brezis M, Rosen S: Hypoxia of the renal medulla—its implications for disease. N Engl J Med 332:647-655, 1995
42. Molitoris BA, Sandoval R, Sutton TA: Endothelial injury and dysfunction in ischemic acute renal failure. Crit Care Med 30:S235-S240, 2002
43. Basile DP, Donohoe D, Roethe K, et al: Renal ischemic injury results in permanent damage to peritubular capillaries and influences long-term function. Am J Physiol 281:F887-F899, 2001
44. Brodsky SV, Yamamoto T, Tada T, et al: Endothelial dysfunction in ischemic acute renal failure: rescue by transplanted endothelial cells. Am J Physiol 282:F1140-F1149, 2002
45. Anagnostou A, Lee ES, Kessimian N, et al: Erythropoietin has a mitogenic and positive chemotactic effect on endothelial cells. Proc Natl Acad Sci U S A 87:5978-5982, 1990
46. Beleslin-Cokic BB, Cokic VP, Yu X, et al: Erythropoietin and hypoxia stimulate erythropoietin receptor and nitric oxide production by endothelial cells. Blood 104:2073-2080, 2004
47. Scalera F, Kielstein JT, Martens-Lobenhoffer J, et al: Erythropoietin increases asymmetric dimethylarginine in endothelial cells: role of dimethylarginine dimethylaminohydrolase. J Am Soc Nephrol 16:892-898, 2005
48. Buemi M, Galeano M, Sturiale A, et al: Recombinant human erythropoietin stimulates angiogenesis and healing of ischemic skin wounds. Shock 22:169-173, 2004
49. Bahlmann FH, DeGroot K, Duckert T, et al: Endothelial progenitor cell proliferation and differentiation is regulated by erythropoietin. Kidney Int 64:1648-1652, 2003
50. Bahlmann FH, De Groot K, Spandau JM, et al: Erythropoietin regulates endothelial progenitor cells. Blood 103:921-926, 2004
51. Patel NS, Sharples EJ, Cuzzocrea S, et al: Pretreatment with EPO reduces the injury and dysfunction caused by ischemia/reperfusion in the mouse kidney in vivo. Kidney Int 66:983-989, 2004
52. Vaziri ND, Zhou XJ, Liao SY: Erythropoietin enhances recovery from cisplatin-induced acute renal failure. Am J Physiol 266:F360-F366, 1994
53. Bahlmann FH, Song R, Boehm SM, et al: Low-dose therapy with the long-acting erythropoietin analogue darbepoetin alpha persistently activates endothelial Akt and attenuates progressive organ failure. Circulation 110:1006-1012, 2004
54. Jaquet K, Krause K, Tawakol-Khodai M, et al: Erythropoietin and VEGF exhibit equal angiogenic potential. Microvasc Res 64:326-333, 2002
55. Fishbane S, Ragolia L, Palaia T, et al: Cytoprotection by darbepoetin/epoetin alfa in pig tubular and mouse mesangial cells. Kidney Int 65: 452-458, 2004
56. Ehrenreich H, Hasselblatt M, Dembowski C, et al: Erythropoietin therapy for acute stroke is both safe and beneficial. Mol Med 8:495-505, 2002
57. Leist M, Ghezzi P, Grasso G, et al: Derivatives of erythropoietin that are tissue protective but not erythropoietic. Science 305:239-242, 2004
58. Fiordaliso F, Chimenti S, Staszewsky L, et al: A nonerythropoietic derivative of erythropoietin protects the myocardium from ischemia-reperfusion injury. Proc Natl Acad Sci U S A 102:2046-2051, 2005
59. Westenfelder C, Baranowski RL: Erythropoietin stimulates proliferation of human renal carcinoma cells. Kidney Int 58:647-657, 2000
60. Feldman L, Wang Y, Rhim JS, et al: Erythropoietin stimulates growth and STAT5 phosphorylation in human prostate epithelial and prostate cancer cells. Prostate 66:135-145, 2005
61. Henke M, Laszig R, Rube C, et al: Erythropoietin to treat head and neck cancer patients with anaemia undergoing radiotherapy: Randomised, double-blind, placebo-controlled trial. Lancet 362:1255-1260, 2003
62. Hardee ME, Kirkpatrick JP, Shan S, et al: Human recombinant erythropoietin (rEpo) has no effect on tumour growth or angiogenesis. Br J Cancer 93:1350-1355, 2005