TRANSPLANT INTERNATIONAL

Transplant International ISSN 0934-0874

REVIEW

Erythropoietin-mediated protection in kidney transplantation: nonerythropoietic EPO derivatives improve function without increasing risk of cardiovascular events

Willem G. van Rijt,[1,2] Harry van Goor,[2] Rutger J. Ploeg[3] and Henri G. D. Leuvenink[1]

1 Department of Surgery, University of Groningen and University Medical Center Groningen, Groningen, The Netherlands
2 Department of Pathology and Medical Biology, University of Groningen and University Medical Center Groningen, Groningen, The Netherlands
3 Nuffield Department of Surgical Sciences, University of Oxford, Oxford, UK

Keywords
erythropoietin, ischaemia/reperfusion injury, nonerythropoietic EPO derivatives, renal transplantation, renoprotection.

Correspondence
Willem G. van Rijt, Department of Surgery, University of Groningen and University Medical Center Groningen, Hanzeplein 1, 9713 GZ Groningen, The Netherlands.
Tel.: +31 50 3619801;
fax: +31 50 3632796;
e-mail: w.g.van.rijt@umcg.nl

Conflicts of interest
All authors declare no conflict of interests.

Received: 31 March 2013
Revision requested: 29 April 2013
Accepted: 27 July 2013
Published online: 22 August 2013

doi:10.1111/tri.12174

Summary

The protective, nonerythropoietic effects of erythropoietin (EPO) have become evident in preclinical models in renal ischaemia/reperfusion injury and kidney transplantation. However, four recently published clinical trials using high-dose EPO treatment following renal transplantation did not reveal any protective effect for short-term renal function and even reported an increased risk of thrombosis. This review focusses on the current status of protective pathways mediated by EPO, the safety concerns using high EPO dosage and discusses the discrepancies between pre-clinical and clinical studies. The protective effects are mediated by binding of EPO to a heteromeric receptor complex consisting of two β-common receptors and two EPO receptors. An important role for the activation of endothelial nitric oxide synthase is proposed. EPO-mediated cytoprotection still has enormous potential. However, only nonerythropoietic EPO derivatives may induce protection without increasing the risk of cardiovascular events. In preclinical models, nonerythropoietic EPO derivatives, such as carbamoylated EPO and ARA290, have been tested. These EPO derivatives improve renal function and do not affect erythropoiesis. Therefore, nonerythropoietic EPO derivatives may be able to render EPO-mediated cytoprotection useful and beneficial for clinical transplantation.

Transplantation of deceased donor kidneys

Delayed graft function (DGF) and primary nonfunction (PNF) are serious complications of renal transplantation. Overall, DGF is associated with a 41% increased risk of graft loss and a 38% increased risk of rejection [1]. In Europe, deceased donor kidneys represent 73% of all transplanted kidneys in 2011 [2]. Thus, improvement of short- and long-term function of transplanted deceased donor kidneys is an important focus in transplantation research.

Renal ischaemia/reperfusion (I/R) injury is a significant cause of reduced short-term function after transplantation. Deceased organ donation can be divided into two types of donation: organs donated after brain death (DBD, deceased brain dead) and after cardiac death (DCD, deceased cardiac dead). Short-term function of kidneys is significantly more compromised in DCD than in DBD-derived kidneys. The incidence of both DGF and PNF is 72% and 23% after DCD compared to 18% and 4% after DBD, respectively. The increased incidence of PNF results in reduced long-term graft survival of DCD kidneys [3].

Despite many important achievements in transplantation, such as improved surgical techniques, better treatment of complications and a profound reduction in kidney rejection, overall graft survival has only marginally increased [4,5]. This phenomenon is probably in part because of the current Achilles' heel in transplantation: the use of large numbers of older and high-risk donor organs that have suffered from substantial I/R injury. As we suspect that future donor resources will not return to the ideal

and young organ donors of the past, but merely focus on marginal DCD donors, better insight in the pathways of injury and repair are mandatory. Prevention and protection in high-risk donor organs against ischaemic injury will be necessary to maintain and hopefully enhance the results in kidney transplantation.

A promising strategy to protect against renal I/R injury is erythropoietin (EPO)-mediated cytoprotection. However, recent clinical trials did not reveal protective capacities of high-dose EPO treatment following renal transplantation. In this review, we will outline the renoprotective mechanism of EPO and discuss the disadvantages of high-dose EPO treatment in renal transplantation. Nonerythropoietic EPO derivatives could translate EPO-mediated cytoprotection to the transplantation clinic without increased risk of cardiovascular adverse events.

Cytoprotective pathway of EPO

Erythropoietin has pleiotropic actions. Besides stimulation of erythropoiesis, EPO also has a local tissue protective function [6–8]. In numerous models of renal I/R injury use of EPO has been shown to have protective effects. EPO improved renal function and reduced inflammation, apoptosis and structural damage [9–15]. EPO treatment preischemia, as well as treatment postreperfusion can be cytoprotective. Protective systemic EPO doses range from 300 to 5000 IU/kg [9–15]. However, following renal I/R improvement of renal function by 5000 IU/kg EPO appears superior compared to 300 IU/kg [12,14]. No studies have been performed to compare different EPO doses following renal I/R.

Maio *et al.* confirmed the protective capacities against renal I/R injury in a DCD transplantation model [16]. As a result of its 'nonerythropoietic' and cytoprotective capacities, EPO became an interesting agent reducing I/R injury and improving short- and long-term function after transplantation.

Erythropoietin was first discovered for its regulatory capacities of erythropoiesis. It induces proliferation and prevents apoptosis of erythroïd progenitor cells via binding to a receptor complex consisting of two EPO receptors ($EPOR_2$) [8]. However, in the past two decades, EPO appeared to have additional distinctive cytoprotective capacities. It plays an endogenous role in limiting local inflammation and tissue damage. These cytoprotective effects are not mediated by binding of EPO to the classic $EPOR_2$ complex, but by binding to a tissue protective receptor complex [17,18]. Immunoprecipitation studies showed that the EPOR is able to form a heteromeric receptor complex ($EPOR_2$-βCR_2) with the β common receptor (βCR). However, binding of EPO to this receptor complex is suggested to induce the cytoprotective pathway of EPO [17]. In neuronal tissue I/R injury results in up-regulation of EPOR expression starting directly after reperfusion. However, as increased EPO expression is delayed by several hours, a window of intervention is created [19]. Renal I/R causes up regulation of the $EPOR_2$-βCR_2 complex in renal tissue [20]. The distribution of cytoprotective receptor complex in renal tissue is not known because of a lack of reliable immunohistochemical antibodies. The binding affinity of the classic $EPOR_2$ complex for EPO is 1–10 pmol/l, while the affinity of the EPOR-βCR complex for EPO is 2–20 nmol/l [21,22]. This means that significant higher doses of EPO are required to induce cytoprotection compared to the stimulation of erythropoiesis.

The tissue protective signalling cascades have been described in various *in vitro* and *in vivo* models. As the classic erythropoietic $EPOR_2$ complex, binding of EPO to the $EPOR_2$-βCR_2 complex causes phosphorylation of janus activated kinase-2 (JAK2) [23]. This results in activation of two main signalling cascades: signal transducer and activator of transcription-5 and phosphatidylinositol 3-kinase/AKT (PI3K/AKT). These signalling pathways induce regeneration, inhibit apoptosis and inhibit inflammation [21]. PI3K/AKT is also able to increase regional blood flow by increasing endothelial nitric oxide synthase (eNOS) activity [24].

In various renal IRI models, the protective effects of EPO have been tested. EPO is able to increase phosphorylation of protective pathways as JAK2, PI3K/AKT and eNOS following renal I/R [14,23,25]. It has been widely shown that EPO administered pre as well as postreperfusion is able to attenuate renal I/R injury [9–16]. Besides improvement of renal function, EPO also has anti-inflammatory and anti-apoptotic capacities. EPO reduces expression of important inflammatory markers, as IL-6 and TNF-alpha [10,11]. Apoptosis and necrosis following renal I/R are reduced by EPO resulting in improved renal morphology [10,12,26]. Structurally, EPO also decreased the activity of TGF-β, indicative of reduced developing fibrosis [27].

Endothelial nitric oxide synthase

Nitric oxide synthase activity is a physiologic regulator of renal function and determinant of glomerular haemodynamics [28]. The direct effect of EPO on renal function [9,15] can be explained by increased activity of eNOS. Following renal I/R injury, eNOS phosphorylation is reduced at 6 h postreperfusion and subsequently normalized after 24 h [29]. The direct enhancing effect of EPO on renal function is presumably the effect of increased eNOS activity. This suggests increasing eNOS phosphorylation by high-dose EPO treatment is most effective in the first 6 h after reperfusion.

Growing evidence points to the important role of endothelial stimulation by protective EPO treatment. As knock-out of the EPOR is lethal because of an inefficient erythropoiesis, a transgenic EPOR knock-out has been developed in which the EPOR is only expressed in haemapoïetic and vascular endothelial cells [30]. Models of cardiac ischaemia or traumatic brain injury showed that EPO is still protective in these transgenic EPOR knock-out mice [31,32]. However, knock-out of eNOS diminishes the protective effect of EPO [24,31]. These studies show the dependence of eNOS enhancement and an important role of endothelial stimulation by EPO. The βCR is integrative in endothelial EPO signalling as it is essential for enhanced phosphorylation of protective signalling cascades like JAK2, AKT and eNOS in bovine aortic endothelial cells [33]. In addition to enhance PI3K/AKT, EPO may also increase eNOS phosphorylation due to an increased AMP-activated protein kinase (AMPK) activity. This regulator of energy metabolism is integrated in EPO signalling via the βCR and inhibition of AMPK reduced eNOS phosphorylation [34].

Recently, a new interaction between the βCR and the vascular endothelial growth factor receptor-2 (VEGFR2) has been described by Sautina *et al.* NO induction by EPO depends on βCR as well as VEGFR2 [35]. This finding underlines the importance of endothelial stimulation for immediate improvement of renal function and supports that EPO is able to preserve density of peritubular capillaries following renal I/R injury [36]. Affinity of the interaction between βCR and VEGFR2 for EPO has not been investigated yet.

In several *in vitro* studies, the role of the βCR in cytoprotection by EPO is shown to be essential [17,35,37]. In a model of spinal cord injury in βCR knock-out mice EPO did not induce cytoprotection [17]. However, Kanellakis *et al.* showed that darbepoietin, a long-working EPO analogue, still is protective against cardiac I/R injury in βCR knock-out mice [38]. Thus, EPO mediated cytoprotection may not be solely dependent of the $EPOR_2$-βCR_2 complex or βCR-VEGFR2 interaction.

Apparently, EPO is able to activate several protective signalling pathways. Further studies are necessary to determine the exact role of each pathway. It is, however, evident that tissue protection is mediated by other receptor complexes than stimulation of erythropoiesis. Enhanced eNOS activation appears to be crucial for improvement of renal function as EPO is not able to ameliorate renal function in eNOS knock-out mice. eNOS activity can be increased by EPO treatment via three signalling cascades. Figure 1 illustrates a scheme of proposed renoprotective pathways. The erythropoietic receptor complex has no protective function, although stimulation of this complex may be responsible for the increased risk of cardiovascular adverse events.

Clinical EPO treatment after clinical renal transplantation

Encouraged by pre-clinical results four clinical trials were initiated in the Netherlands [39], Germany [40], France [41] and the USA [42]. The protective effect of EPO following transplantation of deceased donor kidneys has been investigated in one open label and three double blind, randomized controlled trials. All studies aimed to improve short-term function after transplantation. The end points were incidence of DGF or renal function at 1 month after transplantation. Major differences were seen in inclusion criteria for donor types. In two studies, both types of deceased donors were included [40,42]. Martinez *et al.* included all recipients of a deceased donor kidney with a risk of DGF ≥60% based on the DGF risk index [41]. Aydin *et al.* only included DCD donor kidneys [39]. Statistical power was determined 80% based on either reduced incidence of DGF [39,40,42] or improved renal function [41]. Aydin *et al.* did not meet its powered inclusion as the Data and Safety Monitoring Board stopped the trial because of the slow inclusion rate. However, using the actual DGF rate of 81% in the placebo group, power was recalculated at 98% [39]. Characteristics of these studies are shown in Table 1.

None of the clinical studies showed a significant reduction in DGF (Table 2) or immediate improvement of renal function. The large differences in incidence of DGF between the four studies can be explained by inclusion of different donor types. As secondary end points, these studies used markers of renal function. Aydin *et al.* showed a significant increase in endogenous creatinine clearance at 12 months post-transplantation (EPO vs. placebo: 68 ± 23 ml/min vs. 57 ± 25 ml/min) whilst the other studies did not observe any difference in renal function. An important finding is the increased risk of thrombosis during the first year following transplantation by EPO treatment (EPO vs. placebo: 24.4% vs. 6.4%) in the report by Aydin *et al.* The other studies did not show any differences in adverse events although in three studies EPO was shown to increase haemoglobin levels after transplantation [39–42].

Translation of protective EPO treatment

In preclinical studies, the cytoprotective capacities of EPO have been thoroughly tested in renal I/R or transplantation models as earlier discussed [9–16]. However, the translation appears difficult. Apart from healthy animals and no immunosuppressive treatment in experimental models, there are several factors that may explain the lack of protection and clinical improvement in the recent trials [39–42].

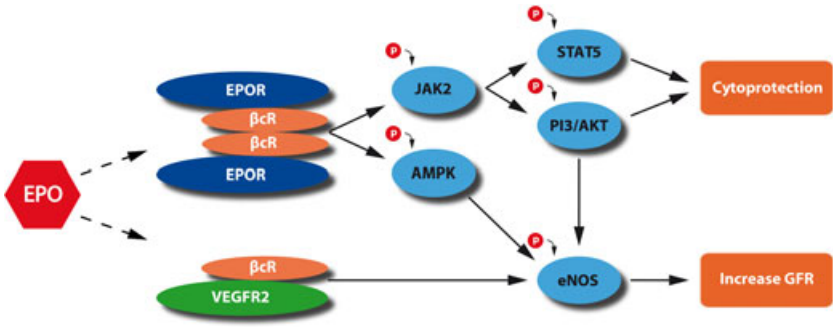

Figure 1 Proposed renoprotective pathway of EPO. EPO is able to activate either the $EPOR_2$-βCR_2 complex or an interaction between the βCR-VEGFR2. Binding of EPO to the $EPOR_2$-βCR_2 complex activates anti-inflammatory, anti-apoptotic and pro-survival pathways. PI3/AKT and AMPK, activated by the $EPOR_2$-βCR_2 complex, and βCR-VEGFR2 interaction, are responsible for increased eNOS phosphorylation by EPO. The direct stimulative effect on renal function is presumably the effect of enhanced eNOS activity. eNOS, endothelial nitric oxide synthase

Table 1. Study characteristics.

	Aydin *et al.* [39]	Hafer *et al.* [40]	Martinez *et al.* [41]	Sureskumar *et al.* [42]
Design	RCT Double blind Single-centre	RCT Double blind Single-centre	Open label Randomized Multicenter	RCT Double blind Single-centre
Treatment	Epoetin-β	Epoetin-α	Epoetin-β	Epoetin-α
Dose	33 000 IU	40 000 IU	30 000 IU	40 000 IU
Control treatment	Saline	Saline	N/A	Saline
Timing	3 24 h post 48 h post	Reperfusion 3 days post-Tx 7 days post-Tx	Pre-surgery 12 h post-Tx 7 days post-Tx 14 days post-Tx	Reperfusion
Donor type	DCD	Deceased donors	Deceased donors DGF risk >7	Deceased donors
Number of patients	C: 45 vs. EPO: 47	C: 44 vs. EPO: 44	C: 52 vs. EPO: 52	C: 36 vs. EPO: 36
Follow-up	12 months	12 months	3 months	1 month
Renal function	GFR C: 57 ml/min EPO: 68 ml/min	eGFR C: 43.6 ml/min EPO: 40.6 ml/min	eGFR C: 44.0 ml/min EPO: 42.5 ml/min	eGFR C: 36.7 ml/min EPO: 37.0 ml/min

DCD, deceased cardiac dead; GFR, glomerular filtration rate; EPO, erythropoietin; RCT, randomized controlled trial.

Table 2. Incidence of DGF.

Incidence of DGF	EPO group (%)	Control group (%)
Aydin *et al.* [39]	76.2	78.3
Hafer *et al.* [40]	23	32
Martinez *et al.* [41]	32	38.8
Sureshkumar *et al.* [42]	41.7	47.2

DGF, delayed graft function; EPO, erythropoietin.
High-dose EPO treatment after renal transplantation did not significantly reduce incidence of DGF.

Timing

Currently, treatment to reduce I/R injury is ethically and practically best applicable in the recipient. Although, protective treatment of donors is increasingly being considered if it does not harm the donor. Most renal I/R injury emerges during the reperfusion phase. Thus, cytoprotective treatment early in the reperfusional phase potentially improves function after transplantation. Based on these practical, ethical and mechanistical reasons, protective EPO treatment focusses on the recipient.

In the four clinical trials timing of the EPO treatment considerably differs (Table 1). Pre-clinical studies showed that high-dose EPO administration is protective when administered between 30 min pre-ischaemia and 6 h postreperfusion [12,14]. There are no studies showing the protective capacities of EPO when administrated more than 6 h postreperfusion. Although timing of treatment in rodents cannot be directly translated to the human, there is definitely no evidence for clinical high-dose EPO treatment from 2 to 14 days after transplantation.

Dosing

Dosing of protective EPO treatment is a difficult issue to allow translation of this treatment from animal work to the clinical situation. In most preclinical I/R models, EPO doses of 1000 IU/kg or higher were tested [9–15]. However, in the clinical trials doses ranging from 30 000 to 40 000 IU were used independently of the weight of the recipient [39–42]. Assuming the average recipient weighs 75 kg, means that recipients were treated with a dose of approximately 500 IU/kg. This is relatively low to induce EPO mediated tissue protection as binding affinities of the erythropoietic- and the tissue protective receptor complex are different. As earlier mentioned, the binding affinity of the classic $EPOR_2$ complex for EPO is considerably higher than the affinity of the $EPOR_2$-βCR_2 complex [21,22]. This means that distinctly higher systemic EPO levels are required to induce the tissue protective receptor complex compared to stimulation of the erythropoiesis. Clinical EPO treatment to stimulate erythropoiesis is dosed at 75–300 IU/kg. Thus, the dose used in the clinical trials, 500 IU/kg, is comparatively low to induce renoprotection.

Non-erythropoietic EPO derivatives

In animals models, no increased risk of high dose, protective EPO treatment is observed as follow-up is relatively short. Besides, recipients of a renal transplant often suffer any kind of co-morbidity, while in preclinical studies healthy animals are used. However, based on preclinical trials high-dose EPO treatment was thought to be safe. As mentioned above, the used dose EPO in clinical renal transplantation trials was 2–10 times lower than dosages in animal models. However, Aydin *et al.* already observed an increased risk of thrombosis in the first year following transplantation [39]. In renal transplantation EPO doses used post-transplantation did not reach protective levels, although the risk of side effects already increased. An increased serum EPO concentration raises the haematocrit and markedly enhances platelet and endothelial activation [8,43]. These mechanisms are causative for the increased risk of cardiovascular adverse events. In cancer patients it has also been shown that EPO treatment to stimulate erythropoiesis already increased thromboembolic events and mortality [44]. Thus, safety concerns about high-dose EPO treatment in renal transplantation are justified and increasing the EPO dose to induce cytoprotection is irresponsible. Besides the risks of cardiovascular events, several clinical trials in anaemic cancer patients suggested a stimulating effect of EPO on tumour progression. Aapro *et al.* elegantly reviewed meta-analyses and there is no evidence for enhanced tumour progression by EPO [45].

To overcome the shortcomings of cytoprotective EPO treatment, nonerythropoietic EPO derivatives have been developed. Tissue protection is mediated by a specific receptor complex and this created an opportunity to develop these nonerythropoietic EPO derivatives. All nonerythropoietic EPO derivatives, which have been tested in models of acute renal injury, will be discussed: asialo-erythropoietin (asialo-EPO), carbamoylated EPO (CEPO), glutaraldehyde EPO (GEPO) and ARA290. These derivatives do not bind to the classic $EPOR_2$ complex. Thus, erythropoiesis or platelet activation is not stimulated. In this way cytoprotection can be induced without increasing risk of cardiovascular adverse events. The effect of nonerythropoietic EPO derivatives on tumour progression has not been investigated. However, an enhancing effect of nonerythropoietic EPO derivatives on cancer is unlikely, as the proposed mechanism of tumour progression by EPO is mediated by the classic $EPOR_2$ complex [45] which is not activated by nonerythropoietic EPO derivatives.

Continuous exposure of precursor red blood cells to EPO is required for stimulation of erythropoiesis, while cytoprotection can be induced by brief exposure. Based on this principle, an EPO derivative with a very short half-life could be protective and would not stimulate erythropoiesis. Enzymatic desialylation of EPO results in asialo-EPO possessing a half-life of several minutes. In renal I/R asialo-EPO attenuated renal dysfunction and improved survival [26]. Although, asialo-EPO does not stimulate erythropoiesis, asialo-EPO still has the same affinity for the classic $EPOR_2$ complex as EPO [18,46]. Therefore, redundant effects of asialo-EPO via this receptor complex cannot be excluded.

Carbamoylated EPO is synthesized by cyanide carbamoylation and GEPO is based on glutaraldehyde modification [18,47,48]. These EPO derivatives distinctly differ on molecular level of EPO and asialo-EPO. Carbamoylation and glutaraldehyde modification reduce charge of lysine residues on EPO molecules. This prevents stimulation of erythropoiesis [49]. *In vitro* and *in vivo* experiments showed that CEPO and GEPO do not affect erythropoiesis [18,48]. Half-life of CEPO and GEPO is approximately 6 h, comparable to half-life of EPO [18]. In several models of renal I/R injury and brain death protective capacities of CEPO have been observed. Depending on AKT phosphorylation, CEPO improves renal function. Apoptosis, tubular injury and structural damage were reduced by CEPO treatment [27,36,50–53]. Furthermore, CEPO also improves angiogenesis, improves renal blood flow and prevents reduced density of peritubular capillaries [36,51,52]. GEPO has only been tested in one I/R model showing preserved renal function and reduced histological damage [48].

The third and newest generation of nonerythropoietic EPO derivatives is ARA290, also known as pyroglutamate

helix B surface peptide. ARA290 is derived from the binding site of EPO to the $EPOR_2$-βCR_2 complex. It mimics the three-dimensional structure of the ligand binding to $EPOR_2$-βCR_2 complex and possesses a half-life of approximately 2 min [37]. This means that ARA290 is not able to bind the erythropoietic $EPOR_2$ complex. The protective capacities of ARA290 have been shown in models of haemorrhagic shock and neuronal injury [54–57]. In renal I/R cytoprotection by ARA290 has been shown in rodent and porcine models [20,25,37,58]. Post-reperfusional administration of ARA290 to 6 h postreperfusion improved short-term renal function, reduced inflammation, reduced apoptosis and reduced structural damage [20,25,58]. Mechanistically, ARA290 is able to increase AKT and eNOS phosphorylation [25]. Inhibition of PI3/AKT diminishes the protective effect of ARA290 indicating the importance of this pathway [20]. As mentioned before, Yang *et al.* showed that renal I/R upregulates $EPOR_2$-βCR_2 expression in renal tissue at 48 h postreperfusion. Interestingly, ARA290 prevents this increase in receptor expression. ARA290 in combination with Wortmannin, a PI3/AKT pathway inhibitor, doubled $EPOR_2$-βCR_2 expression compared to I/R injury [20]. This suggests $EPOR_2$-βCR_2 complex is part of a physiologic cytoprotective effect and inhibition of one of its down-stream pathways results in a further increase in the expression of the cytoprotective receptor complex. We showed in a porcine I/R model that ARA290 is able to improve the glomerular filtration rate in the first 7 days postreperfusion. Furthermore, ARA290 prevented structural damage. In the first 24 h postreperfusion, ARA290 increased urinary nitrite + nitrate concentrations suggesting increased nitric oxide synthase activity [58].

Half-life of the four different EPO derivatives is important to determine timing of treatment. CEPO and GEPO possess a half-life of several hours [18], while the half-life of asialo-EPO and ARA290 is only minutes [37,46]. In I/R injury most damage occurs early in the reperfusion phase and eNOS phosphorylation is reduced in the first 6 h postreperfusion. Therefore, the most optimal window of treatment is in the first 6 h postreperfusion. Depending on the different pharmacokinetics of the nonerythropoietic EPO derivatives timing of treatment should be carefully chosen as differences in half-life will affect moment of treatment.

Asialo-EPO, CEPO, GEPO and ARA290 show comparable protective effects in renal I/R injury to cytoprotective EPO treatment. The major benefit of nonerythropoietic EPO derivatives is that they do not influence the erythropoiesis or platelet activation [37,50]. Therefore, titration to high, cytoprotective levels is possible without an increased risk of cardiovascular events. CEPO and ARA290 are most interesting derivatives as these molecules have no affinity for the classic $EPOR_2$ complex and the renoprotective capacities have already been shown in several renal I/R experiments.

Conclusions

Erythropoietin mediated cytoprotection is promising. However, increased risk of cardiovascular events is a serious concern of high-dose EPO treatment. Especially as cytoprotective levels have not been reached in clinical trials, although the risk of thrombosis already increased. Nonerythropoietic EPO derivatives may be the solution. In preclinical models derivatives like CEPO or ARA290 did not influence erythropoiesis, but retained their protective capacities. These EPO derivatives could be titrated safely to protective levels in the transplantation clinic. Cytoprotective treatment should be timed early in the reperfusion phase.

Only nonerythropoietic EPO derivatives, like CEPO or ARA290, may induce protection without increasing the risk of cardiovascular events. Non-erythropoietic EPO derivatives, administered early postreperfusion, may be able to improve short-term renal function. Hereby, incidence of DGF and PNF following renal transplantation could be reduced. Pre-clinical results warrant further investigation of the renoprotective effects of nonerythropoietic EPO derivatives in renal transplantation.

Funding

None.

References

1. Yarlagadda SG, Coca SG, Formica RN Jr, Poggio ED, Parikh CR. Association between delayed graft function and allograft and patient survival: a systematic review and meta-analysis. *Nephrol Dial Transplant* 2009; **24**: 1039.
2. Oosterlee A, Rahmel A. Annual Report 2011. Eurotransplant International Foundation, 2011.
3. Snoeijs MG, Winkens B, Heemskerk MB, *et al.* Kidney transplantation from donors after cardiac death: a 25-year experience. *Transplantation* 2010; **90**: 1106.
4. Lamb KE, Lodhi S, Meier-Kriesche HU. Long-term renal allograft survival in the United States: a critical reappraisal. *Am J Transplant* 2011; **11**: 450.
5. Meier-Kriesche HU, Schold JD, Srinivas TR, Kaplan B. Lack of improvement in renal allograft survival despite a marked decrease in acute rejection rates over the most recent era. *Am J Transplant* 2004; **4**: 378.
6. Chatterjee PK. Pleiotropic renal actions of erythropoietin. *Lancet* 2005; **365**: 1890.
7. Maiese K, Li F, Chong ZZ. New avenues of exploration for erythropoietin. *JAMA* 2005; **293**: 90.
8. Jelkmann W. Molecular biology of erythropoietin. *Intern Med* 2004; **43**: 649.

9. Forman CJ, Johnson DW, Nicol DL. Erythropoietin administration protects against functional impairment and cell death after ischaemic renal injury in pigs. *BJU Int* 2007; **99**: 162.
10. Hu L, Yang C, Zhao T, *et al.* Erythropoietin ameliorates renal ischemia and reperfusion injury via inhibiting tubulointerstitial inflammation. *J Surg Res* 2012; **176**: 260.
11. Ishii Y, Sawada T, Murakami T, *et al.* Renoprotective effect of erythropoietin against ischaemia-reperfusion injury in a non-human primate model. *Nephrol Dial Transplant* 2011; **26**: 1157.
12. Johnson DW, Pat B, Vesey DA, Guan Z, Endre Z, Gobe GC. Delayed administration of darbepoetin or erythropoietin protects against ischemic acute renal injury and failure. *Kidney Int* 2006; **69**: 1806.
13. 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* 2004; **66**: 983.
14. 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* 2004; **15**: 2115.
15. Solling C, Christensen AT, Krag S, *et al.* Erythropoietin administration is associated with short-term improvement in glomerular filtration rate after ischemia-reperfusion injury. *Acta Anaesthesiol Scand* 2011; **55**: 185.
16. Maio R, Sepodes B, Patel NS, Thiemermann C, Mota-Filipe H, Costa P. Erythropoietin preserves the integrity and quality of organs for transplantation after cardiac death. *Shock* 2011; **35**: 126.
17. Brines M, Grasso G, Fiordaliso F, *et al.* Erythropoietin mediates tissue protection through an erythropoietin and common beta-subunit heteroreceptor. *Proc Natl Acad Sci U S A* 2004; **101**: 14907.
18. Leist M, Ghezzi P, Grasso G, *et al.* Derivatives of erythropoietin that are tissue protective but not erythropoietic. *Science* 2004; **305**: 239.
19. Liu R, Suzuki A, Guo Z, Mizuno Y, Urabe T. Intrinsic and extrinsic erythropoietin enhances neuroprotection against ischemia and reperfusion injury in vitro. *J Neurochem* 2006; **96**: 1101.
20. Yang C, Zhao T, Lin M, *et al.* Helix B surface peptide administered after insult of ischemia reperfusion improved renal function, structure and apoptosis through beta common receptor/erythropoietin receptor and PI3K/Akt pathway in a murine model. *Exp Biol Med (Maywood)* 2013; **238**: 111.
21. Brines M, Cerami A. Erythropoietin-mediated tissue protection: reducing collateral damage from the primary injury response. *J Intern Med* 2008; **264**: 405.
22. Masuda S, Nagao M, Takahata K, *et al.* Functional erythropoietin receptor of the cells with neural characteristics. Comparison with receptor properties of erythroid cells. *J Biol Chem* 1993; **268**: 11208.
23. Breggia AC, Wojchowski DM, Himmelfarb J. JAK2/Y343/STAT5 signaling axis is required for erythropoietin-mediated protection against ischemic injury in primary renal tubular epithelial cells. *Am J Physiol Renal Physiol* 2008; **295**: F1689.
24. Oba S, Suzuki E, Nishimatsu H, *et al.* Renoprotective effect of erythropoietin in ischemia/reperfusion injury: Possible roles of the Akt/endothelial nitric oxide synthase-dependent pathway. *Int J Urol* 2012; **19**: 248.
25. Patel NS, Kerr-Peterson HL, Brines M, *et al.* The delayed administration of pHBSP, a novel non-erythropoietic analogue of erythropoietin. attenuates acute kidney injury. *Mol Med* 2012; **18**: 719.
26. Okada T, Sawada T, Kubota K. Asialoerythropoietin has strong renoprotective effects against ischemia-reperfusion injury in a murine model. *Transplantation* 2007; **84**: 504.
27. Imamura R, Isaka Y, Sandoval RM, *et al.* A nonerythropoietic derivative of erythropoietin inhibits tubulointerstitial fibrosis in remnant kidney. *Clin Exp Nephrol* 2012; **16**: 852.
28. Schlaich MP, Schmitt D, Ott C, Schmidt BM, Schmieder RE. Basal nitric oxide synthase activity is a major determinant of glomerular haemodynamics in humans. *J Hypertens* 2008; **26**: 110.
29. Yamasowa H, Shimizu S, Inoue T, Takaoka M, Matsumura Y. Endothelial nitric oxide contributes to the renal protective effects of ischemic preconditioning. *J Pharmacol Exp Ther* 2005; **312**: 153.
30. Suzuki N, Ohneda O, Takahashi S, *et al.* Erythroid-specific expression of the erythropoietin receptor rescued its null mutant mice from lethality. *Blood* 2002; **100**: 2279.
31. Teng R, Calvert JW, Sibmooh N, *et al.* Acute erythropoietin cardioprotection is mediated by endothelial response. *Basic Res Cardiol* 2011; **106**: 343.
32. Xiong Y, Mahmood A, Qu C, *et al.* Erythropoietin improves histological and functional outcomes after traumatic brain injury in mice in the absence of the neural erythropoietin receptor. *J Neurotrauma* 2010; **27**: 205.
33. Su KH, Shyue SK, Kou YR, *et al.* Beta common receptor integrates the erythropoietin signaling in activation of endothelial nitric oxide synthase. *J Cell Physiol* 2011; **226**: 3330.
34. Su KH, Yu YB, Hou HH, *et al.* AMP-activated protein kinase mediates erythropoietin-induced activation of endothelial nitric oxide synthase. *J Cell Physiol* 2012; **227**: 3053.
35. Sautina L, Sautin Y, Beem E, *et al.* Induction of nitric oxide by erythropoietin is mediated by the beta common receptor and requires interaction with VEGF receptor 2. *Blood* 2010; **115**: 896.
36. Cassis P, Azzollini N, Solini S, *et al.* Both darbepoetin alfa and carbamylated erythropoietin prevent kidney graft dysfunction due to ischemia/reperfusion in rats. *Transplantation* 2011; **92**: 271.
37. Brines M, Patel NS, Villa P, *et al.* Nonerythropoietic, tissue-protective peptides derived from the tertiary structure of erythropoietin. *Proc Natl Acad Sci U S A* 2008; **105**: 10925.
38. Kanellakis P, Pomilio G, Agrotis A, *et al.* Darbepoetin-mediated cardioprotection after myocardial infarction involves multiple mechanisms independent of erythropoietin

receptor-common beta-chain heteroreceptor. *Br J Pharmacol* 2010; **160**: 2085.
39. Aydin Z, Mallat MJ, Schaapherder AF, *et al.* Randomized trial of short-course high-dose erythropoietin in donation after cardiac death kidney transplant recipients. *Am J Transplant* 2012; **12**: 1793.
40. Hafer C, Becker T, Kielstein JT, *et al.* High-dose erythropoietin has no effect on short- or long-term graft function following deceased donor kidney transplantation. *Kidney Int* 2012; **81**: 314.
41. Martinez F, Kamar N, Pallet N, *et al.* High dose epoetin beta in the first weeks following renal transplantation and delayed graft function: results of the neo-PDGF study. *Am J Transplant* 2010; **10**: 1695.
42. Sureshkumar KK, Hussain SM, Ko TY, Thai NL, Marcus RJ. Effect of high-dose erythropoietin on graft function after kidney transplantation: a randomized, double-blind clinical trial. *Clin J Am Soc Nephrol* 2012; **7**: 1498.
43. Stohlawetz PJ, Dzirlo L, Hergovich N, *et al.* Effects of erythropoietin on platelet reactivity and thrombopoiesis in humans. *Blood* 2000; **95**: 2983.
44. Tonia T, Mettler A, Robert N, *et al.* Erythropoietin or darbepoetin for patients with cancer. *Cochrane Database Syst Rev* 2012; **12**: CD003407.
45. Aapro M, Jelkmann W, Constantinescu SN, Leyland-Jones B. Effects of erythropoietin receptors and erythropoiesis-stimulating agents on disease progression in cancer. *Br J Cancer* 2012; **106**: 1249.
46. Erbayraktar S, Grasso G, Sfacteria A, *et al.* Asialoerythropoietin is a nonerythropoietic cytokine with broad neuroprotective activity in vivo. *Proc Natl Acad Sci U S A* 2003; **100**: 6741.
47. Jelkmann W. 'O', Erythropoietin carbamoylation versus carbamylation. *Nephrol Dial Transplant* 2008; **23**: 3033; author reply 3033.
48. Chattong S, Tanamai J, Kiatsomchai P, *et al.* Glutaraldehyde erythropoietin protects kidney in ischaemia/reperfusion injury without increasing red blood cell production. *Br J Pharmacol* 2013; **168**: 189.
49. Satake R, Kozutsumi H, Takeuchi M, Asano K. Chemical modification of erythropoietin: an increase in in vitro activity by guanidination. *Biochim Biophys Acta* 1990; **1038**: 125.
50. Imamura R, Isaka Y, Ichimaru N, Takahara S, Okuyama A. Carbamylated erythropoietin protects the kidneys from ischemia-reperfusion injury without stimulating erythropoiesis. *Biochem Biophys Res Commun* 2007; **353**: 786.
51. Imamura R, Okumi M, Isaka Y, *et al.* Carbamylated erythropoietin improves angiogenesis and protects the kidneys from ischemia-reperfusion injury. *Cell Transplant* 2008; **17**: 135.
52. Coleman TR, Westenfelder C, Togel FE, *et al.* Cytoprotective doses of erythropoietin or carbamylated erythropoietin have markedly different procoagulant and vasoactive activities. *Proc Natl Acad Sci U S A* 2006; **103**: 5965.
53. Nijboer WN, Ottens PJ, van Dijk A, van Goor H, Ploeg RJ, Leuvenink HG. Donor pretreatment with carbamylated erythropoietin in a brain death model reduces inflammation more effectively than erythropoietin while preserving renal function. *Crit Care Med* 2010; **38**: 1155.
54. McVicar CM, Hamilton R, Colhoun LM, *et al.* Intervention with an erythropoietin-derived peptide protects against neuroglial and vascular degeneration during diabetic retinopathy. *Diabetes* 2011; **60**: 2995.
55. Patel NS, Nandra KK, Brines M, *et al.* A nonerythropoietic peptide that mimics the 3D structure of erythropoietin reduces organ injury/dysfunction and inflammation in experimental hemorrhagic shock. *Mol Med* 2011; **17**: 883.
56. Robertson CS, Cherian L, Shah M, *et al.* Neuroprotection with an erythropoietin mimetic peptide (pHBSP) in a model of mild traumatic brain injury complicated by hemorrhagic shock. *J Neurotrauma* 2012; **29**: 1156.
57. Schmidt RE, Feng D, Wang Q, *et al.* Effect of insulin and an erythropoietin-derived peptide (ARA290) on established neuritic dystrophy and neuronopathy in Akita (Ins2(Akita)) diabetic mouse sympathetic ganglia. *Exp Neurol* 2011; **232**: 126.
58. van Rijt WG, Nieuwenhuijs-Moeke GJ, van Goor H, *et al.* ARA290, a non-erythropoietic EPO derivative, attenuates renal ischemia/reperfusion injury. *J Transl Med* 2013; **11**: 9.