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Erythropoetin as a novel agent with pleiotropic effects against acute lung injury

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Abstract

Current pharmacotherapy for acute lung injury (ALI) and the acute respiratory distress syndrome (ARDS) is not optimal, while the biological and physiological complexity of these severe lung injury syndromes requires consideration of combined-agent treatments or agents with pleiotropic action. In this regard, exogenous erythropoietin (EPO) represents a possible candidate since a number of preclinical studies have revealed beneficial effects of EPO administration in various experimental models of ALI. Taken together, this treatment strategy is not a single mediator approach, but it rather provides protection by modulating multiple levels of early signaling pathways involved in apoptosis, inflammation and peroxidation, potentially restoring overall homeostasis. Furthermore, EPO appears to confer vascular protection by promoting angiogenesis. However, only preliminary studies exist and more experimental and clinical studies are necessary for the clarification of the efficacy and potentially cytoprotective mechanisms of EPO action. In addition to the attempts to optimize the dose and timing of EPO administration, it would be of great value to minimize any potential toxicity, which is essential for EPO to fulfil its role as a potential candidate for the treatment of ALI in routine clinical practice. The present article reviews recent advances that have elucidated biological and biochemical activities of EPO which may be potentially applicable for ALI/ARDS management.

Keywords: Erythropoietin· Acute lung Injury· Acute respiratory distress syndrome· ALI· ARDS

Introduction

Clinically, acute lung injury (ALI) and its more severe form, acute respiratory distress syndrome (ARDS), can occur in response to a variety of pulmonary or systemic insults that induce damage of the alveolar-capillary membrane and pulmonary inflammation. This acute condition is characterized by bilateral pulmonary infiltrates, non-cardiogenic pulmonary oedema, impaired gas exchange and need for mechanical ventilation [1-3]. Pulmonary inflammation complicated by a systemic response leads to the release of cytokines, oxidants and other pro-inflammatory mediators that subsequently propel the inflammatory and apoptotic response in the lung [4-10]. About 55% of ALI/ARDS cases can be attributed to direct causes, such as pneumonia or aspiration, whereas indirect causes such as extra-pulmonary sepsis or trauma, account for 20% of the cases. In about 21% of instances, multiple risk factors are recognized while, in 4% of cases, no underlying pathophysiology can be identified [11]. The annual incidence of ALI and ARDS in the United States of America is estimated to be 79 and 59 cases/100,000 population respectively [3, 12], whereas the associated mortality remains high at 30%–50%, despite optimal supportive care [12, 13]. Patients are likely to have prolonged hospital courses with mechanical ventilation, while those surviving often suffer from cognitive deficits or long term disabilities, such as reduced vital capacity, exercise tolerance and physical strength [11-15].

Over the past two decades, a variety of interventions and intensive care strategies have been used in treating patients with ALI/ARDS. Treatment of the underlying cause of injury or disease is essential, along with mechanical ventilation with lung protective strategies, supportive care, and judicious fluid management [3, 13, 16]. Unfortunately, current specific options for treatment are limited, although, the complex pathology of this condition offers an array of potential pharmacological targets such as ventilation/perfusion abnormalities, vascular dysfunction, edema, inflammation, oxidant injury, arterial hypoxemia, surfactant dysfunction and fibrosis [16]. It has been suggested that pleiotropic therapeutic strategies may be more appropriate to restore the disturbed homeostasis during ALI/ARDS. In other words the investigation of new therapeutic approaches should include the use of combined-agent treatments or a distinct substance with multiple actions. In this regard, exogenous erythropoietin (EPO) represents a possible candidate since, over the last years, it has emerged as a multifunctional tissue-protective cytokine with anti-

apoptotic, anti-inflammatory and immunomodulatory properties [17-19].

EPO is an endogenous 30.4-kDa glycoprotein hormone and a member of the type I cytokine superfamily that stimulates red blood cell production in order to increase oxygen transfer and delivery. The circulatory mature protein has a span of 165 amino acids and contains 4 glycosylated chains including 3 N-linked and 1 O-linked acidic oligosaccharide side chains [20]. Reduced arterial oxygen content associated with anaemia or hypoxia is the predominant stimulus for EPO production mainly in the kidneys [18, 20-22]. EPO promotes bone marrow erythroid cell proliferation, differentiation, and survival through its binding to an erythroid progenitor cell surface EPO receptor (EPO-R) [20, 21, 23]. Secondary production of EPO as well as expression of the EPO-R have been also demonstrated on several tissues and organs including brain, heart, lungs, liver, kidneys, vascular endothelium, and lymphoid tissues [18, 21, 22].

The exogenous administration of recombinant human erythropoietin (rhEPO) is presently approved for the treatment of anaemia as a consequence of a variety of disorders. However, it is well-established that EPO also possesses therapeutic properties beyond mere haemopoiesis and therefore, EPO is now considered to have applicability in a variety of disorders, such as cerebral ischaemia, spinal cord injury, head trauma, epilepsy, kidney damage, myocardial infarction and reperfusion injury, and chronic congestive heart failure [17-19]. In agreement with these studies, EPO has been also shown to have significant beneficial effects in experimental models of ALI. The remainder of this article reviews recent advances that have elucidated biological and biochemical activities of EPO which may be potentially applicable for ALI/ARDS management.

ERYTHROPOIETIN AND ALI/ARDS

Around 7% of intensive care patients will eventually develop ALI/ARDS, whereas delivery of critical care to patients with ALI/ARDS accounts for a significant proportion of intensive care unit capacity [11]. In the last decade EPO has emerged as a multifunctional endogenous mediator that participates in a broad array of pathophysiological processes during critical illness. EPO is considered to be relatively deficient in critical illness, since the EPO response to known physiological stimuli appears to be blunted in critically ill patients [24]. Interestingly, local and circulating mediators of critical illness, including prostaglandin, arachidonic acid,

glucocorticoids, reactive oxygen species (ROS) and cytokines such as tumor necrosis factor α (TNF- α), interleukin 1 β (IL-1 β), and interleukin 6 (IL-6), are known to modulate EPO production and the expression of EPO-R [18, 25]. Furthermore, EPO appears to be a survival negative prognostic factor during sepsis, a major risk factor for ALI/ARDS [26, 27]. In patients suffering from septic shock, high serum EPO levels correlate with the severity of organ injury, hypoxia score (PaO₂/FiO₂ ratio) and lactate values [26, 27]. Important to the present review, numerous pre-clinical studies have revealed that several tissues are protected by EPO administration before or following various stressors, which also represent risk factors for ALI/ARDS [17-19] and clinical data also support this concept [28, 29].

Consistent with these findings, several reports in animal models of ALI have documented that EPO administration decreases the severity of lung injury by various etiological factors. EPO appears to protect the integrity of the pulmonary epithelial and endothelial cells and to attenuate the associated pulmonary oedema and deterioration of pulmonary oxygenation function. Treatment with EPO has been proven beneficial in experimental ALI caused by hyperoxia [30, 31], acute necrotizing pancreatitis [32], ischaemia/reperfusion [33, 34], experimental sepsis induced by cecal ligation and puncture [35] or LPS [36-37], zymosan induced non-septic shock [38] and tracheobronchial and pulmonary type II epithelial injury, following traumatic brain injury [39]. In these studies EPO has been found to interact with its receptor (EPO-R) to induce a range of pleiotropic cytoprotective actions. Nevertheless, the potential use of EPO could be limited or even halted by the dose-dependent risk of associated side effects, especially in high-risk groups such as patients with vascular thrombosis, hypertension, congestive heart failure, and neoplasms [18]. Therefore, a careful consideration of appropriate EPO doses is certainly indicated before extending experimental findings to humans or other animal models.

Protection of vascular integrity: Anti-apoptotic and angiogenic action of EPO in ALI/ARDS

The principle mechanism by which EPO may preserve cellular integrity involves the inhibition of cellular apoptosis that seems to represent an early trigger in the pathogenesis of ALI/ARDS [7, 8]. Thus, in a number of tissues and organs, EPO exhibits anti-apoptotic properties secondary to various insults [17-19]. In rats, the

pulmonary apoptosis index was markedly reduced as a result of treatment with EPO prior to ischaemia/reperfusion lung injury [31]. Moreover, EPO prevents LPS-induced apoptosis and eventually promotes the viability of bovine pulmonary artery endothelial cells [40]. Additionally, EPO treatment protects against apoptotic cell death in the lung after induction of experimental endotoxaemia in mice [35], while repeated administration of EPO reduces epithelial cell apoptosis and attenuates bleomycin-induced pneumonitis in mice [41]. Fas-mediated apoptosis of respiratory epithelium [7, 8] was inhibited by EPO in an in vitro model of ALI [42]. This anti-apoptotic effect of EPO was associated with induction of a pro-apoptotic Bcl-x(L)/Bax ratio, that seems to confer protection during ALI by suppressing apoptosis [42]. In the same way, EPO nearly completely restored the marked suppression of the antiapoptotic proteins Bcl-x(L) and Bcl-2 in isolated vessels during rodent endotoxaemia, ultimately resulting in a pronounced improvement of both endothelial vasoconstriction and relaxation [43]. Given the underlying evidence that apoptosis is importantly involved in ALI/ARDS, the fundamental concept emerging is that EPO promotes cell survival and ultimately induces tolerance to the subsequent insult. Unfortunately, the anti-apoptotic properties of EPO have also been reported to enhance tumor progression under some conditions by blocking tumor cell apoptosis [18].

The signaling cascade of apoptosis inhibition after EPO binding to its receptors has not been fully defined, but several molecular signals are reported to be involved in EPO's anti-apoptotic efficacy. Activation of specific EPO-R, results in the activation of Janus tyrosine kinase 2 (JAK2-kinase) and ultimately in the activation of Akt (protein kinase B) and phosphoinositide-3 kinase (PI-3 kinase) phosphorylation [18]. Expression of phosphorylated Akt and Erk, which are thought to mediate the survival signalling pathway induced by EPO, tend to be increased in lung tissues from mice treated with EPO after bleomycin-induced pneumonitis [41]. In addition, EPO may act at the transcriptional level, at least in part, through Akt-mediated modulation of nuclear factor- κ B (NF- κ B) [18]. However, EPO treatment prevented apoptosis, but did not alter the phosphorylation levels of NF κ B in the lung, after LPS administration [35]. Recent data suggest that JNK (c-Jun NH(2)-terminal kinase) signal pathway may also be involved in the anti-apoptotic of EPO against hyperoxic lung injury in neonatal rats [44]. In addition, the cytoprotective effects of EPO have been shown to involve the activation of STAT 3 and STAT 5 as well as the inhibition of caspases 3,

8, and 9 [18]. Similarly, EPO treatment significantly decreased the amount of active caspase-3 protein and caspase-3/7 activity in the lung of endotoxaemic mice [35].

Besides preserving cell integrity, EPO seems to play a dual role in vascular protection by promoting angiogenesis [45, 46]. Over a longer time frame this angiogenesis may also provide indirect cellular protection and promote functional recovery [47]. Based on this notion, EPO treatment might prevent lung injury seen with hyperoxia by stimulating angiogenesis, since EPO treatment significantly increased vascularization in hyperoxia-exposed rats [31]. However, the angiogenic actions of erythropoietin may potentially exacerbate retinopathy or enhance the progression of cancer by assisting with tumor angiogenesis [18].

The angiogenic action of EPO is probably mediated by increased expression of several key molecules in this process such as vascular endothelial growth factor (VEGF) and angiopoietin-1 (Ang-1). EPO seems to interact synergistically with VEGF to potentiate its vascular activity [48]. However, VEGF appears to play a complex role in ALI/ARDS and may have diverse beneficial or even deteriorating actions depending on the stage and the initial cause of the disease [49]. Interestingly, the EPO/EPO-R signaling pathway may somehow modulate Ang-1, since EPO stimulation leads to increased Ang-1 expression [45, 50]. Ang-1, a vascular growth factor involved in later stages of angiogenesis [51], is down-regulated during experimental ALI and has also been shown to attenuate vascular leakage, pulmonary inflammation and apoptosis [52, 53]. Nevertheless, the effect of EPO administration on lung VEGF or Ang-1 levels during ALI/ARDS has not been studied. In light of the limited data, more work is definitely needed to delineate the putative involvement of VEGF and Ang-1 in EPO-mediated effects in the lung.

It is noteworthy that endogenous EPO is a potent physiologic stimulus of endothelial progenitor cell (EPC) mobilization [54, 55]. Endothelial cells carry the EPO receptor and can be stimulated by EPO [46, 54]. Stimulation of endothelial cells by EPO increases cell migration and proliferation, endothelin-1 production and release, enhanced vascular sensitivity to nor-epinephrine, and induction of angiogenesis [54-56]. Thus, EPO may serve as a cytokine promoting the repair process of the injured pulmonary endothelium through the recruitment of bone marrow-derived EPC, and their incorporation into the pulmonary endothelium as well as the stimulation of preexisting pulmonary endothelial cells [54, 55]. Bone marrow-derived EPC are likely to contribute to pulmonary endothelial repair not only by

incorporating into the endothelial cells but also by improving the functions of the resident pulmonary endothelial cells through paracrine effects [57-58]. Table 1 summarizes the experimental data suggesting that EPO confers vascular protection in the setting of ALI.

Anti-inflammatory properties of EPO during ALI/ARDS

Although the pathogenesis of ALI/ARDS is incompletely understood and appears multifactorial, over-exuberant inflammation leading to irreversible lung damage and fibrosis is believed to play a pivotal role in the lung injury process both in experimental models and in patients [1, 2, 59]. Thus, inhibition of the inflammatory response may be one possible pathway of lung injury attenuation by EPO that exhibits anti-inflammatory properties mediated by both hormonal and paracrine modalities [17-19, 60]. In addition, since pulmonary inflammation is a key feature in the pathogenesis of fibrosis in ALI/ARDS, the anti-inflammatory activity of EPO might explain the finding that EPO treatment was associated with decreased pulmonary fibrosis in hyperoxia-exposed rats [30, 31] or bleomycin-treated mice [41, 61].

In agreement with this notion, previous studies have demonstrated that EPO attenuated pulmonary neutrophil accumulation in animals experiencing hyperoxic injury [30, 31], ischaemia/reperfusion injury [33, 34], endotoxemia [36, 37], non-septic shock [38], and acute necrotizing pancreatitis [32]. Markers of neutrophil infiltration such as malondialdehyde levels and myeloperoxidase activity in the lung tissue are also reduced as a result of EPO administration [30, 32, 37]. Leukocyte adhesion ligands such as P-selectin and intercellular adhesion molecule-1 (ICAM-1) are implicated in the activation of endothelial cells and the adhesion and migration of leukocytes to the sites of inflammation during lung injury [62]. Modulation of P-selectin expression, possibly via an influence on TNF- α production, appears to represent a major mechanism underlying the anti-inflammatory actions of EPO in the setting of ALI [63]. Moreover, EPO has been shown to down-regulate intercellular adhesion molecule-1 (ICAM-1) expression [64]. This observation is consistent with the reported ability of EPO to reduce the number of ICAM1-positive pulmonary endothelial cells in animals with bleomycin-induced pulmonary inflammation [61].

Persistent elevations of proinflammatory cytokines (TNF- α , IL-1 β , IL-6, IL-8) are found in the plasma, lavage fluid and lung tissue from patients or animal models with ALI/ARDS and have been shown to be related with similar severity of lung

dysfunction scores and to be predictive of poor clinical outcome [1-3, 6, 59, 65, 66]. In several experimental models of ALI, administration of EPO attenuated the accumulation in circulation, BAL and lung tissue of various cytokines, such as TNF- α , IL-6 and IL-1 β [30, 32, 33, 56, 34, 37]. Furthermore, in an experimental model of ischaemia/reperfusion ALI, pretreatment with EPO also reduced the over-expression of matrix metalloproteinase (MMP)-9 in lung tissue [34], which is considered to be an important factor correlated with the elevation of proinflammatory cytokines and microvascular permeability [67]. It is notable that TNF- α can directly inhibit endogenous EPO production [25]. In this way, the inhibition of endogenous EPO by TNF- α might contribute to the role of this mediator in the pathogenesis of lung injury, and partly explain why exogenously administered EPO is especially beneficial.

Several cellular mechanisms, including the mode of gene regulation and signal transduction, may account for the anti-inflammatory effects of EPO. A growing body of evidence suggests that EPO may act at the transcriptional level, at least in part, through inhibition of NF- κ B [18]. NF- κ B is a transcription factor that has been demonstrated to play a pivotal role in inflammation during ALI by promoting maximal transcription of a wide array of proinflammatory molecules including TNF- α , IL-1 β and other mediators [68, 69]. Interestingly, during LPS induced acute endotoxaemia in rats, pretreatment with EPO attenuated pulmonary inflammation and suppressed TNF- α and IL-1 β overproduction, while the enhancement in NF- κ B activation by LPS was significantly inhibited [37]. It is possible that the mechanism of action of EPO in ALI is via either down-regulation of TNF- α or via modulation of NF κ B induced cell adhesion or both [63].

As a result, it is reasonable to conclude that EPO may attenuate ALI, partly by decreasing the expression of proinflammatory cytokines, such as TNF- α , and inhibiting the accumulation of neutrophils. Yet, data concerning the effect of EPO administration on serum levels of pro-inflammatory cytokines in LPS induced endotoxemia in animals appear to be contradictory. Low-dose EPO pre-treatment deteriorated LPS-induced pulmonary damage in conscious rats and increased serum TNF- α , IL-6, and IL-1 β , along with aggravating endotoxin shock-induced markers of organ injury [70]. More interestingly, a recent study in healthy humans treated with LPS showed that pretreatment with EPO had no direct suppressive effect on TNF- α and IL-6 plasma levels [71]. Furthermore, EPO failed to significantly increase the plasma levels of the anti-inflammatory cytokine IL-10 and to attenuate

the increase in circulating neutrophils [71]. Accordingly, the beneficial action of EPO administration after LPS administration in rats had no effect on pulmonary inflammation, while the phosphorylation levels of NF- κ B in the lung were not affected [35]. Therefore, it has been proposed that the beneficial effects of EPO may be linked to inhibition of apoptosis rather than to the anti-inflammatory properties of EPO [35]. Alternatively, EPO might attenuate cytokine production and inflammation in an indirect way by preventing apoptosis and providing increased resistance of cellular targets to the effects of the noxious insult [70].

Antioxidant properties of EPO during ALI/ARDS

The over-expression and release of reactive oxygen species (ROS), and reactive nitrogen species (RNS) has been implicated as a crucial landmark in the initiation and further development of ALI/ARDS. In response to various inflammatory stimuli, lung endothelial cells, alveolar cells, and airway epithelial cells, as well as activated alveolar macrophages and neutrophils, produce both nitric oxide (NO) and superoxide, which may react to form peroxynitrite, a highly reactive radical [5, 9, 73, 74]. In addition to other effects, EPO has been demonstrated in various tissues to be an antioxidant as it can inhibit free radical production and associated lipid peroxidation [17-19]. Likewise, animal studies have shown that EPO administration led to inhibition of the oxidative stress-associated lipid peroxidation in the lung parenchyma of animals after traumatic brain injury, acute necrotizing pancreatitis or endotoxemia [32, 36, 37, 39]. At the same time, EPO has shown potent ability to suppress the inducible NO synthase (iNOS)-mediated NO production and peroxynitrite formation, thereby attenuating cytotoxicity and vascular dysfunction during experimental shock induced by zymosan or LPS [36, 38, 43]. Besides inhibition of iNOS, EPO is well known to induce expression of endothelial NO synthase (eNOS) [75]. Thus, recovery of the regulation of the microcirculation by eNOS-mediated nitric oxide may also contribute to the protection achieved by EPO treatment [76]. Interestingly, EPO also attenuated the increase in the activity of pulmonary poly-ADP-ribose polymerase caused by oxidative injury during non-septic shock in zymosan-treated mice [38].

It is believed that EPO exerts its anti-oxidative effect either directly as a potent free radical scavenger by the scavenging actions of its sugar moieties, or indirectly by increasing the activities of anti-oxidant enzymes such as superoxide dismutase,

glutathione peroxidase, and catalase [77, 78]. Furthermore, EPO can decrease the plasma iron concentration and increase the ability of plasma to inhibit lipid peroxidation [79, 80]. The inhibitory effect of EPO on iNOS has been shown to require the activation of the phospholipase C γ 1 and protein kinase C pathway [81]. Moreover, in LPS-treated rats EPO has been shown to inhibit the pulmonary activation of NF- κ B, an important redox-sensitive transcription factor necessary for iNOS gene expression [37]. Table 2 summarizes the experimental data that suggest an anti-inflammatory and anti-oxidant effect of EPO during ALI.

DISCUSSION

Taken together, the results of the aforementioned studies demonstrate that the pleiotropic action of EPO at multiple levels apparently contributes to the amelioration of crucial pathophysiological aspects of experimental ALI. Unfortunately, no single animal model of ARDS replicates the complex pathophysiological changes seen in patients [59]. However, these findings provide the rationale for the potential use of EPO as a therapeutic agent in the treatment or prevention of ALI/ARDS. Benefits in survival in human studies of ALI/ARDS have not yet been seen, but a recent multicenter, placebo controlled, double blind study has shown that when administered in critically ill patients with anemia, EPO decreased mortality in trauma patients in spite of failure to decrease the need for red cell transfusions [82]. Promising results have also been reported in a randomized, double-blind, placebo-controlled, two-center trial, which comprised the administration of weekly rhEPO in critically ill and in patients receiving long-term mechanical ventilation [83]. Although not significant, the mortality rates in patients treated with EPO (12%) were lower than in the patients treated with placebo (23%). Thus, larger and more definitive studies are needed in order to establish whether EPO treatment affects clinical outcomes in ALI/ARDS patients. In addition to the attempts to investigate its pharmacodynamics, pharmacokinetics, administration routes and doses, future *in vivo* animal experiments and clinical strategies must also seek to reduce potential adverse effects during EPO treatment.

Experimental findings appear to differ according to the animal model of ALI and the timing of EPO administration. The results in a murine model of endotoxemia indicate that the greatest survival was achieved when EPO was administered 1 hour after the LPS challenge, and that the survival benefit was lost when EPO was

administered 1 hour before the LPS challenge or 3 hours or more after the LPS challenge [35]. By contrast, in an ischaemia/reperfusion model of ALI, the best therapeutic time was a short time (2 h hours) before the procedure, while the induction of ischemic tolerance by EPO seems to be relatively rapid after injection [34]. EPO seems less protective when administered at a same dosage after the onset of reperfusion [34]. Based on this, in the case of lung transplantation, it has been postulated that it would be more beneficial to preservation of lung tissue and lung function if EPO was given to donors [34, 39]. Nevertheless, the previous findings may limit the clinical use of EPO because it appears to have a narrow therapeutic window, and that administration must be started before or very soon after the onset of the initial cause of ALI/ARDS.

Different doses of EPO have been reported to protect lung against variable challenges. Low doses of EPO (400 U/kg; i.p.) have been found to improve hyperoxia-induced lung injury in neonatal rats [31]. The same dose of EPO administered 18 h after cecal ligation and puncture improved the microcirculatory perfusion and tissue energy balance in murine sepsis [84]. However, EPO 500 IU/kg failed to protect from ischaemia/reperfusion induced ALI in rats [34] whereas in the case of LPS (20mg/kg) induced endotoxemia lower doses of EPO (300 U/kg; i.v.) have been reported to aggravate pulmonary damage in rats [70]. Higher doses of EPO than that normally used in the treatment of anemia attenuated lung injuries induced by acute necrotizing pancreatitis (1000 IU/kg; i.m.) [32] or nonseptic shock caused by zymosan in mouse (1000 IU/kg; s.c.) [38]. Contrasting with a lack of effect at low doses, the use of markedly high doses of EPO (3000 U/kg; i.p.), was found to confer lung protection in endotoxic rats after LPS challenge [36]. Likewise, high EPO doses (3000 U/kg; i.p. repeatedly) have been reported to inhibit hyperoxia-induced ALI in neonatal rats [30]. In an experimental model of I/R induced ALI in rats the same dose of EPO (3000 IU/kg i.p.) has been determined as the optimal therapeutic dosage, although doses ≥ 2000 IU/kg appeared to be beneficial [33-34].

Considering the above findings, clearly, most studies reporting beneficial effects used high doses of EPO (1000-4000 IU/kg), that exceed the dose (100–200 IU/kg/week) routinely used for patients with anaemia. Long-term clinical experience in anemic patients with chronic renal failure has shown that EPO is relatively safe and well tolerated, but adverse effects, including hypertension, thrombosis, and blood hyperviscosity are concerns for patients receiving large doses [18]. Furthermore, the

potential enhancement of tumor progression has been another significant concern of prolonged EPO treatment, while pure red cell aplasia represents a rare but serious side effect [18]. However, the recent phase II trial of human stroke treated with EPO used three daily doses of 33,000 IU/patient (approximately 500 IU/kg), and EPO was shown to be safe and did not raise the hemoglobin concentration [28]. Furthermore, EPO at weekly doses of 40,000 IU/patient (approximately 600 IU/kg) was used in intensive care units for critically ill patients to reduce the need for transfusion, and it appears to be safe although mortality was not changed [83]. However, in a more recent clinical trial, EPO at weekly doses of 40,000 IU/patient was associated with an increase in the incidence of thrombosis in patients with trauma, although the mortality was reduced [82]. Taking into account the devastating consequences of ALI/ARDS, it is reasonable to speculate that the beneficial effects of EPO treatment may prove to override the potential harmful effects. Nonetheless, due to increased risks of high doses future strategies must also seek to reduce potential toxicity. The feasibility of a large dose of erythropoietin may be enhanced by the use of carbamylated EPO that lacks erythropoietic activity yet retains the full cytoprotective actions of EPO [85].

In summary, a number of preclinical studies have revealed a pleiotropic beneficial effect of EPO administration in various experimental models of ALI suggesting that EPO may be valuable as a clinical candidate for ALI/ARDS. However, no clinical studies exist to date examining EPO in ALI/ARDS patients to demonstrate a significant effect on survival and thus more experimental and clinical studies are required for the clarification of the efficacy and potentially cytoprotective mechanisms of EPO action. Moreover, the optimal dose has not been clearly determined yet, while specific time constraints for efficacious administration of exogenous EPO as a cytoprotectant may exist. Importantly, future studies should also include attempts to minimize any potential toxicity, which is essential for EPO to fulfill the role as a potential candidate for the treatment of ALI in routine clinical practice.

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Table 1. Summary of EPO actions considered to be beneficial in the preservation of the alveolar-capillary barrier integrity during experimental ALI

Attenuation of lung edema

Preservation of pulmonary epithelial and vascular endothelial cell integrity

Inhibition of pulmonary apoptosis

Restoration of suppressed antiapoptotic proteins.

Modulation of pro-apoptotic and anti-apoptotic signalling pathways.

Promotion of pulmonary angiogenesis

Direct angiogenic effect

Recruitment and incorporation of bone marrow–derived endothelial progenitor cells into the pulmonary endothelium

Modulation of angiogenic factors (VEGF, Ang/Tie system)

Table 2. Summary of anti-inflammatory and anti-oxidative actions of EPO considered to be beneficial during experimental ALI

Anti-inflammatory effects

Attenuated pulmonary neutrophil infiltration

Modulation of leukocyte adhesion ligands (P-selectin, ICAM-1) expression

Decrease of various cytokines (TNF- α , IL-6 and IL-1 β) in BAL and lung tissue

Decrease of various cytokines (TNF- α , IL-6 and IL-1 β) in circulation

Attenuation of the over-expression of matrix metalloproteinase (MMP)-9 in lung tissue

Anti-oxidative effects

Modulation of NF κ B induced cell adhesion and cytokine production

Inhibition of the oxidative stress-associated lipid peroxidation in the lung parenchyma

Attenuation of the increased activity of pulmonary PARP

Inhibition of iNOS expression and peroxynitrite formation in the lung