Nonerythropoietic Properties of Erythropoietin: Implication for Tissue Protection

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Abstract: Erythropoietin (EPO) is used at present in clinical practice to stimulate red cell production. However, a number of reports have emerged suggesting the presence of nonerythropoietic properties for EPO. Chief among them is its ability to confer protection against acute tissue injury. In this report, we briefly review the role of EPO in tissue protection and provide examples of tissue protection using cisplatin-induced kidney injury model. Also provided is a brief description of potential pathways through which EPO may be mediating this effect.

Key Words: cell injury, erythropoietin, tissue protection

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The identification and cloning of erythropoietin (EPO) and the recombinant production of EPO have been a remarkable milestone in medical therapeutics. Since the therapeutic introduction of EPO a few decades ago, it has been used primarily to stimulate red cell production. However, a number of experimental reports during the last 2 decades have suggested that EPO may have other functional properties other than its widely recognized erythroid-stimulating property. A prominent one is its ability to confer protection to nonerythroid cells and tissues against injury partly through its antiapoptotic property. Indeed, several studies have demonstrated that EPO can suppress cell death and modulate organ function in the setting of injury.[1–4] One of the earlier studies was that of Siren et al.,[1] in which administration of EPO both in vitro and in vivo was associated with reduced neuronal apoptosis and improved motor function. Moreover, early on, it was demonstrated that erythropoietic property of EPO can be dissociated from its cytoprotective property. For example, by removing the sialo moiety from EPO molecule, the asialo EPO was shown to confer neuroprotection in the absence of erythropoiesis.[5]

ROLE OF EPO IN KIDNEY PROTECTION

The role of EPO in protecting kidneys against injury has also been demonstrated in several models.[6,7] Using the cisplatin-nephrotoxicity model, it has been shown in the rats that EPO administration before or soon after cisplatin can reduce acute kidney injury.[8] The cisplatin-induced kidney injury is an interesting model because it mimics to a greater extent what happens in the kidneys of cancer patients receiving cisplatin; that is, cisplatin at higher doses as in rat kidneys can cause acute tubular necrosis and apoptosis.[8] It is considered that because intracellular chloride concentration is very low compared with extracellular compartments, cisplatin once enters the tubular cells and tends to lose its chloride moieties.[9] Loss of chlorides leaves the platinum highly unstable, susceptible to free-radical formation. Cisplatin at least in part through radical mechanism causes cell death through both necrosis and apoptosis.[9]

IN VIVO AND IN VITRO EXPERIENCE WITH EPO

In one of our studies, EPO provided a dose-dependent protection against cisplatin-induced apoptosis of human renal proximal tubular cells in culture.[8] In contrast, an inactive EPO, synthesized deliberately with incorrect amino acid sequence to prevent it from binding to the EPO receptor (EPOR) site, had no effect on preventing cisplatin-induced apoptosis, suggesting protection as a function of EPO-EPOR interaction. Similar findings of cytoprotection as in EPO were also reproduced when darbepoetin (DPO), a long-acting EPO, was used in a repeat experiment. Some of these findings were extended to animal models in which rats were injected intravenously with cisplatin 5.5 mg per kg of body weight as a single dose to test the efficacy of erythropoiesis-stimulating proteins (ESPs) (EPO or DPO) as kidney-protective agent.[8] Various dosing and timing regimens were used, and kidney function data and histology were obtained. The control animals injected with saline had no increase in serum creatinine or hematocrit. However, animals injected with cisplatin had a significant increase in serum creatinine, but the rise in serum creatinine was significantly lower in animals pretreated with DPO (Fig. 1). In our study, although protection was consistently demonstrated, renal protection was incomplete, suggesting that ESPs reduce the extent and duration of renal injury, but other mechanisms of injury not amenable to ESPs are present. Of note is that the protection was present even if ESPs were administered after injury. The in situ staining for apoptosis in the kidneys of cisplatin-injected rats showed evidence for extensive apoptotic cell death compared with staining in the kidneys of saline-injected rats. However, the striking finding was the significant reduction in cisplatin-induced renal tubular apoptosis of animals pretreated with DPO. Prevention of necrotic injury by ESPs was less impressive and might explain for the presence of residual injury and incomplete renal protection in the ESP-treated groups.

In further mechanistic studies, EPO, but not inactive EPO, induced significant increase in STAT-5 and Akt phosphorylation, and pretreatment of cells with Janus kinase (JAK) inhibitor AG 490 dose-dependently reversed the protection of EPO against the cisplatin. These findings collectively suggest an important role for EPO-EPOR interaction, and JAK-STAT pathway in EPO afforded cytoprotection against cisplatin.[10,11]

A clinical study by Corwin et al.[12] observed a decrease in the mortality rates in patients admitted to the intensive care unit (ICU) randomized to epoetin alfa (EPO) as compared with ICU patients who were given placebos. This effect was statistically significant in a subset of patients admitted with trauma. There was no difference in the hemoglobin levels and the units of blood transfused among the 2 groups, leading the authors to suggest

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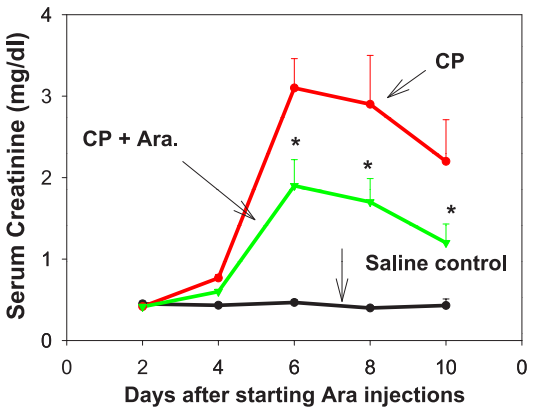
that the observed effect was most likely due to the nonerythropoietic effect of EPO. However, there was significantly higher occurrence of thrombotic and thromboembolic complications in the EPO group.[12] The latter finding suggests that an ideal molecule in this setting is a tailor-made one that is tissue-protective but devoid of thrombotic or erythropoietic properties. Others have also suggested that EPO cytoprotection may stem from its cytokine and antiapoptotic properties.[13,14]

POSSIBLE SIGNALING PATHWAY FOR THE CYTOPROTECTIVE PROPERTIES

Based on immunoprecipitation studies, it has been suggested that part of the tissue-protective properties might emerge through the β common receptor (βCR) pathway (Fig. 2).[15] The canonical EPO pathway involves the binding of 2 EPO ligands with the EPOR homodimeric receptors on cell membranes, which in turn causes the EPOR conformational changes leading to the activation of JAK1-2–STAT 3 or 5A pathway via phosphorylation.[16,17] The phosphorylation and activation of phosphoinositol 3-kinase and subsequently that of Akt kinase also lead to the inhibition of apoptosis. The alternate pathway involving βCR pathway is considered primarily protective and involves the binding of 2 EPO molecules to a heterodimeric receptor complex consisting of EPOR and βCR (Fig. 2), which can lead to the activation of the RAF-MEK-ERK pathway regulating cell proliferation and apoptosis.[18] That EPO's cytoprotection could be mediated through the βCR pathway[19,20] raises the possibility that EPO molecules could be modified to specifically activate the cytoprotective but not the erythropoietic signaling. This is of particular importance since a clinical study[12] had indicated that EPO compared with placebo in a subset of critically ill patients admitted to the ICU might have significantly lower mortality rate. The study also demonstrated as alluded to above that the patients who received EPO also had significantly higher incidence of thrombosis and thromboembolic phenomenon.[12] Therefore, tailoring the EPO molecule to create new molecules that will only confer cytoprotection without erythropoietic or thrombotic property might be of particular use to reduce mortality and morbidities in patients with acute injuries. Dr. Brines and colleagues[21] have developed such a molecule that seems to be devoid of EPO activity, yet in animal and cell culture studies seems to confer cytoprotective properties. Coleman and Brines[13] had presented their related work in this symposium, and their article related to their presentation is published in this journal.



n = 6-7 rats; * P <0.05 vs. the rest on day 6, 8 and 10.

FIGURE 1. Darbepoetin (Ara) reduces cisplatin nephrotoxicity. Ara indicates Aranesp.

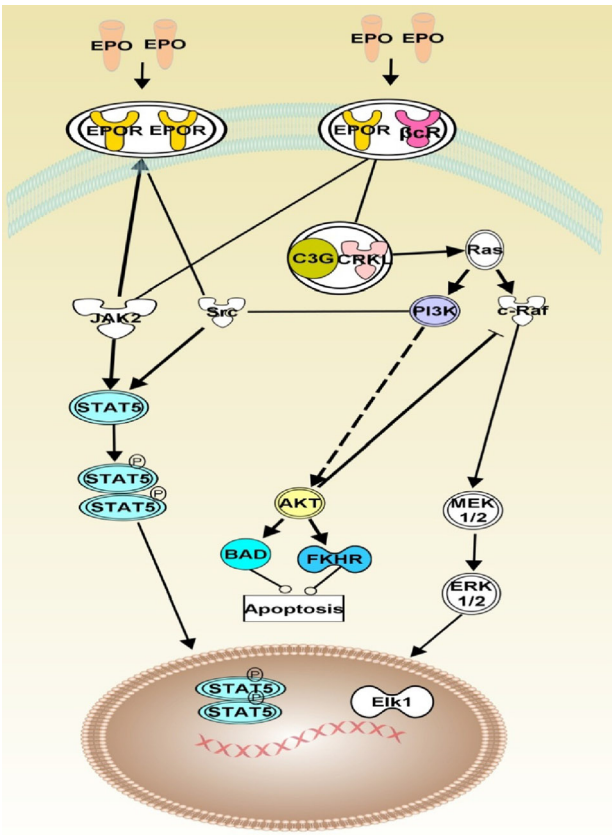


FIGURE 2. Possible signaling pathways (EPO-EPOR and EPO-βCR) mediating EPO's cytoprotection. The alternate pathway involving βCR pathway (right-hand side) is considered primarily cytoprotective without erythropoietic properties and involves the binding of 2 EPO molecules to a heterodimeric receptor complex consisting of EPOR and βCR leading to activation of the RAF-MEK-ERK pathway regulating cell proliferation and apoptosis.

CONCLUSIONS

In summary, EPO and related proteins may have tissue-protective properties in addition to their well-known erythropoietic function. New experimental data suggest that new molecules tailor-made from EPO molecule may provide tissue protection without the complications associated with increased erythropoiesis and prothrombotic state.

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