A Role for Heme Oxygenase-1 in the Antioxidant and Antiapoptotic Effects of Erythropoietin: The Start of a Good News/Bad News Story?

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Abstract
Erythropoietin (EPO) is the major regulator of erythropoiesis. EPO’s actions have been shown to be antiapoptotic and dependent on JAK2 signaling and Akt phosphorylation. These effects serve as link between EPO and heme oxygenase-1 (HO-1). HO-1 is an inducible enzyme with potent antioxidant and antiapoptotic activities which are regulated by Akt signaling. EPO’s ability to alter cellular systems that involve apoptosis and oxidants suggests that EPO treatments are likely to have multiple and different effects which may start a good news/bad news story. Recombinant human EPO is the recognized treatment of choice to address anemia and to stimulate erythropoiesis in chronic renal failure patients, through its antiapoptotic action which likely involves HO-1. On the other hand, EPO treatment to address anemia in cancer patients, while providing significant improvements in cancer patients’ quality of life, its effects on survival are equivocal, likely due to its linkage with HO-1. Two clinical trials of EPO in patients with solid tumors have, in fact, shown specific negative effects on survival. However, EPO’s effect on tumor growth and survival is not uniformly pro growth and pro survival, as EPO may act synergistically with chemotherapy to induce apoptosis. Finally, compounds have been synthesized that do not trigger EPO receptor and thus may allow experimental distinction and, therefore, at least potentially affect at the clinical level the tissue-protective effects of EPO (e.g., antiapoptosis) without provoking its other potentially detrimental effects.

The Good News
The elucidation of the erythropoietin (EPO) mediated control of erythropoiesis remains an ongoing process, but continues to yield findings of considerable scientific and clinical importance.

EPO has been shown to be a major regulator of proliferation and differentiation of erythroid progenitor cells which acts through its inhibition of proapoptotic caspase activation and attenuation of cell death in response to oxidative stress [1, 2]. Mechanistic details of EPO’s antiapoptotic action have been recently clarified with the demonstration that the effects of EPO are dependent on JAK2 signaling. The cytoplasmic portion of the EPO re-
ceptor, in fact, contains a Box 1 motif that upon EPO binding phosphorylates and thereby activates JAK2 [3]. In addition to the antia apoptotic activity via JAK2, EPO affects phosphatidylinositol 3-kinase (PI3K) and Akt (protein kinase B) activity [3–5]. Akt1, Akt2, and Akt3, the three mammalian isofoms, belong to the cAMP-dependent kinase/protein kinase G/protein kinase C superfamily of protein kinases which, once triggered, activates multiple targets with antia apoptotic effects [5]. Interestingly PI3K/Akt-pathway-related responses to oxidative stress and apoptosis have been described at the level of transcriptional regulation of heme oxygenase-1 (HO-1) [6]. HO-1 catalyzes the rate-limiting step in heme degradation, producing carbon monoxide, free Fe^{2+}, and biliverdin. Biliverdin is subsequently reduced to bilirubin by biliverdin reductase. HO-1 is a phase II enzyme induced by oxidative stress [7] and has been shown to possess potent antioxidant and antia apoptotic activities [8]. Both biliverdin and bilirubin display powerful antioxidant properties in vitro, and RNA interference against biliverdin reductase depletes bilirubin and markedly elevates tissue reactive oxygen species levels and causes apoptotic cell death [9]. HO-1 is the inducible isof orm of the three HO isofoms described. HO-1 is increased in response to injurious stimuli such as hyperoxia, hypoxia, as well as exposure to endotoxin and heavy metals, and it has become increasingly evident that HO-1 is a component in an interlocking series of oxidant defenses and signaling systems [7].

Very recently, Salinas et al. [10] have shown that HO-1 is also phosphorylated by Akt which induces an increase in HO-1 activity [10], and PI3K/Akt-pathway-related responses to oxidative stress and apoptosis have been described at the level of transcriptional regulation of HO-1 [6].

That Akt is so clearly involved in EPO’s activity provides evidence linking EPO and HO-1. Studies on endothelial progenitor cells (EPC) have shown that these cells were mobilized by statins which signal via the PI3K/Akt pathway [11, 12], and, in addition, simvastatin’s anti-inflammatory and antia apoptotic effects have been shown to occur through PI3K/Akt induction via HO-1 effects [13]. The increases in antioxidant defenses joined with increased expression of HO-1 upon EPO treatment we noted in hemodialysis patients, together with the high degree of a correlation between hemoglobin and HO-1 expression, suggest a direct non-erythropoiesis-related effect of EPO [14] and assume an increased importance in understanding the basis of EPO’s effects. These effects result in important clinical significance, as it is well recognized that patients with renal failure, whether on renal replacement therapy or not, are subject to increased oxidative stress which underlies their elevated risk of cardiovascular disease, a major complication in these patients [15]. Also noteworthy are the facts that hemodialysis patients who fail to respond to EPO have an improved response to EPO, resulting in improving anemia when treated with carnitine [16], and that very recently carnitine has been shown to increase the number of colony-forming unit-erythroid colonies in mouse bone marrow cell cultures, suggesting that carnitine stimulates erythropoiesis and thereby improves renal anemia [17]. These reports again are consistent with a linkage between EPO, HO-1, and oxidative stress [18], as carnitine has been reported to reduce apoptosis in experimental heart failure [19], and our group [20] reported that carnitine-treated human endothelial cells in culture show increased HO-1 mRNA and protein expression. Moreover, interaction of PI3K/Akt pathway and apoptosis in heart failure has been traced to transcriptional regulation of HO-1, and oxidative-stress-induced apoptosis is known to play an important role in the pathogenesis of heart failure [21]. Other studies examining HO-1 documented its antia apoptotic role in hemopoietic precursor cells in bone marrow [22, 23] and in protection against acute renal failure by EPO through upregulation of HO-1 [24]. Finally, further support for a linkage between EPO and HO-1 comes from other nerythropoietic effects of EPO, such as those on EPC. EPO is, in fact, a potent regulator of EPC, making EPO a key molecule in the process of vascular repair [25]. Interestingly, also statins have been shown to increase number and differentiation of EPCs via PI3K/Akt pathway, and, as noted earlier, statin-induced antia apoptotic and anti-inflammatory-effect-related responses have been shown to occur via PI3K/Akt-mediated induction of HO-1 [6, 13]. All this is noteworthy insofar, as the clinical outcome in patients with myocardial infarction is strongly correlated with the number of mobilized EPCs from the bone marrow, and the number of EPCs significantly correlates with endothelial function and cardiovascular risk factors in patients having no myocardial infarction [26, 27].

Thus there is robust support from a variety of studies, including those from our laboratory, for HO-1’s interrelated roles as both an antioxidant as well as a mediator of the antia apoptotic action of EPO, particularly with respect to EPO’s effect on hemopoietic cells in hemodialysis patients.
The Bad News

The ability of EPO to alter cellular systems that involve apoptosis and oxidants suggests that EPO’s overall effects may not all be assumed to be either selective or benign. This is particularly so given the Jekyll-and-Hyde-like nature of oxidants, wherein they can function to damage biomolecules and tissues as well as to mediate aberrant signals, but are nonetheless critical components involved in normal cell and organ system function and signal transduction. The changes accompanying chronic systemic EPO elevation and their connection to the pathology found remain to be fully explored. However, the growing data regarding EPO’s effects on HO-1 coupled to the studies showing its antiapoptotic effects suggest that more attention may need to be paid to the potential for EPO to reduce or prevent apoptosis, when it is a desired outcome [28]. In fact, a direct growth regulation by EPO in prostate cancer has been shown [29], and evidence that increased autocrine EPO signaling promotes survival in breast cancer cells has also been provided [30]. Arcasoy et al. [29] have, in fact, shown growth regulation by EPO and its receptor in an autocrine or paracrine manner in prostate cancer. Similarly, Acs et al. [30] have provided evidence that increased autocrine EPO signaling induced by moderate levels of hypoxia in breast cancer cells upregulates bcl-2 and bcl-XL, inhibits hypoxia-induced apoptosis, and promotes survival [30].

It is well recognized that EPO treatment provides significant improvements in terms of quality of life in cancer patients with anemia. However, a recent report by the European Organization for Research and Treatment of Cancer (EORTC) [31] indicated that while EPO can be shown to provide significant quality of life improvements in cancer patients with anemia, its effects on survival were equivocal. The EORTC report also examined in depth the results of two specific clinical trials, where EPO’s effect on patient survival provoked specific concerns [32, 33]. The first such trial [32] was a randomized, prospective trial using recombinant EPO and involved patients with metastatic breast cancer. The trial was terminated early due to impaired patient survival that was associated with disease progression in those patients receiving recombinant EPO when compared to patient survival and disease progression of the placebo arm. In the second trial [33], in patients with cancer of head and neck, it was found that EPO treatment was associated with a poorer progression-free survival in those patients randomized into the recombinant human EPO arm.

As is the case for EPO, the connection of EPO and HO-1 is also supported by HO-1 involvement in cancer cell proliferation. HO-1 is, in fact, upregulated in many tumors and plays a critical role in tumor growth [34]. In our laboratory further evidence was found for the involvement of HO-1 in tumor cell growth. We have found, in fact, that doxazosin, an alpha blocker used to treat benign prostatic hyperplasia, decreases HO-1 mRNA and protein levels in human prostate biopsy specimens, providing a mechanism for the proapoptotic actions of doxazosin [35] and further suggesting that HO-1 activity and prostate cell apoptosis are linked [36].

Other potential side effects of EPO continue to be examined, with thrombosis and vascular events being a particular focus [37]. Bohlius et al. [38] have recently reported the results of a systematic meta-analysis of EPO based on twelve trials published up to 2001. Overall, the authors concluded that there was no conclusive evidence of an increased risk of hypertension and thromboembolic events or related complications in cancer patients treated with EPO. However, several reports have described a high rate of thrombotic and vascular events in EPO-treated groups [39]. One potential mechanism for this association is that EPO has been reported to show procoagulant properties that predispose to thrombosis [40, 41].

Given the extensive use of EPO in clinical practice, further investigations specifically addressing the relationship(s) between EPO and HO-1 and their effects on cancer growth/survival are warranted. Of note, in response to the issue of EPO’s effect on cancer patient survival, the EORTC report [31] suggested that ‘the issues of tumor response/progression and survival must be carefully studied in order to provide clear guidelines for the future’. In a recent study, Pajonk et al. [28] have gone a step further and suggested that the EPO use should be restricted in patients suffering from EPO-receptor-expressing cancers.

On the other hand, there is recent experimental evidence that EPO may act synergistically with chemotherapeutic agents [42] and increase tumor cell apoptosis [43] and that HO-1, in addition to be antiapoptotic in some cells, has been found to act as proapoptotic in others [44] and that these effects also link EPO with HO-1, making the entire argument EPO-HO-1/cancer growth and survival being very much in need of further clarification.
Conclusions

EPO actions appear to be linked to oxidative stress mediators and to HO-1 resulting from EPO’s widespread antiapoptotic effects which strongly point to a central role for HO-1 as a mediator of EPO and confirm its status as a major antioxidant/antiapoptotic factor. The recognition of such a central role of HO-1 is likely to be useful in several different conditions. This includes the development of therapeutic strategies aimed at increasing the HO-1 activity and thereby counteracting oxidative stress and apoptosis, specifically in chronic renal failure patients with their increased cardiovascular disease risk. The connection between EPO, HO-1, oxidative stress, and diseases such as cancer and heart disease suggests that the use of EPO to address anemia needs to be carefully assessed, as the perceived benefits may be accompanied, in some conditions such as cancer, by significant negative effects. Our limited understanding of the interaction of EPO and HO-1 and the oxidant and other pathways they modify as well as the apparently contradictory effects for both HO-1 and EPO suggest that EPO effects and its clinical usage are very much in need of further clarification. The future of EPO- and HO-1-related research and clinical modalities is even more exciting, as Leist et al. [45] have published results with compounds that trigger EPO-mediated tissue-protective pathways without cross talk with the hematopoietic system, suggesting that it will be possible to evoke, in specific target cell and organs, EPO’s protective effects (e.g., antiapoptosis), avoiding its other potentially detrimental effects.

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