Novel tissue remodelling roles for human recombinant erythropoietin

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Abstract

rHuEPO (recombinant human erythropoietin) is a haemopoietic growth factor and a primary regulator of erythropoiesis that is used for the treatment of chronic anaemia associated with RA (rheumatoid arthritis). Erythropoietin also appears to modulate a broad array of cellular processes, including progenitor stem-cell development, cellular integrity, angiogenesis and oxidative damage. These diverse activities suggest the exciting possibility of multiple roles for rHuEPO therapy in a variety of disorders other than RA, including cerebral ischaemia, myocardial infarction, chronic congestive heart failure and cancer. Thus it appears that rHuEPO may be a pleiotropic agent, capable of influencing tissue remodelling independently of its established erythropoietic role. Whereas these effects may be largely beneficial, dose-related side effects could have implications for the safe therapeutic use of rHuEPO and its illegal use as a performance-enhancing agent in endurance sports.

Introduction: EPO (erythropoietin) and RA (rheumatoid arthritis)

EPO is a hypoxia-induced, multifunctional tropic factor that is best known in its role as haemopoietic growth factor and primary regulator of erythropoiesis. It is primarily produced by peritubular capillary endothelium of kidney and its principal target is the EPO receptor found on colony-forming-unit erythrocytes [1]. Secondary sources of EPO are synthesized in hepatocytes of healthy adults, and recent studies have identified another two sites of EPO production, the brain and uterus, that are regulated in a tissue-specific manner. The paracrine system is independent of the endocrine system in erythropoiesis and prevents neuronal death, while the uterine EPO induces oestrogen (oestradiol-17β)-dependent uterine angiogenesis [2].

Since the human EPO gene was cloned in 1985, rHuEPO (recombinant human erythropoietin) has been widely administered for treatment of chronic complications, including ACD (anaemia of chronic disease), which is a common feature of active RA [3]. Although, the effect of rHuEPO on cartilage metabolism has not yet been elucidated, there is some evidence that it may be directly involved in the inflammatory process. It has been suggested that one of the reasons for the success of rHuEPO in ACD therapy may be that inflammatory cytokines such as IL-1β, TNF-α (tumour necrosis factor α), and IL-6 may contribute to the pathogenesis of ACD, by inhibiting EPO production [4].

RA, NO (nitric oxide) and ECM (extracellular matrix) turnover

RA is a persistent autoimmune disease, involving chronic inflammation of the diarthrodial joints, which causes cartilage degradation and bone destruction. These changes are influenced by cell–cell interactions and the secretion of MMPs (matrix metalloproteinases), secreted by RA synovial fibroblasts and responsible for degradation of the ECM macromolecules [5]. Joint cells produce chemical mediators, including NO and pro-inflammatory cytokines, such as IL-1β, IL-6 and TNF-α. NO may play a critical role in regulating articular ECM remodelling in RA, through the production of MMPs. Both NO and MMPs are therefore implicated in the pathological processes of inflammatory joint diseases [5]. Through the elaboration of effector signals including cytokines, mesenchymal cells stimulate or suppress inflammation via autocrine and paracrine mechanisms. Moreover, they may play an integral role in the initial phases of synovitis by releasing cytokines that trigger angiogenesis [6]. It has been demonstrated that rheumatoid synovial fibroblasts co-cultured with articular cartilage tissue slices showed IL-1β-stimulated fibroblast invasion of cartilage and may therefore play an important role in the pathogenesis of RA [7]. The detection of NO production by cultured fibroblasts in response to rHuEPO has recently provided a model for investigation of the mechanisms that may control these processes [8].

In order to study the molecular basis for RA, cellular models for NO production and GAG (glycosaminoglycan) release have been established. For example, cultures of articular chondrocytes and synovial fibroblasts from rabbits have been shown to synthesize NO when stimulated with pro-inflammatory cytokines [9]. However, chondrocytes are probably not the only source of NO production in the
diarthrodial joint. Other potential sources include pannus tissue fibroblasts [10] and possibly ligament cells [11].

Therapeutic doses of EPO
No optimum dose for rHuEPO therapy has been established for ACD, mainly owing to the large variation in baseline levels of EPO at the start of therapy and variations in the degree of iron storage in some patients, which may determine hyporesponsive effects. It has been suggested that doses of 50 units/kg three times weekly are sufficient to achieve reticulocytosis but only if the patient has sufficient iron, folate and vitamin B₁₂ stores and no other forms of ongoing inflammation. However, for other anaemias, such as those developed in cancers, AIDS and RA, higher doses (e.g. 150 units/kg or more, 3 times weekly) may be required. The response to therapy (even at higher doses) is variable and in general the higher the patient’s baseline plasma EPO level, the poorer the response to rHuEPO therapy [12].

Effect of rHuEPO on explant models of RA
Little is known concerning the role(s) of rHuEPO within chronically inflamed joints. To address this, our group has developed a PNE (porcine nasal explant) tissue model of RA, which is composed predominantly of chondrocytes, to test the effects of rHuEPO on models of inflamed cartilage. When PNE was stimulated with pro-inflammatory IL-1β, a significant increase in NO and GAGs was detected in the culture medium. Unexpectedly, a similar effect was detected following rHuEPO treatment. Since NO production was abolished by the enzyme inhibitor L-NAME (N⁵-groto-L-arginine methyl ester), this suggested that control of both IL-1β and rHuEPO responses is modulated at the level of nitric oxide synthase (P.J. Coussons, S. Baig, C. Fanutti and R. Grant, unpublished work).

A similar pattern of response was measured when explants were co-cultured with Swiss 3T3 fibroblasts in order to model the inflamed pannus/cartilage interface. In this case, the magnitude of NO and GAGs release was significantly higher than that of PNE monoculture. Collectively, these results suggest that rHuEPO may have a direct, non-erythropoietic (possibly proinflammatory) effect on joint tissue. This effect was detected within a relatively narrow concentration range but was abolished at elevated doses of rHuEPO (P.J. Coussons, S. Baig, C. Fanutti and R. Grant, unpublished work).

Implications for rHuEPO therapy
Recent reports suggest that EPO is a pleiotropic agent, capable of influencing tissue remodelling independently of its established erythropoietic role. For instance, EPO may modulate a broad array of cellular processes that include progenitor stem-cell development, cellular integrity, angiogenesis and oxidative damage. These protective effects may eventually be harnessed for disease therapy in a variety of disorders other than RA, including cerebral ischaemia, myocardial infarction, chronic congestive heart failure and cancer (for review, see [13]).

On a cautionary note, although rHuEPO therapy is usually associated with beneficial effects, especially in ACD therapy, non-erythropoietic effects of rHuEPO could have undesirable consequences. Indeed, it is established that a major side effect of ACD treatment with rHuEPO is the development of hypertension [14].

If pro-inflammatory effects are associated with low doses of rHuEPO, its illegal use, especially in sports, could lead to long-term pathologies. Moreover, as the beneficial effects of rHuEPO on ACD are likely to outweigh other considerations, pro-inflammatory side-effects within joint tissue could conceivably go unnoticed. Finally, although the use of rHuEPO in cancer therapy may improve the efficacy of chemotherapy and radiotherapy, it has been recently pointed out that tumour cells often express EPO-R (EPO receptor). Therefore careful studies are required to exclude fully the possibility that rHuEPO may promote tumour growth [15].

References

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Received 3 August 2005