Chapter 6

Epo Delivery by Genetically Engineered C2C12 Myoblasts Immobilized in Microcapsules

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Abstract

Over the last half century, the use of erythropoietin (Epo) in the management of malignancies has been extensively studied. Originally viewed as the renal hormone responsible for red blood cell production, many recent in vivo and clinical approaches demonstrate that various tissues locally produce Epo in response to physical or metabolic stress. Thus, not only its circulating erythrocyte mass regulator activity but also the recently discovered nonhematological actions are being thoroughly investigated in order to fulfill the specific Epo delivery requirements for each therapeutic approach.

Introduction

The foundations of the present understanding of the hormonal role of Epo were laid by a succession of French scientists during the second half of the 19th century. Bert and his collaborator Jourdanet demonstrated that the physiological effects of gases depend upon their partial pressure and the relationship between tissue hypoxia and the production of erythrocytes was established.1 In 1906 Carnot and De Flandre hypothesized that a circulating factor, namely “hemopoietine”, was responsible for red blood cell production and that in certain situations such as anemia or high altitude, its concentration in the blood increased.2 The term hemopoietine was replaced four decades later, in 1948, by the term erythropoietin introduced by Bondsdorff and Jalvisto, who linked Epo solely with red blood cell production.3 As scientific and clinical experiments started to show promising results in the 1950s, Jacobson and his group established the kidney as the primary site of production of Epo.4 The isolation and purification of Epo required huge efforts and finally in 1977 Miyake et al could successfully purify it to apparent homogeneity from urine collected from patients suffering from aplastic anemia.5

The cloning of the epo gene in 1983, initiation of human recombinant Epo (rHuEpo) therapy in 1985 and approval for its clinical use in 1989, gave rise to a greatly expanded understanding of the biology of Epo and has already been associated with several chronic states. Epo has been widely used in the treatment of anemia that is associated with various chronic conditions. These include end-stage renal disease, malignancy and HIV infection. Epo is also used before selective surgical procedures to reduce blood transfusions, especially in Jehovah’s Witnesses. The scarcity and complications of allogeneic blood transfusions such as allergic reactions, immunosuppression,
alloimmunization, graft-versus-host disease\(^6\) and transmission of viruses and parasites should be carefully considered against the cost and benefits of rHuEpo.\(^7\)

Epo is an acidic glycoprotein consisting of 165 amino acids and a molecular mass of 30-35 kD produced mainly by hepatocytes during fetal stage. After birth, almost all circulating Epo originates from peritubular fibroblast-like cells located in the cortex of kidneys\(^8,10\) under the control of an oxygen-sensing mechanism as proposed by Erslev and Gabuzda\(^11\) where a functional feedback links the rate of red blood cell production to the demand for oxygen by tissues.

The regulation of \textit{epo} gene expression occurs mainly at the transcriptional level by DNA-dependent mRNA synthesis and gene activation. Tissue hypoxia is the main stimulus for Epo production\(^12\) and this mechanism has been thoroughly investigated for many years although \textit{epo} gene expression is not only stimulated when the \(O_2\) capacity (corresponding to the Hb concentration) of the blood decreases, but also when the arterial pO\(_2\) decreases (i.e., at high altitude residence) or when the \(O_2\) affinity of the blood increases.

The mechanisms of degradation of the circulating Epo are still incompletely understood. To a minor degree, Epo may be cleared by the liver and the kidneys. However, there is evidence to assume that Epo is mainly removed from circulation by uptake into erythrocytic and other cells possessing the Epo receptor.\(^13\)

**Therapeutic Applications Beyond Erythropoiesis**

Recently, advances in analytical techniques have enabled to demonstrate that Epo provides its effects not only in the erythroid compartment but also in other non-erythrocytic cells and organs carrying Epo receptors including the brain, spinal cord, retina, reproductive organs, cardiovascular system (cardiomyocytes, endothelium, vascular smooth muscle), skeletal muscle, liver, gastrointestinal tract (gut and pancreas), lung and the kidney,\(^14-26\) which has led to a major revision of the biological role of Epo (Table 1). In addition, in the embryo, Epo is required for cardiac myocyte proliferation.\(^27\) A chance observation by Anagnostou and coworkers\(^28\) suggested the first extra-hematopoietic activities of Epo observing that Epo induced chemotaxis and mitosis of cultured endothelial cells. Endothelial cells respond to local ischemia by producing Epo. Therefore, these cells, distributed universally throughout tissues, could potentially provide Epo-mediated protective function globally.\(^29\) These findings support the idea that in fact, Epo is a more pleiotropic growth and survival hormone than previously thought. Epo/Epo-R interactions have been reported to induce a wide range of cellular responses, including angiogenesis, chemotaxis, mitogenesis, mobilization of intracellular calcium and inhibition of apoptosis,\(^30\) providing cell proliferation, differentiation and survival and consequently tissue protection. However, not only these beneficial effects should be mentioned. In fact, Epo seems to exert both positive and negative effects on tumor biology. As a consequence, further well-controlled studies carried out in xenogeneic models of different types of cancer are essential to determine the ability of Epo to regulate angiogenesis, apoptosis, chemoradiation sensitivity and tumor growth in the presence or absence of concomitant chemoradiotherapy.\(^31-37\)

**Novel Erythropoiesis Stimulating Strategies: Potential New Treatments for Anemia**

Before recombinant human Epo became available for therapy, about 25% of patients with chronic kidney disease needed regular transfusions of red cells. In the light of the therapeutic value of rHuEpo it should be remembered that today’s success has been based on a century of laborious research into the basics of erythropoiesis.\(^38\)

Genetic engineering enabled to produce rHuEpo for the treatment of anemias of chronic renal failure and other diseases. Table 2 gives an overview of novel pharmacological approaches to stimulate erythropoiesis.\(^39-58\)

Regarding clinical setting, a major drawback of this treatment is the requirement of repeated injections, twice or three times weekly. Various efforts have, therefore, been proposed to produce longer-acting erythropoietin analogues that could retain the biological activity of erythropoietin.