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EPO abuse in sport

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Abstract

In many sport disciplines the delivery of oxygen to muscles plays a critical role. Indeed, muscle performance declines during prolonged and intense activity as a consequence of the shift from the aerobic to the anaerobic metabolism with an increase of lactate. To enhance the aerobic capacity two alternatives may be used: increasing either the transport or the delivery of oxygen. In this setting, erythropoietin use is the practice of illicitly using a drug to improve athletic performances. In the present overview, old and newer erythropoietic stimulating molecules are described with a special emphasis on their potential side effects. Direct and indirect detection methods are briefly described with the aim of mentioning their roles and limits with regard to anti-doping strategies. **Keywords:** erythropoietin, sport, erythropoietic stimulating molecules, detection methods, side effects

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Introduction

In many sports disciplines an increase in maximal aerobic power is desirable to improve performance. First studied in the 1930s¹, the determinants of maximal oxygen uptake (VO_2max) were clearly identified as linked to maximal cardiac output, blood volume, total haemoglobin^{2,3}. It was demonstrated that VO_2max is directly correlated to total body haemoglobin, but not to haematocrit value; underscoring the idea that manoeuvres aimed to induce haemoconcentration without increasing the total body haemoglobin were not able to increase VO_2max ³. The last step was the demonstration that increasing total body haemoglobin resulted in an increase in the VO_2max and induced a positive impact on performances⁴. In the late 1980s recombinant erythropoietin (rHuEPO) became available for the treatment of anaemia, and the first studies conducted on end-stage renal disease patients showed that this hormone induced an increase in the total body haemoglobin with a coupled improvement of exercise tolerance⁵. By the early 1990s it became clear that rHuEPO was the preferred drug for athletes seeking to artificially enhance their endurance performance. In fact, from a practical point of view, rHuEPO had several advantages compared to blood transfusions (autologous blood doping), including no need for blood withdrawal, storage, transport, and reinfusion.

Erythropoietin

Erythropoietin (EPO) is a glycoprotein hormone with a molecular weight of 30.4 kDa⁶. After birth, almost all circulating EPO originates from peritubular fibroblast-like cells located in the cortex of the kidneys⁷. Studies demonstrated the presence of a circadian rhythm in serum EPO levels with the lowest mean level at 08:00h, a 42% increase at 16:00h and a 60% increase to the highest at 20:00h, and suggested that the aging process does not influence the physiological diurnal fluctuations but modifies the average daily levels and the amplitude of the diurnal variations^{8,9}. EPO production is solely regulated by tissue oxygenation¹⁰. The discovery of the family of proteins called hypoxia-inducible transcription factors (HIF) has improved the understanding of the mechanisms of the response to hypoxia¹¹. EPO gene expression is induced by HIF^{12,13}.

In addition to the well known erythropoietic effects, it has been demonstrated that EPO has also the effect of lowering plasma volume probably by inducing a downregulation of the renin-angiotensin-aldosterone axis¹⁴. This mechanism of concomitant increased erythropoiesis and lowered plasma volume seems to be crucial in determining the final EPO effect, which results in an increase of the oxygen-carrying capacity of blood.



Other erythropoiesis-stimulating molecules

1. Darbepoietin

The discovery and development of novel erythropoiesis-stimulating protein (NESP, 38,500 D or darbepoietin- α) arose from research into the structural features of EPO. Compared to rHuEPO, darbepoietin- α has increased sialylated carbohydrate content that is associated with a prolonged serum half-life and increased in vivo biologic activity. NESP binds to the Erythropoietin receptor (EPO-R) in an identical manner as native EPO inducing the same intracellular signalling. When comparing the intra-venous pharmacokinetics of NESP and rHuEPO in patients under dialysis, the average terminal half life for NESP was three times longer than for rHuEPO (25.3 vs. 8.5h). The clearance of NESP was significantly lower than that of rHuEPO (1.6 ± 0.3 vs. 4.0 ± 0.3 ml/hr/kg), and the volume of distribution for NESP and rHuEPO were equivalent (52.4 ± 2.0 vs. 48.7 ± 2.1 ml/kg)¹⁵.

2. Continuous erythropoietin receptor activator

More recently, a third-generation molecule, continuous erythropoietin receptor activator (CERA) has been developed. CERA differs from EPOs through its integration of amide bonds between amino groups and a single methoxy polyethylene glycol-succinimidyl butanoic acid polymer (30kDa). This bond leads to prolonged plasma half life of the molecule and to a superior in vivo potency. The increased activity of this molecule, when compared to other rHuEPOs, is not only due to the extended plasma half life, but also to different EPO-R activation kinetics. Indeed, CERA is able to rapidly repeat the attachment to and dissociation from the EPO-R, resulting in a prolonged repetition of EPO-R activation. This mechanism results in a more potent stimulation of erythropoiesis, both in magnitude and duration compared to standard rHuEPOs¹⁶.

3. Hematide

A novel pegylated synthetic peptic erythropoiesis stimulating agent derived from

research on the EPO-mimetic peptides named hematide is of additional interest. Hematide is a dimeric peptide unrelated in sequence to either native or recombinant EPOs, which binds to EPO-R and stimulates erythropoiesis. Moreover, hematide has a prolonged plasma half-life and a slower clearance time compared to other rHuEPOs. Results from preregistrative studies indicate that hematide produces clinically meaningful and lasting increases in haemoglobin levels when dosed once a month without the risk of pure red cell aplasia (PRCA) and it is easy to transport because it is stable at room temperature^{17,18}.

4. HIF stabilising agents

As previously described, HIF plays a central role in controlling EPO gene expression. Researchers have recently demonstrated how cobalt, a relatively rare transition metal with properties similar to iron, may target HIF. Cobalt chloride is a well recognised chemical inducer of in vivo hypoxia-like responses, such as erythropoiesis and angiogenesis. The precise mechanism of this induction is not yet understood. However, the hypoxia-like response probably involves increased DNA binding activity of HIF-1 α , as cobalt stabilises HIF-1 α through generation of reactive O₂ species by a non-enzymatic, non-mitochondrial mechanism. The final result of this induction is enhanced EPO production and more efficient stimulation of the erythropoietic response¹⁹.

5. Prolyl-hydroxylases inhibitors

HIF activity is negatively regulated by prolyl-hydroxylases (HIF-PH) small molecules that appear to be effective inhibitors of HIF-PH, are under development. Two of them (FG-2216 and FG-4592) seemed well tolerated in Phase I studies and produced robust increases in Hb levels. If proven to be safe, these molecules could offer not only the convenience of oral dosing and relatively low cost, but also improved efficacy due to their capacity to stimulate complete erythropoiesis, including iron mobilisation and suppression of negative effects of pro-inflammatory cytokines, such as IL-6 on RBC production²⁰.



Enhanced erythropoiesis and performances

Intense aerobic exercise performed for an extended period of time is closely related to the amount of oxygen that may be transported to the muscles. Several authors have demonstrated the ergogenic properties of rHuEPO administration. Studies performed on animal models showed that following rHuEPO administration exercised rats had higher muscle glycogen, higher free fatty acids, and lower lactate levels when compared with the untreated controls^{21,22}. Those results suggested that muscle increased oxygen availability may affect the energy substrate used during exercise. This implies that rHuEPO administration may result in a lower contribution of anaerobic metabolism to fulfil the energy requirements during exercise.

Although few studies on humans have been performed, available data indicate that the haemoglobin increases induced by rHuEPO administration on athletes may increase VO_2max , time to exhaustion at 95% VO_2max , and improvement in run time performances to significant levels^{5,23}. Since EPO-R is present in endothelium, in smooth muscle cells, and in fractions of the sarcolemma of skeletal muscle fibres, many scientists believe that rHuEPO has a number of additional benefits beyond its role of increasing the concentration of the circulating red cells. The potential role of EPO administration on muscle cells has been investigated for possible additional performance-enhancing properties^{24,25}. At present, rHuEPO administration seems not to have any apparent effect on capillarisation or muscle fibre hypertrophy²⁵. Therefore rHuEPO administration on healthy humans is considered to enhance athletic performance by means of augmenting oxygen transport and increasing maximal oxygen consumption due to augmented systemic and muscular peak oxygen delivery.

Side effects

Documented side effects of rHuEPO use include muscle cramps, upper respiratory infections, headache, hyperviscosity, thrombosis, and hypertension²⁶. Long-term rHuEPO use can also lead to the development of pure red cell aplasia (PRCA)^{27,28}, which occurs from the generation of antibodies against rHuEPO that are able to neutralise native EPO, leading to the absence of red cell precursors in the bone marrow. The development of PRCA in patients with chronic kidney disease receiving rHuEPO seems to be associated with subcutaneous administration of rHuEPO²⁹.

Moreover, in a systematic review of 57 trials on 9353 cancer patients, it has been shown that treatment with rHuEPO increased the risk of thromboembolic events with a relative risk of 1.67³⁰. A variety of underlying mechanisms have been postulated, including increased blood viscosity, increased platelet count and/or adhesion, decreased concentrations of protein S and protein C, and endothelial activation³¹.

Similarly, a number of mechanisms have been implicated in the pathogenesis of rHuEPO-induced hypertension. These include an increase in erythrocyte mass, alterations in neuro-hormonal factors, such as norepinephrine, endothelin, angiotensin II, alterations in nitric oxide and prostaglandin system, and direct effects on vascular smooth muscle cells³².

The reduced tumour control associated with erythropoietin treatment has been reported in recently published studies^{33,34}, and the decreased overall survival shown in five trials³⁵ raises additional considerations when considering erythropoiesis stimulating agent administration. Meta-analysis of this issue has produced conflicting results, and in updated guidelines^{36,37} there is a level I evidence that the target haemoglobin concentration following treatment with erythropoietic proteins should be 12g/dL to reduce the side effects associated with



administration of the drug. The results of these observations should be strongly emphasised in the sport environment where healthy subjects are involved.

Detection methods

The only direct method currently accepted by the World Anti-Doping Agency (WADA) to detect rHuEPO and darbepoietin in the urine of athletes is the "double-blotting" technique³⁸. This method has met serious criticism with regard to its low detection power and because the short half-life of rHuEpo makes it difficult to detect this substance even 3 days after its last injection. Moreover, case reports have shown that detection maybe impossible 12-18 hours following the last injection; this observation is even more significant considering the use of maintenance micro doses of this drug³⁹. Additional criticism involves cases of false-positive testing in an experimental setting⁴⁰ linked to post-exercise proteinuria, where proteins with structural homology to erythropoietin may cross-react with the anti-EPO antibodies⁴¹⁻⁴³. These observations imply that athletes doping with rHuEpo may not be at great risk of testing positive. Some authors agree that the practical implication is that urine testing during competition in the season is of little or no value. The optimum strategy to obtain a positive urine test in users is to require out-of-competition testing during the "boosting" period.

Several indirect methods for the detection of rHuEPO misuse have been published. At the beginning of 1990s, some authors proposed a model based on the analysis of macrocytic, hypochromatic erythrocytes⁴⁴, while others looked at soluble transferrin receptor/ferritin ratio⁴⁵. The lack of sensitivity and/or specificity of those tests led investigators to multiparametric mathematical models for detecting rHuEPO misuse. Different algorithms were developed to identify athletes currently using rHuEPO (ON-model) and those who had taken rHuEPO in the past (OFF-model)⁴⁶⁻⁴⁸. An additional third-generation model has been suggested, utilising the adoption of haematological passports⁴⁹. This strategy is an important

deterrent and information coming from those analyses could be utilised by anti-doping agencies for the identification of athletes to be target-tested. Moreover, several sport federations already exclude athletes from competition if a blood check reveals abnormal hematocrit values (no start). Notably, in all indirect approaches threshold limits must be properly set by taking into consideration inter-individual variations and confounding elements related to exercise-induced modifications in the chosen haematologic parameters.

Summary

EPO-induced increase in total body haemoglobin has been proven to augment $VO_2\text{max}$. This improvement may result in a prolonged time to exhaustion at 95% $VO_2\text{max}$, as well as in a lower contribution of anaerobic metabolism to energy production. Available data confirm that rHuEPOs may be considered the "drug of choice" for those athletes seeking to enhance their endurance performances. In addition, rHuEPOs have a number of practical advantages when compared to blood transfusion techniques, as well as a "limited" detectability. The immediate and long-term side effects should be strongly emphasised, considering that they may occur in young and otherwise healthy subjects. Newer molecules with erythropoietic stimulating properties are already used by athletes as demonstrated by athletes who tested positive for CERA in the 2008 Tour de France. The analysis of blood parameters as well as the adoption of blood passports should be considered a deterrent and should help anti-doping agencies plan effective out-of-competition testing programs. In this regard, problems of inter-technique comparison for reliable results, as well as inter- and intra-individual variability, should be emphasised in future studies.

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