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Monitoring recombinant human erythropoietin abuse among athletes

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ABSTRACT

The illegal administration of recombinant human erythropoietin (rHuEPO) among athletes is largely preferred over blood doping to enhance stamina. The advent of recombinant DNA technology allowed the expression of EPO-encoding genes in several eukaryotic hosts to produce rHuEPO, and today these performance-enhancing drugs are readily available. As a mimetic of endogenous EPO (eEPO), rHuEPO augments the oxygen carrying capacity of blood. Thus, monitoring the illicit use of rHuEPO among athletes is crucial in ensuring an even playing field and maintaining the welfare of athletes. A number of rHuEPO detection methods currently exist, including measurement of hematologic parameters, gene-based detection methods, glycomics, use of peptide markers, electrophoresis, isoelectric focusing (IEF)-double immunoblotting, aptamer/antibody-based methods, and lateral flow tests. This review gleans these different strategies and highlights the leading molecular recognition elements that have potential roles in rHuEPO doping detection.

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1. Introduction

The International Olympic Committee (IOC) has banned the administration of recombinant human erythropoietin (rHuEPO), which is preferred over blood doping to enhance performance, in 1987. Although blood doping increases the oxygen carrying capacity of the blood (Lippi and Guidi, 2000), it poses problems such as allergic reactions or hemolytic crisis; thus, athletes switched to rHuEPO. The illegal use of rHuEPO has becomes rampant due to advances in recombinant DNA technology and protein expression that enabled mass production of the substance. rHuEPO is a mimetic of EPO, which is a glycoprotein hormone and the important erythropoietic growth factor responsible for erythroid differentiation, survival, and proliferation (Fisher, 2003).

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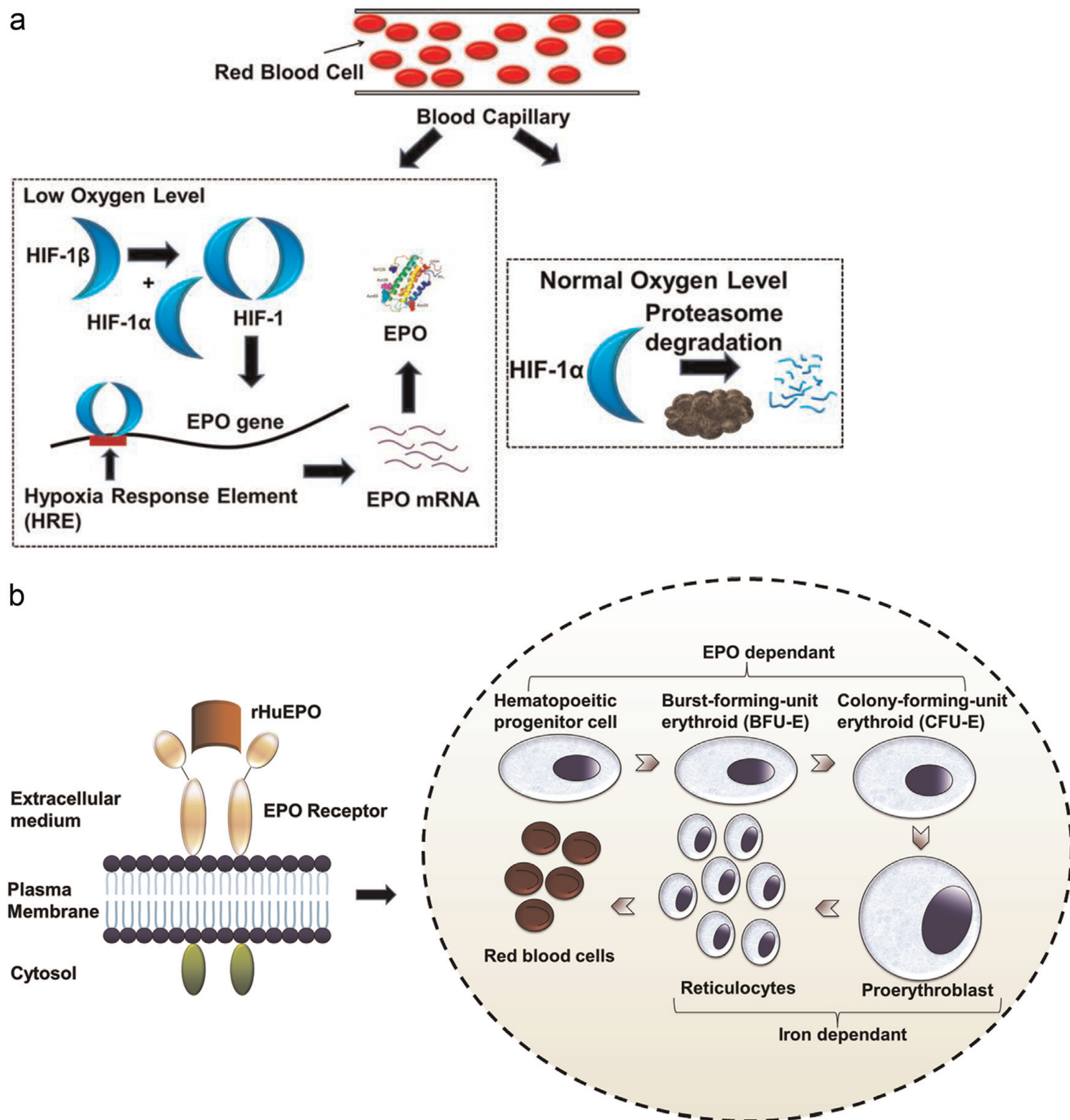

Fig. 1. (a) The level of oxygen affects the synthesis of the HIF-1α subunit, which forms a heterodimer with HIF-1β to generate transcription activator hypoxia inducible factor-1 (HIF-1), which in turn regulates EPO synthesis (Jiang et al., 1996). At the normal oxygen level, HIF-1α has a very short half-life due to its degradation by the proteosome (Huang et al., 1998; Salceda and Caro, 1997). However, under hypoxia (i.e., low oxygen level), synthesis of HIF-1α increases and dimerization with HIF-1β occurs to form HIF-1. The resulting dimer of HIF-1 binds to the hypoxia response element, which is located upstream of the EPO gene in kidney and downstream of the gene in liver (Kochling et al., 1998). This increases the transcription rate of the EPO mRNA that leads to the rise of the EPO level (Ivan et al., 2001; Jaakkola et al., 2001; Kochling, et al., 1998). (b) rHuEPO, a mimetic of EPO, binds to the EPO-receptor and increases the production of RBCs, thereby augmenting the oxygen carrying capacity of blood.

This glycoprotein is encoded by a gene located on chromosome 7, and the majority of EPO (90%) is produced in the kidney (Moore and Bellomo, 2011). EPO is initially synthesized as a polypeptide containing 193 amino acids, of which the first 27 amino acids constitute the signal peptide. Before excretion, these terminal amino acids are removed, resulting in 166 amino acid polypeptide. Oligosaccharide side chains are added at the N-glycosylation sites of the amino acid asparagine at positions 24, 38, and 83. Similar glycosylation also takes place at the amino acid serine located at position 126 (Narhi et al., 1991). These oligosaccharide side chains are required for the in vivo activity of the EPO, as they prevent fast degradation of the EPO in the liver before it reaches the target site (Jelkmann, 2008).

Many factors activate the expression of the EPO gene. The main stimulating factor is tissue hypoxia, a phenomenon whereby the oxygen capacity in the blood and the artery is reduced (Maiese et al., 2004). A low level of oxygen promotes the synthesis of HIF-1α, which dimerizes with HIF-1β to form HIF-1. This dimer binds to the hypoxia response element in the EPO gene and elevates the transcription rate of EPO mRNA, leading to the production of more EPO (Fig. 1a). The oxygen carrying capacity of the blood to the muscles is the major obstacle for performing physical activity for extended periods of time. During exercise, oxygen is very quickly consumed, which greatly limits muscular function. The administration of rHuEPO augments athletic performance by increasing the number of erythrocytes/red blood cells (RBCs), which also results in a dramatic increase of oxygen uptake (VO_{2max}) and ventilatory threshold (VT) (Audran et al., 1999; Rivier and Saugy, 1999) (Fig. 1b).

2. Recombinant human EPO

The first recombinant human EPO (rHuEPO) produced was epoetin alpha (Ashenden et al., 2012; Jelkmann, 2008). Other

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