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Review

Doping in the recombinant era: Strategies and counterstrategies ☆

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Abstract

Advances in recombinant DNA technology have created one of the most powerful weapons in the current doping arsenal: recombinant proteins [Sweeney HL. Gene doping. Sci Am 2004;291:62–9; Unal M, Ozer Unal D. Gene doping in sports. Sports Med 2004;34:357–62]. Recombinant erythropoietin (EPO) and human growth hormone (hGH) are currently being abused but are fortunately detectable either directly by employing isoelectric focusing and immunoassays or indirectly by assessing changes in selected hematopoietic parameters. The detection is technically demanding due to the extent of similarity between the recombinant proteins and their endogenous counterparts. Another issue facing detection efforts is the speed and conditions at which blood samples are collected and analyzed in a sports setting. Recently, gene doping, which stemmed out of legitimate gene therapy trials, has emerged as the next level of doping. Erythropoietin (EPO), human growth hormone (hGH), insulin-like growth factor-1 (IGF-1), peroxisome proliferator-activated receptor-delta (PPAR δ), and myostatin inhibitor genes have been identified as primary targets for doping. Sports clinical scientists today are racing against the clock because assuring the continued integrity of sports competition depends on their ability to outpace the efforts of dopers by developing new detection strategies.

Keywords: Recombinant proteins; Human growth hormone; Erythropoietin; Doping; World Anti-Doping Agency (WADA); Gene therapy; Gene doping

Contents

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Introduction

Doping in various forms has always been a major problem in competitive sports, and it has always been the role of clinical scientists to constitute the main line of defense against doping by developing reliable and practical detection methods. Today, recombinant protein abuse is an evolving form of doping that poses a strong challenge to clinical detection. The World Anti-Doping Agency (WADA) prohibits the use of recombinant proteins which are classified under substance doping [2]. Recombinant proteins threaten to help make good on a promise of the "super athlete" at the cost of sports ethics.

These doping agents are exceptionally threatening to sports and challenging to clinical scientists due to a combination of their incredible performance enhancement potential and the fact that they are essentially the same as their endogenous counterparts. As detailed below, there are documented incidents of abuse of recombinant proteins, and there are also established methods for detecting some of them. However, this remains one of the most challenging tasks in doping detection.

Candidate doping recombinant proteins and current detection methods

The two main recombinant proteins that are currently abused are recombinant EPO (rEPO) and rhGH [3]. Initially, immunoassays generally yielded rather poor results in detection of recombinant proteins since the antibodies used were unable to distinguish between endogenous and recombinant proteins [5]. Table 1 summarizes the main methods available for detection of rEPO and rhGH.

Erythropoietin (EPO)

Increasing oxygen delivery to tissues is important to optimize muscular activity and improve athletic performance, particularly in terms of endurance. Several methods have been shown to increase oxygen delivery to tissues including altitude and hypoxic rooms, blood transfusion, and treatment with erythropoietin (EPO).

Table 1
Detection methods of recombinant proteins

Recombinant protein	Method	Sample type	Reference
EPO	Capillary zone electrophoresis	Physiological fluid	[13]
EPO	Isoelectric focusing (IEF)[a]	Urine	[3,7,12]
EPO	Immunoassay using monoclonal antibody	Blood	[16]
hGH	Immunoassay of isoforms	Blood	[19]
hGH	Pharmacodynamic endpoint monitoring	Blood	[19]

[a] In use since Sydney 2000 Olympics.

EPO is a 165-amino-acid (34 kDa) glycoprotein synthesized by the kidney in response to low blood oxygenation. EPO stimulates erythropoiesis through action on erythroid progenitor cells. The carbohydrate content of EPO is about 40%, a feature which contributed greatly to the detection of rEPO. Glycosylation of EPO is species and tissue-specific and critical for its biological activity. Recombinant and endogenous EPO isoforms have different glycosylation patterns [5]. rEPO is widely used to treat anemia caused by chronic renal disease. EPO was the first recombinant hematopoietic growth factor produced and has been available commercially as a recombinant protein drug since 1989 [5]. Several types of rEPO are commercially available including: Epoetin alpha (Eprex, Janssen-Cilag), Epoetin beta (Neorecormon, Roche), and Darbepoetin alpha (Nespo, Dompè) [5,6]. It is estimated that doping using rEPO was being implemented by 3–7% of the best athletes of endurance sports [7]. Side effects of EPO include hypertension and thrombotic cardiovascular and coronary events. The Sydney 2000 Olympics marked the beginning of the use of effective methods to detect injected rEPO.

Because EPO levels fluctuate over time and among individuals, measuring EPO concentration alone may not be useful to detect doping. Potential abuse of rEPO can best be detected by measurement of five hematopoietic parameters. These are: concentration of serum EPO, hematocrit level, percentage of reticulocytes, percentage of macrocytes, and concentration of serum-soluble transferrin receptors (sTfr) [6,7]. The reference values for these parameters were found to vary by gender, ethnicity, as well as altitude [8].

Two models were developed based on the behavior of each of the 5 parameters during and after controlled treatment with rEPO [9]. The "ON" model is applied during or shortly after rEPO treatment, whereas the "OFF" model is used weeks after termination of treatment [6,9,10]. A simpler approach employs only a combination of hemoglobin level, concentration of serum EPO, and percentage of reticulocytes; it was found to have higher sensitivity in cases of low dose rEPO abuse [6]. For the "ON" model: "ON" score = hemoglobin + 9.74 ln (EPO); OR hemoglobin + 6.62 ln (EPO) + 19.4 ln (sTfr). For the "OFF" model: OFF score = hemoglobin − 60 (reticulocyte percentage)$^{1/2}$; OR hemoglobin − 50 (reticulocyte percentage)$^{1/2}$ − 7 ln (EPO) [11].

If these parameters were unusual, isoelectric focusing (IEF) of urine samples is then employed to provide proof of rEPO abuse [3,12]. The combination of the blood parameters with urine IEF was approved by the International Olympic Committee in 2001 [7]. This test resulted in the forfeiture of medals won by three cross-country skiers in the Salt Lake City