Trends in Analytical Chemistry 57 (2014) 47–63

Contents lists available at ScienceDirect

Trends in Analytical Chemistry

journal homepage: www.elsevier.com/locate/trac

Review

Screening of protein biomarkers for sports doping and veterinary control

Susann K.J. Ludwig [a,*], Leendert A. van Ginkel [a,b], Michel W.F. Nielen [a,c]

[a] RIKILT Wageningen UR, P.O. Box 230, 6700 AE Wageningen, The Netherlands
[b] EU Reference Laboratory, RIKILT Wageningen UR, P.O. Box 230, 6700 AE Wageningen, The Netherlands
[c] Laboratory of Organic Chemistry, Wageningen University, Dreijenplein 8, 6703 HB Wageningen, The Netherlands

ARTICLE INFO

Keywords:
Analytical method
Biomarker discovery
Detection strategy
Doping test
Hormone abuse
Method development
Protein biomarker
Screening
Sports doping
Veterinary control

ABSTRACT

There are similarities between sports doping and veterinary control. Prohibited substances (e.g., anabolic agents and peptide hormones) are similar, and immunoassays and chromatography-mass spectrometry are applied as analytical methods in both worlds. In recent years, detection strategies based on protein biomarkers were successfully developed and adopted in sports control. When measuring biomarkers, the window of detection can be extended due to a prolonged biological response, so a whole range of substances may be tackled in an indirect manner. In view of the similarities in intended biological effects, such as increased muscle mass, we envisage that biomarker-based detection may be adopted veterinary control in future. In this review, we discuss detection strategies based on protein biomarkers for biomarker discovery and method development. With the lessons learnt from successfully implementing biomarker strategies in doping regulations, we advocate adoption in the veterinary world and revision of the current restrictive regulations concerning analytical methods.

Contents

Abbreviations: 2D-DIGE, Two-dimensional differential gel electrophoresis; 2D-GE, Two-dimensional gel electrophoresis; ALS, Acid-labile sub-unit; CERA, Continuous erythropoietin-receptor activators; EASIA, Enzyme-amplified sensitivity immunoassay; ELISA, Enzyme-linked immunosorbent assay; Epo, Erythropoietin; eST, Equine somatotropin; FCIA, Flow-cytometric immunoassay; GC, Gas chromatography; GH, Growth hormone; GHRH, GH-releasing hormone; GHRP, GH-releasing peptide; ICMA, Immunochemiluminescence assay; ICTP, C-terminal telopeptide of Type I collagen; IEF, Isoelectric focusing; IGF, Insulin-like growth factor; IGFBP, IGF binding protein; kDa, KiloDalton; LC, Liquid chromatography; MALDI, Matrix-assisted laser desorption/ionization; MS, Mass spectrometry; ORF, Open-reading frame; PCR, Polymerase-chain reaction; PICP, Procollagen type I C-terminal propeptide; PIIINP, N-terminal propeptide of procollagen type III; rbST, Recombinant bovine somatotropin; rhGH, Recombinant human GH; r-HuEPO, Recombinant human Epo; RIA, Radioimmunoassay; rpST, Recombinant porcine somatotropin; SHBG, Sex-hormone-binding globulin; SPR, Surface-plasmon resonance; SRM, Single-reaction monitoring; TOF, Time-of-flight; uHPLC, Ultra-high-performance liquid chromatography; VEGF, Vascular endothelial growth factor; WADA, World Anti-Doping Agency.

* Corresponding author. Tel.: +31 317 480422.
E-mail address: susann.ludwig@wur.nl (S.K.J. Ludwig).

http://dx.doi.org/10.1016/j.trac.2014.01.005
0165-9936/

1. Introduction

Increasing muscle mass and enhancing performance or productivity are common aims of athletes and in food production. It is well known that muscle growth is facilitated by the use of certain prohibited substances. Therefore, it is not surprising that, during and after major sports events, a substantial number of athletes are accused of doping and also, during routine veterinary controls in food production, the presence of prohibited growth promoters is detected. For both, in sports and food production, there are bans on the use of similar substances, such as anabolic agents (e.g., exogenous and endogenous steroid hormones and clenbuterol), peptide hormones and growth factors [e.g., growth hormone (GH)] [1,2], so similar monitoring and detection methods can be used for their detection.

The best proof of drug abuse is obtained if the abused substance itself is found in the body. Numerous methods for their direct detection are available for a multitude of different possible substances of abuse. These conventional methods are mainly based on chromatographic separation, such as gas chromatography (GC) or liquid chromatography (LC), followed by mass spectrometric (MS) or tandem MS (MS/MS) detection of target ions or ion transitions [3,4]. Also, ligand-binding assays, such as the enzyme-linked immunosorbent assay (ELISA), can be used for their direct detection [5]. Further, bioassays may be used to detect compounds with androgenic or anti-androgenic activity in urine [6].

As an alternative to these conventional direct detection methods, indirect analysis can be used. One approach is to monitor certain biomarkers in the body, the levels of which are specifically increased or decreased after administration of a specific active substance. Thus, the biological effects of an illegal substance are measured and therewith indirect proof of doping can be delivered. Such an approach has several advantages over the classical direct detection:

(1) usually, the biological effect of a substance lasts longer than the presence of the substance itself in body fluids, so the window of detection of doping is expanded;
(2) we can expect that different substances for growth promotion exert similar effects on the body, so biomarker-based detection methods have the potential to detect a whole class of substances, including designer substances with unknown chemical structure and synthetic versions of natural hormones; and,
(3) low-dose mixtures of different banned substances, which might escape from direct detection of each individual substance used, could still be detected by the combined effect that they exert [7].

Biomarkers, which can be used for the effect monitoring described, can be mRNA, metabolites or proteins, which can be analyzed using transcriptomic, metabolomic and proteomic techniques, respectively [7–10].

This review, covering the period from 1997 until 2013, focuses on protein and peptide biomarkers that have been and can be used for the detection of drugs of abuse in sports and veterinary control. Consequently, only endogenous peptides and proteins are considered, the levels of which specifically change after drug administration. The biomarker-based approaches described are not limited to the detection of substances enhancing muscle growth, but also include substances improving performance in athletes and milk production in cattle.

Section 2 outlines the protein-biomarker-discovery pipeline in sports doping and veterinary control. Some of the protein-biomarker-based methods, described in detail in Section 3, have already been implemented in routine doping control. By contrast, so far, no protein biomarker-based method has been successfully implemented for veterinary control, because current regulation has not adopted biomarker-based detection approaches yet. Nevertheless, lessons learnt during the biomarker-identification process for doping control may support and stimulate the development and the acceptance of protein-biomarker-based detection methods in future veterinary control programs, as discussed in Section 4.

2. Techniques used in developing protein-biomarker-based methods

2.1. Process

For the development of biomarker-based methods in sports doping and veterinary control, several phases have to be successfully completed before final implementation of the biomarker-based tests is possible (Fig. 1).

The first phase in the development of biomarker-based methods is the discovery phase, in which candidate biomarkers for substance abuse are identified by untargeted or targeted proteomic approaches. In general, well-controlled treatment studies are performed to obtain samples comprising a limited biological variation. Samples from a treated and an untreated group are compared to create candidate-biomarker lists. Unlike in clinical biomarker studies, where cell culture and animal models can also be used during the discovery phase, in doping and veterinary control, only biofluids or tissues are analyzed, and are also the target sample matrix in the final assay. In any case, easily accessible samples are chosen for analysis, and are mainly urine and blood in sports doping control, and urine, blood and tissue, such as muscle or liver, in veterinary control.

After completion of the first phase, a separate qualification phase, as in clinical biomarker studies, is often unnecessary in doping and veterinary control. The clinical qualification phase is required for two reasons [11]:

first, if cell culture or animal model samples were used during the discovery phase, the discovered biomarkers have to be confirmed in the final sample matrix; and,

second, an analytical method, which will be subsequently used during the following verification phase, is introduced and evaluated.

In contrast, for doping and veterinary control, the final sample matrix is already used in the discovery phase and, if necessary, the alternative analytical method is evaluated in combination with the subsequent verification phase.

During the verification phase, targeted proteomic biomarker analysis is used to confirm the previous findings and to assess