"Fc-based fusion proteins in sports drug testing: Detecting GDF15/Fc and the myostatin inhibitor ActRIIB-Fc by proteomic approaches"

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PROJECT REVIEW

Fc-fusion based drugs are an emerging class of pharmaceuticals which already found their way into competitive sports: EPO-Fc is an erythropoiesis-stimulating agent where the attachment of two immunoglobulin Fc domains to the protein results in a prolonged therapeutic activity due to an increased plasma half-life and enables administration via inhalation. While EPO-Fc can be simultaneously detected with other recombinant erythropoietins by routine doping control assays, there are currently no tests for other doping-relevant Fc-fusion proteins such as the myostatin inhibitor ActRIIB-Fc (ACE031) or the cytokine GDF15/Fc, which is a member of the transforming growth factor beta (TGFβ) superfamily.

The TGFβ superfamily includes several growth factors which are promising therapeutic targets for metabolic disorders and muscular dystrophies. Both TGF-β inhibitors and the cytokines themselves have been pursued as drug candidates. Within this study, a proteomics-based detection assay for emerging Fc-fusion proteins relevant as performance-enhancing agents in sports will be developed. ActRIIB-Fc (ACE-031), a fusion protein composed of the extracellular domain of the human activin receptor type IIB (ActRIIB) and the Fc domain of human immunoglobulin G (IgG), which was found to significantly increase muscle mass and function by inhibition of myostatin and other ligands of ActRIIB, will be used as model compound in addition to GDF15/Fc, a fusion protein of the TGFβ cytokine growth/differentiation factor 15 (GDF15) and the immunoglobulin Fc fragment. Different peptides, mutants and constructs of GDF15 are currently tested as pharmaceuticals for the treatment of different age-related and metabolic disorders.

The proactive development detection assays for therapeutic Fc-fusion proteins, TGFβ cytokines and TGFβ inhibitors is of great interest as several drugs of these categories are already available on the black market as well as for research purposes.

Results and Conclusions:

Cytokines of the TGF-β superfamily as well as their inhibitors are currently being evaluated as potential protein drugs for the therapy of a variety of diseases such as metabolic disorders and muscular dystrophies. The aim of this research project was to develop detection assays for two emerging Fc-fusion proteins potentially relevant as performance-enhancing agents in sports: The TGF-β cytokine GDF-15/Fc and an ActRIIB-Fc fusion protein related to the TGF-β/myostatin inhibitor ACE-031. Two complementary,

multiplexed detection assays for ActRIIB-Fc were developed by using affinity purification in combination with either proteolytic digestion and LC-HRMS, or Western blotting. Both approaches can readily be modified to include other ActRII-Fc fusion proteins such as Luspatercept (modified ActRIIB-Fc). A central and critical aspect of the test methods is the ability of all target analytes to bind to activing-receptor ligands; in case of the model compound GDF-15/Fc, the available reference materials were found to be of substandard quality and w provided as multimer, which significantly interfered with affinity purification process due to the elimination of its binding capability. Consequently, this analyte was not included into the test method validation process but the analyte's characteristics and its potential implementation into flexible and expandable assays was elucidated. Overall, the developed assays proved fit-for-purpose and will expand the range of available test methods for emerging protein therapeutics, particularly those of higher molecular mass.