

“Identification of new metabolites of peptide-derived drugs using a novel Isotope-labeled Reporter Ion Detection strategy”

Mario Thevis, Andreas Thomas, Wilhelm Schänzer (German Sport University, Cologne, Germany)

Project Overview

Compared to the metabolism of low molecular mass drugs (such as anabolic agents, stimulants etc.), the biotransformation of peptide-based drugs after (subcutaneous) administration is largely unknown. Especially for larger peptides and proteins, dedicated in-vitro models simulating the parenteral administration have been missing. Since mass spectrometric methods commonly rely on detailed information about the active drug and particularly its metabolites existing in the circulation, studies providing these data are critical. In the present project it is planned to apply a sophisticated in-vitro model using skin tissue microsomes for prohibited peptides such as synacthen, insulin like growth factor, growth hormone releasing hormone and others. Selected amino acids of the utilized reference peptides will be isotopically labeled, which facilitates and accelerates the identification of formed metabolites by means of diagnostic reporter ions but does not affect the metabolic reactions of the substance of interest. High resolution mass spectrometry enables finally to identify the molecular mass and the amino acid sequence of the formed metabolites, which eventually can serve as target peptides for efficient doping controls using this Isotope-labeled Reporter Ion Detection strategy.

Results and Conclusions

Investigations into the metabolism of peptidic drugs still represents a substantial challenge in doping controls, and the detection of the intact non-metabolized drug (candidate) is the most commonly employed strategy for most of the established detection assays. In contrast to drugs of low molecular mass (*e.g.* anabolic androgenic steroids, stimulants, *etc.*), *in vitro* experimental approaches based on *e.g.* liver microsomes do not simulate appropriately the conditions for peptide and protein metabolism, as these compounds are almost exclusively applied by parenteral routes and alternative approaches have been required.

In the present project, the strategy to use skin tissue microsomes for *in vitro* metabolism studies of prohibited peptide hormones (insulin, synacthen and corticotropin) was shown to be a particularly informative option. Combined with the use of stable isotope-labelled peptides, the identification of resulting metabolites was significantly facilitated due to the formation of diagnostic reporter ions (derived from amino acid-generated immonium ions) still bearing the ^{13}C or ^2H isotope label(s) by means of high-resolution / high accuracy mass spectrometry. Applying this stable Isotope-labeled Reporter

Ion Screening (IRIS) approach to selected prohibited peptides (insulin, synacthen and corticotropin) yielded nearly 20 metabolites for these peptides with truncated amino acid chains. Especially for metabolically less stable peptides such as synacthen, the identification of these new metabolites will support prolonging the detection window in doping control samples. In addition, also for metabolically more stable peptides such as insulin and corticotropin, identified metabolites showed diagnostic potential concerning the differentiation between endogenous secretion and subcutaneous administration. Finally, a generically applicable approach with simplified sample preparation protocol was developed by means of mixed-mode cation exchange solid-phase extraction, which facilitates the preparation of blood samples for doping control analysis without the need for immunoaffinity purification.