“In vivo metabolism studies on growth hormone releasing hormones and their detection by liquid chromatography-mass spectrometry”

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Project overview

Growth homone releasing hormones (GHRH) induce growth hormone (hGH) secretion and are prohibited as performance enhancing agents as they act as classical releasing factors of hGH. The availability and the effects are extensively discussed in several internet blogs and black-market platforms. Three synthetic analogues to GHRH (Geref, Tesamorelin and CJC-1295) have been shown to be available to athletes aiming at undermining the doping control system, whereby Geref and Tesamorelin are approved as therapeutics and CJC-1295 has been in clinical trials. Generally, only limited pharmacokinetic data are available for all three peptide-based drugs and their metabolism, particularly their renal elimination after standard therapeutic administration, is largely unknown. This hinders an effective analysis in blood or urine samples for these compounds due to the lack knowledge about relevant metabolites to screen for.

The main parts of the planned project are thus the characterization of metabolites of all GHRH analogues in a rat model (in-vivo) and human blood specimens (in-vitro). Subsequently, effective target peptides (intact drug or derived metabolites) will be evaluated in different biological fluids allowing to establish an initial test method as well as confirmatory procedure inclusive of all relevant target analytes.

Results and Conclusions

The family of growth hormone releasing hormones is prohibited in sport due to performance enhancing properties, attributed to enhanced endogenous growth hormone production and/or secretion. Most prominent candidates include the single-chain peptidic drugs/drug candidates Geref (Sermorelin), Tesamorelin, CJC-1295 and CJC-1293. Effective doping control analysis is featured by sensitive determination of the most reliable target metabolites in the respective biological fluid (urine or blood). Thus, the knowledge about the metabolism of a prohibited substance is crucial. In this study, detailed analytical information about the target peptides and their potential metabolites after in vitro experiments and literature review was obtained. Most promising analyte candidates for doping control purposes were synthesized and characterised by mass spectrometry. Several sample preparation strategies were evaluated using solid phase extraction, protein precipitation, ultrafiltration prior to immunoaffinity purification (with magnetic beads or MSIA) and liquid chromatographic-mass spectrometric detection. All developed methods showed sufficient sensitivity to cover the

estimated concentrations after administration, and the implementation into existing procedures for other prohibited peptides (insulins, synacthen, etc.) is enabled. The procedures were validated for qualitative result interpretation and considered “fit for purpose” for doping control analysis.