PROJECT REVIEW

"Mass spectrometric characterization and identification of Endogenous and Synthetic Insulins"

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Insulin, a peptide hormone consisting of two disulfide-linked chains composed by 51 amino acids, is part of the endogenous system regulating blood glucose levels by inducing glycogen synthesis. Owing to inconvenient properties of insulin preparations, rapid- as well as long-acting insulins have been developed, which overcome the drawbacks of remedies consisting of recombinant insulin only.

Both classes of synthetic insulins, rapid and long acting analogues, as well as human insulin itself are possibly misused in sports, some reasons of which were discussed in the literature. The testimony of an athlete of having used insulin as well as findings of injection solutions in luggage of cyclists substantiate this suspect, and sensitive, selective and comprehensive procedures are necessary to determine these compounds in doping control samples.

So far, analyses of insulins are mainly performed by means of immunoassays, but also mass spectrometric approaches were demonstrated in the past, which indicate advantages of this technique over immunochemical determination of insulins such as elimination of cross-reaction. The improvements and innovations in soft ionization of large biomolecules by electrospray (ESI) or matrix-assisted laser desorption (MALDI), as well as the production of highly sensitive mass selective detectors enable the development of procedures capable of screening and confirmation of insulin and its new biotechnologically synthesized analogues for doping control purposes.

In order to identify rapid- and long-acting insulins, their mass spectrometric properties after electrospray ionization have to be elucidated. As differences of endogenous and synthetic insulins are limited to exchanges of few amino acids, and one compound is identical with human insulin regarding molecular weight, product ion experiments have to be optimized to efficiently analyze the target compounds in low concentrations in biological fluids.

Furthermore, isolation of insulin and its synthetic counterparts from plasma will be performed by different methods, e.g. solidphase extraction (SPE) and immunoaffinity chromatography (IAC). CommerciaUy available plasma will be fortified with the analytes of interest, extraction recoveries, reproducibility and detection limits will be

determined.

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Results and Conclusions

Synthetic insulin derivatives with modified amino acid sequences such as Humalog Lispro, Novolog Aspart or Lantus Glargine have been introduced to the pharmaceutical market as they provide better controllability, faster or prolonged bioactivity and improved convenience compared to conventional recombinant human insulin preparations. However, the misuse of insulin in sports has been reported manifold, and the international doping control system has required a reliable and robust assay to determine the presence or absence of related prohibited drugs. A qualitative evidence of administered substances is of utmost importance, which is preferably obtained by mass spectrometry. Hence, a top-down sequencing-based assay was developed that allows the detection of synthetic insulin derivatives in human plasma. Therefore, specimens of 2 mL were fortified with three synthetic insulin analogues to simulate plasma levels after subcutaneous administration. A subsequent purification by immunoaffinity chromatography and solid-phase extraction was performed, and extracts were analyzed by microbore liquid chromatography and tandem mass spectrometry. Product ion scan experiments of intact proteins enabled the differentiation between endogenously produced insulin and its synthetic analogues by collisionally activated dissociation of multiply charged precursor ions. This approach has allowed the assignment of individual fragment ions, in particular of those comprising modifications that are originating from C-termini of B-chains, and thus, enabled the unambiguous detection of synthetic derivatives of human insulin in plasma samples. Humalog Lispro contains the same set of amino acid residues as endogenous insulin with one pair having switched positions (B_{28} and B_{29}, lysine and proline). Here, MS/MS data are essential to allow mass spectrometric separation and identification of target analytes. Other synthetic drugs (Novolog Aspart or Lantus Glargine) possess different amino acid compositions and deviate in molecular mass from human insulin, which facilitates their mass spectrometric and chromatographic differentiation. Recoveries of synthetic insulins from plasma aliquots ranged from 91-98%, and detection limits were accomplished at 0.5 ng/mL for all target analytes, which represents plasma levels of insulin for non-fasting normal subjects.

Publications

- Qualitative determination of Synthetic analogues of insulin in human plasma by immunoaffinity purification and liquid chromatography-Tandem mass spectrometry for doping control purposes. Thevis M, Thomas A, Delahaut P, Bosseloir A, Schänzer W. Anal. Chem. 2005, 77:3579-3585.