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Poster presentation

Quantification of cGMP in human plasma by isotope dilution LC-MS/MS

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Background

Cyclic guanosine monophospat (cGMP) is a second messenger activating intracellular protein kinases as part of the nitric oxide (NO)-cGMP pathway. Fast and reliable quantification of cGMP is therefore of major importance for scientific advancement in this field. However, until now only cumbersome immunoassays for the quantification of cGMP are available. Here we present a fast, reliable and specific method for the quantification of cGMP in humane plasma by isotope dilution liquid chromatography-tandem mass spectrometry (LC-MS/MS).

Methods

For optimal precision and accuracy of the assay, a stable isotope labeled internal standard has to be provided. Since such an internal standard is not available commercially, it was synthesized in-house by the conversion of isotope labeled guanosine triphosphat to isotope labeled cGMP by the enzyme soluble guanylyl cyclase (sGC), activated by the NO-donor DEA NONOate.

Plasma sample preparation consisted of the addition of 20 nmol/l of the internal standard, protein precipitation by the addition of perchloric acid, and extraction of unpolar substances from the aqueous phase with cyclohexane. The analytes in the aqueous phase were chromatographically separated by HPLC on a porous graphitic carbon column and detected by electrospray tandem mass spectrometry working in the positive ionization mode.

Results

The chromatographic separation resulted in sharp and symmetrical peaks of cGMP and its stable isotope labeled internal standard at identical retention times, undisturbed by interferences from endogenous substances. No mass spectrometric crosstalk from cGMP to the signal of the internal standard or vice versa was observed. The extraction efficiency was determined to 81 ± 6%. The calibration was linear in the range of 1 to 20 nmol/l. The coefficient of variation was 6.1% at a concentration of 1 nmol/l and 3.4% at 20 nmol/l with accuracies of -8.5% and -1.6% at both concentrations, respectively.

First results with this method were achieved in a preliminary clinical trial enrolling 16 volunteers (age 23 – 55 y, median 44 years, all male) after giving informed consent. After oral application of 110 mg/kg arginine, the cGMP plasma concentrations rose from the baseline of 2.06 ± 0.83 nmol/l to 2.73 ± 1.16 nmol/l after 2 h. The concentrations after 4 h and 24 h were back to baseline with 1.92 ± 0.74 nmol/l and 2.03 ± 1.12 nmol/l, respectively. Although not significant, this rise of cGMP fits in the theory that elevated arginine-levels result in higher NO production, which in turn leads to more cGMP.