MASS SPECTROMETRY AS A TOOL TO DISCOVER CYP PHOSPHORYLATION

G. Redlich[1], E. Langenfeld[1], U. Zanger[2], T. Saussele[2], G. Vacun[3], A. K. Nuessler[4], H. E. Meyer[1], K. Marcus[1]

[1] Medizinisches Proteom-Center, Ruhr-Universität Bochum, Bochum
[2] Dr. Margarete Fischer-Bosch Institute of Clinical Pharmacology, Stuttgart
[3] Institute of Biochemical Engineering, University of Stuttgart, Stuttgart
[4] Fresenius Biotech GmbH, Munich (in cooperation with the Charité, Berlin).

Introduction

CYP2B6 is a Cytochrome P450 isoenzyme of significant pharmacological interest that is responsible for the metabolism of important drug targets including cytostatic Cyclophosphamide or the anti-HIV drug Efaviranz. CYP2B6 shows common genetic polymorphisms between individuals which to date are not well characterized. Indeed, the analysis of the genotype allows no exact prediction of the patients' phenotype concerning drug metabolism. This points to further mechanisms affecting CYP2B6 activity such as protein phosphorylation could be. A correlation between the extent of phosphorylation and the level of enzyme activity was shown for the rat CYP2B6 ortholog CYP2B1 [1]. However, to date the impact of phosphorylation on function and variability is still controversially discussed [2]. Reasons could be, that no large-scale experiments in human were performed yet and that reproduction of such results is difficult, because phosphoprotein analysis is highly challenging. Although several techniques are established, these are far from being routinely applied. Problems result in the fact that phosphate residues of a protein are easily cleavable during sample preparation. Further drawbacks are that in most cases only a few percent of a protein is phosphorylated. Therefore phosphopeptides should be enriched before mass spectrometric analysis. Furthermore the ionization process (in MALDI and ESI) is less efficient for phosphopeptides in contrast to non-phosphorylated ones because of the negatively charged phosphate residues. Beyond this, the unwanted fragmentation of HPO_3 or H_3PO_4 (neutral loss) inside the mass spectrometer can lead to loss of information that the peptide was phosphorylated.

Results / discussion

A new workflow will be described for sensitive and quantitative CYP phosphorylation analysis by state-of-the-art mass spectrometry technology. The experiments are performed mainly on a 4000 QTrap mass spectrometer (Applied Biosystems) using a targeted approach for specific detection as well as quantification of CYP2B6 phosphorylation. By the use of the so called multiple reaction monitoring (MRM) method (Fig.1) very sensitive phosphopeptide detection even in complex mixtures is achieved. It is planned to correlate the obtained data with enzyme activity. For these experiments a large liver tissue bank with more than 300 well-characterized human samples is available (Dr. U. Zanger, IKP Stuttgart, Germany). Further, primary human hepatocytes treated with Rifampicine (as a CYP2B6 inductor) followed by cell lysis at different time-points are analyzed.

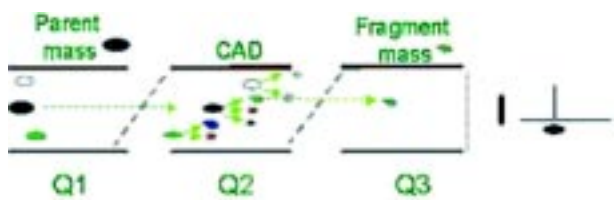

Figure 1. *The use of multiple reaction monitoring in a triple quadrupole instrument. After spraying the analyst mixture into the mass spectrometer a specific parent mass of interest (specific peptide) is selected in Q1 if present. In Q2 the precursor is fragmented by collision-induced dissociation. Q3 is set up to detect a specific fragment mass. If the reporter pair of both peaks in Q1 and Q3 is present, the instrument switches to the measurement of a MS/MS spectrum of the precursor ion to confidently identify the peptide (figure adapted from www.separationsnow.com).*

References

1. Oesch-Bartlomowicz B, Oesch F. Phosphorylation of cytochrome P450: First discovery of a posttranslational modification of a drug-metabolizing enzyme. Biochemical and Biophysical Research Communications 2005; 339: 446-9.
2. Oesch-Bartlomowicz B, Oesch F. Cytochrome-P450 phosphorylation as a functional switch. Archives of Biochemistry and Biophysics 2003; 409: 228-34.