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Multiplexed probing of cytochromes P450 using Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS)

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Cytochromes P450 (CYP) are among the most important enzymes involved in the metabolism of xenobiotics. CYP profiles as co-determinants of adverse effects vary substantially and are subject to xenobiotic-dependent modulations. Hence, profiling methods for CYPs, associated receptors and involved signaling compounds are an important tool for toxicological research. ICP-MS has often been used in recent times for the element specific detection of proteins. On one hand, it can be utilized directly to detect the phosphorus in proteins through which phosphorylation status of proteins could be monitored. On the other hand, proteins of interest could be labeled and indirectly observed concurrently (1).

Considering the different hyphenation techniques for ICP-MS, laser ablation is found to be the most suitable one for SDS-PAGE separated proteins. Laser ablation of blot membranes after PAGE separation gives the required quantitative information of proteins with high accuracy as it has lesser volumes, minimal contamination and is easy to handle compared to gels. Based on these aspects a new laser ablation cell has been designed and adopted (2 and 3). For concurrent determination of proteins in a sample, downstream applications such as immuno-blotting could be useful provided that specific antibodies are available. We use two differentially labeled monoclonal antibodies for detection of CYP1A1 and CYP2E1 by LA-ICP-MS. These CYPs are present in rat liver microsomes after 3-methylcholanthrene and isonicotinic acid hydrazide treatment. In addition expression of the same CYPs from the duodenum of minipigs was analyzed and compared. The CYP1A1 antibody was labeled with Europium via a covalently coupled chelator (DOTA = 1,4,7,10-tetraazacyclo-dodecane-tetraacetic acid) and the CYP2E1 antibody was iodinated. Suitability of the labeled antibodies for detection of blotted microsomal CYPs by LA-ICP-MS and the detection sensitivity will be demonstrated. Simultaneous application of the two differentially labeled antibodies to determine several expressed CYPs will be demonstrated.

References:
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