PO 3.29

Single cell quantification of platinum in chinese hamster ovary cells treated with *cis*-platinum (*cis*-diamminechloroplatinum(II)) using LA-ICP-MS: A new tool for the comparative analysis of single CHO-9 cells by LA-ICP-MS and optical microscopy

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In recent time more and more applications for analytical atomic spectrometry in the broad field of biological research are discovered. The methods for analysis of trace elements bound to biomolecules are advancing fast. About thirty years ago Rosenberg discovered the inhibition of cell division by *cis*-platinum (cis-diamminechloroplatinum(II)). Nowadays c*is*-platinum is used in chemotherapy to treat various kinds of tumours including those of testis, neck, lung, cervix and ovary. It is generally accepted that *cis*-platinum induced cytotoxicity results mainly from adduct formation of $[Pt(H_2O)_2(NH_3)_2]^{2+}$ with the genomic DNA, triggering apoptosis, cell-cycle arrest and the induction of chromosomal aberrations (CA).
Here we describe the combined application of light microscopy and laser ablation inductively coupled plasma mass specrometry (LA-ICP-MS) to individual Chinese hamster ovary (CHO) cells. This approach provides both information about chromosomal aberrations (optical microscopy of metaphase cells) and also information about the uptake of platinum on a single cell level.

Chinese hamster ovary cells (CHO) are exposed over night (20 h) to *cis*-platinum at concentrations from 1,6 to 20 µM. Cells are trypsinised, hypothonically shocked and fixed in methanol/glacial acetic acid (3:1) before they are spread on microscopy slides. Nuclear staining is performed by GIEMSA, which allows light microscopy of interphase nuclei and metaphase chromosomes at a high resolution level. Chromosomal abberations induced by *cis*-platinum are counted and categorized. After transferring the slide into the ablation chamber the cells are retrieved by the imaging facility. All LA-ICP-MS parameters were tuned for maximum sensitivity on phosphorus as well as on platinum. Analysis of phosphorus and platinum content of these single cells is performed afterwards by ablating the complete cell by one single laser pulse using the laser ablation system coupled to an inductively coupled plasma mass spectrometer (LA-ICP-MS). Quantification of these small amounts was performed by comparison of integrals of the ICP-MS signal resulting from the ablation of superficially applied known amounts of a matrix matched standard. The amounts of platinum uptake depending on the concentration in the growth medium can be found in the fg (10^{-15} g) range per cell while chromosomal aberrations of nearly all types can be observed. First results were fundamentals and indicated that this new approach is a capable method for the simultaneous analysis of single cells on an optical and elemental level. Future work will aim on optimization of the detection and on more complex biological questions.