Contribution ID: **66** Type: **Poster**

P58 - Contribution of micro-PIXE to investigate the toxicology of soluble and particulate cobalt on human lung cells

Friday, 11 July 2014 13:00 (1 hour)

The mechanisms of toxicity of metal oxide particles towards lung cells are far from being understood. In particular, the relative contribution of intracellular particulate versus solubilized fractions is rarely considered as it is very challenging to assess, especially for low-solubility particles such as cobalt oxide (Co3O4). We used micro-PIXE analysis to quantify the intracellular particulate and solubilized fractions of Co in human lung cells (BEAS-2B) exposed in vitro to cobalt oxide. Single cells were imaged and elemental quantification was carried out in whole cells, either excluding the particles (giving the solubilized fraction) or including only the cobalt oxide particles (giving the particulate fraction).

Quantitative determination was assessed by simultaneous micro-PIXE and micro-RBS analysis performed with a proton beam of 3.0 MeV energy at AIFIRA facility, CENBG. The proton beam was focused down to a 0.8 µm spot size, resulting in a 350 pA beam current on target, to determine the trace element content (Mg, P, S, K, Ca, Fe, Co and Zn) at the subcellular level.

The Co solubilized fraction determined by micro-PIXE was in good agreement with ICP-MS measurements obtained on bulk analysis of cell lysates. In addition, mico-PIXE analysis enabled to quantify the intracellular particulate fraction of Co which could not be achieved by ICP-MS, since the samples preparation for ICP-MS involved a fraction of extracellular cobalt oxide particles sedimentation together with the cells, preventing the intracellular cobalt particle fraction assess.

Our study shows that cobalt oxide particles, of very low solubility, are readily incorporated by BEAS-2B human lung cells. Combination of micro-PIXE and ICP-MS techniques allowed demonstrating that they are partially solubilized at low pH within lysosomes. Solubilized cobalt was detected within the cytoplasm and the nucleus. As expected from these low-solubility particles, the intracellular solubilized cobalt content is small compared with the intracellular particulate cobalt content, in the part-per-thousand range or below. However, we were able to demonstrate that this minute fraction of intracellular solubilized cobalt is responsible for the overall toxicity. Cobalt oxide particles are readily internalized by pulmonary cells via the endo-lysosomal pathway and can lead, through a Trojan-horse mechanism, to intracellular release of toxic metal ions over long periods of time, involving specific toxicity.

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Session Classification: Poster Session with Cheese and Wine