Metabolism of a new Arsenic based drug against leukaemia: micro-fluorescence and imaging on patient's hair

Acute promyelocytic leukaemia (APL) is a form of adult myeloid leukaemia, characterised by an inhibition of myeloid differentiation leading to an accumulation of the leukaemic cells at the promyelocytic stage of development, severe coagulopathy and high early mortality. Since the early 90's, APL is treated by a combination of standard chemotherapy with all-trans retinoic acid (ATRA), leading to a nearly twofold increase of patient survival. Nevertheless 25% of patients have a relapse after the first treatment, leaving only bone marrow transplantation as an option, although only for the younger relapsed patients. For relapsing patients a new drug is tested, injectable solubilised arsenic trioxyde.

We used the microbeam synchrotron X-ray Fluorescence (μ-SXRF) technique to characterize he Arsenic content of hair after the therapy. The measurements were carried out on beamline ID22. Three kinds of measurement were performed.

By measuring As content along hair length we can achieve a kinetic study to monitor the arsenic content in patient's hairs. Taking into account a mean hair growth rate of 350 μm per day, the 100 μm step used for sampling corresponds to *ca* 7 h of hair growth. We observe a sharp (spanning 3 to 4 points, about 24 hours) rise in As content with the beginning of treatment and a rapid fall down to normal levels at the end of the therapy. It is noteworthy that therapy interruptions are very well reflected to arsenic hair content as seen in figure 1. The results show three successive cycles of increased arsenic content, separated by two periods of lower, although higher than normal, level. The total hair length between the first rise and last fall of arsenic level is *ca* 2 cm, corresponding to an 8 week treatment. This pattern is in accordance with the clinical background of this patient, which has received an As therapy for 40 non-continuous days in a period of two months.

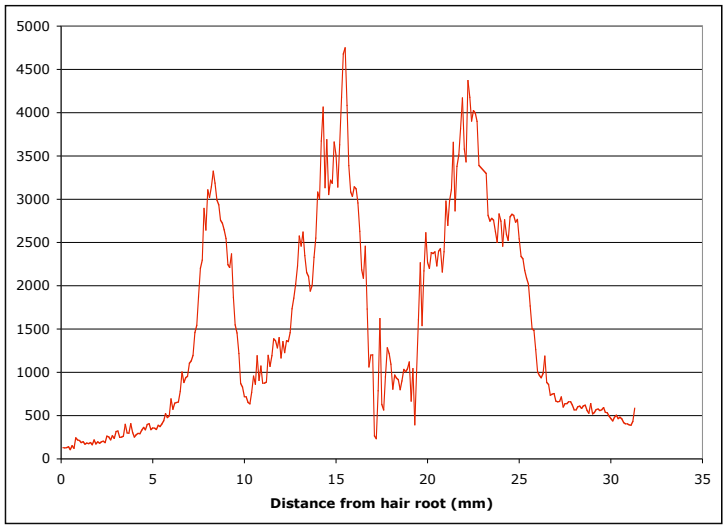

Figure 1. Variation of As content in hair as a function of time. The treatment interruptions are well reflected in the As hair content of patient 2.

Transversal human hair sections (20 micrometer thick) deposited on 4 μm polycarbonate films were analysed. Micro-XRF mapping were performed using a monochromatic beam of energy 14 keV using the Si[111] double crystal monochromator. The beam was focused to 1x3μm^2 with the Kirkpatrick Baez double mirror devices, which deliver 2.0E+11 ph/s at 14 keV. Stepsize was 1*3 μm^2. X-ray fluorescence spectra were collected with a Si(Li) detector. It appears that the arsenic is concentrated at the exterior part of the cortex, just under the cuticle, in a region believed to have lower cystine content than the main cortex (Figure 2).

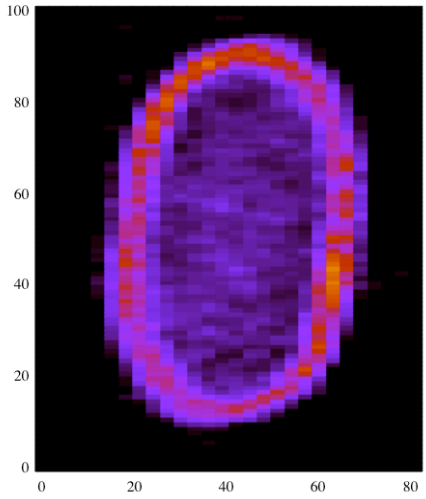

Figure 2. The As is located at the periphery of the cortex.

As chemical speciation was investigated locally from μ-XRF maps by scanning As absorption K-edge (11.863 keV) with energy step of 0.4 eV. Few scans collected in the fluorescence mode were averaged in order to improve the statistic. Micro-XANES spectra recorded at the points of hair with high arsenic content, shows that arsenic is at the As(III) oxidation state, *i.e.* as administered and not oxidised to As(V) as the metabolites found in urine, suggesting the incorporation of As in hair occurs before oxidation, in accordance to the kinetic observation about rapid incorporation after injection. Furthermore, the XANES spectra correspond to an oxygen type (N and/or O) and not sulphur environment, suggesting arsenic is not linked to the keratin cysteine residues, corroborating the 2D cartography results.

Principal publication and authors

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