ESRF	**Experiment title:** Intracellular intercation of anticancer anthracyclines with essential trace metals in cultured cells characterised by micro-SXRF imaging	**Experiment number**: LS-1547
Beamline:	**Date of experiment**: from: 04 may 2000 to: 08 may 2000	**Date of report**: 1st March 2001
Shifts:	**Local contact(s)**: BOHIC Sylvain	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

R. Ortega* and G. Devès* LCNAB, CNRS UMR 5084, Université de Bordeaux 1, BP 120 Le Haut Vigneau, 33175 Gradignan, France

S. Bohic* and A. Simionovici* Micro-FID ID 22, ESRF, BP 220, 38043 Grenoble, France

B. Ménez* and M. Bonnin-Mosbah* LPS, CEA/CNRS, CE Saclay, 91191 Gif-sur-Yvette, France

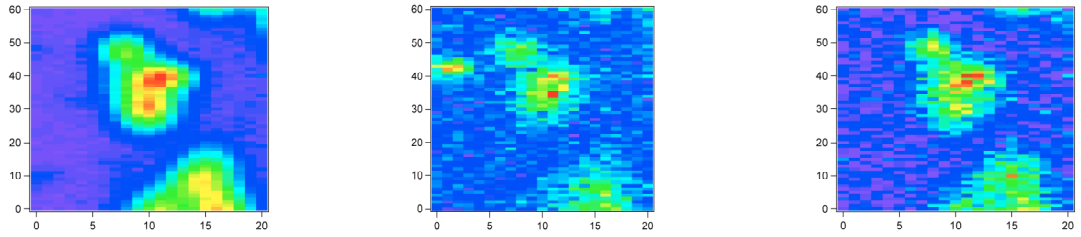
Report:

Chemical studies have shown that doxorubicin, a well established anticancer agent, is a powerful iron chelator and the resultant iron-drug complex is an efficient catalyst of the conversion of hydrogen peroxide to the highly reactive hydroxyl radical. However, the intracellular complexation of doxorubicin with iron is still debated. Using nuclear microprobe analysis, we previously observed in human ovarian cancer cells exposed to 20 µM iodo-doxorubicin (IDX) that iodine and iron cellular distributions were spatially correlated, suggesting a mechanism of intracellular iron chelation by the anthracycline compound. Because maximal plasma drug concentrations in patients are expected around 5 µM, µ-SXRF (Synchrotron X-ray Fluorescence) and XANES (X-ray Absorption Near Edge Spectroscopy) experiments for iron speciation analysis, were performed in this study on cultured cells exposed to pharmacological doses, 2 µM, of IDX or doxorubicin.

Cells were grown directly on thin Formvar films treated with an attachment factor, gelatin gel type B, prepared under aseptic conditions. Exponentially growing cells were exposed first to 25 µM $FeSO_4$ during 20 hours, extensively rinsed with RPMI, and incubated for 4 hours in new complete culture medium. Then cells were exposed to respectively 2 µM doxorubicin, or 5 µM IDX, during 2 hours. Cell monolayers were rinsed with RPMI salt solution before cryofixation in isopentane chilled with liquid nitrogen, and freeze-drying at -30°C.

X-ray microfluorescence of single cells was performed at beam-line ID-22 at the European Synchrotron Radiation Facility (ESRF). A 14 keV 'pink' polychromatic beam and refractive lenses were used resulting in a beam size of 1 µm x 10 m (vertical x horizontal) with a flux of 5.10^{11} photons/s. Fe K-edge X-ray absorption spectroscopy experiments were carried out in fluorescence mode. The sample positioned on a high-resolution X-Y-Z-θ stage and held at 45° to the beam, was observed by a Si(Li) detector placed 10 mm away from the sample. The detector was positioned at 90° to the excitation beam in order to minimize the scattered radiation background. Tantalum slits were used to collimate the incident monochromatic 7.2 keV X-ray beam approximately to 300 µm x 300 µm (around 200 cells wide) with a flux of approximately 10^{10} photons/s. The X-ray beam energy was scanned around the absorption K-edge of iron, between 7.099 and 7.18 keV with 135 steps (0.6 eV step) and 90 seconds acquisition time for cancer cells analysis.

Iodine imaging of cells treated with 5 µM of IDX could be performed in this study and the surface concentrations are reported in Fig 1. SXRF mapping shows an intracellular distribution of trace elements comparable to previous results obtained by micro-PIXE for higher doses of IDX (20 µM). Particularly, the co-localization of iron and iodine within the cell nucleus is observed (Fig. 1b and 1c). This was also found using monochromatic excitation (data not shown) but more than 12 hours mapping were necessary. Using a mean value of freeze-dried cells surface mass (260 µg/cm^2) obtained by RBS, maximum concentrations for cells treated with 5 µM IDX were 10340 ppm for potassium, 274 ppm for zinc, 76 ppm for iron and 580 ppm for iodine.



a) b) c)

Fig 1. Two dimensional elemental imaging of cancer cell treated with 5 µM of IDX and freeze-dried. Cell was mapped with a 14-KeV polychromatic "pink"-beam excitation, step size 1 x 3µm (V x H) and 2.5 sec acquisition time/step, around 2-hour total acquisition time. Distribution within cells of Potassium (a) , Iron (b) Kα X-ray line and Iodine (c) Lβ-line.

In the Fe(III)-doxorubicin compounds, chelation occurs predominantly at the two semiquinone sites with hexagonal geometry. However, the Fe K-edge X-ray absorption spectra from 2 µM doxorubicin exposed cells and control cells were almost similar (Fig. 2). This result could mean that the intracellular iron is not complexed by the drug at 2 µM doxorubicin, or that the method is not sensitive enough to reveal quantitatively small changes in iron coordination, particularly in the present case, with a low counting statistic due to the low intracellular iron content. Finally, a very slight shift in normalized absorption could be noticed at energy > 7.15 keV between 2 µM doxorubicin exposed cells and control cells. Further experiments are required to verify if this shift is of real spectroscopic interest and if it could account for a difference in intracellular iron complexation.

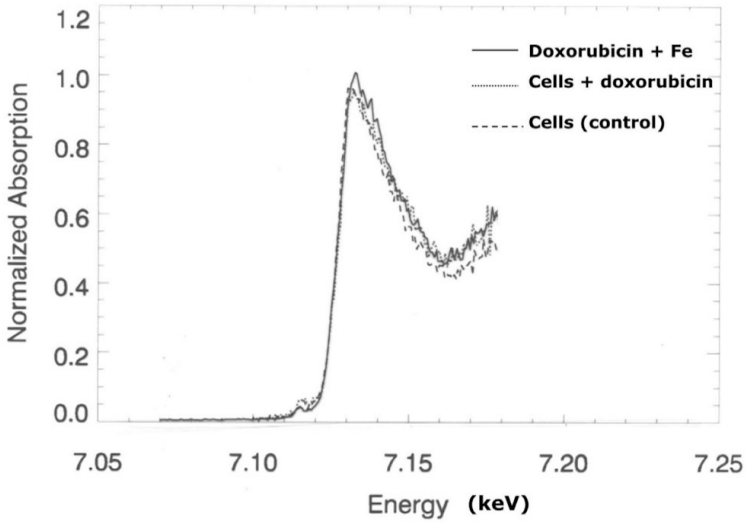


Fig 2. XANES spectra obtained at the absorption K-edge of iron between 7.099 and 7.18 keV with 135 steps (0.6 eV step) and 90 seconds acquisition time for cancer cells analysis (300 µm x 300 µm, around 200 cells wide)

References

Bohic S., Simionovici A., Snigirev A., Ortega R., Devès G., Heymann., Schroer C.G. (2001) Synchrotron hard X-ray microprobe : fluorescence imaging of single-cells. *Applied Physics Letters*, 78, 22, 3544-3546

Ortega R., Devès G., Bohic S., Simionovici A., Ménez B., Bonnin-Mosbah M (2001) Iron distribution in cancer cells following doxorubicin exposure using proton and X-ray synchrotron radiation microprobes. *Nuclear Instruments and Methods in Physics Research*, (in press)