

## **8-Hydroxyquinolines in the Treatment of Cancer: A Key Role for Copper in their Mechanism of Action**

*K. L. Summers,<sup>1</sup> K. B. Gagnon,<sup>1</sup> M. J. Pushie,<sup>1</sup> A. K. James,<sup>1</sup> G. J. Sopasis,<sup>2</sup> N. V. Dolgova,<sup>1</sup> N. J. Sylvain,<sup>1</sup> B. Lai,<sup>3</sup> D. Sokaras,<sup>4</sup> T. Kröll,<sup>4</sup> H. H. Harris,<sup>2</sup> H. K. Nichol,<sup>1</sup> I. J. Pickering,<sup>1</sup> G. N. George<sup>1</sup>*

<sup>1</sup> University of Saskatchewan, Saskatoon, Canada

<sup>2</sup> University of Adelaide, Adelaide Australia

<sup>3</sup> Advanced Photon Source, Argonne National Laboratory, Lemont, USA

<sup>4</sup> Stanford Synchrotron Radiation Lightsource, SLAC National Accelerator Laboratory, Menlo Park, USA

Breakdown of copper homeostasis is associated with many diseases, including genetic copper storage diseases such as Menkes' disease and Wilson's disease, and cancers. Copper has been found to be an essential cofactor in tumor angiogenesis and high tissue or serum copper levels have been found in many human cancers, including prostate, breast, colon, lung, and brain cancers. One potential avenue for developing anti-cancer therapies is through the use of copper chelating drugs, which can induce profound biological changes. 8-Hydroxyquinolines (8HQs) are lipophilic metal ion chelators that have been used as antibacterial treatments since the 1930s. These chelators, clioquinol (5-chloro-7-iodo-8-hydroxyquinoline; CQ) in particular, are of interest as potential drugs in the treatment of some cancers. Because CQ has been proposed to bind and relocate available copper into cells, causing toxic intracellular copper accumulation and thereby promoting cancer cell death, many 8HQs have recently been more accurately described as ionophores. The cytotoxicity of 8HQs in cancer cells appears to be copper-dependent and to increase with hydrophobicity of the 8HQ drug. In this study, structures of Cu(II)-bound 8HQs have been analyzed using a number of spectroscopic techniques, including conventional X-ray absorption spectroscopy (XAS) and High Energy Resolution Fluorescence Detected (HERFD) XAS. Additionally, X-ray fluorescence imaging (XFI) was used to map the elemental distribution in brain cancer cells treated with 8HQs in an attempt to co-localize copper and 8HQs, and aid in the elucidation of the anti-cancer mechanism of action of 8HQs. Our findings suggest that 8HQ drugs that bind copper differently may also have a different anti-cancer mechanism of action, and point to a key role for copper in this mechanism. Understanding the metal binding mechanism of 8HQs and how these drugs alter intracellular metal homeostasis are essential steps in understanding how ionophore therapy compares with chelation therapy and how metal-binding drugs may assist in the treatment of cancers.