ESRF	**Manganese distribution in dopaminergic cells and relationship to Parkinson's disease etiology.**	**Experiment number**: MD343
Beamline: ID21	**Date of experiment**: from: 30/04/2008 to: 06/05/2008	**Date of report**: 13/01/2009
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Parkinson's disease (PD) is a neurodegenerative disorder that leads to the progressive loss of dopaminergic neurons in the substancia nigra pars compacta. This phenomenon is still unexplained, even though some metals, and especially redox metals such as Mn and Fe are suspected to play a major role in the etiology of PD. The aim of this experiment was to determine the chemical element mapping of dopaminergic neurons, PC12 cultured cells, at the sub-cellular level when neurons are treated with environmental neurotoxins such as maneb and paraquat, related to Parkinson's disease (Thiruchelvam et al., 2000). We were especially interested in determining the cellular manganese distribution because at high concentrations manganese has been reported to cause Parkinsonian-like symptoms, known as '*manganism*', and this element is also considered like as potential environmental risk factor for PD and its related disorders (Martin, 2006).

In recent experiments at ESRF we performed X-ray fluorescence imaging of chemical elements on PC12 cells with a 150 nm spatial resolution. Nano-chemical imaging indicates that when cells were exposed at manganese excess this metal is accumulated in cytosol near of nucleus. Our hypothesis, as well as others (Lashuel et al., 2006), is that an alteration in Golgi apparatus can lead a misproduction of vesicle and a misregulation of transporters vesicle into the cell (Carmona et al., 2010). Consequently dopamine, as well as other proteins and ions, is not well stocked and could initiated the production of reactive oxygen species leading to cell death through redox cycling due to the oxidative stress induced by the highly reactive.

Our purpous in this experiment was to determine metal distribution in cells when exposed to environmental toxic products, such as maneb a fungicide composed of manganese : 1,2-Ethanediylbis(carbamodithioato)) (2-))-manganese. PC12 cells exposed to other neurotoxic compounds, like paraquat, another pesticide which does not contain manganese, were also prepared for this experiment but the analyses focused mainly on maneb exposed cells as the results were very interesting and the analysis time limited.

In this experiment, a setup for high spatial resolution X-ray fluorescence microanalysis based on a multilayer lens and a piezo sample stage was used on ID21 beamline, thus leading to the obtention of a photon beam with 0.27 µm x 1.3 µm (v x h) spatial resolution and with 10^9 ph/s flux of photons. The characteristics of the beam, limited in flux, allowed the mapping of biological trace elements (in the µg/g range) but only for few analyses because the mean aquisition time per cell was 10 hours ore more.

X-ray fluorescence microanalysis of PC12 cells enabled to define the element distributions in the main two cellular compartments : nucleus and cytosol. Figure 1 shows an example of analysis: P, S, Cl and K are found in all cellular compartments, whereas Fe is found in small structures in the cytosol confirming the results obtained in previous experiments (Ortega et al., 2007). In PC12 cells exposed to $MnCl_2$, Manganese accumulates in a specific region of cell cytosol, near the nucleus, identified as the Golgi apparatus in a previous study (Carmona et al., 2010).

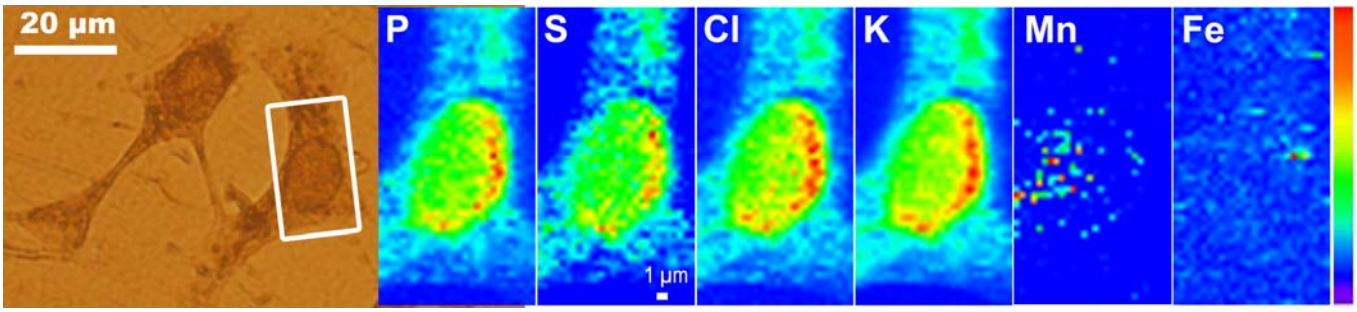

Figure 1: elemental distribution in a PC12 cell exposed to 50 µM of $MnCl_2$ during 24 hours. Scan size is 14 x 26 µm. Color bar ranges from blue to red (min to max) is proportional to the number of X-rays detected.

In figure 2, and for about 4 examples more obtained during the experiment (not shown), a similar distribution was observed showing the same peri-nuclear accumulation of manganese when cells are exposed to Maneb instead of $MnCl_2$.

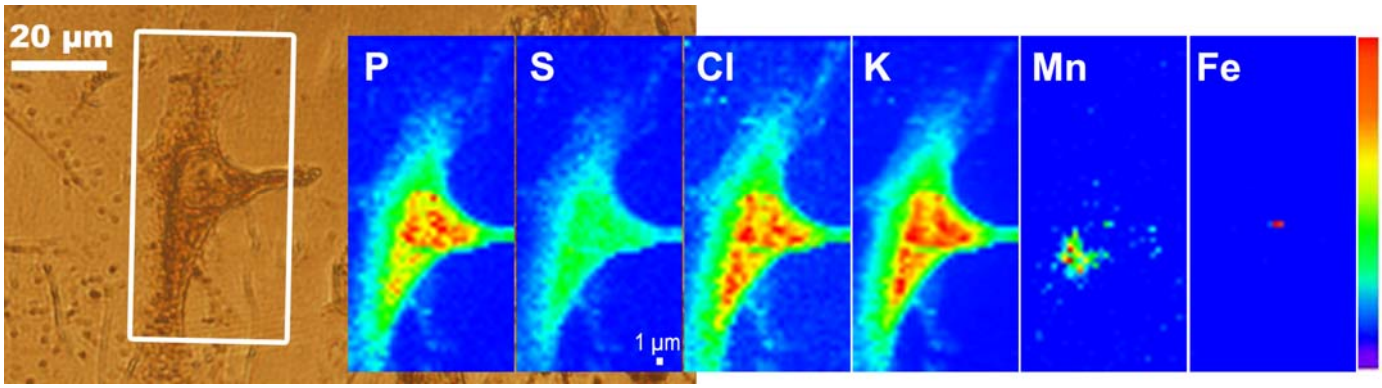

Figure 2: elemental distribution in a PC12 cell exposed to 50 µM of Maneb during 24 hours. Scan size is 32 x 64 µm. **At left:** is the image of cell before irradiation and **at right:** the x-ray fluorescence images. Color bar ranges from blue to red (min to max) is proportional to the number of X-rays detected.

The perinuclear localisation of manganese suggests that this element accumulates within the Golgi apparatus wich are usually located in one side of the cell, close to the nucleus, like confirmed using PC12 cells labelled with fluorescent markers specific to Golgi apparatus, figure 3. It is of paramount importance to understand the neurotoxicity of manganese and the repercution in PD. A similar interaction with Golgi apparatus has been evidenced for alpha synuclein mutant proteins and linked to the pathogenesis of PD (Cooper et al., 2006). The alteration of Golgi apparatus functions would result in a deffective trafficking of secretory vesicles (synthetised by Golgi apparatus) and an altered stockage of dopamine in cells (outside neurovesicles) explaining the specificity of dopaminergic cells neurodegeneration in PD (Lashuel et al., 2006).

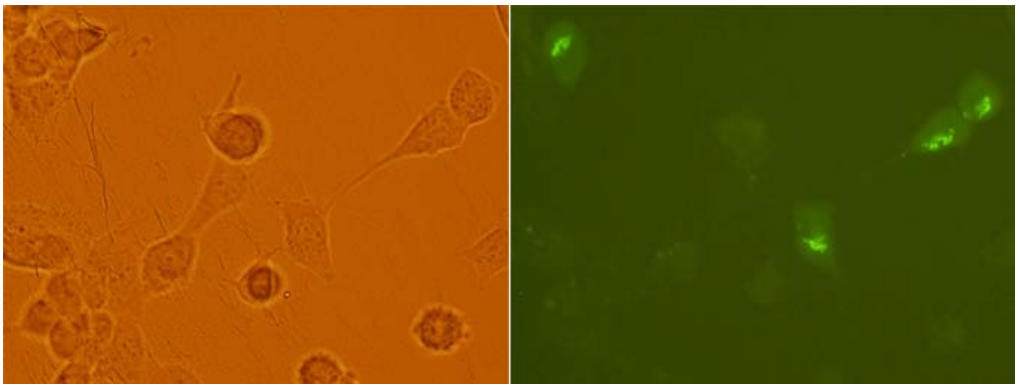

Figure 3: at left: PC12 cells growing in culture medium; at right: the same PC12 cells marked with GFP Golgi apparatus. We can see that Golgi apparatus is located in one side of cells near the nucleus.

References:

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