Superoxide dismutase 2 in human brain: A marker for mitochondria and mitochondrial dysfunction in Parkinson's disease and dementia with Lewy bodies

O. Barnes and J.H.T. Power, Department of Human Physiology, School of Medicine, Flinders University, Bedford Park , SA 5042, Australia.

Mitochondrial dysfunction and oxidative stress are regarded as two of the main mechanisms associated with the pathogenesis of Parkinson's disease (PD) and Dementia with Lewy body disease (DLB). Both PD and DLB are neurodegenerative diseases termed synucleinopathies characterized by the formation of intracellular inclusions termed Lewy bodies. Lewy bodies are aggregations of lipid and protein within the doperminergic neurones within the substantia nigra in PD and within cortical neurones in DLB. There is still debate as to whether Lewy bodies are protective or are detrimental to neurones, although there are several reports that they generate hydrogen peroxide. Cellular antioxidant enzymes offer protection against reactive oxygen species, including free radicals and peroxides, produced as a by-product of oxidative phosphorylation. The mitochondrial antioxidant enzyme superoxide dismutase 2 (SOD2) is a key enzyme in mitochondrial defences as it inactivates the superoxide radical to form hydrogen peroxide during oxidative phosphorylation. Several other enzymes such as glutathione peroxidase 4 (GPx4) and the more recently identified peroxiredoxin 3 and peroxiredoxin 5 inactivate mitochondrial derived hydrogen peroxide. These antioxidants are vital to protect mitochondria from oxidative damage to DNA, lipids and proteins. This project is focussed on the distribution of SOD2 in human brain and the profile of oxidative damage in PD and DLB.

Human brain proteins from PD, DLB and control tissue were separated using PAGE and the molecular forms of SOD2 were determined using Western blotting. The general distribution of SOD2 was examined using light immunohistochemistry and the specific cellular distribution with confocal immunofluorescence with specific cellular markers. SOD2 was co-localized with the Lewy body marker α-synuclein to determine if there was a relationship between Lewy body pathology and mitochondria since we have previously observed mitochondrial components in Lewy bodies using electron microscopy. In addition, SOD2 was co-localized with a marker of oxidative DNA damage (8-hydroxy-2-deoxyguanosine) to determine if mitochondrial or nuclear DNA damage was present in these diseases. A SOD2 ELISA was set up to determine if the level of this enzyme was up or down regulated in PD or DLB.

Western blotting indicated that SOD2 was an abundant enzyme in white and gray matter of all brain regions examined with a molecular weight of 22kD, the predicted molecular weight of SOD2. Light immunohistochemistry indicated that SOD2 was present in most cells with a granular staining consistent with mitochondrial staining. There appeared to be stronger staining in the control brain tissue with the large cortical neurones particularly prominent. A network of granular staining was observed in all tissues consistent with mitochondrial staining in axons. Confocal immunohistochemistry with cellular markers indicated that neurones, astrocytes, microglia and oligodendrocytes were all positive for SOD2, consistent with this enzyme being essential in all cells. White matter in diseased tissue was well stained and many astrocytes were large and activated.

Confocal immunohistochemistry co-localizing α-synuclein and SOD2 in PD and DLB showed that mitochondria were sequested into Lewy bodies in a progressive manner. Lewy bodies were seen to be surrounded with SOD2 positive mitochondria. In neurons with advanced Lewy bodies mitochondrial fragments and SOD2 were present but the mitochondrial integrity appeared to be lost. ELISA results indicated that the level of SOD2 was highest in normal control brain indicating that SOD2 was not upregulated in these diseases. This is in contrast to the cellular antioxidants peroxiredoxin 6 and glutathione peroxidase 1 in these diseases. Staining with a DNA oxidative marker showed that neurones with Lewy bodies did not exhibit mitochondrial nuclear damage. Interestingly, surrounding glial cells showed marked oxidative damage as shown by the nuclear staining.

In conclusion these results show that SOD2 is an abundant antioxidant enzyme in human brain and present in most cells and is an excellent marker for mitochondria. The finding that mitochondria are sequested into Lewy bodies may have serious implications for neuronal survival. The loss of cellular energy production may be the mechanism of cell death in these diseases.