Oxidative Stress in Neurodegeneration Associated to Chronic Kidney Disease: Effect of Indoxyl Sulphate

S. Adesso[1], M. Saturno[1], B. Di Iorio[2], G. Autore[1], S. Marzocco[1]

[1]Dept of Pharmacy, School of Pharmacy, University of Salerno, Italy
[2]Nephrology Division, Medicine Department, Landolfi Hospital, Solofra (AV), Italy

Living cells continually generate reactive oxygen species (ROS) during energetic metabolism. ROS play an important physiological role but their uncontrolled production would be extremely deleterious (Rao et al., 2002). The central nervous system (CNS) is particularly vulnerable to oxidative stress and ROS production has been associated with different neurodegenerative diseases (Jie et al., 2013). Astrocytes are the most numerous component of the glial cells in the CNS and they are involved in different forms of neurodegeneration (Erol 2010). Neurodegenerative complications often occur in chronic kidney disease (CKD), a condition characterized by a progressive loss of renal function and retention of solutes, normally excreted by healthy kidneys. These compounds, called uremic toxins, have deleterious effects in various physiological functions in CKD patients (Brouns et al., 2004). Indoxyl sulphate (IS) is a protein bound uremic toxin, poorly excreted by dialytic process, known as nephro-vascular toxin (Dou et al., 2007) and recently reported as enhancer of inflammatory response and ROS release in macrophages (Adesso et al., 2013). In order to evaluate the effect of IS on astrocytes during oxidative stress induced by inflammatory conditions, C6 cultured rat astroglial cells were treated with IS (60-15 μM) in presence of Lipopolysaccharide from *E. coli* (LPS) plus Interferon γ (IFN). Our results indicate that IS significantly increased ROS release, evaluated by cytometry, respect to C6 cells treated with LPS+IFN alone. In order to study the mechanisms of IS-induced ROS in C6 cells, we analyzed ROS release in presence of diphenyleneiodonium (DPI), a NAD(P)H oxidase inhibitor, and in presence of n-acetylcysteine (NAC). In this experimental conditions DPI and NAC significantly inhibited ROS release induced by IS. IS is a ligand of the aryl hydrocarbons receptor (AhR) and in order to verify even in astrocytes the effect of IS on AhR activation, AhR nuclear translocation was detected by a Laser Confocal Microscope. Our results indicated that IS is able to induce AhR activation in astrocytes in inflammatory conditions, by a mechanism involving ROS release as indicated by DPI addition. IS-induced ROS in C6 cells was measured also in the presence of an AhR inhibitor (CH-223191) and an NF-kB inhibitor (pyrrolidine dithiocarbamate, PDTC). This two inhibitors significantly reduced ROS release. Our results indicate that IS impairs astrocytes migration ability, as evaluated by wound healing assay, by mechanisms also involving ROS release. Nuclear factor (erythroid-derived 2)-like 2 (Nrf2) is a transcription factor that regulates numerous antioxidant and phase II detoxifying enzymes (Kobayashi et al., 2005). By immunofluorescence techniques it has been observed that in our experimental conditions, IS reduced Nrf2 translocation into the nucleus, respect to cells treated with LPS+IFN alone. Nrf2 activation induces the transcription of genes that encode anti-oxidative and detoxifying proteins such as Heme Oxygenase-1 (HO-1). By flow cytometry techniques it has been observed that IS in presence of LPS+INF reduced HO-1 expression, respect to LPS+INF alone. Our results indicate that IS significantly impairs oxidative status in astrocyte and let to hypothesize its contribute to neurological complications observed in CKD.

Rao et al. (2002) *Nutr Neurosci.* 5: 291-309.
Jie et al. (2013) *Int. J. Mol. Sci. 14*, 24438-24475.
Erol (2010) *J. Alzheimer Dis.* 19(1):129-35.
Vanholder et al., (2001). *Int J Artif Organs.* 24(10):695-725.
Brouns et al., (2004). *Clin Neurol Neurosurg.* 107(1):1-16.
Dou et al., (2007). *Journal of Thrombosis and Haemostasis*. 5: 1302–1308.
Adesso et al., (2013). *Plos One*. 8:9 e76778.
Kobayashi et al., (2005). *Antioxid Redox Signal.* 7:385–394.