Indoxyl Sulphate Affects Astrocytes and Microglia Functions: an *ex vivo* Study

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Neurodegenerative diseases represent a growing group of disorders and evidence indicate that neuroinflammation and oxidative stress contribute to pathogenesis of these diseases (Rodriguez et al., 2009). Chronic neuroinflammation is most often detrimental, damaging to neurons and nervous tissue. Microglia and astrocytes are primarily involved in neuroinflammation. In response to a variety of stimuli and pathological events leading to neuroinflammation these cells are activated. Microglia promote astrocytic activation by releasing inflammatory mediators and reactive oxygen species (ROS). On the other hand, activated astrocytes facilitate activation of distant microglia or in some cases can also inhibit microglial signalling (Kingwell et al., 2012).

In patients, neurodegeneration is frequently associated with chronic systemic diseases. For example, chronic kidney diseases (CKD) are a risk factor for neurodegenerative diseases. Uremic toxins have been described as relevant for chronic inflammation and oxidative stress which could be mechanisms of the associated neurodegeneration. Therefore, we will examine the effect of a uremic toxin, indoxyl sulphate (IS), on neuronal cells, focusing our attention on the inflammatory and oxidative stress pathway.

In order to assess the effect of IS on CNS immune cells, during inflammatory response, we used primary astrocytes and mixed glial cell cultures from postnatal day 2 mouse brains. Primary astrocytes cultures were prepared removing microglia by shaking and then using a lysosomotropic agent Leu-Leu-OMe. The effect of IS was evaluated on primary astrocytes and mixed glial cell cultures either by adding the toxin alone or in the presence of Lipopolysaccharide from *E. coli* (LPS) and Interferon γ (IFN).

To evaluate the effect of IS primary astrocytes and in mixed glial cells, cells were treated with IS (15-60 μM) for 1 h and then simultaneously with LPS (1μg/ml)+IFN (100U/ml) for 24h. Then NO production, iNOS and COX-2 expression, TNF-α and IL-6 release and nitrotyrosine formation were evaluated. In these experimental conditions IS significantly increase all pro-inflammatory parameters in primary astrocytes and to a greater extend in mixed glial cell cultures. Moreover, IS increased TLR-4 and Nuclear Factor-kB nuclear translocation, both in primary astrocytes and in mixed glial cell cultures, compared to cells treated with LPS+IFN alone. Because the inflammatory response is associated with oxidative stress conditions and oxidative stress is involved in neurodegeneration, we evaluated, in the same experimental conditions, the effect of IS on ROS release. Our results indicated a significant and concentration-dependent increase in ROS production. This increase was more evident in mixed glial cell cultures compared to primary astrocytes alone. By immunofluorescence techniques, it has been observed that IS can reduce nuclear factor (erythroid-derived 2)-like 2 nuclear translocation, involved in the antioxidant response, and one of its product, Heme Oxygenase-1. Because ROS can affect cellular homeostasis and can influence the cell cycle, we evaluated the effect of IS on cell cycle distribution. Our data indicate that IS affected cell cycle distribution, increasing the G0/G1 and S phases and decreasing G2 phase both in astrocytes and in mixed glial cells.

Our results indicate that IS significantly potentiates inflammatory response and oxidative status mostly in mixed glial cell cultures thus influencing the 'cross-talk' between astrocytes and microglial cells. These results lead to the hypothesis of a significant contribution of IS in the clinically observed neurological complications in CKD.

Rodriguez et al., (2009). *Cell death Differ.* 16(3):378-85.
Kingwell et al., (2012). *Nat Rev Neurol* 8, 475.