Oxaliplatin-induced neurotoxicity: could it correlate with the Neurovascular Unit impairment?

M. Maresca[1], L. Di Cesare Mannelli[1], J.J. Branca[2], L. Micheli[1], B. Tenci[1], E. Lucarini[1], A. Pacini[2], M. Gulisano[2], C. Ghelardini[1].

[1]Department of Neuroscience, Psychology, Drug Research and Child Health, - Neurofarba - Pharmacology and Toxicology Section, University of Florence, Florence, Italy.
[2]Department of Experimental and Clinical Medicine – DMSC - Anatomy Section, University of Florence, Florence, Italy.

Oxaliplatin is a platinum drug with antineoplastic properties used for colorectal cancer. Its dose limiting side effect is represented by neurotoxicity clinically manifested with a severe painful neuropathy. Preclinical studies evidenced that repeated treatment with oxaliplatin lead to intense molecular and morphological changes in the Central Nervous System (CNS). Both protein and DNA oxidation occur in the spinal cord of oxaliplatin-treated rats (Di Cesare Mannelli et al., 2012); furthermore, microglia and astrocytes are activated in spinal and supraspinal areas (Di Cesare Mannelli et al., 2013). To note, studies in rats and non-human primates revealed a low oxaliplatin capability to cross the blood brain barrier (BBB) (Jacobs et al., 2005; Huang et al., 2016). BBB is a crucial structure where astroglia shares dynamic interactions among neurons, endothelial cells and perycites so forming the Neurovascular Unit (NVU). The endothelium of cerebral blood vessels selectively restricts the blood-to-brain paracellular diffusion of compounds; it is mandatory for cerebral homeostasis and proper neuronal function. In this context, pannexin 1, expressed both on astrocyte terminals and the vascular wall, is a large transmembrane channel connecting the intracellular and extracellular space and allowing the passage of ions and small molecules, such as ATP, between these compartments (Kaneko et al., 2015; Munoz et al., 2015). Intriguingly, while, under resting conditions, panx-1 remains closed, a site- specific carboxy-terminal proteolysis by caspase-3 (Engelhardt et al., 2015) irreversibly open the channel, allowing the passage of large amount of ATP and other solutes with size up to 1 kDa (Bao et al., 2004), thus impairing NVU homeostasis (Kaneko et al., 2015).
The purpose of this study was to evaluate the interference of oxaliplatin with the BBB system analyzing the panx-1-dependent mechanism in a rat brain endothelial cell line (RBE4) and in a rat model of oxaliplatin-induced neuropathy. Cells were cultured in growth medium till the confluence and then treated for 48h with a concentration of oxaliplatin ranging between 0.1-100 mM. In order to assess the sublethal dose of oxaliplatin able to activate panx-1 (measured as ATP release) without triggering the apoptotic signalling pathway, we evaluated the cell viability by the MTT assay. Results showed that the sub-lethal concentrations were able to increase caspase-3 activity and ATP release in the culture media, leaving unchanged the pro-apoptotic factor Bax expression levels and suggesting a role of panx-1 in the oxaliplatin-dependent BBB alterations.
These data, together with the evidence that panx-1 is particularly expressed by the epithelial cells of the choroid plexus (Maslieieva and Thompson, 2014), propted us to analyse its expression levels *ex vivo*, in oxaliplatin-treated rats. Oxaliplatin (2.4 mg kg^{-1}, i.p.) was administered daily and on day 14 animals were sacrificed, brain were dissected and cut at bregma levels relative to the lateral, 3rd and 4th ventricle. Immunofluorescence analysis revealed that oxaliplatin treatment led to an intense panx-1 activation (30%) in the choroid plexus throughout ventricles.
Summarizing, these data offer an initial image of the NVU homeostasis impairment induced by oxaliplatin suggesting a novel target to counteract platin neurotoxicity.