Into the unexplored pathophysiological role of the blood brain barrier during oxaliplatin-induced neuropathy

1)Maresca M. 2)Di cesare mannelli L. 3)Branca J. 4)Micheli L. 5)Tenci B. 6)Lucarini E. 7)Trallori E. 8)Awada K. 9)Morucci G. 10)De bock M. 11)Leybaert L. 12)Pacini A. 13)Gulisano M. 14)Ghelardini C.

Dept. of Neurofarba - Unoversity of Florence

Oxaliplatin is an antineoplastic agent currently adopted for the treatment of colorectal cancer. Its dose limiting side effect is represented by neurotoxicity clinically manifested with a severe chronic painful neuropathy. Preclinical data evidenced that repeated treatment with oxaliplatin lead to intense molecular and morphological changes in the Peripheral Nervous System (PNS) as well as in the Central Nervous System (CNS) (Di Cesare Mannelli et al., 2013). To note, oxaliplatin has low capability to cross the blood brain barrier (BBB). In the meanwhile BBB, whose role during neuropathies remains unclear, is a crucial structure where glial cells dynamically interact with neurons and endothelial cells so forming the Neurovascular Unit (NVU). Notably, the cytoplasmic Ca2+ concentration ([Ca2+]i) of the endothelial layer and the extracellular ATP, which is crucially involved in the chronicity of neuropathic pain, are essential for the balance of the BBB homeostasis (De Bock et al., 2013; Bynoe et al., 2015). In this context, Pannexin 1 (Panx-1), a large transmembrane channel, expressed both on astrocyte terminals and the vascular wall, allow the passage of ions and small molecules, such as ATP, between the intracellular and the extracellular compartments. Preclinical data evidenced that Panx-1 blockers are able to revert oxaliplatin-induced neuropathic pain. Intriguingly, if ([Ca2+]i) changes can modulate the conformational state of Panx-1, which usually remains closed under resting conditions, a site-specific carboxy-terminal proteolysis by caspase-3 irreversibly open the channel allowing the passage of large amount of ATP and other molecules, thus impairing NVU homeostasis (Dahl, 2015; Enghelardt et al., 2015).

The aim of this study was to evaluate the interference of oxaliplatin with the BBB system analysing 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 8h, 16h or 24h with a concentration of oxaliplatin ranging between 1-100 µM. The MTT assay allowed us to identify the sub-lethal dose of oxaliplatin able to activate Panx-1 (measured as ATP release) without triggering the apoptotic signalling pathway. Results showed that sub-lethal concentrations were able to increase ([Ca2+]i) as well as the levels of GRP-78 (78 kDa glucose-regulated protein) thus allowing the hypothesis of endoplasmic reticulum impairment. Furthermore, the sub-lethal concentrations of the antineoplastic agent activated the caspase-3 after 8h and 16h treatment leaving unchanged the pro-apoptotic factor Bax expression levels. Extracellular ATP concentration was also increased suggesting a role of Panx-1 in the oxaliplatin-dependent BBB alterations. The immunofluorescent staining revealed derangement of cytoskeleton protein F-actin after 8 or 16h treatment, and relocation of the tight junction protein ZO-1 after 24h treatment with the platinum derivative. These data prompted us to further investigate the BBB oxaliplatin-induced impairment also in vivo. On day 14, animals, daily administered with oxaliplatin (2.4 mg kg-1, i.p.), were sacrificed 15 min after the intravenous injection of 3 or 10 kDa fluorescent-labelled dextran. The amount of the fluorescent dye, which

crossed the BBB, was measured in specific spinal cord and brain areas. Moreover, micro-vessels of the brain circulation were obtained in order to analyse changes in the immunofluorescence signal of activated caspase-3, Panx-1, ZO-1 and F-actin.

Summarizing, these data offer an initial image of the NVU homeostasis impairment induced by oxaliplatin suggesting a novel target to counteract platin neurotoxicity.

References:

Bynoe et al. (2015). Fluids Barriers CNS. 12: 20.

Dahl (2015). Philos Trans R Soc Lond B Biol Sci. 370(1672): 20140191.

De Bock et al. (2013). Prog Neurobiol. 108: 1-20.

Di Cesare Mannelli et al. (2013). J Pain. 14(12): 1585-1600.

Engelhardt et al. (2015). J Membr Biol. 248: 285.