OXALIPLATIN NEUROTOXICITY: MORPHO-FUNCTIONAL APPROACH

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Oxaliplatin (OHP) chemotherapy (CHT) is seriously limited by neurotoxic side effects for whom there is no treatment; this unmet clinical need is in part due to an uncompleted pathogenetic knowledge and, therefore, robust preclinical models are needed to advance patients' care. OHP-induced peripheral neurotoxicity (OIPN) has a peculiar profile: it comprises an acute syndrome and a chronic sensory axonopathy. Acute OIPN is characterized by transient cold-induced paresthesia and cramps, lasting 2-3 days after each administration; acute OIPN has been attributed to a transient ion channel dysfunction. The worse acute OIPN is, the more severe the chronic neuropathy that ensues. Therefore, an OIPN model should be able to reproduce both conditions.
We designed an *in vivo* study to this aim: we compared a control group with a treated group (OHP 3 mg/Kg twice a week over 4 weeks, iv). Nerve excitability testing (NET) was used to assess acute OIPN. Behavioural test, nerve conduction studies (NCS), and neuropathology were used to characterise chronic OIPN; the latter included: morphological/morphometrical assessments of the caudal nerve and dorsal root ganglia (DRG); intraepidermal nerve fiber density (IENFD); spinal cord immunohistochemistry for the transient receptor potential vanilloid type-1 (TRPV1) receptor. Data were collected at the end of treatment and 6 weeks after.
NET allowed us to show that acute OIPN ensued as soon as the first administration and it did not persist after CHT (no NET alterations 1 week after CHT completion). Behavioural tests, NCS, nerve/DRG morphological/morphometrical analysis, and IENFD showed that a mild sensory neuronopathy/axonopathy had ensued at CHT completion and nearly completely resolved at follow-up. Densitometric analysis of TRPV1 immunolabeling in the dorsal horn of the spinal cord at the end of treatment showed an increased density of TRPV1 staining in OHP animals (in lamina I and inner lamina II). This difference was maintained at follow-up.
We showed that acute OIPN (*i.e.*, alterations of ion channels) is transient and chronologically related to OHP administration: it resolves after chemotherapy completion and it does not correspond to neuropathic pain and/or small fiber neuropathy; NET was normal 1 week after CHT completion, whereas IEFND and spinal cord immunohistochemistry showed alterations even 6 weeks after. Acute and chronic OIPN are distinct entities to be carefully and separately considered in future research.

POSTERS SESSION

IN VITRO ANALYSIS OF AN INNOVATIVE BIOMATERIAL

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The objective of the present study is to evaluate biocompatibility and biomimeticity of an innovative membrane with the aim to apply it for repairing somatic and autonomic peripheral nerves in case of traumatic or iatrogenic lesions. Starch- derived (GLUCIDEX®) hyper-crosslinked polymers with suitable mechanical properties were electrospinned as membrane and tested, *in vitro* using immortalized Schwann Cells (RT4-D6P2T cells), for cell survival and proliferation to evaluate the biocompatibility and biomimetic nature of the scaffolds.
RT4-D6P2T cells were cultured i) in direct contact with the membrane, to investigate the interaction with the substrate and ii) in the presence of membrane dissolution products, to test the effect on cell proliferation and organization.

i) Concerning to the adhesion assays, the actin cytoskeleton results more organized in the control group, however, after 24 h, the density and the area occupied by RT4-D6P2T increased.
ii) Several analyzes were conducted using the dissolution products of Glucidex® membranes; the proliferation assay revealed that, after 1, 4 and 7 days of culture, cells maintain proliferative behavior under all conditions tested although a slight decrease, compared to the control, is observed at the first two time points. The actin cytoskeleton profile revealed that cells cultured in conditioned medium have a high organization and generate membrane protrusions, lamellipodia, correlated to cell migration, an important feature of glial cells in support of peripheral nerve regeneration.

Investigating apoptosis and the specific cellular alterations due to Bax, pro-apoptotic protein, and Bcl-2, anti-apoptotic protein, our study revealed that the dissolution products of the membrane are not related with cell death, contrarily, they are associated with good survival. Further investigations are underway to deepen the effect of the dissolution products on expression of gene involved in the regulation of nerve regeneration by Schwann cells.

SATELLITE GLIAL CELLS IN CHEMOTHERAPY-INDUCED PERIPHERAL NEUROPATHY

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Chemotherapy-induced peripheral neuropathy (CIPN) is a frequent side effect caused by many of the most commonly used chemotherapeutic agents, including anti-tubulins (paclitaxel, PTX) and platinum derivatives (cisplatin, CDDP). Due to incomplete understanding of the molecular mechanisms of CIPN, to date no

effective therapy is available. Sensory neurons into dorsal root ganglia (DRG) have been investigated as principal targets of neurotoxicity so far. In this study, we focus on a possible novel target of CIPN, investigating the changes of satellite glial cells (SGCs) in the DRG and their crosstalk with neurons following repeated administration of PTX and CDDP in rats.
Morpho-functional analyses were performed to verify the features of CIPN. Qualitative and quantitative immunohistochemistry, 3D-immunofluorescence, immunoblotting, and transmission electron microscopy analyses were also performed to detect alterations in SGCs and their interconnections.
We demonstrated that after 4 weeks of PTX, but not CDDP treatment, SGCs were strongly activated. A similar activation remained after 4 weeks of follow up, when the painful component of neuropathy, but not the nerve damage, was resolved. In addition, non-physiological connections between SGCs and/or SGC-neuron were evident in PTX rats: we observed activated SGCs surrounding different adjacent neurons and an increase in the intimate contact between SGCs and their associated neurons where a complex and peculiar pattern of glial cytoplasmic projections was present. Moreover, PTX increased the expression of Connexin43 with perineuronal localization and the expression of the adhesion molecule L1-CAM in the cytoplasm and plasma membrane of neurons. We conclude that SGCs may act as principal actors in PTX-induced peripheral neurotoxicity, paving the way for the identification of new druggable targets for CIPN treatment and prevention.

THE ROLE OF PACAP IN AN *IN VITRO* MODEL OF ALS

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Amyotrophic lateral sclerosis (ALS) is an incurable and multifactorial neurodegenerative disease induced by the synergistic action of genetic and environmental factors. It is characterized by the loss of motor neurons (MNs), but not all MNs undergo degeneration: neurons of the oculomotor nucleus, which regulate eye movements, are less vulnerable compared to hypoglossal nucleus MNs. The adenylate cyclase-activating polypeptide 1 (ADCYAP1) gene, encoding for pituitary adenylate cyclase-activating polypeptide (PACAP), was found to significantly up-regulated in the oculomotor versus hypoglossal nucleus suggesting that it could play a trophic effect on MNs in ALS. By using a motor neuron-like hybrid cell line (NSC-34) expressing human SOD1 G93A as an *in vitro* model of ALS, we investigated the role of PACAP following growth factors deprivation. Our results showed that PACAP increases cell viability and prevents epidermal growth factor (EGF) deprivation−induced cell death in NSC−34 cells through EGFR transactivation mediated by protein kinase A stimulation. Overall these data that a deeper characterization of mechanisms involved in PACAP/EGFR axis activation in G93A SOD1 mutated neurons may allow identifying new targets for ALS therapy.

THE BBB PERMEABILITY: HOW MUCH BLOOD FLOW-INDUCED SHEAR STRESS AFFECT IT

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The blood-brain barrier (BBB) is the well-known physiological wall that allow the selective influx and efflux of molecules between the brain parenchyma and the blood circulation. The protagonists of this mechanism are the endothelial cells, assisted by the presence of pericytes and astrocytes. On the other hand, cerebral blood flow is also strictly regulated and its increase can induce shear stress. Indeed, in the past years, neurodegenerative disorders were associated to blood pressure variability. In the present research we evaluated the shear stress effect on rat brain endothelial cell line (RBE4), a widely used BBB *in vitro* model, in order to investigate the putative role of increasing flow in tight junction dislocation. To mimic blood flow in our *in vitro* model, we used the LiveBox2 (LB2) instrument (IVTech S.r.l., Lucca, Italy) that allow to set-up a millifluidic flow, ranging from 50 to 500 µl/min. Briefly, the RBE4 cells were gently seeded on the cover slip of LB2 chamber system, and allowed to growth at least for 24 h. The day after, in order to induce the medium flow on the chamber system, the system was connected to the pump and the appropriate flow rate was set-up. The system was left for 3 days at 37°C, 5% CO2 in humidified atmosphere, then the system was opened and the cells were fixed in cold methanol for 20 min. at 4°C. Immunofluorescent staining for zonula occludens-1 (ZO-1) was performed in order to evaluate the tight junction dislocation. Our results clearly demonstrated that shear stress affect the ZO-1 localization starting from 100 µl/min flow rate. Such a deleterious effect can be hypothesized as a possible mechanism that induces an increase in barrier permeability and therefore allows the entry of harmful substances that alter the brain parenchyma.

Acknowledgments: work supported by #NEXTGENERATIONEU (NGEU) and funded by MUR, National Recovery and Resilience Plan (NRRP), project MNESYS (PE0000006) – A Multiscale integrated approach to the study of the nervous system in health and disease (DN. 1553 11.10.2022); and by Fondazione Cassa di Risparmio di Firenze, Florence, Italy (grant number: 2020.1706).

FUNCTIONALLY INDEPENDENT SUBUNITS OF THE ARCUATE FASCICULUS AND THEIR CONTRIBUTION TO HIGHER-ORDER PROCESSING

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Despite being one of the most studied white matter bundles of the human brain, the anatomical and functional organization of the arcuate fasciculus is still a matter of debate. While earlier anatomo-clinical models of language processing considered the arcuate fasciculus as a unique entity, recent evidence has highlighted the importance of distinct tract segments, each with its specific functional relevance in language comprehension and production.