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SESSION V
PERIPHERAL SYSTEM AND GUT-BRAIN AXIS

MORPHOLOGICAL AND METABOLOMIC CHANGES IN PIPN

Bonomo R[1,2], Canta A[1], Chiorazzi A[1], Carozzi VA[1], Meregalli C[1], Pozzi E[1], Alberti P[1,4], Marmiroli P[1], Skene DJ[3], & Cavaletti G[1,4]

[1]*Experimental Neurology Unit, School of Medicine and Surgery, University of Milano-Bicocca, Via Cadore 48, 20900, Monza, Italy;*
[2]*School of Medicine and Surgery, Kore University of Enna, Enna, Italy;*
[3]*Chronobiology, Faculty of Health and Medical Sciences, University of Surrey, Guildford GU27XH, UK;*
[4]*Fondazione IRCCS San Gerardo dei Tintori, Monza, Italy*

Chemotherapy-induced peripheral neurotoxicity (CIPN) is one of the most common dose-limiting side effects of paclitaxel (PTX) treatment. Many age-related changes have been hypothesized to underlie nerve damage. The results of these studies, however, are inconclusive and other potential biomarkers of nerve impairment need to be investigated. Twenty-four young (2-months) and 24 adult (9-months) Wistar male rats were randomized to either PTX treatment (10 mg/kg i.v. once/week for 4 weeks) or vehicle administration. Neurophysiological and behavioral tests were performed at baseline, after 4 weeks of treatment and 2-week follow-up. Intraepidermal nerve fiber density and nerve morphology/morphometry were analysed. Blood and liver samples were collected for targeted metabolomics analysis. At the end of treatment, the neurophysiological studies revealed a reduction in sensory nerve action potential amplitude ($p<0.05$) in the caudal nerve of young PTX-animals, and in both the digital and caudal nerve of adult PTX-animals ($p<0.05$). A significant decrease in the mechanical threshold was observed only in young PTX-animals ($p<0.001$), but not in adult PTX-ones. Nevertheless, both young and adult PTX-rats had reduced IENF density ($p<0.0001$), which persisted at the end of follow-up period. Targeted metabolomics analysis showed significant differences in the plasma metabolite profiles between PTX-animals and age-matched controls, with triglycerides, diglycerides, acylcarnitines, carnosine, long chain ceramides, sphingolipids, and bile acids playing a major role in the response to PTX administration. Our study identifies for the first time multiple related metabolic axes involved in PTX-induced peripheral neurotoxicity, and suggests age-related differences in CIPN manifestations and in the metabolic profile.

MALADAPTIVE PLASTICITY OF THE NEUROVASCULAR UNIT

De Luca C[1], Virtuoso A[1], Bocchetti M[2], Cirillo G[1], Scrima M[2], Papa M[1,3]

[1]*Laboratory of Neuronal Networks Morphology and System Biology, Department of Mental and Physical Health and Preventive Medicine, University of Campania "Luigi Vanvitelli", 80138, Naples, Italy;*
[2]*Molecular and Precision Oncology Lab.- Biogem Scarl, Ariano Irpino, Italy;*
[3]*SYSBIO Centre of Systems Biology ISBE-IT, Milan, Italy.*

Peripheral nerve injury (PNI) offers a unique model for studying spinal cord plasticity without directly affecting the central nervous system (CNS). The sciatic nerve lesion with sural sparing (SNI) combines motor axon damage and sensory nerve fiber loss, impacting ventral and dorsal horn neurons. The model represents a simultaneous axotomy of motor neurons in the gray matter of the spinal cord and a lesion of the peripheral processes of pseudounipolar neurons in the dorsal root ganglia (DRG). We focused on the neurovascular unit (NVU) within the lumbar spinal cord, examining early changes following SNI in rats. Our results reveal a complex interplay between the coagulation protein thrombin, its receptor PAR-1, and matrix metalloproteinase 9 (MMP9). PAR-1 is initially expressed on neurons and perivascular cells. After injury, it clusters near astrocytic endfeet, where MMP9 can cleave and activate it. MMP9 also alters the spinal extracellular matrix (ECM), particularly the basal lamina, contributing to maladaptive plasticity. Our analysis using immunohistochemistry, RNA sequencing, and RNA scope demonstrates the timely upregulation of MMP9 and its targets. Additionally, we observed changes in tight junctions and channel proteins, primarily at the protein level. Astrocytic water channel aquaporin 4 (AQP4) and gap junction protein connexin 43 (Cx43) become redistributed, and microglia/macrophages infiltrate the spinal cord. The dorsal and ventral horns exhibit distinct responses to the injury. Our findings expand our understanding of the NVU's role in spinal cord damage and highlight the importance of vascular factors in maladaptive plasticity.

IN VIVO STUDY OF CARFILZOMIB-INDUCED NEUROPATHY

Iseppon F[1], Chiorazzi A[1], Canta A[1], Alberti P[1,2], Carozzi VA[1], Pozzi E[1], Cherchi L[1], Fabbro V[1], Rodrigues-Menendez V[1] and Meregalli C[1]

[1]*Experimental Neurology Unit, School of Medicine and Surgery, University of Milano-Bicocca, Monza, Italy;*
[2]*Fondazione IRCCS San Gerardo dei Tintori, Monza, Italy.*

Chemotherapy-induced peripheral neuropathy (CIPN) is a common side effect of cancer treatment with chemotherapeutic agents. 20S proteasome inhibitors such as Bortezomib (BTZ) and Carfilzomib (CFZ) have been approved for treatment of multiple myeloma and other liquid tumors, also include CIPN among their side effects. To date, no effective treatment for this condition has been developed. Observations in patients treated with these drugs showed that BTZ induces a worse neuropathic phenotype when compared with ones treated with CFZ. While the strong BTZ-induced neuropathic symptoms have been replicated in a preclinical setting, there is still no preclinical animal model of CFZ-

induced neuropathy. Our aim is to investigate the behavioral and morphological differences between BTZ and CFZ in terms of nerve damage and fiber loss. Therefore, we first selected a CFZ dose able to guarantee a level of anti-neoplastic activity comparable to that of BTZ in terms of proteasome inhibition as well as good tolerability for the model animals. Then, we treated the animals via intravenous administration of 0.8 mg/kg 2qwx4 of BTZ and 2 mg/kg 2qwx4 of CFZ. Here, we evaluated general toxicity over time as well as the insurgence of neuropathy and neuropathic pain using conduction velocity analyses, dynamic Von Frey tests, and evaluation of intraepidermal fibers density (IENF): all these tests show clear neuropathy developing as early as 2 weeks after the beginning of treatment with BTZ, whereas mice treated with CFZ show only mild symptoms throughout. We next sought to dissect any difference in the morphological and morphometrical features in peripheral nerves between the two treatments. We observed a clear degeneration and loss of axonal fibers in both the caudal and sciatic nerves of BTZ-treated animals, that is already evident at half-treatment (2 weeks), whereas the impact on the CFZ-treated cohort is much less severe and becomes significant only at the end of treatment (4 weeks). Taken together, these results show a clear difference in the neurotoxic symptoms between the two drugs, which reflects in the morphological and functional discrepancies. Therefore, these models are able to reproduce the clinical aspects of CIPN and pave the way for the investigation of the molecular mechanisms that underlie these differences.

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dECM HYDROGELS FOR SUPPORTING NERVE REGENERATION

Metafune M[1], Ciriza J[2], Fernandez-Carro E[2], Muratori L[1], Raimondo S[1]

[1]*Department of Clinical and Biological Sciences, Neuroscience Institute Cavalieri Ottolenghi (NICO), University of Turin, Turin, Italy;*
[2]*Tissue Microenvironment Lab, Institute I3A, University of Zaragoza, Zaragoza, Spain*

Peripheral nerve injury is a clinical condition that severely reduces patient quality of life by damaging sensory and motor functions. In the last decades, tissue engineering has developed a variety of materials that can promote the regeneration of peripheral nerves in case of severe nerve damage. Among these, due to their ability to preserve tissues native environment, stimulate the proliferation and migration of Schwann cells (SC), and provide cues for nerve regeneration, Extracellular matrix (ECM) hydrogels could be a significant advancement in nerve regeneration support systems.
The aim of the present study is to define the possible role of a human decellularized extracellular matrix (dECM) in sustaining peripheral nerve regeneration *in vitro* and then *ex vivo*, in order to develop an innovative strategy in the field of nerve repair. The dECM tested in this study is derived from cadaver human skin and underwent a decellularization protocol to obtain a dECM hydrogel. It was tested *in vitro* on neuronal (NSC34) and glial (RT4-D62PT) cell lines and on primary Schwann cell culture. Proliferation assay was performed on RT4-D62PT SC cell line, using dECM in solution, while primary SC have been cultured to analyze its role in promoting migration, with promising results. To study the interactions of neurons with the extracellular molecules and to evaluate neurite orientation and outgrowth, NSC34 cells were seeded on coverslips coated with dECM, differentiated after 3 days of culture in order to quantify the neurites number and length. The preliminary results showed that this matrix has a significant impact on the proliferation and migration of glial cells, and on axonal sprouting and elongation of motor neurons. The dECM hydrogel will be tested also *ex vivo* on dorsal root ganglia (DRG) and autonomic explants to obtain a multicellular structure that provides a closer approximation to *in vivo* conditions. Further investigations are underway to deepen the effect of the dECM in the activation of molecular pathways related to peripheral nerve regeneration.

THE ROLE OF GUT-MICROBIOTA ON PERIPHERAL NERVES

Pellegrino D[1], Farzin S[1], Cicconetti C[2], Bertone F[1], Oliviero S[2], Basic M[3], Bolsega S[3], Haastert-Talini K[4], Cescon M[5], Gambarotta G[1] and Ronchi G[1]

[1]*Department of Clinical and Biological Sciences (DSCB), University of Torino, Orbassano, 10043, (Torino), Italy;*
[2]*Department of Life Sciences and System Biology, University of Torino, Torino, Italy;*
[3]*Institute for Laboratory Animal Science and Central Animal Facility, Hannover Medical School, Hannover, Lower-Saxony, Germany;*
[4]*Institute of Neuroanatomy and Cell Biology, Hannover Medical School, 30625 Hannover, Lower-Saxony, Germany;*
[5]*Department of Molecular Medicine, University of Padova, Padova, Italy*

Human gut microbiota is the dynamic and complex population of microorganisms (bacteria, fungi, protozoa and viruses), which contributes to tissue homeostasis through a series of physiological functions. The gut microbiota influences not only the gastrointestinal tract, but also a growing list of other organs, leading to the definition of "gut-organ-axes". We have recently demonstrated that also the somatic peripheral nerves and the neuromuscular system depend on the presence of a well-balanced gut microbiota for proper development; indeed, we showed that germ-free mice (GF, mice bred in sterile environment to prevent microbial exposure) and gnotobiotic mice (OMM12, mice stably colonized with 12 specifically defined bacterial strains) have smaller diameter and hypermyelinated axons, together with a dysregulation of pathways critical to development and myelination, compared to control mice (CGM). We therefore analysed peripheral nerves of GF mice colonized with donor complex microbiota at weaning (EX-GF) with the aim to reveal a possible rescue effect. Interestingly, data from stereological and morphometrical analysis showed a significantly thicker myelin sheath and g-ratio of EX-GF nerves in comparison to all other groups. These findings prompted us to further investigate whether gut microbiota impacts other fibre features, such as internodal length and nodes of Ranvier. Preliminary results show an increase in internodal length of GF mice compared to CGM, OMM12 and EX-GF, and longer nodes of Ranvier. Moreover, through RNA-seq analysis, we investigated the gene expression of those genes express in the different regions of the node of Ranvier (node, paranode, juxtaparanode and internode) and we found that several genes are altered in GF, OMM12 and EX-GF, compared to CGM. These preliminary results show that the lack of microbiota leads to morphological and biomolecular alterations in the peripheral nerves, and colonization of GF mice at weaning seems to result in an over compensatory response in the myelination of peripheral axons. Further studies need to be carried out in order to