

NEW THERAPEUTIC USE OF BOTULINUM NEUROTOXIN SEROTYPE A (BoNT/A): A IN REGENERATIVE MEDICINE.

BoNT/A IN REHABILITATION FROM PARAPLEGIA AND TETRAPLEGIA INDUCED BY SPINAL CORD TRAUMA: PRECLINICAL STUDIES.

CLINICALLY RELEVANT MOUSE MODEL OF SPINAL CORD INJURY

Spinal cord injury (SCI) can result from three main causes that ultimately lead to tissue damage: (1) compression caused by spinal discs or bone material pressing against the cord; (2) destruction from direct trauma; and (3) reduction in blood flow from the initial damage i.e., ischemia.

We present, for the first time, an animal model of spinal cord contusion that belongs to the second type of tissue damage. In the context of human data, it is appropriate to reflect on the degree to which the most utilized experimental models mimic the physical processes of the human injury and accurately reveal the variety of chronic pathologies and outcomes seen in human lesions. It is already evident that some surgical procedures, in animals, are far from the reality: i) spinal transection, widely used to assess regeneration but clinically irrelevant, ii) laminectomy before the spinal cord contusion, which reduces the damage and completely excludes the compression and destruction of bone, or iii) a moderate contusion that in mice does not produce a permanent paralysis. We propose a variation of spinal cord contusion in a murine model that mimics accurately the mechanical trauma and makes it possible to reproduce features found in a variety of human SCI pathologies, including a complete absence of motor recovery. Our preclinical model is a realistic tool for investigating both neuroprotection and neuroregenerative strategies as a proof of concept for the treatment efficacy. After trauma, animal hindlimbs are completely paralysed and motor function definitely compromised. From histological point of view they present primary and secondary injury frame.

BoNT/A effects on spinal cord regeneration and locomotor recovery

Slides represent only partial and published (patent WO2016170501_A1) results obtained following BoNT/A spinal administration in SCI mice. However, our research group has obtained important and impressive findings, here summarized.

- A complete motor recovery in 30 days (BMS mouse scale);
- A complete thermal sensitivity and spinal reflex in 10 days (tail flick test);
- BoNT/A long-lasting effects (BoNT/A exerts its action by the cleavage of the protein SNAP25. The visualization of cl-SNAP25 in spinal cord, by immunofluorescence assay, 30 and 60 days after injection, evidenced an active presence of the toxin);
- cells' and tissue localization of the action of BoNT/A and its retrograde transport (we observed the presence of cl-SNAP25 in different cell type, also distal from the lesion site:

neurons-NeuN; astrocytes – GFAP; oligodendrocytes – OLIG1 have been identified as cell targets)

- BoNT/A reduction of astroglial scar (BoNT/A has anti-inflammatory effects and it is able to reduce astrocytes reaction in response to injury, limiting the glial scar formation that constitutes a physical barrier to axon regeneration);
- BoNT/A effect on microglia activation (BoNT/A modulates immune response of microglia favouring the phagocytic process of cell debris clearance);
- BoNT/A reduction of apoptosis (BoNT/A induces neuroprotection, promotes neurons and oligodendrocyte survival – reduced level of Caspase 3, a protein involved in cell death);
- BoNT/A reduction of inflammatory events (excitotoxicity, a phenomenon induced by excess of glutamate release, leads to neuroinflammation and cell death. BoNT/A reduces glutamate release);
- BoNT/A promotion of remyelination and regeneration (upregulation of different myelin proteins and stem factors);
- BoNT/A reduction of metabolic alterations, events determining the prognosis, induced by SCI (as in patients, mice after SCI show altered levels of glycemia and triglycerides , which are influenced by BoNT/A administration).

Other results were obtained from collaborations to the project:

- efficacy of BoNT/A in a mild murine model of SCI. This model has allowed of evaluating the effects of BoNT/A on neuropathic pain development, at the same time, the Motor Evoked Potentials (MEPs). MEP evaluation is a sensitive method to study the function of motor descending pathways; in animals with spinal cord injury MEP amplitude correlates with the amount of spared/regenerated tissue. All together these data demonstrate the ability of BoNT/A to prevent neuropathic pain insurgence and to facilitate regeneration.
- Analysis of quadriceps femoris muscle demonstrates that the murine model successfully reproduces muscle atrophy, being evident that SCI induces also neuromuscular junction degeneration and a metabolic switch toward oxidative phenotype. BoNT/A counteracts the muscle atrophy and reduces muscle molecular alterations. Moreover, the activation and proliferation of interstitial cells in paralyzed muscle and genes involved in atrophy have been investigated.