BOOSTING ENDOGENOUS MECHANISMS FOR NEURAL PROTECTION AND REPAIR AFTER ACUTE DAMAGE TO THE NERVOUS SYSTEM

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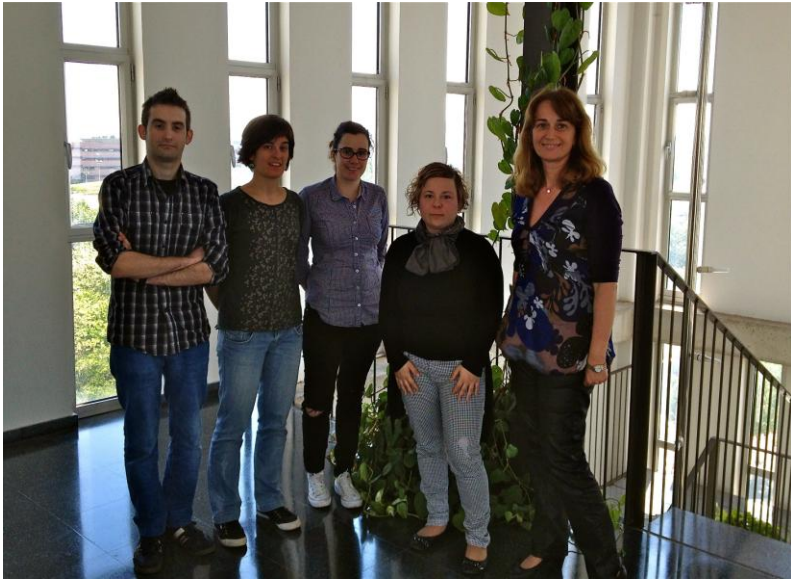Institut de Neurociències, Faculty of Medicine UAB (Universitat Autònoma de Barcelona)

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1. Summary

The main objective of this coordinated project was to deepen scientific knowledge of the endogenous mechanisms of neuroprotection, which are activated naturally in damaged neurons. We have revealed some of the molecular mechanisms of neuroprotection activated in motoneurons (MNs) of the spinal cord when subjected to pulling of its axon by trauma to the peripheral nerve (peripheral nerve lesion, PNL).

This has enabled us to discover and patent a new drug combination that exerts neuroprotection, anti-inflammation and regeneration of the nervous system in a trauma model as severe as the root avulsion of peripheral nerve in rats.

In addition, we have also discovered that the AAVrh10 serotype of adenoassociated viral vectors, when inserted intrathecally or intracranially, efficiently transduces spinal MNs and hypothalamic neurons respectively. This has allowed us to explore its therapeutic potential to overexpress the protein SIRT1, a deacetylase of histones, which is involved in cellular responses to adverse environments. We have observed that this therapy was able to promote neuroprotection in a model of PNL and reduced the hemorrhagic transformation (HT) in a model of brain ischemia.
The results showed that the overall strategy has been successful enough to establish new therapeutic options for CNS injury.

2. Results

The vast majority of injuries occur after neural contusion, tension, compression or ischemia. Avulsion and traction of the peripheral nerves are common forms of damage and are associated with osteoarticular lesions, especially most common fractures and dislocations related to back and hip injury. Peripheral nerve has great tensile strength but very limited elasticity. Therefore, violent traction mechanisms may cause vey extensive nerve damage. For example, when a person is projected from a motorcycle and his back hits the ground, it can produce a strong traction of the components of the brachial plexus, which form the nervous connections from the hand towards the neck. The consequent disconnection requires reimplantation by urgent surgery. However, opportunities for success depend on time after injury, since at that moment, neurons

start to degenerate retrogradely. There are currently no neuroprotective agents for this degeneration on the market so it constitutes an urgent demand since without this treatment surgery is usually avoided. Other less severe injuries may occur by simple compression of the nerve, which usually happens in a variety of situations, such as by inappropriate body postures. One of the most frequent cases in this category is carpal tunnel syndrome. In this syndrome, the pressure on the median nerve in the wrist causes neuropathy characterized by slight discomfort, tingling sensations, difficulties of hand movement and pain.

The recovery from traumatic injuries depends primarily on the severity of the axonal injury and its proximity to the CNS. Spinal cord MNs can regenerate their axons by initiating molecular programs shortly after injury. Yet, glial scar tissue that forms at the interface between the spinal cord and the affected nerve root acts as a barrier preventing axonal regeneration. Thus, only about half of the axons are able to penetrate distally. In humans, the distance for axonal growth is probably even higher than in animal models, up to a rate of 1 mm per day. This extensive length implies difficulties for sustained axonal growth as time goes by and meanwhile the muscle begins to atrophy. Therefore, a small number of axons end up reaching their destination and reinnervate the muscle with some success, which is highly insufficient for functional recovery. In this regard, the lesions affecting the lower limbs tend to be more difficult to recover, in part because the muscles are more distant and therefore need more time for nerve regeneration. Yet, no suitable drugs can be found at clinics nowadays to accelerate this regeneration.

Another type of acute brain injury is cerebral ischemia. Stroke is one of the most frequent cerebrovascular diseases and occurs when the brain stops receiving blood, either by thrombosis, embolism or by bleeding. Rehabilitation allows some recovery after a stroke episode but in many cases patients are disabled, with for example a loss of speech, depression or motor deficits. The brain critically relies on blood flow that brings oxygen and glucose necessary to maintain neural function. Cerebral ischemia triggers a series of events that can lead to cell death. Initially it produces energy depletion that induces an ischemic cascade with the release of molecules that produce oxidative stress and excitotoxicity amongst others; then a local inflammatory response takes place, which can exacerbate brain damage. One of the most feared complications of stroke is hemorrhagic transformation (HT), which occurs in more than 10% of

patients. Its role in the prognosis of patients is arguable, but symptomatic hematoma is associated with a worse outcome. Currently, the use of thrombolytic treatment promotes tissue reperfusion, but at the same time it increases the risk of extravasation. Therefore, it is essential to know the pathophysiology and risk factors for HT.

As we mentioned above, there is no current treatment as a neuroprotective therapy for any of these pathologies. We believe that this may be partly because the scientific rationales used so far have been excessively simplistic and based on the alterations of one gene-one target to design new therapies. This basic approach is still very important to build up more complex knowledge trees that bring us closer to reality. But today, with the development of bioinformatics and computational tools that allow the powerful management of databases, we can start working on more integrative approaches for therapy design.

In this sense, our goal was to expand the knowledge of the neurodegenerative mechanisms and compare it with that for the endogenous mechanisms of neuroprotection. In our initial hypothesis, we wondered whether we could potentiate the endogenous mechanisms of self-protection that neurons naturally engage as an effective manner to enhance neuroprotection. Therefore, our main goal was to devise and explore new therapeutic strategies for traumatic injuries or damage to the nervous system by ischemia. We have worked on two strategies: one based on a platform for discovery of combinatorial drug repositioning and another based on gene therapy to control the expression of any gene. Particularly, we focused on Sirtuin 1 (SIRT1) that exerts epigenetic modifications through deacetylation of histones and transcription factors.

To achieve the first objective, we devised an experimental design based on a comprehensive and computational analysis of empirical data collected from animal models with sciatic nerve root avulsion. This model was chosen for the long history of experimental knowledge of our group. This allowed us to focus very well on suitable time points of sample collection to carry out analysis of changes in protein levels (proteomics) in response to nerve root avulsion (resulting in MN death) in comparison with the response of MNs, which, after a cut-suture of the sciatic nerve, can survive and regenerate. By computational analysis of this data we discovered both the

mechanisms involved in endogenous neuroprotection as well as the mechanisms responsible for neurodegeneration. Moreover, we used this data in sophisticated model of computational analysis to develop mathematical models of protein network associated to each physiopathological condition. Mathematical modeling and the use of tools based on artificial neural intelligence for the learning of the biological molecular behavior allowed us to ask the system for the drug combinations that convert one molecular map (degeneration) into the other (regeneration). We found different drug combinations among which we selected 3 with highest scores.

Selective pharmacology was validated for the treatment of PNL. We found a promising drug combination that was neuroprotective, exerted anti-inflammatory effects and switched surviving MNs towards a pro-regenerative profile. We confirmed this profile using a model of crush nerve injury and we found that the treatment induced accelerated nerve regeneration, and improved muscular reinnervation and functional recovery of injured animals.

The second strategy was to explore the potential use of gene therapy to treat trauma and ischemia. First we analyzed and established the most efficient viral vector. We found that the use of AAVrh10 was the best platform since it specifically transduced MNs in the spinal cord when injected intrathecally, and several nuclei of the brain when injected intracranially, including the hypothalamus which was a target for the ischemia model. We then produced a viral vector AAVrh10 to allow the overexpression of SIRT1 that plays an important role in neuropathologies. In neurodegenerative diseases, SIRT1 is involved in the degradation of aggregated proteins that are hallmarks in the brain of Alzheimer's patients for example. SIRT1 has a key role in delaying the deterioration that occurs during cell aging due to its anti-oxidant promoting effects. Furthermore, overexpression of this protein within the hypothalamus contributed to control the glycemic balance and reduced age-associated dysfunctions (such as sleep and physical activity impairments) through a systemic effect. Therefore, we set up a model to study the effect of hypothalamic SIRT1 on ischemia outcome. In our model of ischemia reperfusion, we observed that SIRT1 overexpression in the hypothalamus prevented post-ischemic HT. In our model of root avulsion, overexpression of SIRT1 led to a 60% increase in MN survival and to an anti-inflammatory effect.

Regarding the advances in scientific knowledge, we have described the molecular mechanisms involved in the retrograde neurodegenerative process as well as the endogenous neuroprotection one. Concerning the mechanism of action of SIRT1, we found that the most important thing was the balance between its activity with respect to other parameters such as cellular energy resources associated to each pathological condition. For instance, in vitro, cells facing an excitotoxic insult need a SIRT1 activity adjusted to the evolution of the lesion to achieve neuroprotection whereas to cope with unfolded protein related death, SIRT1 silencing is preferable. However, as previously mentioned, we achieved appropriate intracellular SIRT1 levels with the use of AAVrh10 in vivo to confer neuroprotection.

3. Relevance and Implications

The relevance of this project is primarily in the possibility of the therapeutic use of pharmacological agents or viral vectors developed in the treatment of ischemic injuries and trauma to the peripheral nerves.

In the case of the pharmacological approach, we believe that their clinical application in both severe trauma of peripheral nerve as in the most moderate cases of compression injuries such as carpal tunnel syndrome or disc hernias could be relatively fast since they are repositioning drugs already known to be safe for human use.

Our proposal for gene therapy is of great clinical potential because we have developed a platform able to transduce a high percentage of MNs in mouse models of diseases. Through a single intrathecal administration, a practice that is usually performed at hospital outpatient clinics, the vector expands into the nervous system through the cerebrospinal fluid. Biosafety of this strategy is relevant not only for the route of administration but also for the amount of vector needed to obtain an efficient transduction, which is much lower (10-20 times less) than what is necessary for intravenous administration. In short, the vectors developed in this project can establish the basis for the proposal of gene therapy clinical trials in the future.

4. Generated Literature

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