Translational Neuroscience

From the bench to bedside: Secondary spinal cord injury, ischemic penumbra after stroke, neural regulation of appetite, microglia in Rett syndrome, signaling pathways in peripheral nerve regeneration

Brendan M. Fong, Jason S. Hauptman

Department of Neurosurgery, Geffen School of Medicine at UCLA, Los Angeles, CA, USA

E-mail: Brendan M. Fong - BrFong@mednet.ucla.edu; *Jason S. Hauptman - jhauptman@mednet.ucla.edu

*Corresponding author

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CX43 CHANNELS: A NOVEL TARGET FOR MINIMIZING SECONDARY INFLAMMATORY RESPONSES IN SPINAL CORD INJURY

Key points:
1. Adenosine triphosphate (ATP) appears to be a key mediator in spinal cord injury.
2. ATP spread after spinal cord injury is reliant on the presence of connexins, which form gap junctions between astrocytes.
3. Mice lacking these connexins show greater functional recovery following spinal cord injury.

Secondary injury in spinal cord injury has been postulated to be due to an inflammatory response propagated by excessive ATP release in surrounding traumatic areas. In this study, the authors explain that the mechanism behind the spread of ATP relies upon the presence of connexin43 (Cx43) channels. Connexins are hemichannels which, when connected with another hemichannel, form gap junctions between two neighboring cells. This allows for the transfer of small molecule nutrients and neurotransmitters that are necessary for cell–cell survival and communication. In order to study the effects of connexins on ATP release secondary to spinal cord injury, the authors utilized astrocytes lacking Cx43. ATP levels were detected and measured in both control and CX43 knockout (KO) mice after spinal cord injury. The researchers found that ATP levels, astrogliosis, and microglia activation were reduced in the KO mice lacking Cx43. Furthermore, the researchers assessed functional recovery of the mice by measuring compound action potentials (CAPs). They found that Cx43 KO mice exhibited improved preservation of spinal cord conduction than CX43 WT mice after spinal cord injury. This work suggests that Cx43 plays an important role in secondary inflammatory responses after spinal cord injury. With future research, Cx43 may be able to be targeted with an inhibitor and a neuroprotective treatment could be utilized in humans to minimize post-traumatic inflammation.

PSD-95 INHIBITOR MAY DECREASE THE ISCHEMIC PENUMBA FOLLOWING STROKE

Key points:
1. Saving the ischemic penumbra following stroke continues to be a major scientific endeavor.
2. PSD-95 inhibitors have shown promise in reducing stroke volumes in rodents.
3. Here, the PSD-95 inhibitor resulted in reduced stroke volumes on MRI and improved functional...
outcomes when administered in a variety of treatment paradigms, including in combination with rt-PA. With no effective pharmacological treatments available, the ischemic penumbra has remained a conundrum in the treatment of stroke. In this study, the authors utilized a peptide termed Tat-NR2B9c. This peptide interrupts the protein–protein interactions of PSD-95, a scaffolding protein that connects with NMDA receptors (NMDARs) to build neurotoxic signaling pathways. Since the use of this PSD-95 inhibitor has shown to be neuroprotective in rodent models, the authors felt that it may also be effective in non-human primates. In this study, macaque monkeys were randomized to receive either placebo or the PSD-95 inhibitor 1 h after the onset of 90-minute middle cerebral artery occlusion (MCAO). The macaques received MRI with perfusion imaging to measure the ischemic volume and an MR angiography to confirm reperfusion. Animals were then assessed at 24 h and 30 days. Interestingly, they found that within the first 24 h, the treatment group exhibited 55% smaller infarct volumes compared with placebo as measured by DWI imaging. Furthermore, the authors noted a 70% reduction in infarct volume as measured by T2-weighted MRI at the 30-day evaluation period. The authors also proceeded to examine functional status using a variety of neurological assessments. They found that the treatment group exhibited improved stroke scores and motor tasks. Interestingly, the authors also proceeded to test the rate of infarct volume change by repeating the same experiment except with a permanent MCAO. They found that the treated group had a twofold reduction in the rate of DWI hyperintensity development. In the setting of MCAO for 90 minutes, the authors noted that reperfusion with recombinant tissue plasminogen activator (rt-PA) may provide a synergistic benefit when used with Tat-NR2B9c. The authors studied this by administering Tat-NR2B9c to macaques 1 h after MCAO for 4.5 h (at which point rt-PA is not routinely used in humans). Using the same methods of analysis, they found similar results with decreased infarct volumes and improved stroke scores. Since intravenous rt-PA is beneficial up to 3 h after stroke, the authors also hoped to demonstrate that administration of Tat-NR2B9c at 3 h after stroke would be beneficial. They found that despite delayed treatment and prolonged ischemic injury, the treatment group showed significant decreases in infarct volumes as compared with placebo controls. As a whole, these results indicate that administration of Tat-NR2B9c in non-human primates in the right setting can be neuroprotective. Given the similarity between humans and higher-order primates, Tat-NR2B9c seems like an excellent candidate for translation from the bench to the bedside. In a human clinical trial, we would have better means of fully examining functional status in conjunction with MRI data and perhaps we could improve the treatment of stroke. It remains unclear if Tat-NR2B9c could be utilized synergistically with the gold standard of rt-PA.

THE ROLE OF MICROGLIA IN ARRESTING RETT SYNDROME

Key points:
1. Autism spectrum disorders are a significant source of neurological morbidity in children.
2. Rett syndrome, which is an X-linked autism disorder, may be linked to abnormalities in microglia.
3. In a rodent model of Rett syndrome, irradiation followed by bone marrow transplantation resulted in improvements in disease severity.
4. This is a fascinating example of microglia–neuronal interactions, and potentially opens the door to a whole new avenue of neurological therapeutics.

Rett syndrome is an X-linked autism spectrum disorder characterized by retarded growth, tremor, apnea, impaired locomotion, and shortened life expectancy. Pathophysiologically, Rett syndrome is caused by a mutation of Meep2, which leads to production of dysfunctional methyl-CpG-binding protein and ultimately leads to neuronal dysfunction. By utilizing male Meep2-mutated mice as a model, the authors of this study show that microglia likely play a role in the pathophysiology of Rett syndrome. These mice show the hallmark phenotype of Rett syndrome. Using the hypothesis that cells derived from the bone marrow may play a role in the physiology of the disease, the authors irradiated the mice on day 28 of life, when the neurological symptoms first appear. These mice then had their marrow rescued by a syngeneic bone marrow transplant from normal mice, whose cells were labeled with a green fluorescent protein (GFP). They found that this transplant resulted in longer life spans, increased growth, increased brain weight, improved gait and tremors, and improved breathing, when compared to controls.

A similar paradigm was then used in females with a single X-linked Meep2 mutation (i.e., heterozygous, with a more delayed phenotype than the male mice). Similar bone marrow transplantation resulted in the same phenotypic improvements in the mice as was seen in the male mice earlier. Since it has been shown previously that whole-body irradiation followed by bone marrow transplantation results in engraftment of microglia in the brain parenchyma, the authors examined the brains of treated animals to see if there were GFP-positive cells engrafted. As expected, the mice showed GFP-positive cells that were positive for CD11b but negative for GFAP or NeuN, suggesting they were myeloid and not neuronal or glial. Interestingly, these engrafted cells had evidence of normal Meep2, while the surrounding cells did not have (suggesting that normal Meep2 was not somehow transferred to nearby cells, thus producing the treatment effect).

To understand the role of these engrafted microglia, the
authors repeated the irradiation experiments but covered the head in lead shielding, thus allowing for restoration of peripheral myeloid cells but not engraftment of parenchymal microglia. As expected, these animals did not show improvements in their disease phenotype. Then, using transgenic approaches, the authors selectively expressed MeCP2 in the myeloid cells of MeCP2-mutated mice. These animals, which were never irradiated or transplanted, also had improvements in their disease phenotype.

The authors then turned attention to the mechanisms underlying microglia dysfunction in MeCP2-deficient mice. They found that the microglia did not respond to immunogenic stimuli appropriately and had a decreased ability for phagocytosis. Assuming that microglial dysfunction then leads to an abnormal accumulation of apoptotic debris, they found that only the transplanted microglia (in irradiated mice) had evidence of intact phagocytosis of these cellular remnants. To prove that this mechanism was necessary for the disease improvement seen in transplanted mice, the authors applied annexin V (a drug that blocks brain phagocytosis) and found that the transplanted mice failed to improve phenotypically. Thus, the authors conclude that it is indeed the impaired phagocytic ability of microglia that leads to the Rett syndrome pathology. This is a well-done, logical set of experiments that shed light on a complex neurodevelopmental disease, which has been poorly understood. Exploring neuroimmunological function will prove to be a key in unlocking many different aspects of brain pathology, including potential therapeutics.

**PERIPHERAL NERVE REGENERATION AND THE ERK-SIGNALING PATHWAY**[4]

**Key points:**

1. The ERK-signaling pathway plays a crucial role in neural degeneration and repair following peripheral nerve injury.
2. When ERK is activated even in the absence of injury, a process similar to Wallerian degeneration occurs even in the absence of direct nerve injury with inflammatory mediators released by Schwann cells.
3. Understanding and targeting ERK signaling will be critical for developing therapy for peripheral nerve regeneration.

When peripheral nerves are injured, they undergo Wallerian degeneration in which axons downstream to the point of injury degenerate. Furthermore, Schwann cells return to their progenitor-like state and help the surrounding macrophages clear apoptotic remnants. An intense inflammatory response occurs in which the brain nerve barrier is disrupted and axons regenerate proximal to distal using Schwann cells as a framework. Since previous research has shown increased ERK-signaling activity in Schwann cells after nerve injury, the authors of this paper utilized transgenic mice containing tamoxifen (TMx)-inducible Raf-kinase/estrogen receptor fusion protein (RafTR) under a myelinating Schwann cell-specific promoter. Essentially, this system enabled the authors to specifically target myelinating Schwann cells and activate the Raf/MEK/ERK signaling pathway in vivo with the addition of tamoxifen. Interestingly, they found that activation of the ERK pathway led to the same process initiated during Wallerian degeneration, but in the absence of axonal injury. Furthermore, ERK induction was similar in magnitude, in comparison to axonal injury controls. The authors also noted that transgenic mice which received tamoxifen for 5 days began exhibiting behavior consistent with demyelinating disorders. To prove that breakdown of the blood nerve barrier actually occurred without direct nerve injury and after ERK activation, the authors utilized Evans blue, a dye that collects within the endoneurium and the perineurium of peripheral nerves. They found that after 4–5 days of tamoxifen treatment, transgenic mice showed complete breakdown of the nerve sheath. The authors proceeded to perform a battery of experiments including RT-PCR to confirm downregulation of myelin-specific gene expression after ERK activation and nerve sections to look for physical changes within the nerves. Consistent with their hypothesis, the authors found increased numbers of macrophages, mast cells, neutrophils, and T cells in tamoxifen-treated animals, which is similar to the cellular milieu seen after axonal injury. Of note, the authors found that fibroblasts were not increased after tamoxifen treatment, yet they are upregulated after normal axonal injury. The authors then proved that substances secreted by ERK-activated Schwann cells initiated this inflammatory response by utilizing microarrays to generate a collection of cytokines such as MEGF10, GDNF, and VEGF. Though this inflammatory response generated by ERK activation and axonal injury is essential for nerve regeneration, prolonged inflammatory responses can lead to secondary injuries. With further research, a small molecule ERK pathway inhibitor could be used to accelerate peripheral nerve regeneration in peripheral neuropathies such as diabetes, traumas, or even genetic disorders. With a further understanding of the ERK pathway, perhaps similar experiments could be created to understand central axonal injury.

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