Magnetic Targeting of Multifunctional Nanoparticles as Strategy to Modulate Neuroinflammation after Spinal Cord Injury

Cinzia Stigliano\(^1\), Elvin Blanco\(^3\), Phillip G. Popovich\(^2\), Philip Horner\(^1\)

\(^1\)Center for Neuroregeneration, Houston Methodist Hospital Research Institute Houston, TX 77030, \(^2\)Department of Neuroscience, The Ohio State University, Columbus, Ohio, \(^3\)Department of Nanomedicine, Houston Methodist Hospital Research Institute Houston, TX 77030
Background

The inflammatory response after injury is fundamental for tissue debris clearance, remodeling and repair. However, when inflammation occurs after spinal cord injury (SCI), it causes expansion of the initial tissue damage with additional progressive neuronal and glial death. The local immune response involves resident microglia, infiltrating macrophages, reactive astrocytes, and endothelial cells. Interesting, on one hand, immune cells produce substances with detrimental effect, including pro-inflammatory chemokines/cytokines, reactive oxygen species and proteases. On the other hand, macrophages/microglia and activated astrocytes have been implicated in protection and regeneration after SCI. Prevention of secondary, immune mediated injury appears to be susceptible to pharmacological intervention. Selection of non-traditional drugs capable of minimizing deleterious inflammation without invalidating the beneficial role of immune cells after SCI is a key factor for a strategic modulation of the neuroinflammation. In this challenge, nanomedicine offers innovative strategies to develop tailored therapies capable of targeting specific cells and tissues where neuroinflammation resides.
Objectives

- Design multifunctional magnetic Nanoparticles (NPs) for selective uptake by CNS macrophages;
- Use magnetic targeting to localize the NPs at the injury site of spinal cord after intrathecal injection;
- Modulate neuroinflammation with targeted drug delivery to CNS macrophages at injury site minimizing CNS toxicity.
Results:

In-vitro NPs Characterization

Multifunctional NPs are spherical with a diameter of 100 nm and comprise: i) a poly(lactic-co-glycolic acid) (PLGA) core, capable of loading drug molecules for anti-inflammatory therapy and iron oxide nanospheres (SPIOs, 5 nm) for magnetic targeting to the site of injury and MRI imaging; and ii) a cyclodextrin external shell for increased cell uptake and loading of optical dyes for fluorescence imaging or synergistic drugs.
Results:

In-vitro NPs internalization tests

The cellular uptake efficiency in vitro has been tested on macrophages and microglia. Fluorescent NPs have been incubated with J774.A1 for 24 h and imaged with confocal microscopy. In order to quantify the internalization after stimulation, BMDM were treated with LPS and incubated with fluorescent NPs; the internalization was quantified by high-content imager. Similarly, NPs internalization was tested over-time on primary culture of microglia.
Results:

Imaging of Magnetic Targeting of NPs in SCI murine model

Fluorescent NPs were intrathecally injected 6 days after injury (T9). Magnet was externally located at injury site before the injection (Magnet mice group). Ex-vivo imaging of brain and spinal cord was performed 24h post-injection with IVIS system. Flow Cytometer has been used to quantify the accumulation of fluorescent NPs in macrophages/microglia (CD45+high; F4-80+) at the lesion site of sc compared to control sc.
The localization of NPs into spinal cord and brain was assessed via histology for all mice groups. Prussian blue staining was performed to detect iron-loaded NPs. The images document the presence of NPs (blue stain) exclusively at the injury site. Off-target regions of spinal cord and brain did not present NPs.

Immunofluorescence revealed the co-localization of fluorescent NPs (red) with Iba1+ macrophages/microglia. The NPs were detected at the injury site into spinal cord.
Spinal cord injuries compromise the integrity of the central nervous system and often cause permanent loss of bodily function. Attenuation of the damage can radically change the life expectations of the affected population, for which there are no therapeutic options to improve their condition. Although there is an incomplete understanding of the divergent role of the immune system in pathology and neuro-regeneration, modulation of neuroinflammation represents an innovative therapeutic strategy to reduce damage and create a microenvironment favorable for regeneration. To date, this amenable therapy does not yet exist. Theranostic nanoparticles represent an innovative system to magnetically guide and confine the therapy at the site of injury, and to achieve effective administration of drugs to the target macrophages and activated microglia. Theranostic nanoparticles, a rising trend in nanomedicine, may open new opportunities for both imaging and therapy in SCI.
Conclusion:

- Multifunctional nanoparticles 100 nm in size were successfully synthetized with reproducible method and with appropriate physical-chemical characteristics. Size and morphology are estimated via DLS and SEM analysis. TEM imaging has been performed to evaluate the expected composition.

- In-vitro study demonstrated no cell toxicity and high internalization in macrophages and microglia over-time. The correlation between internalization rate and activation of macrophages has been investigated.

- Intrathecal injection of NPs in mice after SCI was well tolerated, and the NPs were stable in CSF. The NPs were clearly delivered at injury site; they penetrated the SC tissue and were accumulated in CNS macrophages. Dose, half-live of NPs in CSF, and NPs distribution over-time have to be investigated.

- For future directions, anti-inflammatory drug will be loaded into the NPs, and effect on CNS macrophages and neuroinflammation will be tested in vivo.