Nanoparticle-mediated Topical Delivery of Methylprednisolone After Contusion Injury to the Spinal Cord

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Introduction

Spinal cord injury affects over 11,000 persons a year in the United States. Acute treatment typically involves the systemic administration of high-dose methylprednisolone (MP), to suppress inflammation and limit secondary injury. However, the use of MP systemically also has adverse side effects[1] due to its high dose (at least 30mg/kg). The objective of this study was to develop a topical system to locally deliver MP into the lesion site in a minimally invasive manner, in order to significantly reduce the systemic delivery related side effects, and increase delivery efficiency. To achieve this goal, MP was conjugated to Texas-red (to visualize the distribution of the delivered MP in the spinal cord), encapsulated by biodegradable nanoparticles (NPs - henceforth referred to as Tx-MP-NP) and embedded into agarose gel which acts as carrier for degradable NPs. Tx-MP-NPs embedded in agarose gels were then topically delivered at the site of the contusion-injured spinal cord and animals were perfused two days after delivery. In this report, we demonstrate that the topically administered Tx-MP-NPs successfully delivered MP to the lesion site, and significantly decreased the number of macrophages and the expression of secondary injury related proteins including calpain and iNOS when compared to saline-NPs.

Materials and Methods

Preparation of MP-TR conjugates: Methylprednisolone (MP) and Texas red cadaverine were conjugated to allow for visualization of the MP in and around an injured spinal cord, and their purity was verified using LC mass spectroscopy (Tx-MP).

Preparation of MP-NP and Saline-NP: The Tx-MP encapsulated in PLGA (50:50) based NPs were fabricated by modification of the double emulsion method. Saline encapsulated NPs (Saline-NP) were also fabricated as an experimental control.

Preparation of agarose gel carriers for nanoparticle localization to lesion site: Agarose gels (0.6%, w/v in PBS) were prepared containing either Tx-MP-NPs or Saline-NPs, by mixing 2 mg of nanoparticles with 500 µL 0.6% SeaPlaque agarose in 1x PBS and allowed to cool and gel.

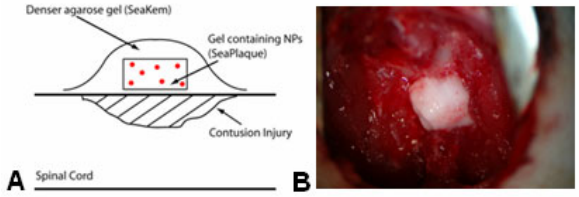

Figure 1. A) Schematic showing the gel containing Tx-MP-NPs placed on top of the spinal cord. B) Picture showing the gel placed on the top of the spinal cord after contusion injury

In vitro characterization of MP release

Release profile: In order to characterize the Tx-MP release from the NPs, a release study was conducted as follows: Tx-MP-NP and Saline-NP embedded agarose gels were prepared as described above, 1xPBS added on top, and the gels were incubated at 37°C. Every 24 hours samples were collected from each well and the absorbance was read at 247 nm to quantify MP released in the preceding 24 hours. Samples were collected for 8 days, and the release profile was plotted.

Bioassay: To verify that the Tx-MPs are as functional as MP alone a bioassay was performed. Agarose gels were prepared containing either Tx-MP-NPs, MP-NPs, or Saline-NPs. Microglia harvested from postnatal day 3 rat pups were activated with LPS and agarose gels carrying the three different types of NPs were placed on top. The amount of nitrite in the media in each condition was measured each day.

In vivo study

Contusion injury and topical Tx-MP delivery: Adult male rats (n=5 for each condition, 200-230g) were used for contusion injury. The 9 and 10th thoracic spinal cord segment were exposed by laminectomy. The contusion injury was performed using an Infinite Horizon (IH) spinal cord injury device (Precision Systems & Instrumentation [PSI], Lexington, KY). Either the Tx-MP-NP embedded agarose gel or Saline-NP embedded gel was topically placed on the lesion site (Figure1). The gels were secured at the lesion site by adding a second, denser layer of agarose gel on the top (Figure 1A). After topical administration of Tx-MP-NPs or Saline-NPs, the muscle and skin were closed. Two days after injury, the animals were perfused transcardially and the spinal cords were longitudinally cryosectioned.

Distribution of Tx-MP in the spinal cord: The cryosectioned spinal cord tissues were examined under fluorescent microscope. To confirm the Texas-red signal we observed in the tissue was specific, the tissues were treated with $CuSO_4$.[3] The distribution of delivered Tx-MPs within the cord was quantified using Image-pro software.

Immunohistochemical analysis: To examine the therapeutic effects of the locally delivered MP, serial sections were incubated with the following primary antibodies: ED-1 to identify macrophage/reactive microglia, Calpain Ab to identify calpain protein, and inducible nitric oxide synthase (iNOS). The number of ED-1$^+$ cells were counted and averaged by Image-pro software and the reactivity of Calpain and iNOS in and around lesion site was quantified (see in Jain et al[2].)

Results and Discussion

The *in vitro* studies verified the Tx-MP was released from the gel carriers for at least four days. Microglia that had been treated with Tx-MP-NPs had significantly lower NO production than those that had been treated with Saline-NP. Figure 2A demonstrates that in the Tx-MP treated animals, the Tx-MP was able to diffuse into the injured spinal cord, as evidenced by the Texas-red signal in boxes 1 and 2.

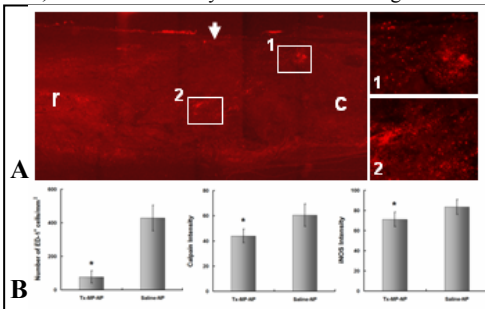

Figure2. A) Representative Tx-MP diffused spinal cord (longitudinal section). Numbered images are magnified from A. r=rostral, c=caudal. Arrow indicates impact site. 2) Quantitative comparison of ED-1+ cells, Calpain, and iNOS reactivity between Tx-MP-NP and Saline-NP treated animals. *P<0.01.

Furthermore, Tx-MP had a therapeutic effect as early as 2 days following injury (Figure 2B). The number of ED-1$^+$ cells in the injured spinal cord of the animals treated with Tx-MP was significantly lower than in those which had been treated with saline. Likewise, the reactivity of Calpain and iNOS are significantly reduced in the animals treated with Tx-MP compared with those treated with saline.

Our results suggest that the novel topical MP delivery system we have developed is an effective way of delivering MP to a spinal cord contusion injury. By encapsulating the MP in PLGA, we have accounted for gradual, sustained release of significantly low dose of MP (100 times lower than dose for systemic delivery). By embedding the NPs in thermoreversible agarose gels, we have designed a minimally invasive, in situ gelling topical delivery system for MP therapy after SCI. We suggest that localized and low dose MP delivery can significantly reduce the adverse effects associated with systemic administration of MP.

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