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Local delivery of minocycline from metal ion-assisted self-assembled complexes promotes neuroprotection and functional recovery after spinal cord injury

Zhicheng Wang [a], Jia Nong [a], Robert B. Shultz [a], Zhiling Zhang [a], Taegyo Kim [b], Veronica J. Tom [b], Ravi K. Ponnappan [c], Yinghui Zhong [a, *]

[a] School of Biomedical Engineering, Science and Health Systems, Drexel University, 3141 Chestnut Street, Philadelphia, PA 19104, USA
[b] Spinal Cord Research Center, Department of Neurobiology & Anatomy, Drexel University College of Medicine, Philadelphia, PA 19129, USA
[c] Department of Orthopaedic Surgery, Drexel University College of Medicine, Philadelphia, PA 19102, USA

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ABSTRACT

Many mechanisms contribute to the secondary injury cascades following traumatic spinal cord injury (SCI). However, most current treatment strategies only target one or a few elements in the injury cascades, and have been largely unsuccessful in clinical trials. Minocycline hydrochloride (MH) is a clinically available antibiotic and anti-inflammatory drug that has been shown to target a broad range of secondary injury mechanisms via its anti-inflammatory, anti-oxidant, and anti-apoptotic properties. However, MH is only neuroprotective at high concentrations. The inability to translate the high doses of MH used in experimental animals to tolerable doses in human patients limits its clinical efficacy. In addition, the duration of MH treatment is limited because long-term systemic administration of high doses of MH has been shown to cause liver toxicity and even death. We have developed a drug delivery system in the form of hydrogel loaded with polysaccharide-MH complexes self-assembled by metal ions for controlled release of MH. This drug delivery system can be injected into the intrathecal space for local delivery of MH with sufficient dose and duration, without causing any additional tissue damage. We show that local delivery of MH at a dose that is lower than the standard human dose (3 mg/kg) was more effective in reducing secondary injury and promoting locomotor functional recovery than systemic injection of MH with the highest dose and duration reported in experimental animal SCI (90–135 mg/kg).

1. Introduction

Traumatic spinal cord injury (SCI) causes partial or complete loss of motor, sensory, and autonomic functions below the injury site. The initial trauma is followed by a wave of secondary injury cascades that leads to progressive tissue damage and cavitation [1], resulting in deleterious functional loss. Thus, therapies that can limit secondary injury will reduce the extent of disability resulting from SCI and improve functional recovery. Many mechanisms contribute to the secondary injury, including inflammation, cellular damage from free radicals such as reactive oxygen species (ROS) and nitric oxide (NO), glutamate excitotoxicity, calcium influx, ischemia, hemorrhage, and edema [1–3]. However, current treatment strategies are highly specific, usually targeting only one or a few elements in the injury cascades, and have been largely unsuccessfully in clinical trials. Minocycline hydrochloride (MH) is a clinically available antibiotic and anti-inflammatory drug that also exhibits potent neuroprotective activities [4–6]. It has been shown to target all of the aforementioned secondary injury mechanisms via its anti-inflammatory, anti-oxidant, and anti-apoptotic properties [2,7–14]. Consequently, MH was considered the highest scoring neuroprotective therapy for SCI in a recent systematic review of pre-clinical data [15].

A number of studies have shown that systemic administration of MH for 1–5 days significantly reduced secondary injury and improved functional recovery in experimental animal models of SCI [2,3,7,16,17]. However, the doses of MH used in these studies (90–135 mg/kg/day for 1–5 days) are much higher than that used in a recent Phase II clinical trial (12–22.5 mg/kg/day for 7 days [18]) and the standard human dose of 3 mg/kg/day [19]. The clinical trial

* Corresponding author. Tel.: +1 215 895 1559; fax: +1 215 895 4983.
E-mail address: yz348@drexel.edu (Y. Zhong).

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shows that intravenous (IV) administration of MH for 7 days only resulted in 2.3 μg/mL MH in the cerebrospinal fluid (CSF) at steady state. While this concentration is sufficient for the anti-inflammatory effect [17,20,21], it is still far below the fully neuroprotective level of 35–75 μg/mL (1.5–50 μg/mL for mitochondrial cell death pathways [22] and glutamate toxicity [23], 10–40 μg/mL for hemorrhage-induced toxicity [13], and 35–75 μg/mL for excitotoxicity and Ca^{2+} influx [11], in a dose-dependent manner). Thus, it is likely that the modest clinical benefit observed in the phase II trial is more related to MH's anti-inflammatory effect rather than its direct neuroprotective effect. Although the potentially sub-optimal treatment dosing regimen in the clinical trial was suggested to be safe, one patient displayed elevated liver enzymes, indicating hepatocellular toxicity [18]. Thus, further increasing the systemically delivered dose of MH to reach its full neuroprotective capacity may not be safe for human patients. In addition, clinically the duration of MH treatment must be limited because long term systemic administration of high doses of MH has been shown to cause morbidity, liver toxicity, and even death [7,24]. Local delivery of MH could potentially expose the injured spinal cord tissue to high local concentrations of MH that systemic administration cannot safely achieve and prolong the duration of MH treatment, while avoiding the deleterious side effects from systemic exposure. However, no study on local delivery of high concentrations of MH to promote neuroprotection after SCI has been reported; this may possibly be due to the limitations of current drug delivery systems.

MH is a highly water-soluble small-molecule drug (MW 494 Da). It is released very quickly (less than 24 h) from hydrophilic drug delivery systems such as hydrogels [25,26]. In addition, MH degrades rapidly in aqueous solution, especially at body temperature [27]. Thus, pumps cannot be used for continuous intrathecal delivery of MH. Poly (lactic-co-glycolic acid) (PLGA) microspheres or nanoparticles have been used for sustained release of MH [28,29]. However, the low drug loading efficiency for MH (up to 1.92%) makes it impossible to administer a sufficient amount of drug in the limited intrathecal space for local delivery. A recent study reported the development of PEGylated poly-ε-caprolactone-based nanoparticles (PCL) for intraparenchymal delivery of MH to modulate inflammation after SCI [30]. However, the drug loading efficiency of PCL is also low (0.1%) [31], which limits the amount of MH that can be applied locally for effective neuroprotection. MH can form positively charged chelates with divalent metal ions including Ca^{2+} and Mg^{2+} [27,32]. Utilizing this property, one study reported encapsulating MH into polyion complex (PIC) micelles through electrostatic interaction between MH-Ca^{2+} chelates and negatively charged carboxymethyldextran-*block*-poly (ethylene glycol) (CMD-PEG) [27]. Although the loading efficiency of MH in the PIC micelles was high (50%), drug release only lasted for 24 h.

For optimum treatment effect, the dose and duration of local MH release should match the progression of secondary injury. Studies have shown that loss of grey matter was usually completed within 24 h, and loss of white matter extended up to 7 days after SCI [36,37]. Approximately 7 days after injury, a delayed wave of oligodendrocyte apoptosis was observed to occur in the white matter. The number of apoptotic cells decreased by 3 weeks, and apoptosis was nearly complete at 6 weeks [36,38–40]. This delayed oligodendrocyte apoptosis, triggered by inflammation and withdrawal of trophic signals after axonal loss [1,36,41,42], causes demyelination of surviving axons and subsequent conduction deficits or failure [1,43]. It has been shown that a low concentration of MH (0.5 ng/mL) was sufficient to inhibit the production of neurotoxic molecules by reactive microglia that lead to apoptosis of oligodendrocytes [17]. Thus, a release profile with high doses of MH release for 7 days for neuroprotection, followed by low doses of MH release for 3–6 weeks targeting chronic inflammation and delayed oligodendrocyte apoptosis potentially matches the progression of secondary injury.

We have developed novel dextran sulfate (DS)-MH complexes self-assembled by metal ion-assisted interaction for sustained release of bioactive MH [33]. DS is a negatively charged biocompatible polysaccharide that has high binding affinity for metal ions [34]. We found that Ca^{2+} or Mg^{2+} ions could induce formation of insoluble complexes between DS and MH [33]. The strong metal ion-assisted interaction enabled high drug loading efficiency (45.2%) and stable long term MH release. In the present study, we encapsulated the complexes into injectable agarose hydrogel so that the drug delivery system can remain localized in the intrathecal space at the injury site (Fig. 1A). This route of drug delivery is preferred over epidural or intraparenchymal delivery because it bypasses the dura mater as a diffusion barrier and is not anticipated to cause any additional tissue damage [35]. However, as we previously reported, MH release from DS-MH complexes was slow due to the strong metal ion-assisted interaction between MH and DS [33]. As a result, we found that encapsulating the complexes into agarose hydrogel resulted in stable low-dose MH release for 37 days. To develop a formulation that can release high doses of MH for effective neuroprotection after SCI, in this study we investigated the factors that control MH release and developed a formulation capable of releasing high doses of MH at the acute stage for neuroprotection, followed by low doses of MH at the chronic stage targeting chronic inflammation and delayed apoptosis of oligodendrocytes. We quantified MH release *in vivo* and found that local delivery of MH at a dose of 1.3 mg/kg generated significantly higher MH concentration in the local spinal cord tissue than intraperitoneal (IP) injection of 495 mg/kg MH over 5 days. This is the first time that high concentrations of MH could be delivered to the local neural tissue at a human safe dose. Using a clinically relevant rat unilateral cervical contusion injury model, we investigated the efficacy of local delivery of MH using this formulation in promoting neuroprotection, immunomodulation (by assessing inflammation and microglia/macrophage polarization), and locomotor functional recovery.

2. Materials and methods

2.1. Fabrication of DS-MH complexes within agarose hydrogel

For slow-release formulation, 2.4 mg/mL DS (500 kDa, Sigma-Aldrich) solution was prepared in 2× Hank's Balanced Salt Solution (HBSS) supplemented with 28.8 mM $MgC1_2$. Agarose (SeaPlaque, Lonza) was subsequently dissolved in the DS solution at 70 °C at a concentration of 3% (w/v). 2 mg/mL minocycline solution was prepared in deionized (DI) water. After the agarose in DS solution was cooled down to 37 °C, an equal volume of MH solution was added to the agarose-DS solution and well mixed. DS-MH complexes were formed within the agarose solution. Then the mixture solution was allowed to gel by cooling at 4 °C for 30 min.

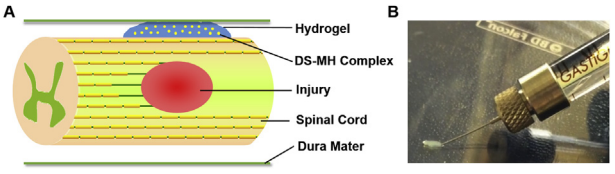

Fig. 1. Agarose hydrogel loaded with DS-MH complexes can be injected into the intrathecal space for controlled local delivery of MH. (A) Schematic illustrating intrathecal injection of complex-loaded hydrogel to bypass the diffusion barrier of dura mater. (B) The complex-loaded hydrogel can be easily injected from a Hamilton syringe.