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Injection of SDF-1 loaded nanoparticles following traumatic brain injury stimulates neural stem cell recruitment

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ABSTRACT

Recruiting neural stem cell (NSC) at the lesion site is essential for central nervous system repair. This process could be triggered by the local delivery of the chemokine SDF-1. We compared two PLGA formulations for local brain SDF-1 delivery: SDF-1 loaded microspheres (MS) and SDF-1 loaded nanoparticles (NP). Both formulations were able to encapsulate more than 80% of SDF-1 but presented different release profiles, with 100% of SDF-1 released after 6 days for the MS and with 25% of SDF-1 released after 2 weeks for NP. SDF-1 bioactivity was demonstrated by a chemotactic assay. When injected in mouse brain after traumatic brain injury, only SDF-1 nanoparticles induced NSC migration to the damage area. More neuroblasts (DCX+ cells) could be visualized around the lesions treated with NP SDF-1 compared to the other conditions. Rostral migratory stream destabilization with massive migration of DCX+ cell toward the perilesional area was observed 2 weeks after NP SDF-1 injection. Local injection of SDF-1-loaded nanoparticles induces recruitment of NSC and could be promising for brain injury lesion.

1. Introduction

Neural stem cells (NSC) have been considered a promising therapy for neurodegenerative diseases, since they have the abilities to generate new neurons and replace cells damaged by disease. NSC can also supply growth factors to the remaining brain parenchima and stimulate intrinsic repair (Vishwakarma et al., 2014).

However, endogenous neurogenesis is usually insufficient for promoting complete central nervous system (CNS) repair. Although progresses have been made, proper recruitment and integration of new neuroblasts to the injured tissue is still challenging (Dibajnia and Morshead, 2013).

SDF-1 is an 8-kDa protein critical to stem cell migration and can be used to direct stem cells to a desired site (Yellowley, 2013). SDF-1 and its receptor, CXCR4, were shown to play a crucial role in NSC migration after brain injury (Xue et al., 2014). SDF-1 is also thought to be angiogenic and neuroprotective (Mao et al., 2014; Wang et al., 2015).

SDF-1 injection in a trauma brain injury (TBI) model in rats ameliorated blood- brain barrier disruption, attenuated neuronal degradation and apoptosis and repressed inflammatory response (Sun et al., 2014). SDF-1 gene therapy ameliorated white matter injury and increased oligodendrocyte progenitor proliferation and migration in a stroke model in mice (Li et al., 2015). SDF-1 delivery was showed to be beneficial for different therapeutic applications, such as wound healing (Henderson et al., 2011; Rabbany et al., 2010), bone regeneration (Fujio et al., 2011; Ho et al., 2015) and myocardial infarction (Segers et al., 2007; Zhang et al., 2007).

A strategy to improve NSC recruitment at a TBI lesion and, therefore CNS repair, could be to locally deliver SDF-1. However, when directly injected into the brain parenchyma, SDF-1 is quickly degraded by metalloproteinase (MMP) 2 and 9 (Segers et al., 2007). To increase SDF-1 half-life or availability at the injection site, SDF-1 has been modified to make it resistant to MMP cleavage (Segers et al., 2007) or cells have been genetically engineered to secrete high concentrations of SDF-1 (Zhang et al., 2007). However, although stem cells engineered to overexpress SDF-1 successfully ameliorated cardiac function when injected in the heart (Zhang et al., 2007), lack of control over the dose and timing of SDF-1 secretion is an issue (Cross and Wang, 2011). Therefore, we propose to encapsulate SDF-1 in polymeric particulate systems that can protect the protein while allowing its controlled and sustained release at the site of injection (Cross and Wang, 2011).

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Two type of polymeric carriers can be used to encapsulate and deliver SDF-1: nanoparticles (NP) and microparticles (MP). MP range is from 1 μm to 1 mm while NP size is below 1 μm. Particle size has an impact on drug loading and release but also on behavior once injected *in vivo*. MP has a higher loading capacity and once locally delivered, tend to remain at the site of injection. NP smaller size makes them adapted to systemic delivery, crossing of biological barriers (including blood brain barrier) and to cell internalization (Hines and Kaplan, 2013; Makadia and Siegel, 2011). Degradation of larger particles is usually faster compared to smaller ones, impacting significantly drug release (Dunne et al., 2000).

Thus, we hypothesized that SDF-1 loaded particles would stimulate recruitment of NSC at the injection site and thus contributed to CNS repair. Our objective was to evaluate the effect of the carrier (nano- or micro-particle) on SDF-1 encapsulation, release and ultimately NSC migration *in vivo*.

2. Materials and methods

2.1. SDF-1 encapsulation

2.1.1. SDF-1 loaded microspheres (MS SDF-1)

Fifty two mg of PLGA RG 502H (Sigma-Aldrich, St. Lois, USA) were solubilized in 1.5 ml of dichloromethane (DCM) (Fischer, Bishop, UK). Fifty μl of aqueous phase containing 5 μg of murine rSDF-1 (Peprotec, Rocky Hill, USA) and 45 μg of BSA were added to the organic phase and sonicated (Branson, Missouri, USA) at 40 W for 15 s on ice to form the first emulsion. 50 ml of a 5% Polyvinyl alcohol (PVA) solution (Acros, New Jersy, USA) was added to the first emulsion and homogenized (ultra turrax T25, Sigma) for 45 s at 8000 rpm to form the second emulsion. The second emulsion was added to 30 ml of 1% PVA under stirring and was incubated at room temperature (RT) for at least 2 h to allow DCM evaporation. MS SDF-1 were washed 3 times by centrifugation at 5000 rpm for 10 min (RT) in deionized water. Samples were lyophilized.

MS SDF-1 size was measured by laser diffraction by HELOS (Sympatec, Pennington, USA) (n = 3).

Encapsulation efficiency (EE) and Drug loading (DL) were measured by dissolving 10 mg of dried MS in 200 μl of 0.1 M NaOH (Sigma) and heated at 37 °C for 15 min. Two hundred microliter of PBS were then added and the resulting solution was centrifuged at 10,000 rpm for 10 min at RT. The supernatant was collected and SDF-1 was quantified with ELISA.

EE and DL were determined as follow:

$$\mathrm{EE}(\%) = \frac{\text{SDF} - 1 \text{ dosed in 1 mg MS} \times \text{total weight in mg of MS} \times 100}{\text{SDF} - 1 \text{ introduced in the formulation}}$$

$$\mathrm{DL} = \frac{\text{SDF} - 1 \text{ dosed in 10 mg of MS}}{\text{10 mg of MS}}$$

2.1.2. SDF-1 loaded nanoparticles (NP SDF-1)

Fifty mg of polymer (25 mg PLGA 502H + 25 mg PLGA- PEG Resomer d50155) were dissolved in 1 ml of DCM (Fischer, Bishop, UK). Fifty microliter of aqueous phase containing 5 μg of SDF-1 and 45 μg BSA were added to the organic phase and sonicated at 70 W for 15 s on ice to form the first emulsion. Two millilitre of a 1% sodium cholate (Sigma) solution were added to the first emulsion and sonicated at 70 W for 15 s on ice. The second emulsion was added drop by drop in a 0.3% sodium cholate solution under stirring and was incubated at 37 °C for at least 1 h for DCM to evaporate. SDF-1 NP were washed 3 times by centrifugation at 10,000*g* for 45 min (RT) in deionized water. NP were re-suspended in 400 μl of 20% sucrose and kept frozen till use.

NP SDF-1 size, polydispersity index (PDI) and zeta potential were measured by dynamic light scattering (NanoSizer Zeta Series, Malvern Instruments, UK). EE and DL were measured as described for MS SDF-1 by dissolving 10 μl of NP in 200 μl 0.1 M NaOH.

EE and DL were determined as followed:

$$\mathrm{EE}\ (\%) = \frac{\text{SDF} - 1 \text{ in } 10\mu\text{l NP} \times 40\ (\text{total volume} = 400\ \mu\text{l}) \times 100}{\text{SDF} - 1 \text{ introduced in the formulation}}$$

$$\mathrm{DL} = \frac{\text{SDF} - 1 \text{ in } 10\mu\text{l of NP}}{10\ \mu\text{l of NP}}$$

2.2. Scanning electron microscopy (SEM)

The shape and surface of the dried micro and nanoparticles were observed by SEM, using a Quanta 650 FEG mycroscope (FEI, Thermo Fischer, Waltham, USA).

2.3. SDF-1 in vitro release

One mg of MS SDF-1 or NP SDF-1 was resuspended in 200 μl of PBS (n = 3). Samples were incubated at 37 °C and at given times, were centrifuged at 10,000 rpm for 30 min at RT. Supernatants were collected and frozen until further analysis.

SDF-1 was quantified using a Human ELISA kit (Peprotec) that has 100% cross-reactivity with murine SDF-1, following supplier specifications. Standard curve was done with murine SDF-1.

2.4. In vitro T lymphocyte migration assay

Impact of SDF-1 formulation was evaluated by incubating Jurkat cells with conditioned medium (n = 3). Two mg of MS SDF-1 were added to 2 ml of migration buffer (RPMI (Gibco, Massachusetts, USA) supplemented with 0.5% bovine fetal serum (Sigma)) and were incubated for 4 h at 37 °C. Based on the SDF-1 MS release curve obtained *in vitro*, predicted concentration was 41 ng/ml. Similarly, 200 μl of NP SDF-1 were added to 2 ml of migration buffer and incubated for 4 h at 37 °C. NP were used directly after production due to massive aggregation after freeze-drying, even in presence of 20% sucrose (data not shown). Predicted concentration based on the NP SDF-1 *in vitro* release curve was 85 ng/ml. Samples were then centrifuged and 600 μl of the supernatants (conditioned media) were placed in 24 well plate. A Transwell cell insert (8 μm pore, Greiner-Bio-One, Kremsmünster, Austria) was placed in each well containing the conditioned medium. Then, 400,000 Jurkat cells (200 μl) were added in the apical chamber of the inserts. Migration buffer alone was used as negative control and a 100 ng/ ml SDF-1 solution was used as a positive control. Cells were incubated at 37 °C for 4 h in a 5% CO2 incubator. Transwell inserts were then removed and the number of cells that migrated in the basolateral was measured by counting on a Neubauer chamber.

Table 1
Properties of PLGA MS and PLGA NP.

Particle type	MS	NP
Size	4,83 ± 0,33 μm	167,9 ± 0,38 nm
PDI	na	0,08 ± 0,04
Zeta Potencial	na	−8,27 ± 0,97 mV
Encapsulation efficiency	89,5 ± 4,5%	84,5 ± 0,1%
Drug loading	89,5 ± 4,5 ng/mg MS	106 ± 13 ng/mg NP

MS: microsphere, NP: nanoparticle, SDF-1: stromal derived factor 1, na = not availiable, PDI = polidispersity index (mean ± SD, n = 3).