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Activation of the niacin receptor HCA_2 reduces demyelination and neurofilament loss, and promotes functional recovery after spinal cord injury in mice

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ABSTRACT

After spinal cord injury (SCI), there is an acute phase of alternatively activated (M2) macrophage infiltration, followed by a long-lasting phase of classically activated (M1) macrophage accumulation in the wound, which is believed to derail healing and compromize organ functions. Thus, agents which are able to modulate macrophage phenotypes may provide significant benefits to SCI patients. In the present study, we demonstrate that the niacin receptor HCA_2 is specifically expressed on the cell surface of M1 but not M2 macrophages. Treatment of M1 macrophages with niacin (300 μM) resulted in down-regulation of the p65 NF-κB phosphorylation, associated with a marked decrease in the levels of M1 markers, including CD86, IL-12, and IL-6, and a significant increase in the expressions of M2 markers, such as CD206, IL-10, and IL-13, suggesting that niacin causes a shift of M1 to M2. Moreover, treatment of the M1-oligodendrocyte precursor cell (OPC) co-cultures with niacin markedly promoted the expression of myelin binding protein (MBP). After SCI in C57/BL6 mice for a week, a marked accumulation of M1 macrophages, which expressed HCA_2 receptor, was evident in the wound. Treatment of the SCI mice with niacin (100 mg/kg) resulted in a dramatic decrease in the number of M1 macrophages and a significant increase in the number of M2 macrophages in the wound. This was associated with a robust inflammation resolution, attenuation of demyelination and neurofilament loss, and significant improvement of locomotor function. Thus, HCA_2 receptor may serve as a therapeutic target to promote post-SCI recovery.

1. Introduction

Spinal cord injury (SCI) is followed by influx of inflammatory blood-borne cells and activation of endogenous cells (Kroner et al., 2014). Recent studies have indicated that different macrophage subtypes can arize during the course of SCI (Kigerl et al., 2009; David et al., 2015). While the ratio of the classically activated (M1) to the alternatively activated (M2) macrophages is roughly equal acutely after SCI, M1 macrophages dominate the wound area within a few days (Kigerl et al., 2009). M1 macrophages produce pro-inflammatory cytokines and neurotoxins, which cause secondary injury after SCI (Horn et al., 2008; Gensel et al., 2009; Busch et al., 2011). In contrast, M2 macrophages phagocytose the myelin debris known to inhibit axonal regeneration (Popovich et al., 1999; Giger et al., 2010; Sun et al., 2010), and they also release protective cytokines to promote neuronal regeneration and tissue repair (Bomstein et al., 2003; Schwartz, 2010; David et al., 2011). Thus, the delayed M1 to M2 transition in the wound may lead to impaired healing, and modification of the SCI microenvironment to increase the M2/M1 ratio may have a neuroprotective effect.

The hydroxycarboxylic acid 2 (HCA_2) receptor is a G-protein-coupled receptor (GPCR) also known as protein up-regulated in macrophages by IFN-γ (PUMA-G; murine ortholog), HM74A (human ortholog), and HCA_2 receptor (Schaub et al., 2001). Recent studies have demonstrated an involvement of HCA_2 receptor in inflammation (Digby et al., 2012; Zandi-Nejad et al., 2013). HCA_2 receptor is expressed by macrophages (Feingold et al., 2014), and its ligand nicotinic acid (niacin) exerts anti-inflammatory effects in a number of tissues, including but not limited to blood vessel, colon, kidney, and brain (Cho et al., 2009; Kwon et al., 2011; Wu et al., 2012; Rahman et al., 2014; Singh et al., 2014; Ganji et al., 2015). Treatment with niacin significantly inhibits TNF-α, IL-6, IL-12, and IL-1β production

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LPS-stimulated wild-type murine bone marrow-derived macrophages (BMMs) but failed to do so in Hca2$^{-/-}$ BMMs (Zandi-Nejad et al., 2013). Similar inhibitory effects of niacin on the production of pro-inflammatory cytokines in stimulated macrophages have also been reported elsewhere (Si et al., 2014; Zhou et al., 2014). Moreover, a recent study showed that HCA_2 receptors activated a neuroprotective subset of macrophages in an ischemic stroke model (Rahman et al., 2014), implying a potential role of HCA_2 receptors in modulating Macrophage phenotypes. However, direct evidence is lacking and it is unclear whether activation of this receptor attenuates secondary injury after SCI.

In the present study, we demonstrate that niacin induced the shift of M1 to M2 phenotype, and promoted oligodendrocyte differentiation in the M1-oligodendrocyte precursor cell (OPC) co-cultures. In the wound of SCI mouse model, a predominant expression of HCA_2 receptors in the infiltrated macrophages was observed, and niacin treatment resulted in a robust shift of M1 to M2 macrophages, associated with a robust inflammation resolution, attenuation of demyelination and neurofilament loss, and significant improvement of locomotor function. Thus, activation of HCA_2 receptor may offer therapeutic benefits to SCI patients.

2. Materials and methods

2.1. Animals

The experiments were carried out in male C57BL/6 mice (20–25 g) from Jackson Laboratory (Bar Harbor, ME, USA). All animals were housed according to standard animal care protocols and maintained in a pathogen-free environment at the Changan Hospital. All procedures were performed in accordance with the Changan Hospital Institutional Animal Care and Use Committee, and followed the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978).

2.2. Spinal cord injury and drug treatment

All surgeries were performed under aseptic conditions. For contusion injury, an Infinite Horizon Impactor (PSI) was used on adult C57BL/6 male mice (20–25 g) as described previously (Luchetti et al., 2010). Briefly, under deep anesthesia with isoflurane (Primal Healthcare, Andhra Pradesh, India), mice received a dorsal laminectomy at the T9 position. A controlled force-defined impact at 50 kdyne was delivered to the exposed cord with a stainless steel impactor tip after securing the lateral processes of T8 and T10. After the impact, the mouse was visually inspected for evidence of adequate impact, primarily for bruizing of the cord, and muscles were sutured and the skin stapled with surgical clamps. Sham animals received a laminectomy without weight drop. The mice were subcutaneously injected with 0.5 ml of buprenorphine (0.1 mg/kg), 0.5 ml of enrofloxacin (2.5 mg/kg), and sterile saline at 2.5 ml/50 g weight. Saline and enrofloxacin were administered daily for a week. The mice underwent gentle bladder expression twice daily until they were voiding on their own. Weight was monitored daily until 14 d post-injury (hereafter denoted as dpi), and then weekly for the duration of the experiments (42 dpi). A 10% weight loss was typically observed after injury, and a high caloric nutrient paste (Nutrical; Evsco) was provided ad libitum as a dietary supplement.

To examine the phenotype of macrophages infiltrated in the wound, 7 days after the injury, SCI mice (n=10) or control (sham) mice (n=10) were killed and their spinal cord used for immunohistochemistry. To evaluate the effect of niacin on the pathology after SCI, immediately after SCI, half of the mice (n=60) were fed with niacin (100 mg/kg, b.i.d.) (Sigma-Aldrich, St Louis, MO, USA) by oral gavage once daily for 4 weeks. The other half of animals (n=60) were fed with vehicle (control) for the same time period. Animals were killed by decapitation at 3 h, 1 day, 3 days, 1 week, 2 weeks, and 4 weeks after the SCI, respectively (n=10 per time point per group), and spinal cord tissues were used for histology analysis. To evaluate the effect of niacin on locomotor activity, the SCI animals treated with niacin (n=10) or vehicle (n=10) were used for the behavioral test. In total, 160 mice were used for these experiments.

2.3. Assessment of locomotor behavior

Hind-limb motor function was evaluated using the Basso Mouse Scale (BMS) rating system (Basso et al., 2006). The open field was a round table that was 20.32 cm high and 1.524 m in diameter situated in a quiet testing room with normal lighting. The day before surgery, the mice were tested to obtain baseline pre-op values, which showed no difference between the four different test groups. After injury, each mouse was observed at 1, 3, 7, 14, 21, and 28 days to evaluate differences in functional recovery among different drug treatments. The extent of force applied in the contusion injuries was chosen to deliver moderate-severe thoracic contusions (50 kdyne) so as to allow some degree of weight-supported stepping in recovery. This also ensured that there was some extent of impairment in hindlimb function during over-ground walking.

Two individuals who were blind to the treatment groups simultaneously scored hindlimb locomotion for 4 min per mouse using the score sheet from Basso et al. (Basso et al., 2006). If the scores differed between the individuals, the lower score was taken. To summarize the steps involved in BMS testing, it was first decided whether the mouse was plantar stepping when it was placed in the open field and the timer was initiated. If not, then ankle movement of dorsal stepping was evaluated and appropriately scored. If there was plantar stepping, then the frequency of stepping and coordination was evaluated as described previously (Basso et al., 2006).

2.4. Immunohistochemistry

Following deep anesthesia, transcardial perfusion was performed, followed by fixation with 4% paraformaldehyde in phosphate-buffered saline (PBS). The spinal cords were dissected out and post-fixed in the same fixative for a few hours. The tissue samples were immersed in 10% sucrose in PBS at 4 °C for 24 h, and 30% sucrose in PBS for 24 h. Segments of the spinal cord (cord segments T8 to T12) were embedded in optimal cutting temperature compound (Sakura Finetek, Torrance, CA, USA) and cut on a cryostat into serial axial or sagittal frozen sections 10 μm thick. The sections were serially mounted on glass slides, and fixed with 2% paraformaldehyde in PBS for 5 min, rinsed in PBS, and stored at −80 °C.

For immunofluorescence staining, frozen sections were permeabilized with 0.1 mol/l Tris-HCl buffer (pH 7.6) containing 0.3% Triton X-100. The following primary antibodies were diluted in commercial diluent (Antibody Diluent with Background Reducing Components; Dako Cytomation, Carpinteria, CA, US) and applied overnight at 4 °C: rabbit anti-Iba-1 (1:500; Wako Pure Chemicals); mouse monoclonal anti-CD86 (1:200; Wako Pure Chemicals); rabbit anti- HCA_2 receptor (1:200; Santa Cruz Biotechnology); mouse monoclonal anti-Arg-1 (1:200; Millipore Bioscience Research Reagents); mouse monoclonal anti-MBP (1:200; Santa Cruz Biotechnology); mouse monoclonal anti-NF-H (1:200; Santa Cruz Biotechnology); monoclonal anti-GFAP (1:200; DakoCytomation). The sections were then incubated for 1 h at room temperature