el Targeting Nanotherapeutics to Improve Functional Recovery and Reduce Side Effects after Acute Spinal C Injury

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INTRODUCTION

Spinal cord injury (SCI) is a catastrophic medical problem that leads to loss of sensory, motor, and autonomic functions. To date, no therapy has been proven to be efficacious in fully restoring neurological functions post SCI. Systemic high-dose methylprednisolone (MP) treatment improves neurological recovery after acute SCI in both animal and human. The utility of high-dose systemic MP in acute SCI remains controversial due to its modest effect on functional recovery and the accompanying adverse effects. To overcome the limitation of MP therapy, we have developed a *N*-(2-hydroxypropyl) methacrylamide copolymer-based MP prodrug nanomedicine (Nano-MP) that can selectively deliver MP to the inflamed SCI site when administered systemically in a rat model of acute SCI, aiming to improve the therapeutic efficacy of MP while minimizing unwanted distribution to and actions on other tissues, thereby, reducing untoward side effects.

Methods

Nano-MP was synthesized (Fig 1). Both complete spinal cord transection and moderate contusion SCI models were used. Immediately after SCI, a single dose of Nano-MP was administered to the SCI rats via intravenous (i.v.) injection. Unconjugated MP and vehicle were subjected to a bolus i.v. injection and followed by 24-h infusion with the Bracken protocol.

RESULTS

After a single i.v injection, Nano-MP preferentially accumulated to the SCI injury site, sequestered (Fig 2) and retained mainly by CD11$^+$ infiltrating inflammatory cells. The Nano-MP significantly inhibited lipid peroxidation (Fig 3) and inflammation of the injured spinal cord, resulting in reduced neuronal damage and enhanced neuroprotection (Fig 4) after acute SCI. Compared to conventional i.v delivery of free MP, Nano-MP administration provided better functional improvement after acute SCI in rats (Fig 5) and had far fewer systemic adverse side effects—that is, less carbohydrate intolerance (Fig 7) , reduced muscle atrophy (Fig 6 & 8) and bone loss (Fig 9) .

CONCLUSION

Nano-MP is a promising drug candidate that is more effective and safer than standard MP therapy and it may be translated as a new treatment for acute SCI patients with improved functional recovery, social independence, and quality of life.

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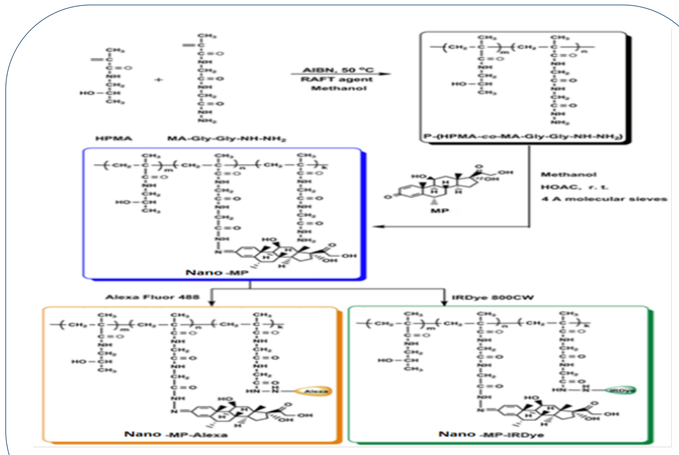
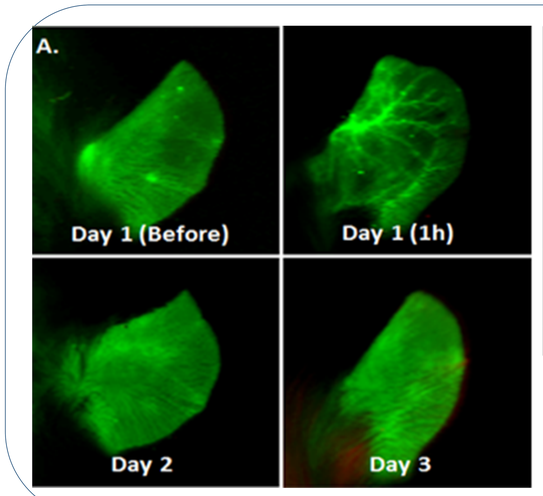


Fig 1. Synthesis of HPMA copolymer-based methylprednisolone prodrug nanomedicine (Nano-MP) and labeling with fluorescent dyes



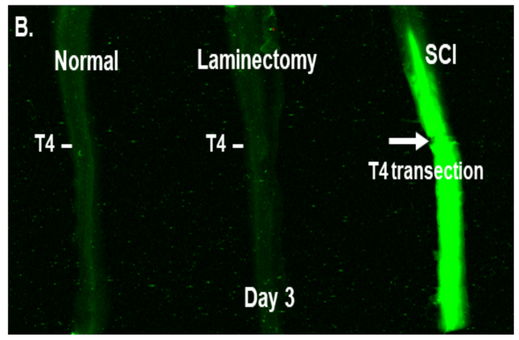


Fig 2. The selective uptake of Nano-MP in the injured spinal cord. (A) Detection of Nano-MP in vivo at different time points post-injection. Male rats underwent complete T4 spinal cord transection or laminectomy only. Nano-MP-IRDye (1 mg/rat) was injected via tail veins immediately after SCI. Blood vessels in the rat ear were visible soon after injection. At days 2 and 3, blood vessels were not detectable in the rat ear. (B) Nano-MP was detected in the injured spinal cord at day 3, but not that in sham or laminectomy-only rats. Images were taken using a LI-COR imaging system (X4). N=10.

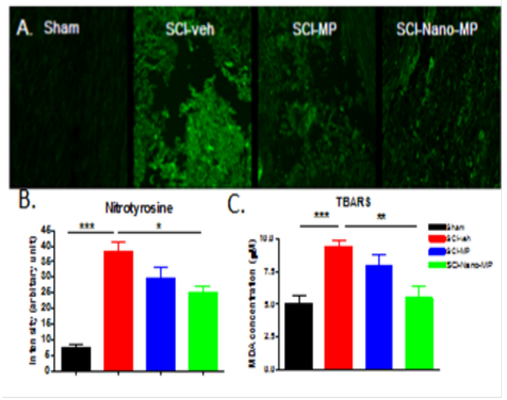


Fig 3. Nano-MP attenuated SCI-induced oxidative stress and lipid peroxidation in the spinal cord. A-a. Representative images showing the nitrotyrosine level in the spinal cord (longitudinally sectioned) and fluorescent intensity quantification (**A**-b). **B**. Malondialdehyde (MDA) level was measured using TBARS assay. Data are expressed as Mean ± SEM. n=5-6/group. *p<0.05, **p<0.01, ***p<0.001 by one-way ANOVA. Scale bar= 40μm.

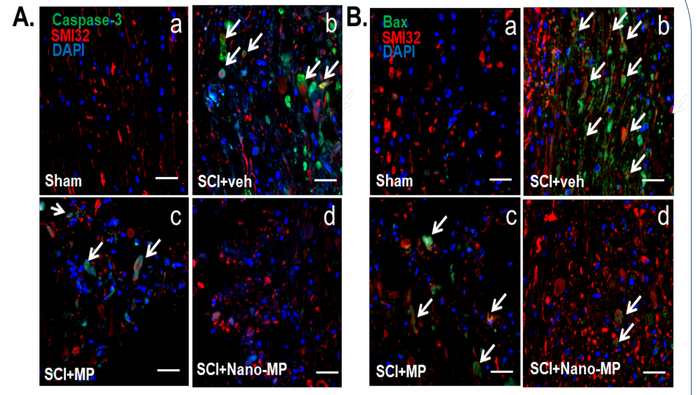


Fig 4. Nano-MP decreased SCI-induced motor neuron apoptosis in the spinal cord after acute SCI. Representative images show the caspase-3 (**A**) or Bax (**B**) ***immunofluorescence*** staining in the spinal cord (longitudinally sectioned, n=5-6/group). Caspase-3 or Bax stain in green; SMI32 (red) and DAPI (purple) stain motor neuron and nucleus, respectively. Scale bar= 20μm. Arrow, co-localization.

Fig 5. Functional recovery evaluated by the BBB scale after SCI. Vehicle, free MP or Nano-MP was administered immediately after SCI. n=3 each group; *p<0.05, **p<0.01, unpaired *t*-test.

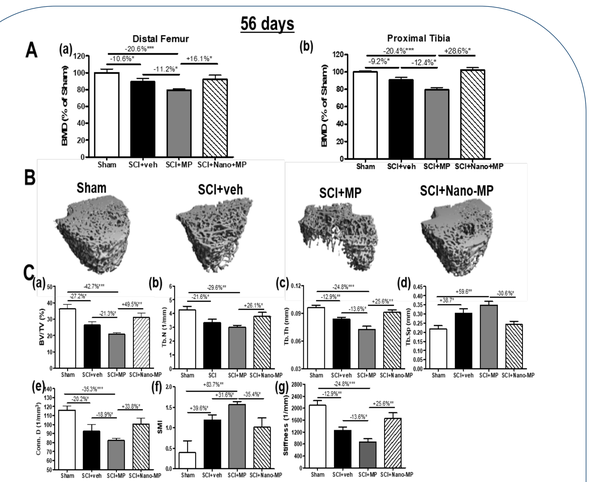


Fig 6. Systemic MP, not Nano-MP, reduces body weight and muscle mass (A) Body weight. (B) Weights for skeletal gastrocnemius. Data are expressed as Mean±SEM. *p<0.05, ***p<0.001 versus the indicated group by one-way ANOVA plus Newman-Keuls post hoc test. n=10-12 per group.

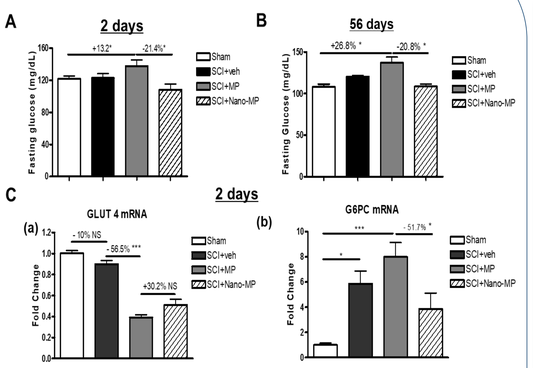


Fig 7. Glucose metabolism. Changes of fasting glucose at 2 days (**A**) or 56 days (**B**) after SCI. Levels of glucose metabolism-related mRNA GLUT 4 and G6PC (**C**) in gastrocnemius muscle are shown 2 days after SCI. *n* = 12/group except the SCI+MP (n=8). Data are expressed as mean ± SEM. Significance of differences was determined using one-way analysis of variance with a Newman–Keuls test post hoc. * *P* < 0.05, ** *P* < 0.01 and *** *P* < 0.001 versus the indicated group; NS, no significant difference.

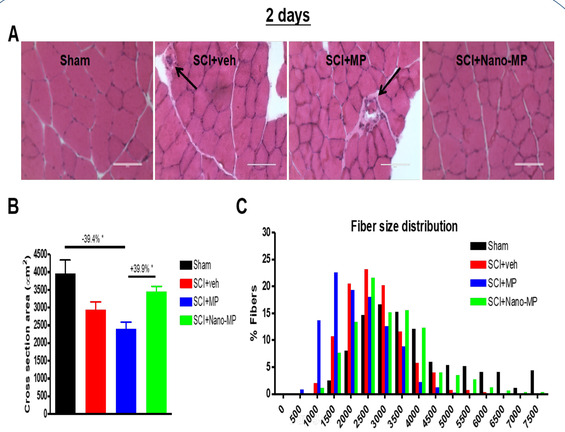


Fig 8. Nano-MP administration reduces MP-associated muscle structure defects after acute SCI. (**A**) Representative Hematoxylin-eosin-staining images of gastrocnemius muscle sections are shown. The arrows indicate necrotic fibers or fibers invaded by inflammatory cells. Bar= 100μm. (**B**) Quantification of fiber cross-sectional area (fCSA). (**C**) Fiber size distribution between experimental groups. Data are expressed as mean ± SEM. *n* = 6–8/group. Significance of differences was determined by using one-way analysis of variance with a Newman–Keuls test post hoc. * *P* < 0.05 and ** *P* < 0.01 versus the indicated group.

Fig 9. Nano-MP administration reduces MP-associated impairment on bone integrity at 56 days after acute SCI. (**A**) Areal bone mineral density (aBMD) measurements in each animal group are shown at the distal femur (a) and proximal tibia (b). (**B**) Representative micro-CT 3D images of trabecular microarchitecture are displayed. (**C**) Measurements are shown for: (a) BV/TV, (b) Tb.N, (c) Tb.Th , (d) Tb.Sp, (e) conn.D , and (f) SMI. Bone stiffness (g) was estimated from μFEA. Data are expressed as mean ± SEM. *n* = 6–7/group. Significance of differences was determined by using one-way analysis of variance with a Newman–Keuls test post hoc. *P < 0.05, **P < 0.01 and ***P < 0.001 versus the indicated group. NS: not significant.