Superoxide Dismutase-Loaded Porous Polymersomes as Highly Efficient Antioxidant Nanoparticles for Osteoarthritis Therapy

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Introduction

Oxidative stress and the reactive oxygen species (ROS) have important roles in osteoarthritis (OA) development.[1] Scavenging ROS by exogenous antioxidant enzymes could be a promising approach for OA treatment. However, the direct use of antioxidant enzymes, such as superoxide dismutase (SOD), is challenging due to a lack of effective drug delivery system. This study utilized a highly efficient antioxidative nanoparticle based on SOD-loaded porous polymersome nanoparticles (SOD-NPs) for delivery of SOD to mouse knee joints and tested their therapeutic efficacy in OA mice.

Methods

SOD-NP synthesis

SOD was encapsulated within the aqueous interior of polymersomes made from a mixture of two amphiphilic diblock copolymers, 75 mol% poly (ethylene glycol)-polybutadiene copolymer (PEG-PBD) and 25 mol% poly (ethylene glycol)-poly (propylene oxide) (PEG-PPO) (Figure 1A). The retention assay was performed by one-time intra-articular (IA) injection of 10 μl IRDye 800CW-labeled SOD or SOD-NPs, the fluorescence intensity in the joint was quantified over a period of 28 days.

Cartilage and Synovium explants

Cartilage and synovium explants were obtained from human knee joints after total knee arthroplasty surgery. Explants were treated with PBS (Untreated), IL-1β in combination with PBS, empty NPs, SOD or SOD-NPs for 8 days.

Cell culture

Chondrocytes and synovial fibroblasts were harvested from mouse knee joints by enzymatic digestion and subjected to viability and flow analysis

Animals

All animal work was approved by the Institutional Animal Care and Use Committee at the University of Pennsylvania. Male C57Bl/6 mice at 3 months of age received destabilization of medial meniscus (DMM) at right knees. They were then divided into 4 groups, receiving 10 μl PBS, empty NPs, SOD (500 U/mL), or SOD-NPs (500 U/mL) IA injections once every 2 weeks for 12 weeks (n = 6 mice/group). An additional group of mice (n = 6) received sham surgery with PBS injections.

Histology

Knee joints were processed for paraffin sections followed by H&E, Safranin-O/fast green (SO/FG), 8-OHdG, Mmp13 and Adamts5 staining. MicroCT- Femurs were scanned from the epiphyseal end at a ± μm resolution by microCT 35. The subchondral bone plate thickness (SBP. Th) of distal femoral end was quantified.

Statistics

Data are expressed as means±SEM and analyzed by one way ANOVA and unpaired, two-tailed Student's t-test.

Results

SOD-NPs up to 500 U/mL (SOD concentration) did not affect viability of mouse chondrocytes (Figure 1B). Joints receiving IRDye 800CW-labeled SOD-NPs injection had much higher fluorescence intensity than those receiving IRDye 800CW-labeled SOD injection. Moreover, the retention of SOD-NPs in DMM joints was longer than that in healthy joints (Figure 1C). Interestingly, biodistribution assay indicated that SOD-NPs were largely retained in synovium and minimally located at articular cartilage surface (data not shown). In vitro, synovial fibroblasts endocytosed SOD-NPs and their production of ROS marker H2DCFDA after TNFα treatment was greatly reduced by SOD-NPs co-treatment (Figure 1D). SOD-NPs treatment protected cartilage (data not shown) and synovial explants (Figure 2A-F) from IL-1β-induced OA-like degeneration. IA delivery of SOD-NPs attenuated DMM-induced OA cartilage erosion and degeneration (Figure 3A, B), synovitis (Figure 3C,

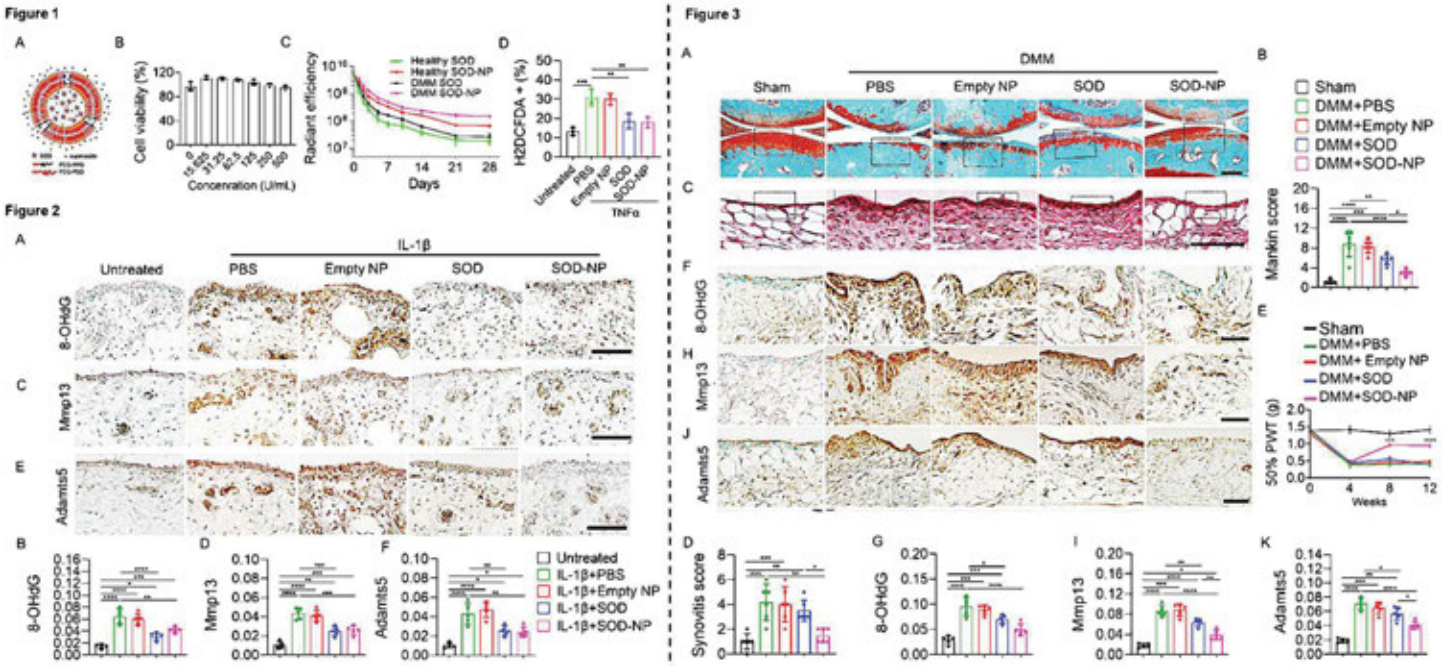

Figure 1. SOD-NP synthesis and characterization. (A) Schematic diagram of SOD-loaded polymersomes. **(B)** The cell viability of primary chondrocytes after incubation with SOD-NPs at various concentrations. (n = 3/group). **(C)** Quantitative analysis of time course radiant efficiency within knee joints over 28 days after IA injection of IRDye 800CW–labeled SOD or SOD-NPs. (n = 6/group) (D) Measurement of H2DCFDA levels in mouse SFs after treatment with TNFα plus PBS, empty NP, SOD, or SOD-NP for 24 h. (n = 3/group).

Figure 2. SOD-NP protects synovium explants from OA injury. (A, C, E) Representative IHC images of 8-OHdG, Mmp13 and Adamts5 in human synovial explants with indicated treatment. Scale bar, 100 μm. **(B, D, F)** Semi-quantitative evaluation of 8-OHdG, Mmp13 and Adamts5 amount represented as IOD/area. (n = 5/group).

Figure 3. SOD-NP attenuates joint destruction in DMM induced mouse OA model. (A) SO/FG staining of knee joints at 12 weeks after surgery. Scale bars: 200 μm. **(B)** The OA severity of knee joints was measured by Mankin score. (n = 6/group). **(C)** von Frey assay at 4, 8 and 12 weeks after DMM surgery. PWT: paw withdrawal threshold. (n = 6/group). **(D)** HE staining of synovium tissue (Black boxed areas). Scale bar: 200 μm. **(E)** Synovitis scores were quantified. (n = 6/group). **(F, H, J)** Representative IHC images of ROS marker 8-OHdG, Mmp13, Adamts5 in synovium tissue. Scale bar: 50 μm. **(G, I, K)** The amounts of 8-OHdG, Mmp13, and Adamts5 in synovium tissue were quantified as integrated optical density to area (IOD/area). (n = 6/group). *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.

D), subchondral bone plate sclerosis (data not shown), and joint pain (Figure 3E). Mechanistically, SOD-NPs treatment significantly reduced amounts of 8-OHdG (ROS marker, Figure 3F, G), Mmp13 (Figure 3H, I) and Adamts5 (Figure 3J, K) in synovium of DMM knees. On the contrary, SOD or empty NPs alone did not alter OA progression after DMM. Similar results were also obtained when SOD-NPs treatment starts at 4 weeks after DMM surgery (data not shown).

Discussion

The therapeutic utility of the antioxidant enzyme SOD is largely hindered by inadequate delivery, stability, and retention at its intended site of action, due to rapid degradation and/or clearance. Our study demonstrates that SOD-loaded porous polymersomes are more efficacious than free SOD in treating OA. Besides the breakdown of articular cartilage, synovial inflammation is also an important risk factor in OA initiation and progression. Our data showed that SOD-NPs can be endocytosed into synovial fibroblasts, leading to attenuated ROS reaction and proteinase production, as well as reduced synovitis symptoms and OA pain relief. Targeting key aspects of synovium inflammation holds great promise for OA therapy.

Significance

This proof-of-principle study demonstrates the therapeutic efficacy of SOD-loaded porous polymersomes for OA treatment.

References

1. Lepetsos P, Papavassiliou AG. ROS/oxidative stress signaling in osteoarthritis. *Biochim Biophys Acta* 2016;1862:576–91.