Antibody-Based Targeting of Elastic Matrix Regenerative Nanoparticles to Aortic Aneurysms

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Statement of Purpose: Abdominal aortic aneurysms (AAAs) are characterized by proteolytic breakdown and poor auto-regeneration and repair of elastic fibers within the aorta wall. We previously showed PLGA nanoparticles (NPs) loaded with doxycycline (DOX) to inhibit elastolysis and also stimulate elastic matrix neoassembly. Elastolysis is mediated by matrix metallo-proteinases (MMPs), specifically MMPs-2 and -9. We showed that at low micromolar doses, DOX continues to inhibit MMP activity as it does at much higher, systemic clinical doses, but also stimulates new elastin crosslinking. We thus seek to deliver DOX at such low doses in a steady and sustained manner from biodegradable polymer nanoparticles, within the AAA wall. Our NP formulations are surface modified with cationic amphiphiles that uniquely impart pro-elastogenic and anti-proteolytic effects separate from the released DOX. Since cathepsin K, a serine protease, is known to be highly upregulated in the AAA wall, in this study, we investigate efficacy of active NP targeting to the AAA wall via incorporation of pendant cathepsin K antibodies (Abs) on the NP surface, and implications to DOX-NP-imparted functional benefits.

Methods: Doxycycline hyclate (DOX)-encapsulating polylactic co glycolic acid (PLGA) NPs were formulated by a double emulsion solvent evaporation technique using didodecyldimethylammonium bromide (DMAB); 0.25% w/v) as a surfactant. Cathepsin K antibodies (Ab) were covalently conjugated to the NP surface by an EDC linking chemistry. Ab attachment was confirmed using a fluorescent secondary Ab. Ab fluorescence was compared to the fluorescence due to an Alexa 633 fluorophore encapsulated within the NPs to estimate efficiency of Ab binding to the NPs. Fluorescence due to the Abs was monitored over 2 weeks in aqueous medium to assess continued retention of the bound Abs. Lack of changes to NP size and positive charge were confirmed via dynamic and phase analysis light scattering respectively, and Ab-based NP binding to cathepsin K overexpressing TNF-α-activated rat aneurysmal SMCs determined. Effects of improved NP localization within these cell layers to elastic matrix neoassembly, fiber formation and MMP production & activity were quantified using a Fastin assay, immunofluorescence/morphometry, western blots and gel zymography respectively. The targeting capabilities of cathepsin K Ab-modified NPs was determined in vitro, ex vivo, and in vivo. In vitro experiments were performed on cells isolated from rat aneurysmal tissue and ex vivo experiments were performed on elastase-injured porcine carotid arteries. Wall uptake and in situ retention of IR-dye encapsulated Ab-modified NPs was assessed using whole tissue fluorescence imaging. In vivo experiments were conducted in rats using an elastase-injured model. Two weeks later, aneurysm formation was confirmed and cathepsin K overexpression at the AAA site confirmed relative to a healthy upstream aorta segment (control). In replicate rats, cathepsin K Ab-modified NIR dye-loaded NPs were infused via tail vein injection, 2 weeks after AAA formation and their retention within the AAA wall and biodistribution in other organs determined by FMT.

Results: Covalent conjugation of cathepsin K Ab to the DOX-NPs enhanced NP binding to AAA SMCs and maintained levels of DOX release, augmented elastic fiber assembly and crosslinking and diminished proteolytic activity. In the in vitro and ex vivo cases, the cathepsin K Ab-modified NPs exhibited an increase in binding to cathepsin K Ab (Figure 1). Conjugating the cathepsin K Ab on the NP surface increased NP binding to the artery wall by 2-fold. Initial outcomes of on-going in vivo NP delivery studies show the tail vein injected cathepsin K-Ab-modified NPs to bind and be taken up for retention in the medial layer of the AAA wall for at least 1wk; limited biodistribution of the NPs was observed in other organs.

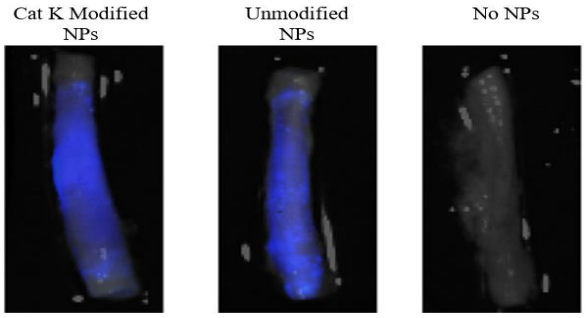

Fig 1. Pseudofluorescence of NP localization in elastase treated porcine carotid arteries.

Conclusion: Utilizing the expression of cathepsin K in AAAs is an effective modality to target NPs to the AAA wall. We have demonstrated an increase in NP binding and retention within the AAA wall with minimal biodistribution. Intimate NP binding to the cell results in more effective pro-elastogenic, anti-MMP, and pro-elastin crosslinking effects due to the NP/drug. Future studies will assess therapeutic efficacy of our NPs in restoring elastin homeostasis in the AAA wall to slow, arrest or regress AAA growth in the same animal model.

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