Cathepsin-K Targeting Nanotherapeutics for Regenerative Repair of Abdominal Aortic Aneurysms

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Statement of Purpose: Abdominal Aortic Aneurysms (AAA) involve slow dilation and weakening of the aortic wall due to chronic breakdown of matrix proteins, such as elastin, via an overexpression of matrix metalloproteases (MMPs), specifically MMP-2 and -9. Auto-regenerative repair of disrupted elastic matrix by smooth muscle cells (SMCs) at the AAA site is intrinsically poor and together with chronic proteolysis prevents buildup of new, intact elastic matrix to enable AAA growth arrest or regression to a healthy state. Oral doxycycline (DOX) therapy has been shown to inhibit MMPs to slow AAA growth, but has systemic effects and inhibits new elastin deposition within AAA tissue, diminishing prospects for restoring elastin homeostasis and thus arresting/regressing AAA growth. We have thus developed cationic amphiphile (DMAB)-modified polylactic-co-glycolic acid (PLGA) NPs with pro-elastogenic and anti-proteolytic properties for localized, sustained DOX delivery within AAA tissue at a much lower dose that we have found to augment elastin regenerative repair. To provide greater specificity of NP targeting, in this study we conjugated the DOX-NP surface with an antibody against cathepsin-K, a lysosomal protease that is highly overexpressed within AAA tissue. In this study, we sought to investigate if cathepsin-K Ab conjugation of the NP surface improves NP –cell binding, its effects on DOX release and how cathepsin-K presence and improved NP-cell interactions impact pro-elastogenic and anti-proteolytic effects of the DOX-NPs.

Methods: Aneurysmal rat aortic smooth muscle cells (EaRASMCs) were isolated from rat AAAs at 21 days post-induction by elastase (40 U/ml, 30 min) infusion, and primary cells passaged twice prior to use in experiments. DOX-NPs containing 2% w/w DOX were prepared using a double emulsion solvent evaporation method using 0.25% w/v diodecyldimethylammonium bromide (DMAB) as a surfactant. Mean hydrodynamic size and surface charge of the NPs was measured in a DLS and Zeta Potential machine. The NPs were either used in experiments as such or following surface conjugation with a monoclonal rabbit anti-rat cathepsin K Ab (H-50; Santa Cruz, Inc.). The antibody was conjugated via a) covalent reaction (EDC linking chemistry) and b) adsorption, both over a range of time periods (1, 5, and 24 hours). Confocal microscopy and UV-Vis spectroscopic analysis of fluorescence associated with NPs conjugated with an Alexa 546-fluorophore tagged cathepsin K Ab using either the two conjugation methods above were used to compare Ab conjugation efficiency. Cathepsin-K expression in TNF-α (100 ng/ml)-stimulated EaRASMC cultures was compared to unstimulated EaRASMCs and healthy rat aortic SMC (RASMC) cultures using western blot and immunofluorescence (IF) imaging. Subsequent experiments based on IF imaging, sought to correlate binding efficiency of cathepsin K Ab-conjugated NPs to cathepsin-K expression by EaRASMCs. DOX release profiles for conjugated and unconjugated NPs was compared over a time-span of 60 days with released DOX quantified using spectrophotometry. To assess if improved targeting of cathepsin-K Ab-conjugated NPs improved their pro-elastogenic and anti-proteolytic effects, TNF-α-stimulated EaRASMCs (n = 6/condition) were cultured with no NPs (control 1), DOX-loaded unconjugated DOX-NPs (control 2), and conjugated DOX-NPs and the following assessed: MMP-2,-9 expression and activation (western blot & gel zymography), elastic matrix deposition (Fastin assay and IF imaging), elastin crosslinking (ELISA for desmosine) cell prolifeartion (DNA assay), and elastic fiber formation and cellular internalization of NPs (TEM).

Results: Cathepsin-K Ab conjugation did not alter size and charge of PLGA NPs. Covalent conjugation for 5 h at room temperature was deemed most efficient for cathepsin-K conjugation. Confocal microscopy showed a the NPs to be uniformly conjugated with the cathepsin-K Ab. TNF-α stimulated EaRASMCs showed significantly higher expression of cathepsin-K expression compared to unstimulated EaRASMCs and RASMCs. Binding of AB-conjugated NPs correlated positively to cathepsin-K expression, with the maximal binding seen inTNF-α stimulated EaRASMCs. DOX release profiles of cathepsin-K-conjugated DOX NPs were similar to unconjugated DOX NPs in exhibiting initial burs release over x h, achieving stead state release by x days and continuing the same for at least 40 days; significant differences in release amounts were noted. Although biochemical analysis for comparing pro-elastogenic and anti-proteolytic effects of Ab-conjugated and unconjugated DO-NPs are still ongoing, imaging outcomes indicate that the functional benefits to matrix regeneration due to cationic amphiphile surface-functionalized DOX NPs is maintained upon cathepsin-K Ab conjugation.

Conclusions: Our studies indicate the cathepsin-K Ab conjugation is a useful modality to improve targeted binding of our DOX NPs to cytokine-activated aneurysmal SMCs within AAAs, and does not compromise the pro-elastin regenerative and anti-proteolytic effects due to the combination of our unique cationic amphiphile-functionalized nanocarriers and DOX released therefrom.

References: Lederle FA. Ann Intern Med 1997;126;441-9. Thompson SG. BMJ 2009;338;2307. Bartoli MA. Ann Vasc Surg 2006;20;228-36. Chang WYC. Am J Physiol Lung Cell Mol Physiol 2010;299;L393-400. Sivaraman B. Acta Biomaterialia 2013;9;6511-25. Sukhova GK. J Clin Invest 1998;102;576-83. Sukhova GK. Annals of the New York Academy of Sciences 2006;1085;161-9. Keegan P. Mol Cell Biochem 2012;367;65-72.