Suppression of abdominal aortic aneurysms using targeted nanoparticles that deliver an MMP inhibitor

Aniqa Chowdhury, Nasim Nosoudi, Naren Vyavahare
Department of Bioengineering, Clemson University, Clemson, SC 29634, USA.

Statement of Purpose: Abdominal aortic aneurysm (AAA) is weakening and ballooning of the abdominal aorta that can be fatal if not treated. The development of AAA is caused by the degradation of elastin in the arterial wall by matrix metalloproteinases (MMPs). Thus, preservation of elastin in arterial segments offers potential for the treatment of AAAs. Hydroxamate-based MMP inhibitors, such as marimastat, solimastat, and batimastat (BB-94) have been shown to reduce MMP activity and medial degradation [1]. We propose a method to deliver BB-94 to the site of AAA with targeted poly D, L-lactide (PLA) nanoparticles (NPs) conjugated with an anti-elastin antibody as a treatment to prevent aneurysmal growth.

Methods: Poly D,L-lactide (PLA) NPs loaded with BB-94 were prepared by the solvent-diffusion based nano-precipitation method. Three different batches containing ratios of 5:1, 10:1, and 15:1 polymer to BB-94 were prepared. Traut's reagent (34 µg, G-Biosciences, Saint Louis, MO) was used for thiolation of 10 µg of rabbit anti-rat elastin antibody (United States Biological, Swampscott, MA), and the mixture was incubated in HEPES buffer (20 mM, pH=9.0) for an hour at room temperature. Thiolated antibodies were rinsed with HEPES buffer and were added to NPs (4 µg antibody per 1 mg NPs) and incubated overnight for conjugation. AAA was induced in the rat abdominal aortic region in 19 male Sprague-Dawley (SD) rats (5-6 weeks old) by perivascular application of 0.50 mol/L calcium chloride by placement of $CaCl_2$ soaked cotton gauze on the aorta for 15 minutes [2]. NPs either blank or BB-94 loaded and conjugated with elastin antibody (10 mg/kg of body weight) were injected in tail vein of rats at 10 days after injury and once a week for additional four weeks (3 injections total). Few rats received NPs loaded with DIR dye to study particle tracking. After 38 days, the rats were euthanized and thoracic and abdominal aortic tissue segments were explanted. The tissue segments were subsequently embedded in paraffin and 5 µm sections were mounted on glass and heated overnight to adhere the tissues. Afterwards, the slides were deparaffinized with xylene and graded ethanol and stained with Verhoeff-van Gieson (VVG) for elastic fibers, Alizarin Red S with a Light Green SF counterstain for calcification, H&E for tissue morphology, immunohistochemistry for M1 macrophages. Aneurysmal development was studied by measuring initial external diameter at the time of injury and final aortic diameter at the time of euthanasia and calculating the percent increase.

Results: H&E staining showed significant inflammation in the EL-NP-BLANK group (Fig 1A), while less inflammation and greater structural integrity was present in the BB-94 treated group (EL-NP-BB-94, Fig 1E). VVG staining showed that the elastic lamina was greatly damaged in the control group (Fig 1B) while better elastin preservation was found in the BB-94 treated group (Fig 1F). Alizarin Red S staining revealed substantial medial calcification (stained red) in the EL-NP-BLANK (Fig 1C) group, while calcification was reduced in the EL-NP-BB94 group (Fig 1G). Immunohistochemistry for M1 macrophages (pinkish red) showed high activity specifically in the adventitia in the blank NP group (Fig 1D) while there is very little macrophage activity in BB94 group (Fig 1H).

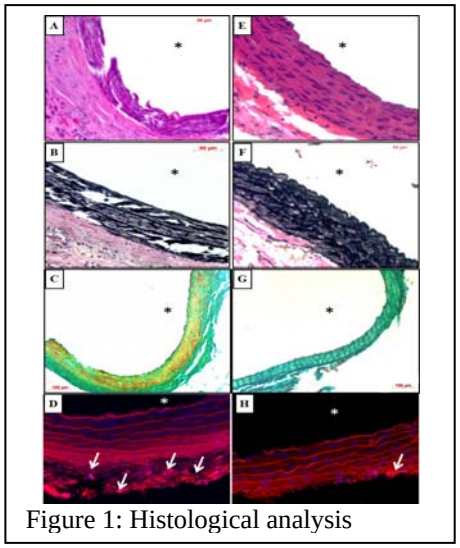

Figure 1: Histological analysis

When outside aortic diameters were compared to the diameter before aortic injury, the control group with blank NPs showed a large increase in diameter (269.5±56%), while significant suppression was observed in the BB-94 treated group (EL-NP-BB-94) (40.25±26%) (Fig 2C), suggesting that the targeted BB-94 delivery inhibited aneurysmal development.

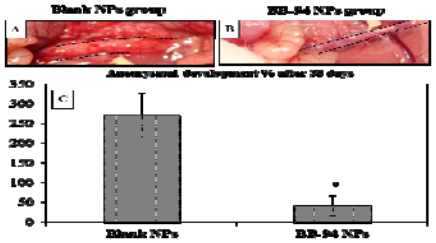

Figure 2: Aneurysmal development

Conclusions: This study demonstrates that the targeted delivery of MMP inhibitor BB-94 to the site of AAA injury effectively inhibits MMPs to reduce elastic lamina damage and suppress aneurysmal development *in vivo* in a $CaCl_2$ mediated injury rat model.

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References:

1. Kadoglou, N.P., et al., Current Medical Research and Opinion®, 2007. **20**: p. 419-432
2. Isenburg, J.C., et al., Circulation. 2007. **115** :p. 1729-1737.