Toward *In Situ* Tissue Engineering of Elastic Matrix Structures in Doxycycline-Stabilized Vascular Aneurysms

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Statement of Purpose: Abdominal aortic aneurysms (AAA) are characterized by a degradation of elastic matrix due to inflammation-associated increases in levels of proteolytic MMPs. This ultimately leads to local vessel wall weakening, expansion, and rupture. At this time, Doxycycline is used clinically to inhibit MMPs within AAAs[1] to arrest aneurysm growth. However, restoring the vessel to its healthy architecture and size has still not been achieved, since such regression demands that new elastic matrix be regenerated. This is challenged by the poor elastin regenerative capacity of adult cells. Our lab has previously identified a combination of exogenous TGF-β1 and hyaluronan oligomers (HA-o) that can synergystically increase elastin synthesis, matrix yield, elastic fiber formation, and maturation by healthy rat aortic smooth muscle cells (RASMCs). Our current approach uses inhibition of MMPs in combination with elastogenic factors in aneurysmal RASMC culture to regenerate new elastic matrix structures.

Methods: Primary culture aneurysmal RASMCs were isolated from adult Sprague-Dawley rats in which aneurysms had been induced with $CaCl_2$ and were used at passage 6 for all experiments. Cells were cultured for 21 days in DMEM, in DMEM with exogenous factors (5 ng/mL TGF-β1, 2 µg/mL HA-o), and with doxycycline (5 µg/mL, 0.5 µg/mL, 51 ng/mL) with and without factors at n=6 per group. Conditioned culture medium (CCM) was collected throughout the study. Cell layers were harvested at 21 days. The Fastin assay was used to test for alkali soluble and insoluble matrix elastin in the cell layer and for tropoelastin in the CCM in order to quantify the elastic matrix produced by the cells under different treatments. DNA assay was used to assess cell proliferation. Elastin and fibrillin were visualized with polyclonal rabbit anti-mouse antibodies to elastin and fibrillin using FITC-conjugated donkey anti-rabbit IgG secondary antibodies. Nuclei were visualized with DAPI. SEM and TEM were preformed to investigate the elastic matrix ultrastructure.

Results: Doxycycline and exogenous factors were not cytotoxic nor did they affect cell viability or morphology. Factors did not affect SMC proliferation. At the highest dose of doxycyline (5 µg/ml) (corresponding to serum levels) cell proliferation was inhibited. Cell proliferation was unaffected in groups with doxycycline combined with factors. Tropoelastin levels were unaffected by doxycyline when delivered alone or with factors. Though modest, alkali-soluble and insoluble matrix elastin accumulation was enhanced by the elastogenic factors. The highest doxycycline dose (5 µg/ml) inhibited elastic matrix accumulation, likely by inhibiting cell proliferation (Fig. 1). The doses that correspond to therapeutically effective doxycycline concentrations in AAA tissues (51 ng/ml) had no effect on elastic matrix accumulation. In cultures receiving both TGF-β and HA-o, elastic matrix accumulation was enhanced relative to cultures receiving the corresponding dose of doxycycline alone. This increase was statistically significant ($p < 0.05$ vs. doxycycline only) except at the highest doxyxycline dose ($p = 0.26$). Immunofluorescence data reinforced these findings and showed that elastin accumulation was limited to underneath and immediately surrounding cells in the highest doxycycline dose.

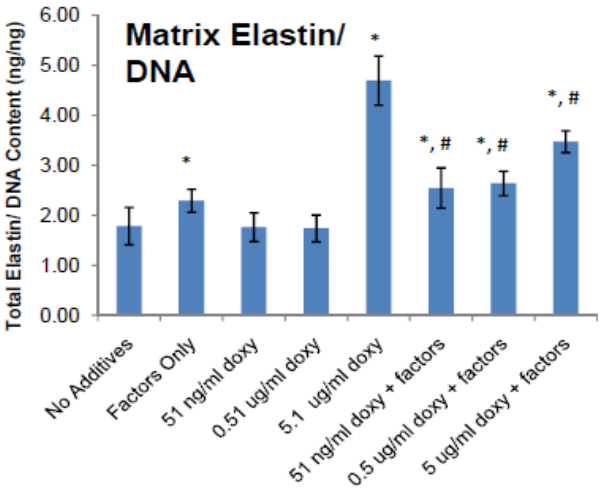

Figure 1. Elastin accumulation in cultures of aneurysmal RASMCs. * represents significance of difference vs. non-additive cultures, and # vs. doxycycline only cultures of identical dose.

Conclusions: The results show that appropriate levels of doxycycline do not impact elastic matrix accumulation, though they do not increase elastin accumulation. Oral dose levels of doxycycline significantly suppress elastin accumulation as well as cell proliferation. The elastogenic effect of HA-o and TGF-β is preserved when administered with doxycycline at doses similar to aortal tissue concentrations known to effectively inhibit AAA growth. Net accumulation of elastic matrix on a per cell (measured as ng of DNA) basis was increased by exogenous factors and decreasing MMPs. At lower doxycycline doses, HA-o and TGF-β significantly increased net elastic matrix accumulation on a per cell basis relative to doxycycline-only cases. This suggests that the exogenous factors and doxycycline show a therapeutic effect when used together. Further optimization of these factor doses and doxycycline is necessary before use *in vivo.*

References: (Lindeman JHN. Circ. 2009;119:2209-2216.) [Funding: NIH/NIBIB EB006078-01A1, NIH/NIBIB HL092051-01A1, NIH/NIBIB HL092051-015 awarded to A Ramamurthi and NIH/NIBIB C06RR18823 awarded to L Dooley.]