Enhanced Intracellular Peptide Delivery with pH-responsive, Endosomolytic Nano-Polyplexes to Prevent Intimal Hyperplasia in Human Saphenous Vein Grafts

Evans BC[1], Hocking KM[2], Brophy CM[2], Duvall CL[1]
Vanderbilt University [1]Department of Biomedical Engineering, [2]Division of Cardiovascular Medicine

Statement of Purpose: Coronary artery bypass grafting with autologous conduits remains the standard treatment for multi-vessel coronary heart disease. However, almost half of these saphenous vein grafts fail within the first 18 months due to intimal hyperplasia (**IH**)[1]. IH is mediated by the activation of MAPKAP Kinase II (**MK2**) in vascular smooth muscle cells (**VSMCs)** due to the cellular stress that occurs during surgical transplantation[2]. MK2 activation results in vasoconstriction and pathological VSMC proliferation, migration, and excess ECM production. Preliminary data suggest that intracellular delivery of a peptidic MK2 inhibitor (**MK2i**) with a cell penetrating peptide (**CPP**) can reduce vasoconstriction and subsequent IH in human saphenous vein (**HSV**)[3], but the efficacy of this approach is limited due to peptide sequestration within endo-lysomal vesicles that are trafficked for exocytosis or lysosomal degradation[4]. To overcome this barrier, we have synthesized and characterized a library of pH-responsive, endosomolytic polyplexes for the intracellular delivery of an MK2i peptide and improvement of vein graft patency.

Methods: A poly(propylacrylic acid) (**PPAA**) homopolymer (M_n = 22,000, PDI = 1.47) was synthesized via RAFT polymerization. A CPP-MK2i fusion peptide (YARAAARQARA–KALARQLGVAA) was synthesized through standard FMOC chemistry and purified via reverse-phase HPLC. The CPP-MK2i peptide and PPAA polymer were mixed at a range of charge ratios (CR, defined $[NH_3^+]/[COO^-]$) from 10:1 to 1:10 to form polyplexes. The size and zeta potential of the polyplexes were determined by dynamic light scattering (DLS) analysis. A CR of 1:3 was chosen as the optimal formulation for further study (hydrodynamic diameter = 110.9 ± 6.89 nm, ζ = -11.9 ± 3.18 mV). PH-responsive, endosomolytic behavior of the polyplexes was assessed through a red blood cell hemolysis assay. *In vitro* MK2 inhibition was assessed through downstream inhibition of Interleukin-6 (IL-6) in TNFα-stimulated HCAVSMCs. HSV explants were obtained from consenting human patients and graft vasorelaxation of 1 mm thick vein ring segments was assayed *ex vivo* using a muscle bath / force transducer. Rings were treated for 2 hours with polyplexes, peptide, or controls and subsequently contracted with phenylephrine (10^{-6} - 10^{-7} M) and relaxed with cumulative log doses of sodium nitroprusside to determine % relaxation. Additional vein rings were cultured for 14 days, fixed, embedded in paraffin, sectioned, stained with Verhoeff-van Gieson, and used to quantify intimal and medial thickness to assess ability of polyplex treatments to abrogate graft IH.

Results: Polyplexes (CR=1:3) demonstrated dissociation into individual polymer and peptide unimers at pH 6.8, demonstrating an effective means for release of the peptide from the polyplex in the endosome. Polyplexes showed no hemolytic behavior at PH 7.4 or 6.8 but showed switch-like, robust hemolysis at pH 6.2 and 5.6, suggesting that the polyplexes will display pH-dependent endosomolytic activity and enable peptide cytosolic delivery (**fig. 1A**). Polyplexes showed enhanced inhibition of TNFα induced IL-6 secretion in a human IL-6 ELISA compared to the peptide alone. HSV explants treated with polyplexes showed significantly more relaxation than the peptide alone, and achieved the same level of relaxation at $1/10^{th}$ of the dose of peptide (100 µM vs 10 µM, **fig. 1B**). Finally, polyplexes demonstrated an enhanced ability to prevent IH compared to the peptide alone showing decreased intimal thickness and intimal/medial ratio after 14 days of organ culture compared to the peptide alone at equivalent concentrations (**fig. 1C**).

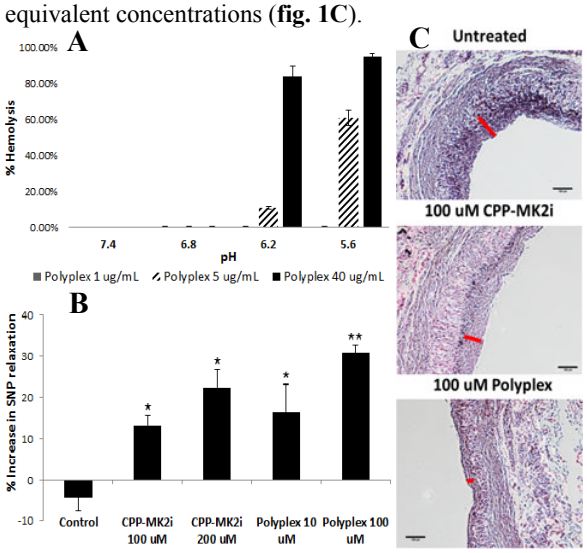

Figure 1: A) Hemolysis assay. **B**) HSV SNP induced relaxation following 2 hr treatment. **C**) HSV histology, red bars indicate intimal thickness. Scale bar = 100 µm.

Conclusions: We have synthesized pH-responsive, endosomolytic polyplexes capable of cytosolic peptide delivery. We have also proven the ability of these polyplexes to enhance the bioactivity of an intracellular-acting MK2i peptide both *in vitro* and *ex vivo*. Our results suggest that PPAA-MK2i polyplexes have strong potential to be used during cardiac and peripheral artery bypass surgeries to prevent IH and to improve long-term graft patency. Studies are currently underway to characterize intracellular trafficking of the polyplexes and to better elucidate the molecular mechanisms underlying the enhanced relaxation and reduced IH observed in HSV.

References:

[1] Alexander et al. *Jama.* (2005); 294(19):2446-2454
[2] Raingeaud et al. *J Biol Chem.* 1995;270(13):7420-7426.
[3] Lopes et al. *J Vascular Surgery*. 2010;52(6):1596-1607.
[4] Duvall et al. *Mol Pharm.* 2010;7(2):468-476.