Rapamycin-loaded uPA-mediated Dendrimer Nanoparticles for Inflammation Modulation and Plaque Stabilization in Atherosclerosis

Hsin-Yin Chuang[1], Huari Kou[1], Yue-Wern Huang[2], Hu Yang[3]
[1]Linda and Bipin Doshi Department of Chemical & Biochemical Engineering, Missouri University of Science and Technology, Rolla, MO, 65409, United States
[2]Department of Biological Sciences, Missouri University of Science and Technology, Rolla, MO, 65409, United States
[3]Joint Department of Biomedical Engineering, Marquette University and Medical College of Wisconsin, Milwaukee, WI, United States

Statement of Purpose: Atherosclerosis is a chronic inflammatory disease and the major underlying cause of cardiovascular morbidity and mortality.[1] Current lipid-lowering drugs reduce systemic cholesterol but provide limited benefits in stabilizing vulnerable plaques.[2] High expression of urokinase-type plasminogen activator receptor (uPAR) has been reported in atherosclerotic lesions.[3] In this work, we designed a urokinase plasminogen activator (uPA)-mediated dendrimer-based nanoplatform, G5PM-uPA/RA, to deliver rapamycin (RA) specifically to plaques. The system aims to attenuate plaque burden, suppress inflammation, and promote plaque stabilization.

Methods: *Synthesis of G5PM-uPA/RA*: As shown in Figure 1, 1) NHS-PEG-maleimide was conjugated to PAMAM dendrimer G5 surface to obtain G5PM. 2) RA was loaded into G5PM by 12 h stirring. 3) Cross-linking with DSP linker via flash nanoprecipitation using a four-inlet vortex mixer (MIVM) to yield G5PM/RA nanoparticle. 4) uPA peptide ligand was attached via thiol-maleimide chemistry. *Characterization of nanoparticles:* TEM and DLS. *Cellular uptake:* FITC-labeled nanoparticles in RAW 264.7 macrophages were examined by a confocal microscopy. RA concentrations in macrophages were analyzed by LC-MS. *Biodistribution studies:* the AMI HTX imaging system was used to analyze IRDye800cw-labeled dendrimer. *In vivo studies: Ldlr*$^{-/-}$ mice fed with high-fat diet were used as an atherosclerosis model. Atherosclerotic plaques were analyzed by Oil Red O (ORO), H&E, Masson's Trichrome (MT), and immunofluorescence staining, along with ImageJ quantification.

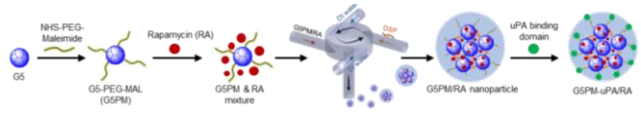

Figure 1. Synthesis of G5PM-uPA/RA nanoparticles.

Results: Figure 2 showed the uniform size (123.9 ± 3.3 nm, PDI = 0.3) of G5PM-uPA/RA and its enhanced uptake in macrophages compared to non-targeted controls. Figure 3 demonstrated the accumulation of G5PM-uPA/RA in aortic arch and arteries, and excreted primarily via hepatic route. Figure 4 showed that G5PM-uPA/RA accumulated selectively in aortic plaques, reduced plaque area, necrotic core size, TNF-α and IL-6 secretion, and caspase 3 activation, and increased fibrous cap thickness after 4 weeks of treatment, compared to free drug and non-targeted groups.

Conclusions: G5PM-uPA/RA demonstrated efficient drug loading, stable morphology, and enhanced macrophage uptake *in vitro*. *In vivo*, G5PM-uPA/RA selectively accumulated in aortic plaques and effectively reduced plaque area, necrotic core size, and inflammatory cytokine expression, while increasing fibrous cap thickness. These results highlight the therapeutic potential of G5PM-uPA/RA to achieve both anti-inflammatory and plaque-stabilizing effects, offering a promising approach to address residual cardiovascular risk.

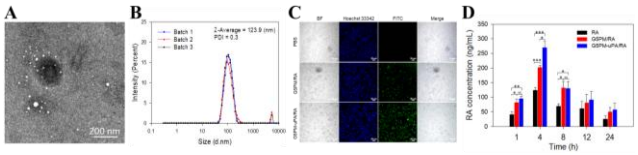

Figure 2. G5PM-uPA/RA showed improved cellular uptake and RA delivery in RAW 264.7 cells than non-targeted G5PM/RA. (A) TEM, (B) DLS, (C) confocal images, (D) LC-MS results; magnification: 20X; blue: nuclei, green: FITC-labeled nanoparticles.

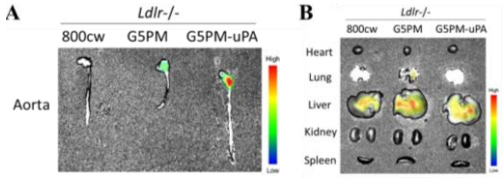

Figure 3. G5PM-uPA showed targeted capacity on uPAR-highly expressing atherosclerotic plaque. Biodistribution of IRDye800cw-labeled nanoparticles in (A) aorta and (B) major organs at 24 h postinjection.

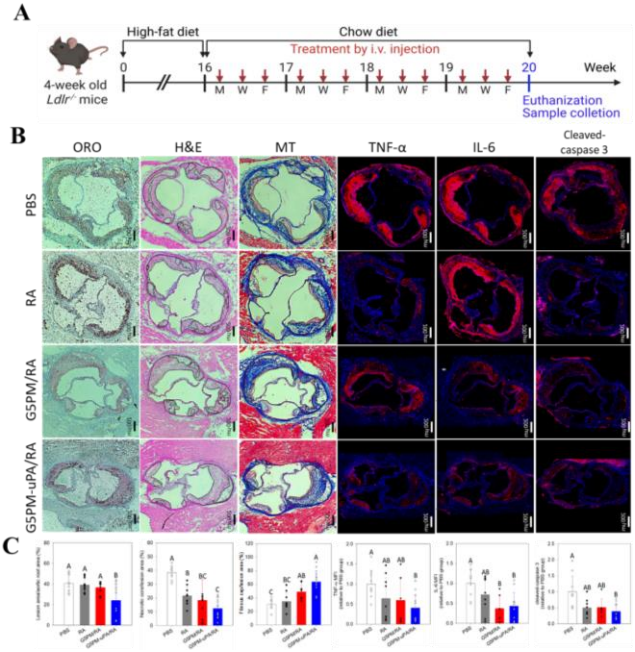

Figure 4. *In vivo* studies in an atherosclerosis mice model. (A) Timeline, (B) representative-stained aortic root sections from atherosclerosis mice, (C) quantitative analysis. Magnification: 4X.

Acknowledgement: NIH R01HL140684; Kummer I&E Doctoral Fellowship.

References:

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2. Yurtseven, E., et al. J Atheroscler Thromb, 2020. 27(9): p. 909-918.
3. Khare, H.A., et al. Atherosclerosis, 2022. 352: p. 103-111.