Nanoparticle targeting to reverse aortic calcification in a modified mouse model of adenine-induced chronic kidney disease

Saketh R.Karamched, Xiaoying Wang, Naren Vyavahare.
Department of Bioengineering, Clemson University, Clemson, SC 29634, USA.

Statement of Purpose: Vascular calcification, in particular elastin-specific medial arterial calcification (MAC) is a salient feature in chronic kidney disease (CKD). MAC is an active mineralization process occurring mostly as linear calcium deposits along elastic lamina. It causes increased pulse wave velocity, pulse pressure and isolated systolic hypertension contributing to increased arterial stiffness. MAC further involves vascular smooth muscle cells (VSMCs) switching their phenotype to osteoblast-like cells. Previously, we have shown that osteogenic transdifferentiation of VSMCs occurs during culture on hydroxyapatite surfaces and on calcified vascular elastin. VSMCs return to their normal phenotype when calcific environment was removed. We have also demonstrated that MAC can be removed by delivering a chelating agent, Ethylene diaminetetraacetic acid (EDTA) loaded in nanoparticles targeted to the sites of calcification [1]. Lineage tracing studies will be useful to investigate the fate of VSMCs *in vivo* after removal of calcification by EDTA. Since mice need to be used in lineage tracing studies, there is a need to establish a mouse model where CKD-related MAC is manifested and can be targeted. This study investigates whether a modification of the well-known dietary adenine model of chronic kidney disease is useful to induce MAC in mice as adenine in the diet alone was not effective.

Methods: Eight-week old C57BL/6J mice were purchased from Jackson Laboratories and were fed with a specially customized diet containing 0.2% adenine to induce renal failure. The mice were divided into three groups after 6 weeks of adenine feeding: One group received IP injections of Vitamin D3 (100 ng/kg body weight) thrice a week for three weeks. Second group of mice were placed on 0.2% adenine diet containing high phosphate concentration (1.8%) for 4 weeks [2]. The third group of mice continued to receive the 0.2% adenine diet only. An additional control group of mice were maintained on standard rodent diet throughout the duration of the experiment. To track progression of the disease, mice were imaged at regular intervals *in vivo* using a high-resolution Bruker Skyscan 1176 micro-CT system (Bruker, Billerica, MA). Mice were also imaged with a high resolution Vevo2100 Ultrasound scanning system to visualize the aorta (VisualSonics, Toronto, Canada). DiR dye (Biotium Inc., CA, USA) loaded bovine serum albumin (BSA) (SeraCare, MA, USA) nanoparticles (NPs) were prepared and coated with an anti-elastin antibody to target calcified elastin in the aorta. NPs were injected through tail vein of mice. After allowing the NPs to circulate for 24 hours, mice were euthanized and individual organs including aorta were imaged to capture DiR fluorescence using IVIS® Lumina XRMS (PerkinElmer, MA). Organs were then collected and stored for further analysis. Serum was also collected for biochemical analysis.

Results: Initial results suggest that vitamin D3 indeed contributed to formation of MAC while adenine feeding alone did not induce any calcification despite the presence of renal damage. DiR fluorescence was observed in mice fed with 0.2% adenine diet followed by three weeks of vitamin D3 IP injections, while no fluorescence was seen in mice fed with 0.2% adenine only. Mice fed with standard diet also did not show any signal as expected. It should however be noted here that the high P group mice are still in maintenance and have not reached their end point yet. It is anticipated that they will have calcified aortas and targeting can be achieved in the high P group as well [2].

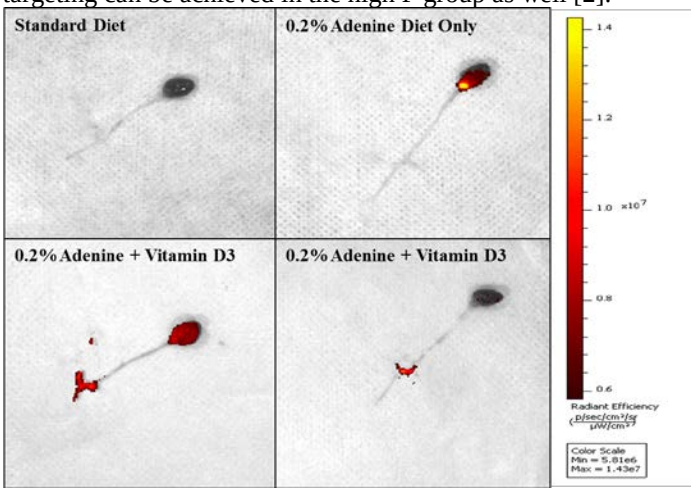

Figure 1. Nanoparticle targeting to calcified aorta in mice fed with adenine supplemented by IP injections of vitamin D3. No fluorescence to indicate targeting is seen in either mice given standard diets or even mice with adenine diet only.

Conclusions: We were able to modify the well-established adenine-model of chronic kidney disease in mice to induce MAC. Targeting of calcified elastin was achieved in vitamin D3 supplemented mice and it is anticipated that the high P fed mice will also manifest calcifications that can be targeted. Based on histological and other downstream analysis, the better modified model amongst the two will be chosen to study reversal of calcification through targeted delivery of chelating agents and VSMC lineage tracing studies.

References:

1. Lei. Journal of Controlled Release. 2014; 196: 79-86
2. Tani. Scientific Reports. 2017; 7:1

Acknowledgement- This research was partially funded by NIH grants- R01HL133662, P20GM103444, and Hunter Endowment at Clemson University