Induction of Cardiovascular Calcification in Non-transgenic Mice via a Single Injection of Pcsk9 Adeno-associated Viral Vector

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Background: Studying atherosclerotic calcification in vivo requires mouse models with genetic deletion of low-density lipoprotein receptor (Ldlr) or apolipoprotein E. A previous study showed a rapid induction of atherosclerosis by proprotein convertase subtilisin/kexin type 9 (PCSK9) in mice. Here, we hypothesize that this method is a useful in vivo tool to study cardiovascular calcification in non-genetically modified C57BL/6 mice.

Results: 10 week old C57BL/6 mice received a single tail vein injection of recombinant adeno-associated viral vector (AAV) encoding PCSK9 (rAAV8/D377Y-mPCSK9). Ldlr-/- and saline injected C57BL/6 mice served as controls. Mice consumed a high-fat, high-cholesterol (HF/HC) diet for 15-20 weeks. PCSK9 and total cholesterol serum levels were significantly increased within one week after injection and maintained for 20 weeks (cholesterol: 82 mg/dL to 820 mg/dL, p<0.01; PCSK9: 0.14 µg/ml to 20 µg/ml, p<0.01). Total cholesterol levels remained 20-30% lower than those of of Ldlr-/- mice. Atherosclerotic lesion size was similar between PSCK9 and Ldlr-/- mice. Saline injected mice did not show any lesions. Plaque collagen content was 31.9±6.6 in PCSK9 mice and 62.9±16.6 in Ldlr-/- mice at 15 weeks of HF/HC diet (p=0.01). However, by 20 weeks, the PCSK9 mice had 57.9±18.6 plaque collagen, suggesting a different stage of plaque progression. Fluorescence reflectance imaging of a near infrared calcium tracer in intact arteries detected 0.4±0.4 aortic calcification in PCSK9 mice and 9.7±1.6 in Ldlr-/- mice at 15 weeks of HF/HC diet (p=0.01); by 20 weeks, the PCSK9 mice had 5.3±1.0 aortic calcification. Tissue non-specific alkaline phosphatase activity positive lesion area was 7.9±4.0 and 8.3±2.6 in PCSK9 mice and 10.8±2.5 and 12.7±1.7 in Ldlr-/- mice at 15 and 20 weeks, respectively. Immunofluorescence analysis demonstrated accumulation of CD68 and RUNX2-positive cells in the plaques of PCSK9 mice similar to Ldlr-/- mice. Conclusion: While injection of recombinant AAV encoding PCSK9 into C57BL/6 mice induces atherosclerotic calcification with slower sclerotic plaque remodeling compared to Ldlr-/- mice, it may serve as a useful tool to study cardiovascular calcification in mice independent of their genetic background.

Disclosure Block: