ABSTRACT SESSION I: ARTERIAL REMODELING AND DISCOVERY SCIENCE FOR VENOUS DISEASE

Elastic Fibers of the Internal Elastic Lamina Are Unraveled But Not Created With Expanding Arterial Diameter in Arteriogenesis

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Objective: Outward remodeling of the arterial wall is essential during arteriogenesis to grow small arterioles into conductance vessels. Prior reports suggest that internal elastic laminae (IEL) are degraded and rebuilt in arteriogenesis. However, it is unclear whether new elastic fibers can be synthesized in adult arteries. We sought to understand the IEL changes that occur in arteriogenesis.

Methods: Rats age 8 to 12 weeks underwent femoral artery ligation (FAL) with distal arteriovenous fistula (AVF) placement to enhance collateral growth. Rats were fed β-aminopropionitrile in drinking water to inhibit lysyl oxidoreductase (LOX). Rats were humanely killed at 2 days to 12 weeks and arteries were harvested (n = 5 per time point) for multiphoton microscopy and quantitative measurement of desmosine content. Human collateral arteries from amputation specimens were also analyzed for comparison.

Results: At 2 days, FAL+AVF treated profunda femoral arteries (PFA) showed maximal vasodilation, but IEL retained its normal structure (Fig). At 4 weeks, PFA diameters increased over contralateral sham-operated PFAs (mean 255 ± 18.5%) and IEL unraveled into a loose web of elastic fibers which persisted at 12 weeks. This IEL pattern was also seen in human collaterals. Despite PFA size increase, elastin content trended downward (sham, 0.41 ± 0.07 ng; AVF, 0.31 ± 0.06 ng; $P = .058$). LOX inhibition resulted in severe fragmentation of IEL in PFAs and abnormally thickened elastic fibers in third-order collaterals.

Conclusions: In arteriogenesis, the IEL unravels and remains weblike for up to 12 weeks after FAL+AVF, a pattern also observed in human collaterals. Despite the growth in arterial diameter, the elastin content was not increased, suggesting new elastic fibers are not created. Stabilization of IEL changes seemed to be mediated by LOX. Further dissection of the mechanisms of arterial remodeling in arteriogenesis will allow us to harness this adaptive process for therapeutic purposes.

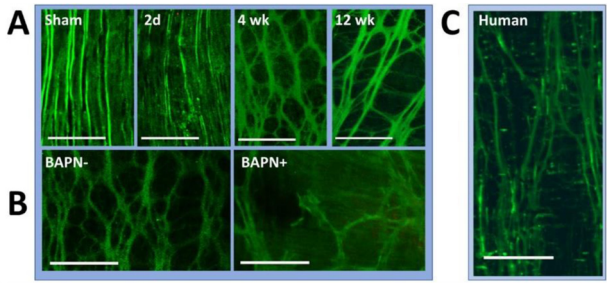

Fig. A, Structural changes in profunda femoral arteries (PFA) internal elastic laminae (IEL) occurring over 12 weeks. **B**, β-Aminopropionitrile + treated rats demonstrate fragmentation of elastic fibers within the IEL. **C**, Sample of human arterial collateral demonstrating similar alterations of IEL to those obtained within our model. Bar = 50 μm.

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A Synthetic Resolvin Analogue (Benzo-Rvd1) Attenuates Vascular Smooth Muscle Cell Migration and Neointimal Hyperplasia

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Background: Persistent inflammation following vascular injury drives neointimal hyperplasia. Specialized lipid mediators (SPM) play a critical role in the process of resolution. We investigated the effects of a novel synthetic SPM on vascular cells and in a model of rat carotid angioplasty.

Methods: Human venous vascular smooth muscle cells (VSMC) and endothelial cells were used in toxicity, migration, proliferation, and nuclear factor-κB activation assays. A model of rat carotid angioplasty was used to evaluate the effects of Benzo-RvD1 (BRvD) applied externally via 25% Pluronic gel. Drug concentration was measured at 3 days after injury via liquid chromatography mass spectrometry, and effects on vessel morphometry were examined at 14 days after injury.

Results: BRvD demonstrated no significant cytotoxicity, and modest antiproliferative activity on VSMC at 100 nmol/L. BRvD significantly attenuated VSMC migration across a range of concentrations (0.1-100 nmol/L) that was equivalent or better than 17R-RvD1, a naturally occurring SPM (Fig 1). BRvD (10-100 nmol/L) inhibited nuclear factor-κB translocation in cytokine-stimulated endothelial cells by 12% to 21% ($P <$ .01), similar to 17R-RvD1. Following external delivery, BRvD was detectable in rat carotid tissue at 3 days (mean, 0.17 pg/mg; n = 3) after injury. Periadventitial treatment with BRvD reduced carotid neointimal thickness at 14 days compared with controls, with similar efficacy to 17R-RvD1 (Fig 2).

Conclusions: BRvD is a synthetic analogue of the SPM 17R-RvD1 that demonstrates similar in vitro and in vivo efficacy to inhibit neointimal hyperplasia as its naturally occurring cognate. The enhanced stability of BRvD may provide therapeutic advantages for antirestenosis strategies.

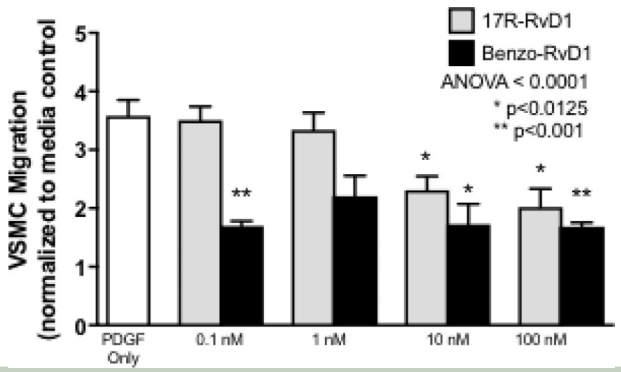

Fig 1.

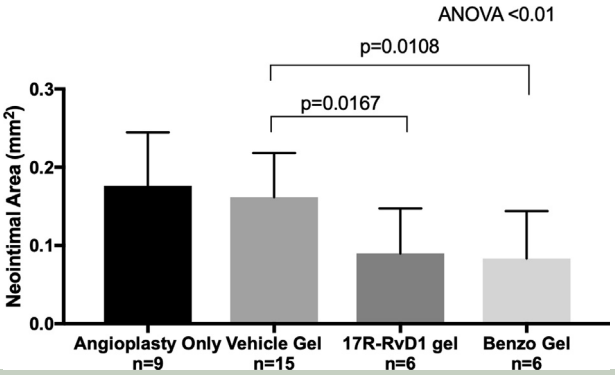

Fig 2.

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Programmed Death Ligand-1 Regulates T Cells and M2 Macrophages to Control Wall Thickening During Arteriovenous Fistula Maturation

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Background: Vascular remodeling during arteriovenous fistula (AVF) maturation is characterized by infiltration of T cells and macrophages. We have previously shown that M2 macrophages play an important role in AVF maturation to reduce inflammation and promote wall thickening. Although T cells can activate macrophages, little is known about T-cell regulation of macrophage function during AVF maturation. Programed death ligand 1 (PD-L1) induces regulatory T cells (Treg) to suppress other T cells and is expressed in endothelial cells. We hypothesized that PD-L1 induces Treg accumulation in the AVF to promote AVF maturation.

Methods: We used the mouse aortocaval AVF model. Intraperitoneal injection of PD-L1 antibody (3 times per week) was used to inhibit PD-L1; control was matched IgG2 isotype antibody. Helper T-cells (Th)-1, Th2, Treg, macrophage accumulation in the AVF was assessed by immunofluorescence with their specific markers. Vascular wall thickening was assessed by elastin van Gieson stain.

Results: Inhibition of PD-L1 significantly increased accumulation of Th1 (15.9 cells/hpf vs 8.6 cells/hpf; $P < .05$) and Th2 (18.0 cells/hpf vs 9.7 cells/hpf; $P < .05$), but decreased Treg (3.8 cells/hpf vs 11.2 cells/hpf; $P < .05$) cells compared with control. PD-L1 significantly inhibited accumulation of TGM2$^+$ (M2) macrophages (5.8 cells/hpf vs 15.7 cells/hpf; $P < .05$) and CD206$^+$ (M2) macrophages (2.6 cells/hpf vs 13.2 cells/hpf; $P < .05$), but not iNOS$^+$ (M1) macrophages (8.8 cells/hpf vs 10.0 cells/hpf; $P = .23$) or tumor necrosis factor-α^+ (M1) macrophages (9.8 cells/hpf vs 12.3 cells/hpf; $P = .29$). There was less wall thickening in AVF treated with PD-L1 antibody compared with control (8.2 μm vs 17.55 μm; $P < .01$) as well as reduced AVF maturation ($P = .03$; n = 16-17; Fig).

Conclusions: Inhibition of PD-L1 is associated with reduced vascular wall thickening as well as less Treg and M2 macrophage accumulation in the maturing AVF. These results suggest that PD-L1 induces Treg cells to promote M2 macrophage accumulation during AVF maturation.

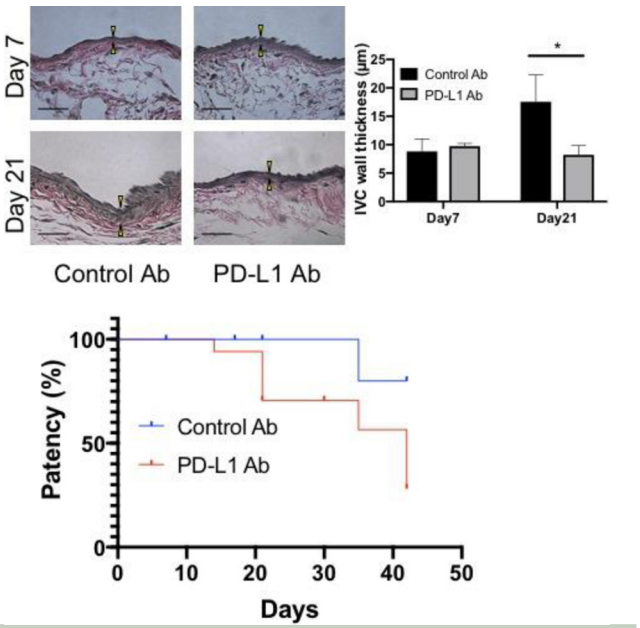

Fig.

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TRPC6 Depletion Results in Loss of Myocardin and Phenotypic Modulation in Vascular Smooth Muscle Cells

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Objective: The contractile phenotype of vascular smooth muscle cells (VSMC) is regulated by expression of the master transcription factor, myocardin. Loss of myocardin is observed during VSMC phenotypic switching and within the hyperplastic neointima after vascular intervention. We have shown that depletion of the nonvoltage gated calcium-permeable channel, canonical transient receptor potential 6 (TRPC6) channel, promotes VSMC phenotypic modulation and more severe carotid stenosis following wire injury. The goal of this study was to determine if TRPC6 regulates VSMC phenotype by altering expression of myocardin.

Methods: Myocardin expression was assessed in primary aortic wild-type (WT) and TRPC6$^{-/-}$ VSMC as well as common carotid artery (CCA) explants from WT and TRPC6$^{-/-}$ mice. Acute TRPC6 knockdown was performed in immortalized mouse VSMC (MOVAS cells) using small interfering RNA. Functional alteration of VSMC was assessed in vitro using a wound healing assay.

Results: Myocardin mRNA levels were significantly reduced in primary aortic TRPC6$^{-/-}$ VSMC compared with primary aortic WT VSMC (mean 5.53×10^7 fold reduction; n = 4; $P < .001$). Myocardin protein was also reduced in TRPC6$^{-/-}$ primary aortic VSMC compared with WT, although not to the same degree (mean, 2.03-fold reduction; n = 3; $P = .001$). Myocardin protein was significantly decreased in whole tissue explants of TRPC6$^{-/-}$ CCA versus WT CCA (55.5% reduction; n = 4 mice per genotype; $P = .007$), demonstrating that significant differences in myocardin expression were present in vivo. In MOVAS cells, acute small interfering RNA-mediated TRPC6 knockdown induced a 44.7% decrease in myocardin protein (n = 3; $P = .044$). Wound healing assays showed a significant increase in the number of migrating TRPC6$^{-/-}$ VSMC compared with WT