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Early Atherogenesis: Aquaporin-1 & Pressure Affect Intima Thickness and Water Transport Across Rat Aorta Walls

ABSTRACT

Early *atherogenesis*, atheroma or plaque formation, is believed to begin with the blood-pressure-driven advection of the large *Low Density Lipoprotein* (LDL) cholesterol particles from the blood across the endothelium into the next vessel wall layer, the subendothelial intima (SI). LDL advects around rare endothelial cells (ECs) whose intercellular junctions are temporarily big enough for LDL passage. LDL can get stuck in the SI by binding to SI extracellular matrix (ECM). The rate of such binding presumably depends on LDL's local SI concentration. A slow, blood-pressure-driven flow of water and small molecules from the blood across the entire endothelium should dilute this concentration and slow LDL binding.

To explain the variation of aortic wall hydraulic conductivity with pressure, Y. Huang et al. (1997, 1998) proposed a hypothesis and later confirmed experimentally that pressure can compress the sparse SI, causing the ECs to partially block the internal elastic lamina's *fenestra* holes, water's putative entrance into deeper wall layers. Recent further work in our laboratory (Toussaint, Nguyen and Xue) found that endothelial cell (EC) expresses a water channel protein called *aquaporin-1* (AQP1) that allows some of this water flow to traverse *through* the ECs, as well as around them via *tight junctions*. They found that blocking or reducing functioning AQP1 numbers lowers the critical blood pressure to compress the SI and reduce the flow. Conversely, a theoretical model developed by another former student (Joshi) in our group predicted that increasing EC AQP1 expression shifts this critical pressure to higher values and thus creates a pressure regime in which increased AQP1 increases the trans-endothelial water flow by decompressing the SI. Raval in our lab experimentally demonstrated that increasing AQP1 indeed increases this flow. Our goal in this work is to show that this flow increase results from increased AQP1 decompressing the SI. If that is the case, it would suggest that such an increase might noticeably slow early plaque formation.

We utilize 10 μM Forskolin, a cyclic-AMP upregulator known to increase the expression of AQP1 in the membranes of some cell (Raval showed this for ECs) to raise the water permeability of ECs. We fix, section and stain rat aortas treated either with 10 μM Forskolin in $8.5*10^{-4}$ % of DMSO solution in water or $8.5*10^{-4}$ % of DMSO solution in water as a blank. Fixation is done at four different pressures, including at least one that should be in the regime of interest. Analyzing transmission electron micrographs, we assess the mean SI thickness as a function of treatment and transmural pressure.

Preliminary results suggest SI thicknesses vs. blood pressure for both Forskolin and blank treatments follow theoretically predicted trends. The SI thickness for a sample treated with Forskolin at 50 mmHg exhibits a ~135 nm expanded SI. As the theory predicts, at higher pressures (e.g 100 mmHg), Forskolin only expands the SI ~20 nm, since at 100 mmHg, pressure alone can compress the SI. We are currently obtaining more data to draw firmer conclusions.