Effects of OxLDL on the Viscoelastic Properties of Vascular Smooth Muscle Cells

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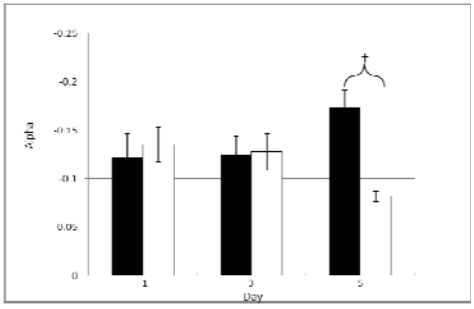
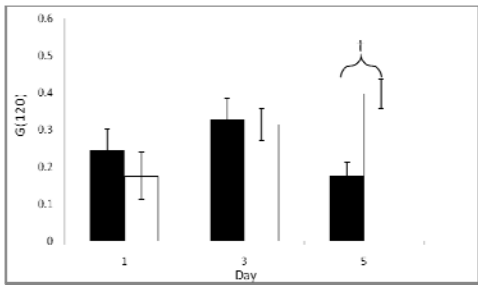
Statement of Purpose: In the current study, it was hypothesized that oxidized low-density lipoprotein (oxLDL) alters the viscoelastic properties of VSMCs through cytoskeletal and morphological changes. To test this hypothesis, we examined the effects of oxLDL on cellular morphology, actin and microtubule distribution, and cellular viscoelastic behavior. Oxidized low-density lipoprotein is believed to be one of the most significant factors in vascular disease development [1]. Among the numerous observed effects of oxLDL, has been the alteration of cytoskeletal structure in vascular smooth muscle cells (VSMCs) [2, 3]. Given that the cytoskeleton plays a dominant role in governing mechanical properties in most cell types, alterations to cytoskeletal composition can result in changes to cellular deformation in response to mechanical loading. Research in our lab has shown that an atomic force microscope (AFM) can be used to measure stress relaxation behavior of living VSMCs [4]. Employing AFM stress relaxation measurements, we quantified VSMC viscoelastic changes in response to oxLDL, and correlated those changes with cytoskeletal alterations. Data from this study will contribute to our goal of gaining a better understanding of the effects of solid contact between endovascular stents and VSMCs.

Methods: Rat aortic smooth muscle cells (P4 to P6) were seeded onto glass coverslips at a density of 10,000 cells/coverslip and cultured in DMEM with 10% FBS for 1 day, followed by culture in serum free DMEM for 3 days. OxLDL, obtained from Biomedical Technologies,Inc (Stoughton, MA) was then added to the media at a concentration of 0.05 mg/ml for periods of 1,3, and 5 days. The oxidation level of the OxLDL was 17.7 nmoles of MDA/mg protein. Control groups remained in serum free media only. At each time point, actin and microtubules were imaged using fluorescence and confocal microscopy, and cells were mechanically tested using AFM stress relaxation (1 μm step strain held for 120 seconds). A 5 μm spherical probe (Novascan, Ames, IA) with a cantilever spring constant of 0.12 N/m was employed for all stress relaxation tests. Stress relaxation data were fit using both the quasilinear viscoelastic (QLV) reduced relaxation function G(t) and a power-law model (At^{α}). For the former, the percentage of relaxation at the end of each 120 sec. test, G(120), was used for statistical comparison, while for the latter, the power-law exponent, α, was used. In addition, apparent elastic moduli were measured using AFM indentation, and force curve were fit with the Hertz model.

Results: After 5 days of exposure, oxLDL-treated VSMCs had significantly greater rate ($p<0.001$) and percentage ($p=0.001$) of stress relaxation compared to controls, as quantified using the power-law exponent, α, and percentage relaxation, G(120), respectively (Fig. 1). In addition, oxLDL-treated VSMCs exhibited significantly lower apparent elastic moduli ($p=0.003$), as measured via Hertzian indentation analysis. Fluorescent imaging revealed that oxLDL-treated VSMCs exhibited decreased actin stress fiber content compared to controls, as well as substantial microtubule rearrangement.



(a)



(b)

Figure 1. (a) Power-law exponent, α, of oxLDL-treated (black) and control (white) VSMCs at 1,3, and 5 days; (b) G(120) of oxLDL-treated (black) and control (white) VSMCs at 1,3, and 5 days.

Conclusions: Data from the current study indicate that oxLDL can induce significant changes to the viscoelastic properties of VSMCs *in vitro*. After 5 days of exposure, oxLDL-treated VSMCs displayed fewer actin stress fibers and greater microtubule dispersion. These cytoskeletal changes were associated with significant changes in viscoelastic measurements. When compared to controls, oxLDL-treated VSMCs showed significantly increased relaxation rate and percentage (alpha and G(120)) at 5 days, with a non-significant trend of decreased relaxation over time for controls. Furthermore, apparent elastic modulus was significantly lower in the oxLDL group. These data point to increasing fluid-like behavior from prolonged oxLDL treatment, with the opposite effect occurring in control cells, possibly due to serum withdrawal. The observed cytoskeletal and morphological changes are similar to those commonly observed in atherosclerotic lesions and restenosis.

References:

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