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Nonlinear optical microscopy of Murine Abdominal Aortic Aneurysm

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ABSTRACT

Abdominal aortic aneurysm (AAA) is a cardiovascular disease characterized by dilation and weakening of the vessel wall. AAA rupture is responsible for approximately 14,000 deaths annually in the United States [1]. Nonlinear optical (NLO) microscopy presents new possibilities for analyzing AAA tissue samples from murine models. Common NLO techniques are two-photon excitation fluorescence (TPEF), which detects the intrinsic autofluorescent properties of elastin, and second-harmonic generation (SHG), which is specific for collagen fibrils. Elastin and collagen, two major extracellular matrix components, help the aortic wall withstand internal pressure. Murine AAAs were created through 1) subcutaneous continuous systemic infusion of angiotensin II (AngII) in hyperlipidemic apolipoprotein E-deficient mice and 2) by intraluminal infusion of elastase (low 0.5 U/ml and high 25 U/ml concentrations) into the infrarenal aorta of rats [2]. We imaged aneurysmal and control tissue using TPEF and SHG and compared the resulting images to sections stained with standard elastin and collagen markers. TPEF images revealed disorganized elastin sheets and SHG images indicated collagen turnover after aneurysm formation. We quantified the relative degree of elastin degradation and collagen content in the aortic media within a user-defined area on sections stained with Verhoeff-van Gieson (VVG) or Masson's trichrome (MTC), as well as on TPEF and SHG images. Our analysis with VVG-stained sections shows that elastin content in AAA tissue is significantly decreased by 64% in AngII models (P=0.02), by 34% in low concentration elastase models (P=0.07), and by 99% in high concentration elastase models (P=0.03), relative to control aortic tissue.

KEYWORDS

Aneurysm, two-photon excitation fluorescence, second-harmonic generation, angiotensin II, elastase

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