The Effect of Oxidation Using Ferric Chloride on the Mechanical Response of Porcine Aortas

Beth Stephen, Bryan W. Davis, Theresa A. Good, L.D. Timmie Topoleski
University of Maryland Baltimore County

Statement of Purpose: The primary function of arteries is mechanical, conducting blood through the body. Thus arteries, and their structure-function relationships, need to be studied as biomaterials. Collagen and elastin are the two primary load-bearing components of arteries and account for their nonlinear mechanical responses (1). To understand the material behavior of the arteries, it is necessary to understand how various processes affect the overall mechanical response of the arteries and the individual affect on the collagen and elastin components.

Arteries *in vivo* are subjected to oxidative processes. Reactive oxygen species (ROS) produced by oxidation reactions accumulate over time, causing an exponential increase in molecular oxidative damage with age (2). Oxidative damage caused by ROS has also been linked to cardiovascular diseases (3). While the biochemical effect of oxidation on arteries has been studied, the effect of oxidation on the mechanical response of the arteries is not known. Since the mechanical behavior of the arteries governs the arterial function, it is important to understand how oxidation affects the mechanical response. This knowledge will improve our understanding of arterial function in health and disease and assist in the development of tissue engineered scaffolds.

Methods: Porcine aorta samples were obtained from a local abattoir immediately following slaughter. The aortas were transported to the lab, cleaned of connective tissue, cut into dog-bone shaped specimens along the longitudinal axis of the aorta, and stored in phosphate buffered saline (PBS). An oxidizing agent, 67mM $FeCl_3$ prepared in PBS, was used to treat the cut specimens. Treatment consisted of placing the aortic specimens in the $FeCl_3$ solution for a prescribed time (2 hr, 4 hr, 8 hr, or 24 hr) and then rinsing in PBS to remove any residual $FeCl_3$ prior to mechanical testing. A control group of untreated specimens were maintained in PBS solution until testing. A total of 39 specimens were tested in 5 groups. All testing was completed within 2 days of slaughter. Specimens were tested using a custom-built uniaxial test apparatus designed for testing arterial samples. Samples were preconditioned for 15 load-unload cycles and immediately tested to failure using a strain rate of 0.05mm/sec. Load and displacement data were collected for each specimen during testing.

Stress-stretch graphs were produced for each specimen, where stretch is defined as the stretch ratio (λ), that is, λ = (final length/initial length) of the specimen. The maximum stress (σ_{max}) and stretch ratio at maximum stress ($\lambda_{max\text{-}stress}$) were calculated for each specimen.

Results: Table 1 summarizes the results for each test group. There was no significant difference in the σ_{max} ($p=0.11$) or $\lambda_{max\text{-}stress}$ ($p=0.10$) values between treatment groups. Graphs for the control group and 24 hr treatment group are shown in Figure 1.

Table 1: Summary of test results by treatment group.

Treatment Time (hours)	n	σ_{max} (MPa) Mean ± SD	$\lambda_{max\ stress}$ (mm/mm) Mean ± SD
0 (Control)	8	0.756 ± 0.258	2.23 ± 1.24
2	7	0.597 ± 0.305	2.07 ± 1.14
4	8	0.959 ± 0.272	2.03 ± 1.10
8	8	0.748 ± 0.143	2.07 ± 1.12
24	8	0.889 ± 0.084	2.18 ± 1.15

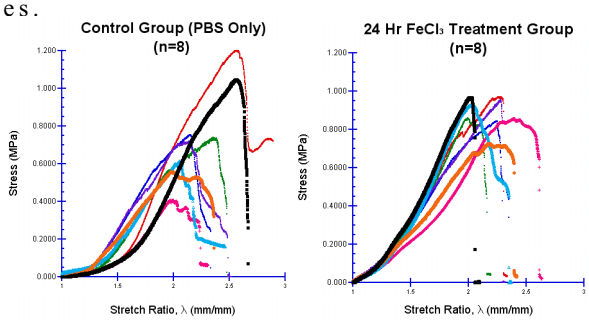

Figure 1: *Results for control group (left) vs. 24 hour treatment group (right). Comparison of the graphs shows no significant change in* σ_{max} *or* $\lambda_{max\text{-}stress}$ *but shows a decrease in the curve's toe region, indicating an effect on the elastin response of the artery.*

Conclusions: The control group (Figure 1) displays a typical arterial mechanical response. Each curve in this group shows an initial toe region characterized by low stiffness that transitions to a stiffer response at higher stretch. The initial toe region is thought to be associated with the elastin response of the material and the higher stiffness response is attributed to the collagen, consistent with other studies (1). In each treatment group, the 24 hr group is presented in Figure 1 as an example, the toe region is greatly reduced in size or absent and the transition to the higher stiffness collagen response occurs sooner. These results indicate that the oxidation treatment may affect the elastin component of the artery resulting in a faster transition to the high stiffness collagen response. There was no significant change in either the σ_{max} or $\lambda_{max\text{-}stress}$, indicating that the collagen response was not affected by the oxidation treatment. These results are consistent with age-related changes in the arterial mechanical response, characterized by elastin degradation and an overall increase in arterial stiffness. Future work will characterize these results using a constitutive model and examine the affect of oxidation on the isolated components.

References: (1) Roach MR and Burton AC. Can. J. Biochem. Physiol. 1957. 35(8): 681-690.
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