The Effect of Pentagalloyl Glucose on the Wall Mechanics and Inflammatory Activity of Rat Abdominal Aortic Aneurysms

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An abdominal aortic aneurysm (AAA) is a permanent localized expansion of the abdominal aorta with mortality rate of up to 90% after rupture. AAA growth is a process of vascular degeneration accompanied by a reduction in wall strength and an increase in inflammatory activity. It is unclear whether this process can be intervened to attenuate AAA growth, and hence, it is of great clinical interest to develop a technique that can stabilize the AAA. The objective of this work is to develop a protocol for future studies to evaluate the effects of drug-based therapies on the mechanics and inflammation in rodent models of AAA. The scope of the study is limited to the use of pentagalloyl glucose (PGG) for aneurysm treatment in the calcium chloride rat AAA model. Peak wall stress (PWS) and matrix metalloproteinase (MMP) activity, which are the biomechanical and biological markers of AAA growth and rupture, were evaluated over 4 weeks in untreated and treated (with PGG) groups. The AAA specimens were mechanically characterized by planar biaxial tensile testing and the data fitted to a five-parameter nonlinear, hyperelastic, anisotropic Holzapfel–Gasser–Ogden (HGO) material model, which was used to perform finite element analysis (FEA) to evaluate PWS. Our results demonstrated that there was a reduction in PWS between pre- and post-AAA induction FEA models in the treatment group compared to the untreated group using either animal-specific or average material properties. However, this reduction was not statistically significant. Conversely, there was a statistically significant reduction in MMP-activated fluorescent signal between pre- and post-AAA induction models in the treated group compared to the untreated group. Therefore, the primary contribution of this work is the quantification of the stabilizing effects of PGG using biomechanical and biological markers of AAA, thus indicating that PGG could be part of a new clinical treatment strategy that will require further investigation. [DOI: 10.1115/1.4040398]

Keywords: pentagalloyl glucose, aneurysm, fluorescence imaging, finite element analysis, biomechanics

Introduction

An abdominal aortic aneurysm (AAA) is a local pathologic expansion of over 50% of the original diameter of the abdominal aorta, typically occurring below the renal arteries. AAA rupture leads to a mortality rate of up to 90% when it ruptures. While extensive literature has documented the pathogenesis, evolution and rupture of AAA [1–4], prevention or suppression of AAA in a clinical setting remains an unsolved research question. The key

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events that characterize AAA growth are the underlying changes in extracellular matrix composition and the local mechanical weakening of the AAA wall. The wall weakening leads to a compensatory increase in collagen synthesis, which triggers increased collagenase activity and a subsequent imbalance of proteolytic enzymes. This upsets the balance between proteases and their inhibitors, resulting in elastin degradation and a consequent release of immunoglobulins and macrophage recruitment. The subsequent event is a production of matrix metalloproteases (MMP) and cytokines, which in turn activate other cells to produce degenerative enzymes. This cycle continues, resulting in further degradation of the elastic lamellae of the medial layer, thus weakening the wall and leading to an expansion of the abdominal aorta and the consequent development of an AAA [5].

Animal models have been used to study the mechanism of AAA formation. Rodent models of AAA have been developed using chemical, genetic, and graft models [6] and several markers identified to represent different stages of AAA evolution. These can be associated with the inflammatory changes in the aortic wall or with its devolving mechanical integrity. MMPs that are synthesized from proenzymes become activated during a proteinase-mediated cleavage of the aminoterminal peptide. Such high MMP-9 activity has been found in AAA tissue specimens compared to their normal aortic tissue counterparts [7]. In addition, targeted deletion of MMP-2 and MMP-9 in mice resisted AAA formation, whereas infusion of macrophages reconstituted AAA. Thus, it is logical that the concentration of inflammatory biomarkers at the aneurysm site can be a potential predictor of AAA expansion and rupture risk. In addition, the noninvasive evaluation of biomechanical markers such as peak wall stress (PWS) and peak wall rupture risk index have given valuable insight into the assessment of AAA rupture [8,9]. Since PWS was found to be highly sensitive to interuser variability during segmentation, the 99th percentile wall stress, which signifies the peak stress after eliminating the AAA surface area containing 1% of the highest stresses, can be used as a viable alternative [10].

In the present work, a chemical model based on the periaortic application of calcium chloride was used to induce AAA generation through inflammation and calcification. One of the advantages of AAA induction in animal models is the ability to evaluate the efficacy of experimental drug therapies. To this end, we have studied the effect of pentagalloyl glucose (PGG), an elastin and collagen-binding polyphenol, on rat AAA suppression. PGG, when applied periaortally, has been shown to bind to elastin within the aortic wall, increase elastic fiber deposition, and thereby stabilize AAA in rats [11]. Recently, an attempt to successfully load PGG in nanoparticles for targeted delivery was made; a reduction in calcification, MMP activity, and elastin degradation was demonstrated in PGG-treated rats [12].

While there are extensive studies on AAA rupture risk assessment and interventional treatments, little work has been done on the suppression of AAA growth, which would be of great clinical interest. Accordingly, the main goal of this work is to develop a protocol for future studies to evaluate the effects of drug-based therapies on the mechanics and inflammation in rodent models of AAA. The mechanics was quantified by evaluating the first principal stress on the AAA wall. The inflammation was studied by observing the differences in MMP activity across untreated and PGG-treated rats. By employing a unique combination of research tools, the scope of this work is based on the use of PGG for aneurysm treatment in the calcium chloride rat AAA model. Through a successful reduction in AAA rupture biomarkers, we implemented a protocol that can be used to evaluate the efficacy of any drug-based therapy for AAA stabilization.

Materials and Methods

Subject Population and Abdominal Aortic Aneurysm Model. Six male Sprague–Dawley adult rats of approximately 354 ± 23 g were used in this study. They were divided into

untreated (rats 1–3) and treated groups (rats 4–6). A 3D time-of-flight multislab gradient echo sequence was used to acquire boundaries of the aortic lumen with the following settings: repetition time/echo time = 201/4.72 ms, field of view = $(120 \text{ mm})^3$, flip angle = 41 deg, matrix = $(256)^3$, and number of excitations = 1. All rats were subject to AAA generation by periaortally application of calcium chloride (CaCl_2). While the untreated group had saline applied, the treated group was subject to PGG application. As part of the survival surgery, the animals underwent laparotomy under isoflurane anesthesia to expose the anterior surface of the abdominal aorta. A gauze applicator presoaked in 0.5 M CaCl_2 was placed on the exposed abdominal aorta for 15 min. For the treated group, a gauze presoaked in PGG was applied periaortally at body temperature (37°C) for 15 min before applying CaCl_2 . AAA induction was done at a suprarenal location for rats 1–4 and at an infrarenal location for rats 5 and 6. Following the survival surgery, in vivo fluorescence imaging was carried out to assess the inflammatory activity in all aortas using an in vivo Imaging System (IVIS, PerkinElmer, Waltham, MA). Prior to imaging, the MMPsense 750 FAST probe (20 nmol per animal) with excitation and emission wavelengths of 749 nm and 775 nm, respectively, was injected intravenously via the tail vein. This probe is an activatable agent that fluoresces only when it encounters MMPs and has been shown to activate in murine aneurysmal tissue [13]. The key element in the structure of the probe is the MMP-cleavable peptide sequence, which induces fluorescence upon cleavage. During the follow-up phase, which was 28 days after the survival surgery, all rats were again subjected to μMRI using the same protocol settings described earlier. IVIS imaging was carried out in situ following nonsurvival surgery. The aorta was exposed while imaging by epi-illumination. Postimaging, the rats were sacrificed and the entire aortas harvested for further destructive mechanical testing. All surgeries, imaging, postoperative care, and euthanasia were approved by and performed in accordance with the policies of the Institutional Animal Care and Use Committee of the University of Texas Health at San Antonio.

Mechanical Testing. The aortas were cut into specimens of 0.5 cm^2 area and the thickness was measured using a micrometer (Mitutoyo America Corporation, Aurora, IL). The specimens were immersed in phosphate buffer solution at 37°C and mounted on a CellScale planar biaxial tensile testing machine (Waterloo, Canada) with two 1.5 N load cells. The specimens were preconditioned by stretching up to 25% for 2 cycles. Equibiaxial tensile tests were carried out at a strain rate of 0.0075 s^{-1} . Force–displacement curves were obtained in both the circumferential and axial directions.

Biomechanical Data Analysis. *Constitutive modeling:* Cauchy stress and strain were calculated for each specimen from the corresponding force–displacement data. The artery was modeled as a nonlinear, elastic, thick-walled cylinder with two layers: intima-media and adventitia. A nonlinear, hyperelastic, anisotropic Holzapfel–Gasser–Ogden (HGO) material model with five parameters [14–17] was fit to the experimental data such that its strain energy density function is given by the below equation:

$$\Psi = \Psi_{\text{iso}} + \Psi_{\text{aniso}},$$

$$\text{where } \Psi_{\text{iso}} = \frac{c}{2}(I_1 - 3), \text{ and}$$

$$\Psi_{\text{aniso}} = \sum_{\text{layer}=M,A} \sum_{i=4,6} \frac{k_1}{2k_2} \left\{ \exp \left[k_2 \left(I_i^{\text{layer}} - 1 \right)^2 \right] - 1 \right\} \quad (1)$$

such that $c, k_1, k_2 > 0$, where c, k_1 , and k_2 are model parameters and I_i^{layer} are the i th invariants of the Cauchy stress tensor per artery layer. The fourth and sixth invariants include the fiber directions of the collagen fiber bundles implicitly, given by the

β_M and β_A angles (M : media, A : adventitia). We have assumed that the Neo-Hookean parameters, c_M and c_A , which characterize the isotropic behavior of the ground matrix in the media and adventitia, are equal to each other, i.e., $c_M = c_A$. Likewise, we have also assumed that the components k_{1A} , k_{1M} and k_{2A} , k_{2M} which characterize the exponential behavior of the collagen fibers in the adventitia and media are also equal to each other, i.e., $k_{1A} = k_{1M} = k_1$; $k_{2A} = k_{2M} = k_2$.

For planar biaxial testing with no shear, the deformation gradient, $\mathbf{F}$, is defined as

$$\mathbf{F} = \begin{pmatrix} \lambda_t & 0 & 0 \\ 0 & \lambda_z & 0 \\ 0 & 0 & \lambda_r \end{pmatrix}$$

where λ_m ($m = t, z, r$) represents the stretch ratio in the circumferential, axial, and radial directions, respectively. Assuming incompressibility of the tissue

$$J = \det(\mathbf{F}) = \lambda_t \lambda_z \lambda_r = 1$$

hence

$$\lambda_r = \frac{1}{\lambda_t \lambda_z}$$

and the invariants

$$I_1 = \lambda_t^2 + \lambda_z^2 + \frac{1}{\lambda_t^2 \lambda_z^2}; \quad I_4 = I_6 = \lambda_t^2 \cos^2 \beta_j + \lambda_z^2 \sin^2 \beta_j (j = M, A)$$

The Green Lagrange strain tensor is given by

$$\mathbf{E} = \frac{1}{2}(\mathbf{F}^T \mathbf{F} - \mathbf{I}) = \frac{1}{2} \begin{pmatrix} \lambda_t^2 - 1 & 0 & 0 \\ 0 & \lambda_z^2 - 1 & 0 \\ 0 & 0 & \frac{1}{\lambda_t^2 \lambda_z^2} - 1 \end{pmatrix}$$

and the second Piola-Kirchhoff stresses are calculated as

$$\begin{aligned} S_t &= \frac{\partial \psi}{\partial E_t} = c \left(1 - \frac{1}{\lambda_z^2 \lambda_t^4} \right) \\ &\quad + \sum_{j=M,A} 4k_1 \cos^2 \beta_j (I_4 - 1) \exp \left[k_2 (I_4 - 1)^2 \right] \\ S_z &= \frac{\partial \psi}{\partial E_z} = c \left(1 - \frac{1}{\lambda_t^2 \lambda_z^4} \right) \\ &\quad + \sum_{j=M,A} 4k_1 \cos^2 \beta_j (I_4 - 1) \exp \left[k_2 (I_4 - 1)^2 \right] \end{aligned}$$

From these, the Cauchy stress tensor, $\mathbf{C} = (1/J)\mathbf{F}\mathbf{S}\mathbf{F}^T$, is determined by Eqs. (2) and (3) in the circumferential and axial directions, respectively,

$$\begin{aligned} C_t &= c \lambda_t^2 \left(1 - \frac{1}{\lambda_z^2 \lambda_t^4} \right) \\ &\quad + \sum_{j=M,A} 4k_1 \lambda_t^2 \cos^2 \beta_j (I_4 - 1) \exp \left[k_2 (I_4 - 1)^2 \right] \end{aligned} \quad (2)$$

$$\begin{aligned} C_z &= c \lambda_z^2 \left(1 - \frac{1}{\lambda_t^2 \lambda_z^4} \right) \\ &\quad + \sum_{j=M,A} 4k_1 \lambda_z^2 \cos^2 \beta_j (I_4 - 1) \exp \left[k_2 (I_4 - 1)^2 \right] \end{aligned} \quad (3)$$

Optimization: An optimization strategy that utilizes both genetic and the Levenberg-Marquardt algorithms was used to identify the

best set of material model parameters that can characterize the tissue response to biaxial stretch. Multi-objective genetic algorithm-based optimization simultaneously minimizes two objective functions that are the sum of the squares of the differences between experimental and model-predicted Cauchy stresses in the circumferential and axial directions, respectively. The tradeoff between the two objective functions can be represented using a Pareto plot in the objective function space. The mean of the resulting set of material model vectors that satisfy additional constraints imposing c , k_1 , $k_2 > 0$ was used as the initial guess for the Levenberg-Marquardt algorithm, which aims to minimize the sum of the two components of the objective function. This strategy yields reproducible, unique sets of specimen-specific material model parameters, which were used subsequently for finite element analysis (FEA).

Three-Dimensional Reconstruction and Biomechanical Evaluation.

An in-house segmentation and geometry quantification script (AAVASC) was used to segment the contrast-enhanced μ MR images of the aorta pre- and postsurgery. The segmentation algorithm for the lumen was based on differences in contrast due to intensity gradients [18]. A surface was lofted over the point cloud-derived curves generated from AAVASC, thus creating a stereolithography file of the lumen using SOLIDWORKS (Dassault Systèmes SOLIDWORKS Corp., Vélizy-Villacoublay, France). Extensions were added to both ends of the model after which a hexahedral volume mesh of the aortic wall was generated using ANSYS ICEM (Canonsburg, PA) with approximately 154,000 linear hexahedral elements. This mesh size was obtained after conducting a mesh sensitivity analysis in which meshes in the range of 32,000 to 302,000 linear hexahedral elements were tested. The thickness of the wall was obtained from animal-specific wall thickness measurements performed with the explanted aortas. At any cross section, the volume mesh has three elements across the thickness of the wall, where the outermost element represented the adventitia and the two inner elements represented the media. Thus, a two-domain aortic wall mesh representing the media and adventitia was generated. The mesh was later input to FEBIO (Scientific Computing and Imaging Institute, Salt Lake City, Utah), an open-source FEA solver. The HGO constitutive material model, given by Eq. (1), was assigned to each domain using the animal-specific parameters described in Table 1, which were obtained from the optimization algorithm. All translational and rotational degrees-of-freedom (DOFs) were fixed at the inlet and outlet surface nodes, and a pressure of 120 mmHg was applied normal to the nodes on the lumen surface. Distribution maps of the first principal stress were obtained using Postview (the postprocessing module of FEBIO). The maximum of the first principal stress was quantified for each FEA model, pre- and postsurgery. A flowchart describing the processes involved in the estimation of these biomechanical metrics is shown in Fig. 1.

To evaluate the effect of geometry alone, pointwise-averaged Cauchy stress-stretch curves were generated from the animal-specific data in the circumferential and axial directions. The material model parameters corresponding to these curves were obtained from the optimization algorithm and used in the same

Table 1 Subject-specific constitutive material parameters obtained from the genetic and Levenberg-Marquardt optimization algorithms

Group	Subject No.	c (N/cm ²)	k_1 (N/cm ²)	k_2	β_M (deg)	β_A (deg)
Untreated	1	35.7	1.660	0.60	99.0	97.9
	2	0.83	0.220	3.04	124	12.6
	3	10.1	1.550	0.52	0.00	69.3
Treated	4	3.40	0.301	2.09	73.4	131
	5	1.68	0.137	2.86	66.5	8.00
	6	1.79	0.178	2.81	25.1	25.5
Pointwise average		3.63	0.216	2.51	58.2	24.0

Fig. 1 Flowchart illustrating the processes involved in the biomechanical assessment of rat AAA specimens

aforementioned finite element meshes for each animal's aorta. Similarly, PWS was quantified for each FEA model pre- and postsurgery.

Biological Activity Assessment. The images acquired using the IVIS imaging system were processed with the LIVING IMAGE software (PerkinElmer, Waltham, MA). Flux, defined as the number of photons emitted per second from a region of interest (ROI), is the primary measure of fluorescence from the IVIS images. Radiance, which is the flux normalized by the projected area and solid angle, was also evaluated within the ROIs. Three types of zones are identified in these IVIS images, namely (1) damaged zone: where the AAA was generated in the aorta (suprarenal or infrarenal location); (2) healthy zone: the healthy counterpart of the damaged zone in the same animal (suprarenal location in a rat with an infrarenal AAA and vice versa); and (3) reference zone: the top left corner of the image. Five ROIs each are chosen in the damaged and healthy zones, and one ROI is chosen as the reference zone in each image, as shown in Fig. 2.

The following metrics were used to assess MMP activity:

(a) absolute values of flux and radiance

(b) damaged to healthy ratio (DHRatio)

$$= \frac{\text{flux or radiance in the damaged zone}}{\text{flux or radiance in the healthy zone}}$$

(c) damaged to reference ratio (DRratio)

$$= \frac{\text{flux or radiance in the damaged zone}}{\text{flux or radiance in the reference zone}}$$

(d) normalized damaged to healthy ratio (nDHRatio)

$$= \frac{\text{flux in damaged zone} - \text{flux in reference zone}}{\text{flux in healthy zone} - \text{flux in reference zone}}$$

All the possible combinations of the aforementioned metrics were evaluated based on flux and radiance values computed at each ROI.

Statistical Analysis. Two sample student *t*-tests were performed to compare the untreated and treated groups of the eight metrics based on flux and radiance using $\alpha = 0.05$ for significance. Since multiple ROIs were extracted from the same image, the *t*-statistic obtained from the two-tailed unpaired student *t*-test was evaluated based on the actual degrees-of-freedom instead of the inflated degrees-of-freedom to avoid pseudoreplication.

Results

Biomechanical Assessment. The animal-specific material constants, which were obtained by least square fitting of the Cauchy

Fig. 2 IVIS image showing location of ROIs and the corresponding flux in the damaged (infrarenal) and healthy (suprarenal) zones in a treated subject. The outline of the aorta is shown with dashed lines.

Fig. 3 Cauchy stress versus stretch in the circumferential and axial directions (a) in an exemplary AAA specimen. Experimentally evaluated Cauchy stress was compared to that predicted using the HGO material model parameters derived from the optimization algorithm (b) using pointwise-averaged material model parameters.

stress formulation to the biaxial testing data for each AAA individually, are summarized in Table 1. A comparison of experimental and predicted stress–stretch curves for an exemplary AAA specimen is presented in Fig. 3(a). The Cauchy stress–stretch curves in the circumferential and axial directions while using pointwise average material parameters are illustrated in Fig. 3(b). The animal-specific and point-wise average material parameters were used as input to the FEA models resulting in distribution maps of first principal stress as shown in Fig. 4 for two exemplary AAA, one each for the untreated and treated groups. PWS was evaluated for all animals pre- and postsurgery using both animal-specific and average material parameters. Figure 5(a) shows the percentage change in mean PWS between pre- and postsurgery geometries in the untreated and treated groups. Using animal-specific material properties results in a 27% increase in mean PWS in the untreated group and 54% decrease in mean PWS in the treated group. Conversely, using average material properties results in a 5% decrease in mean PWS in the untreated group and a 27% decrease in mean PWS in the treated group between pre- and postsurgery geometries. Figure 5(b) illustrates the mean percentage difference between pre- and postsurgery PWS (DPWS). While using animal-specific material properties, we obtained a 32% increase in mean DPWS for the untreated group and a 3% decrease in mean DPWS for the treated group. Likewise, we obtained a 3% decrease in mean DPWS in the untreated group and a 25% decrease in mean DPWS in the treated group while using average material properties.

Biological Activity Assessment. IVIS images showing the distribution of flux based on fluorescence are shown in Fig. 6. The value for each ROI corresponds to the flux in that zone (the values of radiance in the same ROI are not explicitly displayed in the figure). The four metrics used to assess MMP activity based on flux and radiance are displayed in Fig. 7. The bar plots represent the mean of these metrics observed in untreated and treated groups. There is a significant reduction ($p < 0.05$) in the means of the

Fig. 4 Coronal view of the distribution of first principal stress (N/cm²) on the AAA wall of untreated ((a) and (b)) and treated ((c) and (d)) specimens pre-AAA ((a) and (c)) and post-AAA ((b) and (d)) induction surgery. (Orientation—A: anterior; P: posterior; H: head; and F: foot).

absolute, DHratio, and n DHratio metrics based on flux and radiance in the treated group compared to those in the untreated group. However, there is no significant increase in the means of DRratio based on flux and radiance in the treated group compared to those in the untreated group.

Discussion

The CaCl_2 model and its various modifications have been widely used as chemical models of AAA generation in rodents [19,20]. The mechanism behind the formation of AAA in this model is mainly driven by a combination of the breakdown of elastin [7,11,19,21] and simultaneous increase in inflammatory infiltrates such as macrophages and leukocytes [7,20]. Isenburg et al. [11] reported a mean reduction in aortic elastin greater than 50% in diseased rats. In addition to the quantitative reduction, it was also observed that the elastic fibers were flattened and fragmented in the AAA tissue specimens. This chronic degeneration of elastin is associated with an imbalance of MMPs and tissue inhibitor of metalloproteinase in the aortic extracellular matrix [22]. These two processes lead to weaker walls and increased hemodynamic stresses, until the mechanical integrity of the aortic wall is compromised. Prevention and stabilization of AAA by way of inhibiting elastin degradation and attenuating AAA expansion using PGG was also reported by Isenburg and colleagues [11].

The main goal of this work was to develop a protocol for future studies to evaluate the effects of drug-based therapies on the mechanics and inflammation in rodent models of AAA. In the present work, we hypothesized that periadventitial treatment of PGG stabilizes AAA by reduction of PWS and MMP activity. The former was assessed with planar biaxial tensile testing in combination with constitutive modeling of stress and strain behavior, and optical fluorescence imaging in vivo. The choice of the constitutive model used to characterize mechanically the AAA wall is based on its ability to represent the isotropic response of the ground matrix and the anisotropy introduced by the collagen fiber bundles. The in-house optimization code used to identify animal-specific material model parameters was based on a genetic algorithm, which consistently results in a unique set of reproducible parameters. This is a major advantage compared to the usage of Levenberg–Marquardt algorithm alone, which results in parameters dependent on an initial guess.

Effect of Pentagalloyl Glucose Treatment on Peak Wall Stress. The importance of PWS as a biomechanical marker of AAA growth and rupture has been interrogated in this study. Two sets of first principal stress distributions were generated, each based on the use of animal-specific material parameters and average material parameters, respectively. Since the AAA tissue specimens were unavailable prior to the AAA induction surgery, FEA was performed at both day 0 and 28 using the animal-specific material properties obtained at the end of the study. Thus, any differences in PWS that are seen over this timeline for each animal correspond to differences exclusively due to the change in abdominal aorta geometry pre- and postsurgery. While using animal-specific material properties, in the untreated group, we observed a mean PWS of 21.6 N/cm^2 on day 0 and 27.5 N/cm^2 on day 28, which leads to a 27% increase in mean PWS. In the treated group, we observed a mean PWS of 21.6 N/cm^2 on day 0 and 9.9 N/cm^2 on day 28, yielding a 54% decrease in mean PWS. This is in accordance with our hypothesis, which suggests that PGG treatment prior to AAA induction leads to a reduction in PWS compared to the corresponding untreated models.

While using average material parameters, in the untreated group, we observed a mean PWS of 12.6 N/cm^2 on day 0 and 11.9 N/cm^2 on day 28, which leads to a 5% decrease in mean PWS. In the treated group, we observed a mean PWS of 14.4 N/cm^2 on day 0 and 10.6 N/cm^2 on day 28, which leads to a 27% decrease in mean PWS. Although we noticed a slight reduction in PWS in the

untreated group, we see that the percentage difference between mean PWS pre- and postsurgery in the PGG-treated group is significantly lower than that in the untreated group. This is also in agreement with our hypothesis, suggesting the effect of PGG treatment on decreasing PWS. The relative effect of constitutive material model parameters on PWS is evident due to the consistent underestimation of the percentage change in mean PWS while using average material parameters compared to animal-specific material parameters.

From a different perspective, we observed that while using animal-specific material parameters, there is a 32% mean increase in PWS in the untreated group, whereas a 3% mean decrease in PWS in the treated group. However, while using average material parameters, we noticed a 3% mean decrease in PWS in the untreated group versus a 25% mean decrease in PWS in the treated group. The percentage changes in mean PWS and mean percentage change in PWS are both in agreement with the proposed hypothesis. In Fig. 5, the difference between untreated and treated groups due to average material properties shows solely the effect of geometry on biomechanics, whereas the difference between untreated and treated groups due to animal-specific material properties shows the combined effect of geometry and constitutive material parameters. While calculating the percentage change in mean PWS, we see that the effect of material properties is greater than that of geometry, although these changes are not statistically significant due to the small sample size of this study. Conversely, while evaluating average percentage change in PWS, the effects of geometry and material properties are similar. Thus,

Fig. 5 (a) Percentage change in mean peak wall stress and (b) average (and standard error of the mean) percentage change in peak wall stress pre- and post-AAA induction surgery in untreated and treated groups while using average and subject-specific material model parameters

Fig. 6 IVIS images showing fluorescence maps representing MMP activity in an exemplary untreated (a) and treated (b) rat AAA. Warmer colors indicate higher flux, and hence, higher MMP activity. The outline of the aorta is shown with dashed lines in both subjects.

we infer that first principal stress as a biomechanical marker of AAA in the rat CaCl_2 model is driven by changes in abdominal aorta geometry experienced after AAA induction surgery.

Effect of Pentagalloyl Glucose Treatment on Matrix Metalloproteinase Activity. Increased MMP activity has been identified as an important marker of AAA growth and rupture. In this study, MMP activity was measured based on flux and radiance identified within ROIs in the IVIS images. The rationale for choosing five ROIs each in the damaged and healthy “zones” of the rat aorta is to mitigate noise due to flux and radiance measured from surrounding tissue. By using different combinations of ROIs in the evaluation of the nondimensional MMP activity metrics (i.e., DHratio, DRratio, and nDRratio), we also minimize the sensitivity to regional variations of flux and radiance within the damaged and healthy zones.

We have hypothesized that MMP activity decreases with the periadventitial application of PGG. To this end, we obtained a significant reduction in the ratio of absolute values of flux and radiance in the damaged and healthy zones in the PGG-treated group compared to those in the untreated group. Similar observations of statistically significant reduction of MMP activity in the treated group can be made with respect to two other nondimensional metrics—DHratio and nDRratio. These measurements are in accordance with our hypothesis, inferring lower inflammatory

activity in the abdominal aorta after topical application of PGG. Nevertheless, noteworthy is that the DRratio of flux and radiance in the treated group is higher than that in the untreated group, although not statistically significant ($p = 0.098$). A possible explanation for this finding is the inconsistency in the location of the AAA among the treated group animals. Rat 4, which belongs to the treated group, was noted to have higher values of flux and radiance in the diseased zone compared to the other treated rats. Moreover, this was the only treated group animal with a suprarenal AAA. Therefore, since we have only one reference ROI, but five diseased ROIs, any DR ratio essentially reflects a measure of the absolute variation in the diseased zone’s flux or radiance. Consequently, any major variation in absolute values (as observed in rat 4’s diseased flux and radiance) is reflected in its DR ratio, resulting in a significant increase in the mean of the DR ratio of the treated group.

Our findings are consistent with a recent study by Nosoudi et al. [12,23], who performed targeted delivery of PGG nanoparticles to the AAA. Similar to our results, they had observed reduced MMP activity and inflammatory infiltration, in addition to a reduction in elastin degradation. Due to reduced turnover of elastin compared to collagen, the binding between PGG and elastin has been observed to remain intact during the observation period. The work of Nosoudi et al. was based on the remodeling of the aortic wall, which is consistent with the results of the

Fig. 7 Mean and corresponding standard error of mean values of MMP-based metrics derived from flux and radiance in untreated and treated subjects: (a) mean absolute values of flux and radiance, (b) mean ratio of flux and radiance in diseased and healthy ROIs, (c) mean ratio of flux and radiance in diseased and reference ROIs, and (d) mean ratio of normalized flux and radiance in diseased and healthy ROIs. Note that the absolute values of flux and radiance in the untreated group have been scaled down by a factor of 10^4 .

biological analysis that we report in this study. The new aspect in our study is the simultaneous evaluation of the biomechanical and biological parameters.

Limitations and Recommendations for Future Work. Further research should be based on a longitudinal study involving the evolution of biomechanical and inflammatory markers of targeted PGG treatment with a larger sample size. In addition, a regional correlation between first principal stress and metrics of MMP activity could give more insight into the interactions between biomechanical and biological processes involved in AAA growth and suppression. Noteworthy is that the small sample size did not allow us to establish statistical significance of the effect of PGG treatment on biomechanics. Moreover, the AAA were not generated in the same region of the abdominal aorta in all animals due to surgical complications. Material properties used to characterize the behavior of the AAA were derived only from the postsurgery experimental data, although the same properties were used to describe the mechanical response of the presurgery FEA models. Hence, any differences observed in PWS pre- and postsurgery are exclusively due to geometry variations on an animal-specific basis. Concerning the IVIS system, molecular imaging was performed in situ, which led to considerable noise and fluorescence in the soft tissues adjacent to the AAA, since the probe is not targeted to the diseased aorta. This shortcoming can be addressed to a considerable extent by imaging the aortas ex vivo. In spite of the aforementioned limitations, the present study is clinically relevant in that it demonstrates the effectiveness of PGG in suppressing AAA by means of biomechanical and biological analyses. The protocol described in this work, combining biomechanics and molecular imaging, can be used as part of a strategy for evaluating experimental therapeutics.

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