Journal of Biomechanics 46 (2013) 91–96

Contents lists available at SciVerse ScienceDirect

Journal of Biomechanics

journal homepage: www.elsevier.com/locate/jbiomech
www.JBiomech.com

# Biaxial and failure properties of passive rat middle cerebral arteries

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ARTICLE INFO

*Article history:*
Accepted 10 October 2012

*Keywords:*
Traumatic brain injury
Rat cerebral arteries
Passive biaxial mechanical properties

ABSTRACT

Rodents are commonly used as test subjects in research on traumatic brain injury and stroke. However, study of rat cerebral vessel properties has largely been limited to pressure–diameter response within the physiological loading range. A more complete, multiaxial description is needed to guide experiments on rats and rat vessels and to appropriately translate findings to humans. Accordingly, we dissected twelve rat middle cerebral arteries (MCAs) and subjected them to combined inflation and axial stretch tests around physiological loading conditions while in a passive state. The MCAs were finally stretched axially to failure. Results showed that MCAs under physiological conditions were stiffer in the axial than circumferential direction by a mean ($\pm$standard deviation) factor of 1.72 ($\pm$0.73), similar to previously reported behavior of human cerebral arteries. However, the stiffness for both directions was lower in rat MCA than in human cerebral arteries ($p<0.01$). Failure stretch values were higher in rat MCA ($1.35\pm0.08$) than in human vessels ($1.24\pm0.09$) ($p=0.003$), but corresponding 1st Piola Kirchhoff stress values for rats ($0.42\pm0.09$ MPa) were considerably lower than those for humans ($3.29\pm0.64$ MPa) ($p<0.001$). These differences between human and rat vessel properties should be considered in rat models of human cerebrovascular injury and disease.


## 1. Introduction

Disruption of cerebral blood vessel function leads to stroke and is a common outcome of traumatic brain injury (TBI). In the United States, 52,000 deaths result from over 1.7 million cases of TBI annually (Faul et al., 2010); the number of deaths is over 140,000 per year for stroke (Lloyd-Jones et al., 2010). Total annual costs associated with TBI and stroke have been estimated at 60 (Faul et al., 2010) and 65.5 (Rosamond et al., 2008) billion dollars, respectively.

Efforts to better prevent and treat cerebrovascular injury and disease are, in part, dependent upon a more complete understanding of vessel mechanics. This includes definition of blood vessel injury thresholds as well as characterization of relationships between applied forces and vessel function. Experiments on human cerebral vessels have revealed some characteristics of these tissues (Busby and Burton, 1965; Hayashi et al., 1980; Monson et al., 2008; Scott et al., 1972), but the limited availability of human specimens is a barrier to additional research.

Rodents are commonly used as models for both TBI and stroke (Coulson et al., 2002, 2004; Gonzalez et al., 2005; Hajdu and Baumbach, 1994; Hogestatt et al., 1983). Rodent cerebral vessels have also been studied in isolation, but the mechanical properties of these vessels have not been fully characterized. Emphasis has mainly been on circumferential behavior within the physiological loading range (Coulson et al., 2002, 2004; Hajdu and Baumbach, 1994). A more complete definition of these properties is an important step toward addressing questions more specific to cerebral vessel injury and disease. Accordingly, the aim of this research was to define the biaxial and failure properties of passive rat middle cerebral arteries (MCAs) and to compare these characteristics to those of previously studied human cerebral vessels.

## 2. Methods

### 2.1. Vessel dissection and cannulation

The MCA was dissected from 12 male Sprague Dawley rats ($389\pm41$ g). All procedures met requirements established by the Institutional Animal Use and Care Committee at the University of Utah. Rats were anesthetized with isoflurane and exsanguinated via cardiac perfusion using Hank's Buffered Saline Solution (HBSS; KCl 5.37, $KH_2PO_4$ 0.44, NaCl 136.9, $Na_2HPO_4$ 0.34, D-Glucose 5.55, $NaHCO_3$ 4.17; concentrations in mM), followed by a 1% nigrosin dye (Sigma-Aldrich, St. Louis, MO) HBSS solution. The dye enhanced visibility of the artery and its branches during dissection. MCA side branches were ligated with individual fibrils from unwound 6-0 silk suture, and the vessel was cannulated with glass tip needles and secured with 6-0 silk suture and cyanoacrylate glue. Lack of calcium in the HBSS ensured a passive response.

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0021-9290/$ - see front matter 
http://dx.doi.org/10.1016/j.jbiomech.2012.10.015

### 2.2. Experimental apparatus and methodology

Mechanical testing methodology was similar to that described previously (Monson et al., 2008). Briefly, the needles on which the MCA were mounted, and the associated fixtures, were attached to a custom vertical linear stage (Parker Automation, Cleveland, OH). The upper fixture was suspended from a 250 g capacity load cell (Model 31 Low, Honeywell, Golden Valley, MN) through an X–Y stage (MS-125-XY, Newport, Irvine, CA) that allowed for correction of any needle misalignment. The lower fixture was mounted to the stage via a vertical, low friction sled supported by a voice coil actuator (MGV52-25-1.0, Akribis, Singapore). Displacement of this actuator moved the lower fixture vertically along the sled track, axially stretching the MCA. A narrow, glass water bath, filled with HBSS and surrounding the MCA and needles, was attached to the lower fixture. Specimens were viewed via a digital video camera (PL-A641, Pixelink, Ottawa, Canada) equipped with a zoom lens (VZM 450i, Edmund Optics, Barrington, NJ). Vessels were perfused with HBSS originating from a syringe attached to a computer-controlled linear actuator (D-A0.25-AB-HT17075-4-P, Ultra Motion, Cutchogue, NY) and passing through the lower fixture, the mounted MCA, and the upper fixture. Pressure was determined by averaging the signals of pressure transducers (26PCDFM6G, Honeywell, Golden Valley, MN) located at each end of the vessel. Test control, as well as data and video acquisition, were accomplished using a custom LabVIEW program (National Instruments, Austin, TX). Actuator positions were given by digital encoders (resolution 1.0 μm).

Following mounting of the MCA, it was preconditioned by oscillating the luminal pressure (6.7–20 kPa; 50–150 mmHg) for five cycles while length was held constant at various sub-failure axial stretch values up to $\lambda_z \approx 1.2$. The zero-load length (corresponding with $\lambda_z = 1.0$) of each MCA was then estimated as that length where the measured axial load began to increase during an unpressurized (0.25 kPa to maintain an open lumen) axial stretch test. This was followed by a series of six sub-failure, quasi-static, biaxial sequences, consisting of three inflation tests at constant axial stretch ($\lambda_z \approx 1.1$, 1.15, 1.2) and three axial stretch tests at constant luminal pressure (20, 13.3, and 6.7 kPa). Finally, the vessel was stretched axially to failure, at a pressure of 13.3 kPa, to study deformations relevant to TBI.

### 2.3. Data analysis

Data from the encoders, load cell, and pressure transducers were recorded at 100 Hz. Noise observed in the load cell readings was smoothed using the SAE J211 filter (SAE, 1995). Images were acquired at 3 Hz, and current outer diameter ($d_e$) was measured via image analysis software (Vision Assistant; National Instruments, Austin, TX). Similarly, the reference diameter ($D_e$) was measured from an image taken from the zero load length test at $\lambda_z = 1.0$. Since images were obtained at a lower rate than the other signals, additional diameter data were defined using interpolation to allow one-to-one correspondence. Following the method defined by Wicker et al. (2008), wall volume was determined at six different configurations (each combination of $\lambda_z \approx 1.1$, 1.15, 1.2 and pressure $p_i = 0.25, 10.6$ kPa) by measuring inner diameter ($d_i$), $d_e$, and vessel length ($l$) in the images and calculating the associated wall volume of a tube. The average wall volume ($V$), along with the incompressibility assumption, was used to calculate $d_i$ from the measured $d_e$ in subsequent analysis, including the reference inner diameter ($D_i$) from $D_e$ (Eq. (1)).

$$\boldsymbol{d_i} = \sqrt{\boldsymbol{d_e^2} - 4\boldsymbol{V}/(\pi \boldsymbol{l})} \tag{1}$$

Vessels were assumed to be homogeneous circular cylinders, with mid-wall stretch defined by Eqs. (2)

$$\boldsymbol{\lambda_\theta} = \left(\frac{\boldsymbol{d_i} + \boldsymbol{d_e}}{\boldsymbol{D_i} + \boldsymbol{D_e}}\right) \tag{2a}$$

$$\boldsymbol{\lambda_z} = \left(\frac{\boldsymbol{l}}{\boldsymbol{L}}\right) \tag{2b}$$

where the subscripts $\theta$ and $z$ refer to the local cylindrical coordinates in the circumferential and axial directions, respectively. Enforcing equilibrium in the two directions results in the mean Cauchy stresses defined in Eqs. (3)

$$\boldsymbol{T_\theta} = \boldsymbol{p_i}\left(\frac{\boldsymbol{d_i}}{\boldsymbol{d_e} - \boldsymbol{d_i}}\right) \tag{3a}$$

$$\boldsymbol{T_z} = \frac{\boldsymbol{\lambda_z}}{\boldsymbol{A}}\left(\boldsymbol{F_z} + \frac{\pi}{4}\boldsymbol{p_i}\boldsymbol{d_i^2}\right) \tag{3b}$$

where $F_z$ represents the experimental axial force. Residual stresses were not considered due to large variations in measured opening angle.

As a first step in characterizing response in the axial and circumferential directions, stress–stretch data around approximate in vivo conditions were examined. Axial in vivo length was determined for each sample by observing the pressure-axial force response during preconditioning tests (Van Loon, 1977). Circumferential in vivo stiffness was defined as the slope of the stress–stretch curve in the inflation test with an axial stretch value closest to the axial in vivo stretch. An exponential function was fit through the data around the point corresponding to a pressure of 13.3 kPa (100 mmHg), and the value of the function's derivative at 13 kPa was taken as circumferential stiffness. Axial in vivo stiffness was defined in a similar manner from the axial stretch test conducted at a luminal pressure of 13.3 kPa, with the location where stiffness was calculated corresponding to the in vivo length. Note that stiffness is used here as a measure of the resistance of the vessel structure to deformation, rather than as an intrinsic tissue property.

A hyperelastic constitutive model, based on a phenomenological approach, was additionally applied to parameterize the biaxial response of the vessels based on the data from inflation tests. Data from axial stretch tests were not included since they resulted in slight unloading in the circumferential direction during axial stretch, inconsistent with requirements of pseudoelasticity (Humphrey et al., 1990). We applied the Fung-type strain energy function with the zero-load state as the reference configuration shown in Eq. (4) (Fung, 1993)

$$W = \frac{1}{2}c(e^Q - 1) \tag{4}$$

where $Q = c_1E_{\theta\theta}^2 + c_2E_{zz}^2 + 2c_3E_{\theta\theta}E_{zz}$, $W$ represents the strain energy per unit mass of the material, $E_{\theta\theta}$ and $E_{zz}$ refer to the Green strains in the circumferential and axial directions, and $c$ (units kPa), $c_1$, $c_2$ and $c_3$ are material parameters. Shear strains were ignored owing to the axisymmetric loading conditions and observed response. Incompressibility was enforced directly (Humphrey, 2002). The associated mean Cauchy stress values are shown in Eq. (5)

$$t_{\theta\theta} = \lambda_\theta^2\frac{\partial W}{\partial E_{\theta\theta}} = \lambda_\theta^2 c(c_1E_{\theta\theta} + c_3E_{zz})e^Q \tag{5a}$$

$$t_{zz} = \lambda_z^2\frac{\partial W}{\partial E_{zz}} = \lambda_z^2 c(c_2E_{zz} + c_3E_{\theta\theta})e^Q \tag{5b}$$

where $t_{\theta\theta}$ and $t_{zz}$ correspond to the theoretical Cauchy stress values in the circumferential and axial directions, respectively. Radial stresses were assumed to be small based on findings from our previous work on human arteries (Monson et al., 2008). Mean values of $t_{\theta\theta}$ and $t_{zz}$ were calculated for comparison to experimentally derived stresses (Eqs. (3)). Material parameters $c$, $c_1$, $c_2$ and $c_3$ that best fit the experimental data were computed by employing a nonlinear regression routine (fminsearch) in MATLAB (Mathworks, Natick, MA) to minimize the objective function $f$ (Eq. (6))

$$\boldsymbol{f} = \sum_{\boldsymbol{i}=1}^{\boldsymbol{N}}\left[\left(\frac{\boldsymbol{t_{\theta\theta}} - \boldsymbol{T_\theta}}{\boldsymbol{T_\theta}}\right)_{\boldsymbol{i}}^2 + \left(\frac{\boldsymbol{t_{zz}} - \boldsymbol{T_z}}{\boldsymbol{T_z}}\right)_1^2\right] \tag{6}$$

where $N$ represents the total number of data points, and the stresses are defined in Eqs. (3) and (5). Previously collected data from human cerebral arteries were similarly fit for comparison.

Two-tailed, unpaired $t$-tests were used to determine the significance of any differences between determined values, with $p < 0.01$ indicating statistical significance.

## 3. Results

Twelve arteries were successfully tested. Mean ($\pm$standard deviation) unloaded outer diameter and length of these specimens were 0.25 ($\pm$0.02) and 2.11 ($\pm$0.51) mm, respectively. Typical response is shown in Figs. 1 and 2 and is qualitatively similar to what we have previously reported for biaxial behavior of human cerebral arteries.

Rat MCA in vivo stretch was 1.10 ($\pm$0.03) and 1.27 ($\pm$0.11) for the axial and circumferential directions, respectively. At these stretch levels, axial stiffness was 1.14 ($\pm$0.37) MPa and circumferential stiffness was 0.73 ($\pm$0.29) MPa ($p = 0.006$), resulting in a ratio of 1.72 ($\pm$0.73) between the two directions; the ratio ranged from 1.05 (M5, Fig. 4) to 3.30 (M6, Fig. 4). For clarity, the rat data used to determine the axial and circumferential in vivo stiffnesses are identified as $S_{13}$ and the inflation test having the lowest value of axial stretch, respectively, in Figs. 3 and 4. The inflation test having the lowest value of axial stretch was selected as it was consistently closest to the in vivo stretch. However, in most cases it was not exactly at the in vivo axial stretch since this value could only be precisely determined during post-processing.

The Fung-type constitutive model fit the rat MCA data well, as shown for a representative data set in Fig. 5. Further, best-fit material parameters showed that $c_2$ was consistently larger than $c_1$ ($p < 0.001$) (Table 1). These best fit parameters, along with the