Spatial comparison between wall shear stress measures and porcine arterial endothelial permeability

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Submitted 18 September 2003; accepted in final form 29 December 2003

Atherosclerotic plaques primarily form in the large arteries, such as the coronary arteries, the aorta, and the major branches of the aorta. More specifically, the plaques arise preferentially in geometrically complex regions of the vasculature, such as branches and bends. It has been suggested that fluid mechanical forces in these regions are responsible for the localization of the disease. Generally, branch sites and regions of curvature experience a complicated blood flow field containing regions of low and high mean shear stress as well as areas of flow reversal and possible flow separation. In adult human vessel branches, lesions are more likely to form along the outer wall of the vessel branch, where the wall shear stress is relatively low (4, 21). However, the specific fluid dynamic variables responsible for the initiation of the disease have not been identified. Additionally, the mechanisms by which the fluid environment induces a pathological state are not well understood.

Elevated endothelial permeability to macromolecules such as LDL is associated with the development of atherosclerotic lesions (27, 30). Numerous investigators have sought to determine the relation between fluid dynamic shear stress and endothelial permeability. A recent review by Oggunrinade et al. (28) summarizes the results of in vivo and in vitro experiments having this specific aim. Although in vitro experiments provide useful information regarding cellular function, the environment in such experiments is significantly different from the in vivo setting. It would therefore be most desirable to examine the relation between shear stress and the permeability of the endothelial lining in a living organism, preferably one with a physiology and predilection for atherosclerosis comparable to that of humans.

In vivo experiments are confounded by the complexity and variability of the vascular tree; as a consequence, at any selected arterial region, the shear distribution varies among individuals (10, 11, 15, 22, 24). Previous in vivo work relating permeability to shear stress used shear estimates based on the assumption of Hagen-Poiseuille flow (2, 23) or on fluid dynamic computations in simplified geometries (1).

The experimental protocol reported in this study appears to be the first to correlate macromolecular uptake on a site-by-site basis with the pulsatile wall shear stress distribution in the same vessel segment. The proximal portion of the external iliac arteries at the porcine aortic trifurcation was chosen as the area of interest, because this region has a complicated blood flow field with a range of wall shear stresses and is known to be susceptible to lesion formation (7, 12). The porcine model is desirable, because the progression of atherosclerosis is similar in humans and swine (29). The spatial variation of macromolecular permeability was assessed by quantifying postmortem the distribution of the albumin marker Evans blue dye (EBD) in en face tissue segments. The shear stress distribution was obtained from computational fluid dynamic calculations in a computational region derived from an injection cast obtained postmortem.

METHODS

Experimental animal protocol. All procedures were performed in accordance with an established protocol approved by the governing Institutional Animal Care and Use Committee. Three female swine (56–72 kg mass) were preanesthetized with intramuscular injection of Telazol (6 mg/kg; tiletamine HCl + zolazepam) and xylazine (2 mg/kg). During surgery, anesthesia was maintained with an intravenous administration of pentobarbital sodium (13.2 mg/kg·h⁻¹) or by inhaled isoflurane. The femoral arteries were surgically exposed, and perivascular flow probes (Transonic Systems, Ithaca, NY) were placed around each femoral artery to monitor the flow.
blood flow rate. EBD was administered intravenously at a 1:1 molar concentration ratio with blood serum albumin. The EBD was allowed to circulate for 90 min before the animals were killed with an intravenous injection of Euthasol (pentobarbital sodium + phenytoin).

Immediately after the animals were killed, the arterial tree was flushed with saline to clear the vessels of blood and EBD. Casts replicating the infrarenal aorta and the iliac-femoral arteries of each animal were made in the following manner (Fig. 1). Silicone casting material (Polycast RTV-880, Polycast Industries, Bay Shore, NY), supplemented with 20% (wt/wt) 1/32-inch milled glass fibers (Fibre Glast, Brookville, OH), was injected at 100 mmHg into the abdominal aorta just proximal to the renal artery ostia. Once the casting material had cured, the rigid cast and surrounding tissue were carefully excised from the animal and placed in neutral buffered formalin overnight. The arterial tissue was removed from the cast with a dorsal cut. The tissue was pinned out for quantitation of the distribution of EBD uptake using en face photographic densitometry (13).

Mesh generation and computational analyses. Each cast was laser scanned by Product Development Technologies (Lincolnshire, IL) to generate a three-dimensional cloud of ~150,000 points representing the luminal surface. A custom mesh generator was used to create a three-dimensional eight-node brick element mesh representation of the terminal aorta and the common internal, external, and circumflex iliac arteries. A typical mesh (Fig. 2) contained 175,968 volume elements and 18,016 surface elements. Mesh sensitivity tests were performed by iterative refinement of the mesh until further refinement did not substantially change the computational results. The computational region was noncompliant.

The measured femoral artery flow rates were used to estimate the flows through the iliac and circumflex arteries and aorta by means of
flow relations established previously (18). The porosities of porous plugs placed at the vessel termini were adjusted to obtain the desired flow partition (9). Pulsatile laminar flow calculations were performed using the finite-element code FIDAP (Fluent). A uniform inlet velocity profile and no-slip conditions at the rigid walls were applied. The modeled aortic segment was sufficiently long that a well-developed velocity profile existed immediately proximal to the trifurcation. The fluid was assumed to be Newtonian, with a kinematic viscosity of $3.3 \times 10^{-6}$ m$^2$/s (3.3 cS). The inlet flow waveform (Fig. 3) was typical for the iliac region, had a period of 0.75 s, and was discretized into 60 time steps. An iliac artery waveform was applied at the (aortic) inlet so that, in this rigid model, the waveforms in the iliac vessels had the appropriate shape. The mean Reynolds number based on the diameter at the inlet averaged 885 for the three cases, and the average Womersley number was 8.3. Four cycles were computed to obtain temporal convergence.

Mapping of wall shear data and tissue images. The three-dimensional distribution of the computed wall shear stress in the right and left external iliac arteries, from the trifurcation to the deep femoral ostia, was mapped to a two-dimensional image by a numerical transform that simulates the cutting and pinning out of the tissue. Similar to the tissue cut line, the mathematical cut line was made along the dorsalmost edge of the region to ensure registration with the photographs used for EBD quantitation. After the transformation was performed, the two-dimensional image was visually inspected to confirm that the anatomic features of the vessel were in the proper location, with the circumflex ostia and flow divider used as landmarks. An affine transformation based on selected geometric features of the vessel (13) was used to deform the transformed shear image and the en face photographs of the tissue to a common template.

The greatest variation in shear stress was observed in the segment of the iliac artery proximal to the circumflex branch, and this region was selected for further study (16). There were many more pixels available for correlation distal to the region studied. However, the optical density (OD) and shear stress in this region were fairly uniform and necessarily close to the average for the entire segment. We did not want to overweight our correlation with midrange data, which could mask the influence of the data at the high and low ends of the shear distribution. Therefore, the distributions of selected shear stress measures in the proximal iliac segment were compared on a pixel-by-pixel basis ($\sim 5,000$ pixels total) with the OD distribution of the templated image of the EBD-stained tissue. Each pixel represents $\sim 1 \times 10^{-7}$ m$^2$ (0.1 mm$^2$) of tissue area. The OD measures albumin uptake during the dye exposure and is proportional to local permeability (14). Unpublished observations confirm that the amount of free EBD in the blood is negligible within seconds after EBD administration and that the EBD binding sites in the wall are far from saturation. To account for possible differences in experimental dye loading and variability between animals, the measured OD values in each artery were normalized by the mean OD for the entire vessel.

RESULTS

The transformed and templated images displaying the mean (i.e., time-average) wall shear stress magnitude in the proximal segment of the left and right iliac arteries from the initial case are shown with the corresponding templated tissue OD images in Fig. 4. The highest mean shear stress, depicted by the darkest spot in the shear images (Fig. 4, B and D), occurred near the flow divider in both arteries. The mean shear stress in this region was 50–95 dyn/cm$^2$. However, patches of much

Fig. 3. Typical aortic inlet flow wave used for pulsatile flow calculation.

Fig. 4. Templated shear and optical density images for 1 case. A and C: templated optical density maps in left and right iliac arteries, respectively. B and D: corresponding templated maps of time-average shear stress for left and right iliac arteries, respectively. Increasing gray level indicates increasing shear and increasing optical density. Arrows, direction of flow and location of flow divider.
lower shear (10–15 dyn/cm²) are immediately adjacent to this region. The nearly white region opposite the flow divider experienced mean shear stresses <5 dyn/cm². Visual inspection of the images in Fig. 4 suggests an inverse relation between OD and mean shear stress. There was less EBD staining at the flow divider in both iliac arteries, as evidenced by the lighter spots in the OD images. More prominent staining exists opposite the flow divider in the region of lowest calculated wall shear stress. Similar EBD staining patterns were also observed in the four iliac arteries from the latter two cases.

To enable a more quantitative presentation of the data, the pixels from each of the six shear images were sorted into three bins on the basis of their mean shear values. To avoid a disproportionate weighting of pixels at the extremes of the shear range, each bin was allotted an equal number of pixels. After the data were sorted into bins, the average OD and shear stress for the pixels within each shear bin in each vessel were calculated. The resulting normalized OD-time-average shear stress relation for the six arteries is shown in Fig. 5. The dependence of normalized OD on time-average wall shear stress magnitude \( \langle \tau \rangle, \text{dyn/cm}^2 \) can be described by a power law model (Fig. 5)

\[
OD = 1.39 \times \langle \tau \rangle^{-0.118}
\]

The variables were transformed into logarithmic form for linear regression analysis. The resulting \( P \) and \( R^2 \) values were 0.0014 and 0.484, respectively. Varying the number of data bins per case from 3 to 10 had minimal impact on the values of \( P \) and \( R^2 \).

The possible influence of changes in the shear field during the cardiac cycle on EBD uptake was investigated by calculating several time-dependent shear measures at each pixel location in the template. These measures are listed in Table 1, which presents the cross-correlation matrix among the time-dependent parameters and the time-average shear stress. Only the oscillatory shear index (OSI) (17) was not found to be highly correlated with the time-average shear stress. Correlations of OD with the remaining measures were similar to Fig. 5 and are not reported here. OSI, a measure of the variation in the direction of the shear vector during the cardiac cycle, was calculated for each pixel on the basis of its standard definition

\[
OSI = \frac{1}{T} \left( 1 - \frac{\int_0^T \tau(t) \, dt}{\int_0^T |\tau(t)| \, dt} \right)
\]

where \( T \) is the duration of the cycle. The OSI images were also templated to facilitate comparison with EBD uptake, and the numeric data were binned similarly to the time-average shear data. The relation between normalized OD and OSI for the six arteries is plotted in Fig. 6 and can also be fit to a power law model

\[
OD = 1.17 \times OSI^{0.032}
\]

When the above equation was transformed into logarithmic form, \( P = 0.012 \) and \( R^2 = 0.333 \).

**DISCUSSION**

The results presented here compare the spatial distribution of shear stress in an anatomically correct geometry with endothelial permeability to macromolecules in the same artery, under normal conditions and in an animal similar to humans. These data reflect the subtle nuances in arterial geometry neglected by idealized models. Although the spatial distribution of shear

<table>
<thead>
<tr>
<th>Time-Avg Shear</th>
<th>Pulse Shear</th>
<th>Maximum Shear</th>
<th>Minimum Shear</th>
<th>OSI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pulse shear</td>
<td>0.92</td>
<td>0.96</td>
<td>0.98</td>
<td></td>
</tr>
<tr>
<td>Minimum shear</td>
<td>0.82</td>
<td>0.71</td>
<td>0.72</td>
<td>-0.34</td>
</tr>
<tr>
<td>OSI</td>
<td>-0.03</td>
<td>0.17</td>
<td>0.14</td>
<td></td>
</tr>
<tr>
<td>Max temporal gradient</td>
<td>0.92</td>
<td>0.97</td>
<td>0.98</td>
<td>0.63</td>
</tr>
</tbody>
</table>

Values are Pearson correlation statistics for cross-correlation of each of the time-dependent shear measures against the other shear measures. Perfect correlation is indicated by 1 or -1. Maximum shear and minimum shear, largest and smallest shear stress experienced at a given location during the cardiac cycle, respectively; pulse shear, difference between maximum and minimum shear; Max temporal gradient, largest value of time derivative of shear stress experienced at each location; OSI, oscillatory shear index.
stress is predicted well, the magnitude of the calculated shear stress is affected by some of the assumptions made in the calculation. Iliac artery flows were estimated from the measured femoral artery flows using relations developed from laparoscopic flow studies (18), in which both flows were measured simultaneously. Animal variability in the iliac-to-femoral flow ratio introduces ~10% uncertainty in the flow rate through the iliac arteries and a comparable uncertainty in the calculated shear stress in these arteries. Minor errors are also introduced into the shear calculation by assuming non-compliant vessels and Newtonian blood flow. Non-Newtonian effects should not be appreciable in this geometry, because the vessels considered in this study are relatively large, the hematocrit (~30%) is relatively small, and the shear rates in this region are fairly high. The error attributable to compliance is more significant than that attributable to rheology. In a cast of a human aortic bifurcation, the introduction of compliance raised the time-average shear rate in locations where the shear rate is high and lowered the shear rate in locations where the shear rate is low (8). Thus the areas in our computation identified as high and low shear sites may have been exposed to more extreme values than those calculated here.

The inverse relation between shear stress and arterial endothelial permeability seen here represents the basal endothelial permeability of a conduit artery under normal levels of shear stress. Some experiments using cell culture (19, 31), excised tissue (3, 26), and in vivo procedures in the microvasculature (25) have found that permeability increases with acute increases in shear stress. Taken together, these observations may indicate separate adaptive and chronic cellular responses to shear stress or different responses in functionally different segments of the circulation. Research performed in the authors’ laboratory supports the hypothesis that the arterial endothelium undergoes a period of adaptation, characterized by a transient increase in permeability, in response to changing levels of shear stress (13). It is possible that adaptation could play a role in the microcirculation measurements. In the present study, care has been taken to maintain the natural flow rate through the arteries of interest during the course of the experiment. Thus the permeability measured in these arteries should be representative of adapted cells.

This study supports the hypothesis that regions of low shear stress promote the development of atherosclerotic lesions via an increase in lipoprotein uptake and that a certain level of shear stress is beneficial to the endothelium. Growing evidence from cellular and molecular biology experiments also supports this claim. Shear stress has been shown to modify the expression of proteins associated with cellular permeability. Vascular endothelial growth factor and occludin are thought to increase and decrease the permeability of vascular endothelium, respectively. After physiological shear stress was applied to excised porcine carotid arteries for 24 h, the expression of vascular endothelial growth factor decreased while the expression of occludin increased (6). The development of atherosclerotic lesions is also associated with regions of cell turnover caused by a higher apoptotic rate and increased cell proliferation (5). The absence of consistent cell junctions in these regions facilitates paracellular transport of macromolecules and, thus, compromises the permeability barrier of the endothelium. Fluid flow has been shown to decrease the level of apoptosis in cultured cells and in ex vivo organ culture (20).

It has been suggested (21) that regions exposed to a high OSI are more susceptible to lesion formation. The slight positive dependence of endothelial permeability on OSI observed in this study is consistent with this notion. Endothelial cells normally elongate and align in the direction of primary flow. This process is characterized by the remodeling of the cytoskeleton into cell-spanning stress fibers and the formation of cell-cell junctions at the stress fiber termini. However, endothelial cells in regions exposed to a strongly oscillatory flow do not align. The lack of a dominant signal in a principal direction may prevent the cells from forming a regular cytoskeletal network with strong cell-cell junctions, resulting in highly permeable, intercellular gaps.

Mean shear stress and oscillatory flow may act in combination to influence permeability. As shown in the APPENDIX, the near-wall residence time in vascular flows is inversely proportional to the quantity \((1 - 2 \times \text{OSI}) \langle \tau_n \rangle\). This composite function was correlated against OD using the same approach employed in the separate correlations of \(\langle \tau_n \rangle\) and OSI. Owing to the very low values of OSI in these vessels, the result was not noticeably different from that obtained using shear alone.
Thus the permeability variation can be explained as a shear effect or a residence time effect with equal confidence. Correlations in geometries experiencing larger variations in OSI might allow a distinction to be made between the roles of shear stress and residence time in modulating vascular permeability in vivo.

Although permeability was found to depend on the time-average shear stress and OSI, the regression analysis indicates that these shear measures do not explain all the variation in the data. Therefore, it is likely that other parameters play a role in determining the spatial permeability of the endothelium. Considering the variety of fluid forces in the vasculature, it is possible that the endothelial permeability is regulated by a complex interaction of spatially varying hemodynamic parameters.

**APPENDIX: A PHYSICAL INTERPRETATION OF OSI**

The definition of OSI used here is that proposed by He and Ku (17)

$$\text{OSI} = \frac{1}{2} \left( 1 - \frac{\int_0^T \tau_w dt}{\int_0^T |\tau_w| dt} \right)$$

(A1)

where $T$ is the duration of the cycle and $\tau_w(t)$ is the instantaneous shear stress vector.

The OSI varies from zero, when the instantaneous shear vector is collinear with the time-average vector throughout the cardiac cycle, to 0.5, when $\int_0^T \tau_w dt = 0$. This definition has been used by many authors to describe the oscillatory nature of vascular flows. Buchanan et al. (1) sought correlations between OSI and segment averaged values of white blood cell densities at the rabbit aortoceliac junction, but the variation in OSI was too small to reveal any relation that may have existed.

Although the OSI can identify regions of flow reversal, it is insensitive to shear magnitude. Sites where the time-average shear is low may be sites of significant OSI but not necessarily; low shear can result from flow expansion without any local flow reversal beyond that which may follow from pulsatility alone. Similarly, strongly oscillatory flows can exhibit the same OSI as very slow flows with the same waveform. It seems unlikely that endothelial cells sense OSI per se. These considerations suggest that OSI might better be employed in combination with other shear measures, rather than as a stand-alone index of the flow. We suggest here a simple formulation, based on residence time, that does this.

The importance to the atherosclerotic process of the residence time of solutes and formed elements of the blood in the neighborhood of vascular endothelium is well appreciated, and we will not elaborate on it here. Residence time is a relative concept, because all nonadherent particles in the flow are moving and, thus, have zero “residence time” at any location. The relative residence time at a particular site can be expressed in terms of the Cartesian distance, $\delta(y)$, that a fully entrained particle a small distance $y$ from the wall travels during a cardiac cycle; the relative residence time, $t_r$, of such a particle is proportional to $\delta^2$. Near the wall, the excursion of the particle is small enough that the spatial variation in shear can be neglected, and

$$\delta(y) = \left( \int_0^y u(y') dy' \right) = \frac{y}{\mu} \int_0^y \tau_w dy$$

where $\mu$ is viscosity. Solving Eq. A1 for $\int_0^T \tau_w dt$ and substituting into Eq. A2

$$\delta(y) = \left( \frac{y}{\mu} \right) (1 - 2 \times \text{OSI}) \int_0^T |\tau_w| dt$$

(A2)

The average shear stress magnitude ($\tau_w$) is $1/T \int_0^T |\tau_w| dt$, from which

$$\delta(y) = \left( \frac{y}{\mu} \right) (1 - 2 \times \text{OSI})(\tau_w)$$

(A3)

For any arbitrarily small value of $y$, the first term on the right-hand side of Eq. A3 is a constant, so

$$t_r \sim \left( (1 - 2 \times \text{OSI}) (\tau_w) \right)^{-1}$$

In this formulation, OSI acts to modify the effect of time-average shear on the relative residence time at a site. When the OSI is small, it has little effect on the residence time, but as it approaches its limit of 0.5, it can have an increasingly important influence on this quantity and, presumably, on near-wall interactions as well. In flows where OSI can be large at some sites, the product $(1 - 2 \times \text{OSI})(\tau_w)$ may be a useful measure of the shear environment for correlative purposes that incorporates the level of the shear and its oscillatory character.

**ACKNOWLEDGEMENTS**

We thank Ellen Dixon-Tulloch for surgical assistance. Present address of A. L. Hazel: Dept. of Mathematics, University of Manchester, Manchester, UK.

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