Science in China Series C: Life Sciences

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Springer

Ex vitro experimental study on concentration polarization of macromolecules (LDL) at an arterial stenosis

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To verify the previous theoretical prediction that the disturbed flow distal to a stenosis enhances lipid accumulation at the blood/arterial wall interface, we designed a canine carotid arterial stenosis model and measured *ex vitro* the luminal surface concentration of bovine serum albumin (as a tracer macromolecule) by directly taking liquid samples from the luminal surface of the artery. The experimental results showed that due to the presence of a filtration flow, the luminal surface albumin concentration c_w was higher than the bulk concentration c_o as predicted by our theory. The measurement revealed that the luminal surface concentration of macromolecules was indeed enhanced significantly in regions of the disturbed flow. At Re = 50, the relative luminal surface concentration c_w/c_o was 1.66 ± 0.10 in the vortex region, while the c_w/c_o was 1.37 ± 0.06 in the laminar flow region. When Re increased to 100, the c_w/c_o in the vortex flow region and the laminar flow region reduced to 1.39 ± 0.07 and 1.24 ± 0.04, respectively. The effect of the filtration rate, v_w, on the luminal surface concentration of albumin was remarkably apparent. At Re = 50 and 100, when $v_w = 8.9 \pm 1.7 \times 10^{-6}$ cm/s, c_w in the vortex region was 77% and 52% higher than c_o respectively, meanwhile when $v_w = 4.8 \pm 0.6 \times 10^{-6}$ cm/s, c_w in the vortex region was only 66 % and 39% higher than c_o respectively. In summary, the present study has provided further experimental evidence that concentration polarization can occur in the arterial system and fluid layer with highly concentrated lipids in the area of flow separation point may be responsible for the formation and development of atherosclerosis.

concentration polarization, atherosclerosis, arterial stenosis, flow disturbance

Because the endothelium of the artery displays low permeability to plasma proteins, the filtration flow across the artery wall may cause 'concentration polarization' of lipids, a well-known mass transport phenomenon, at the blood/wall interface with the lipids increasing in concentration from the bulk value towards the interface[1—4]. Lipid infiltration into the arterial wall should depend on the concentration of lipids at the blood/vessel wall interface, as the blood vessel wall is directly exposed to the luminal surface lipid concentration.

Because of regional differences in the near-wall blood flow velocity, blood pressure, and vascular permeability, the atherogenic lipid concentration at the luminal surface may vary according to location in the arterial tree. Therefore it is hypothesized that these local variations in the luminal surface lipid concentration may contribute to the localization of atherosclerosis[5].

To test this hypothesis, the concentration of bovine serum albumin (as a tracer macromolecule) at the luminal surface of the canine carotid artery was measured by directly taking liquid samples from the luminal surface of the artery[6]. The results confirmed that concentration polarization could indeed occur in the arterial

Received November 27, 2006; accepted March 24, 2007
doi: 10.1007/s11427-007-0068-3
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Supported by the National Natural Science Foundation of China (Grant Nos. 10632010, 10572017, and 30670517)

system[6]. Then, the transport of atherogenic lipids (LDL) in an arterial stenosis with a semi-permeable wall was numerically studied. The numerical analysis predicted lipid accumulation at the blood/arterial wall interface, with the highest concentration occurring at the flow separation point right distal to the summit of the stenosis[7]. It is believed that the fluid layer with highly concentrated lipids in the area of flow separation point may be responsible for the formation and development of the arterial stenosis.

To verify the theoretical analysis mentioned above, we designed an *ex vitro* experiment and directly measured the luminal surface concentration of bovine serum albumin (as a tracer macromolecule) in an arterial stenosis introduced to the canine carotid artery. This paper will describe the experiment and present the results.

1 Methods and materials

1.1 Model flow study

Prior to albumin concentration measurement, the flow through the test stenosis was first observed by flow visualization. A 15 cm-long segment of common carotid artery was obtained from an adult mongrel dog. The artery was cannulated *in situ* and the two cannulae were fixed rigidly onto a metal frame to hold the vessel at *in vivo* length. A stenosis with 75% area constriction was introduced to the artery segment by using a length of 3-0 silk suture. The artery segment with the stenosis was rendered transparent according to the technique described by Karino et al.[8]. Then the transparent stenosis was mounted to a head-tank overflow perfusion system to observe its flow pattern. The perfusion fluid used in this flow study was methyl salicylate[8] (oil of winter green) to keep the arterial segment transparent. A small amount of neutrally buoyant latex microspheres with diameters of 50－120 μm (Duke Scientific Corp., Palo Alto, CA) was added to visualize the flow in the stenosis. The vortex lengths were measured and recorded at various Reynolds numbers.

1.2 Albumin concentration measurement

1.2.1 Preparation of artery segments. Common carotid arteries were obtained from adult mongrel dogs of both sexes (25－30 kg). Handling of animals was in compliance with "Principles of Laboratory Animal Care in China". The dogs were anesthetized by intravenous injection of sodium pentobarbital (32 mg/kg). After administering heparin (0.5 mg/kg) intravenously, the common carotid arteries were exposed and the surrounding tissue was gently removed. An approximately 10 cm-long segment of the artery was cannulated. The artery was thoroughly examined for leakage *in situ* by pressurization of the vessel through a reservoir placed 100 cm above the artery and filled with Krebs solution (concentrations in mmol/L: NaCl, 118; KCl, 4.7; $NaHCO_3$, 25; KH_2PO_4, 1.2; $MgSO_4$, 1.2; $CaCl_2$, 2.5; glucose, 11). Any points on the vessel that leaked were sealed by coagulating with an electrical fulgurator. The vessel was then flushed slowly to wash out any remaining blood. The two cannulae were fixed rigidly onto a metal frame to hold the vessel at *in vivo* length. The cannulae were then each advanced approximately 2 mm into the vessel, enabling their tips to lay beyond any regions liable to have been damaged during the excision procedure. After a ligature was tied around each cannula as close to the tip as possible, the cannulated vessel was dissected from the body. During the entire procedure, Krebs solution was constantly applied to the outer surface of the vessel to prevent it from drying.

Two arterial segments were fixed under pressure with a 10% formalin solution. The first one was at 100 mmHg, the second at 200 mmHg. The vessels were then sectioned to measure the wall thickness of the vessel. The wall thickness was later used to determine the inner diameters of the test vessels for the calculation of Reynolds number in the perfusion study and the calculation of filtration rate across the vessel wall (see *Filtration Rate Measurement*). It was confirmed that the arteries measured had a thickness of 0.2±0.05 mm.

1.2.2 Perfusion solution. In the perfusion study, freshly prepared albumin Krebs solution was used. Bovine serum albumin (Sigma Chemical Co., St. Louis, MO, USA) was dissolved in the Krebs solution at a concentration of 1.0 mg/mL (density, 1.005 g/cm^3; viscosity, 0.0116 g/cm·s). The pH value of the solutions was adjusted to 7.4.

1.2.3 Perfusion system. Figure 1 shows a schematic drawing of the experimental perfusion system. The harvested artery with the metal frame was immersed in the Krebs solution and then connected horizontally to the overflow head-tank perfusion system. A pressure gauge was connected to a pressure tab located immediately proximal to the blood vessel to monitor the pressure in the blood vessel. A microliter syringe (Hamilton Com-

pany, Reno, Nevada, USA) was used to retrieve liquid samples at the solution/arterial wall boundary through a sampling needle of 31-gauge (diameter = 0.28 mm, length = 50 mm). The microliter syringe had a total volume of 10 μL with an accuracy of ± 0.2 μL. The sampling needle was inserted into the lumen of the test artery through the specially designed distal cannula so that the needle was placed horizontally on the luminal surface of the artery. Care was taken to make sure that the needle was placed parallel and directly on the luminal surface of the test artery.

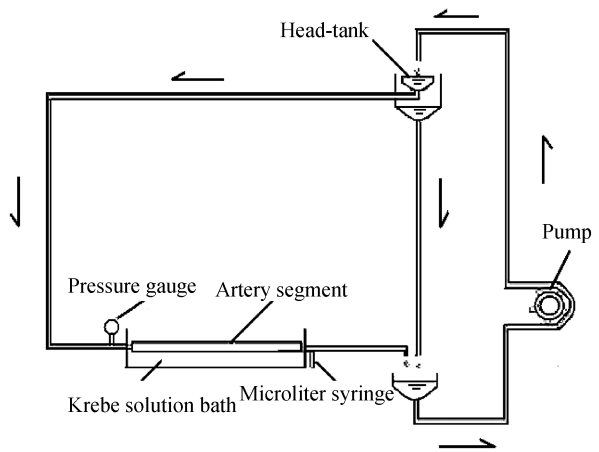

Figure 1 Schematic drawing of the experimental perfusion system. The overflow head-tank provides a steady flow to the canine carotid artery segment.

1.2.4 Measurement of albumin concentration. A standard procedure was followed throughout the entire experiment. The pressure in the perfusion system was gradually increased to 100 or 200 mmHg. The test blood vessel was checked again for leakage. Thereafter, a length of 3-0 silk suture was passed under the artery, and was tied so that the carotid artery was constricted to about 25% of its original area. The distal length of the test vessel after the stenosis was 5.0 cm. The albumin solution was perfused through the blood vessel and recirculated by a rotary blood pump between a collecting reservoir and the overflow head-tank (Figure 1). The excess flow from the overflow head-tank was returned by gravitational force to the collecting reservoir via overflow tubing. The height of the head-tank and the resistance of the outlet tubing were adjusted so that a chosen flow rate (therefore Reynolds number) through the test blood vessel could be obtained while the pressure within the vessel was maintained at 100 or 200 mmHg. Flow rate was measured using a graduated cylinder and a stopwatch. For each pressure level (100 or 200 mmHg), the outer diameter of the test artery was measured to determine the inner diameter of the test vessel so that the Reynolds number could be calculated. The perfusion study was carried out at Reynolds numbers of 50 and 100 for each pressure level (100 mmHg or 200 mmHg). When the flow became stabilized, 10 μL of the liquid sample at the solution/arterial wall boundary was extracted using the microliter syringe. For each Reynolds number, liquid samples were taken from various locations from the test vessel as shown in Figure 2: 1) at a location of 3 diameters from the flow reattachment point measured in the flow visualization study; 2) a location right after the stenosis, and 3) at intervals of 1.5 mm in the vortex flow region. For each location, a total of 5 samples were taken. The interval between two sample retrievals was 5 minutes to allow the flow to become restabilized. During the experiment, the flow rate and pressure in the blood vessel were monitored constantly. The experiments were all carried out at a room temperature of 23 ± 1℃.

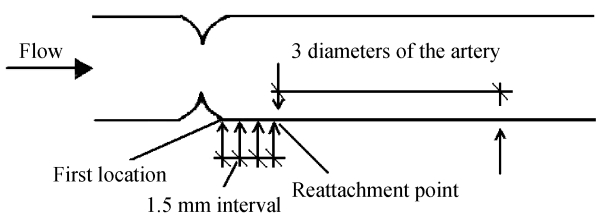

Figure 2 Sampling locations. The first location was right after the stenosis. At this location, the sampling needle was placed as close to the stenosis as possible.

A modified colorimetric method described by McKnight[9] was used to determine the albumin concentration of the samples. According to McKnight, this method is very sensitive and can be used to detect proteins as low as 0.1 μg. The detailed procedure for the measurement of albumin concentration of the retrieved liquid samples can be found in our previous study[6].

1.2.5 Filtration rate measurement. The same experimental procedure described by Deng et al.[10] was followed for the measurement of the filtration rate across the walls of test arteries.

2 Results

2.1 Observation of flow in stenosis model

Flow patterns around the stenosis were observed at four

different flow rates (Reynolds numbers). For all Reynolds numbers studied, a vortex formed distal to the stenosis. In this flow observation study, the vortex length was determined by measuring the distance between the stenosis and the reattachment point of the vortex. The measurement of the normalized vortex length is illustrated as a function of Reynolds number in Figure 3. The vortex length was rendered dimensionless by dividing by the inner diameter of the arterial segment. As evident from Figure 3, the normalized vortex length increased linearly with increasing Reynolds numbers. At the Reynolds numbers of 50 and 100 used in the albumin concentration measurement experiments, the normalized vortex lengths were approximately 1.5 and 3.0, respectively.

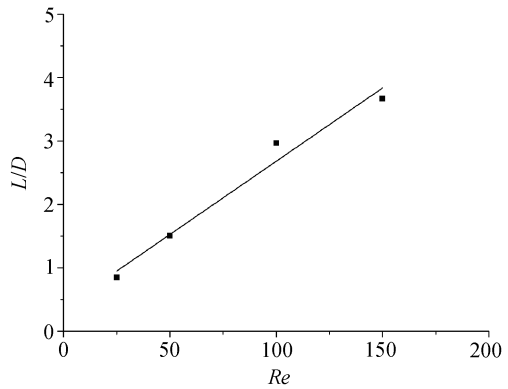

Figure 3 Normalized vortex length against *Re*. *L*, Vortex length; *D*, inner diameter of the artery.

2.2 Measurement of albumin concentration

Figure 4 gives the comparison of the measured albumin concentration of the liquid samples retrieved from the vortex region and the laminar flow region under a transmural pressure of 100 mmHg, at two *Re* numbers (*Re* = 50 and 100).

The filtration rate measured under this pressure level was $4.67 \pm 0.73 \times 10^{-6}$ cm/s. All of the data were normalized with the bulk concentration, c_o. From Figure 4, it is apparent that the relative luminal surface concentration, c_w/c_o, in both the vortex flow region and the laminar flow region, was higher than 1.0, indicating that concentration polarization occurred in the arterial stenosis. The relative luminal surface concentration in the vortex region was significantly higher than that in the laminar flow region (P<0.005) for both *Re* studied. At *Re* = 50, the c_w/c_o was 1.66 ± 0.10 in the vortex region, while the c_w/c_o was 1.37 ± 0.06 in the laminar flow region. When *Re* increased to 100, the c_w/c_o in the vortex flow region and the laminar flow region reduced to 1.39 ± 0.07 and 1.24 ± 0.04, respectively.

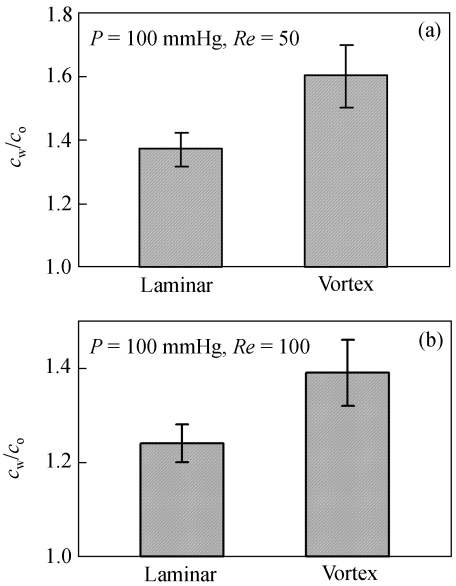

Figure 4 Experimental results (n = 8) for the arteries under a transmural pressure of 100 mmHg ($v_w = 4.67 \pm 0.73 \times 10^{-6}$ cm/s). All of the data were normalized with the bulk concentration, c_o. The relative luminal surface concentration in the vortex region was significantly higher than that in the laminar flow region (P<0.005) for both *Re* studied.

Figure 5 shows the results measured under a transmural pressure of 200 mmHg. The filtration rate measured under this pressure level was $8.9 \pm 1.7 \times 10^{-6}$ cm/s. Again, the relative luminal surface concentration in the vortex region was significantly higher than that in the laminar flow region (P<0.005). By comparing Figures 4 and 5, it could be seen that the effect of the filtration rate, v_w, on the luminal surface concentration of albumin was remarkably apparent. For instance, at *Re* = 50 and 100, when $v_w = 8.9 \pm 1.7 \times 10^{-6}$ cm/s, c_w in the vortex region was 77% and 52% higher than c_o respectively, meanwhile when $v_w = 4.8 \pm 0.6 \times 10^{-6}$ cm/s, c_w in the vortex region was only 66 % and 39% higher than c_o respectively.

3 Discussion

By directly taking liquid samples from the luminal surface of an arterial stenosis, we measured *ex vitro* the luminal surface concentration of bovine serum albumin (as a tracer macromolecule) in the disturbed flow region of the stenosis and compared it with the measured luminal surface concentration of bovine serum albumin in the laminar flow region father downstream of the steno-

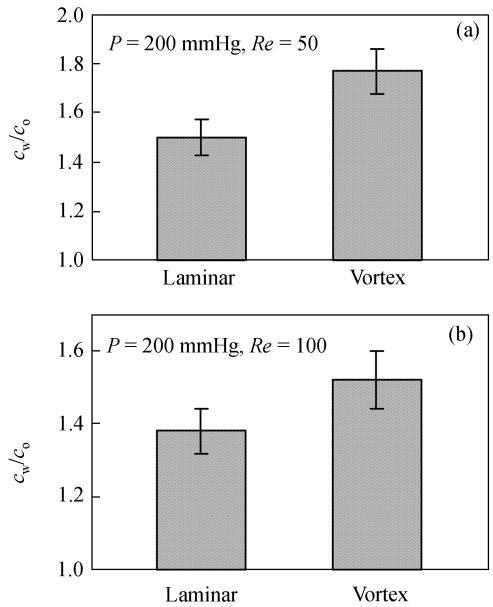

Figure 5 Experimental results (n = 8) for the arteries under a transmural pressure of 200 mmHg (v_w = 8.9 ± 1.7 × 10^{-6} cm/s). All of the data were normalized with the bulk concentration, c_o. The relative luminal surface concentration in the vortex region was significantly higher than that in the laminar flow region (P<0.005) for both Re studied.

sis. The results demonstrated that due to the presence of a filtration flow, macromolecules accumulated at the blood/arterial wall interface, leading to the phenomenon of concentration polarization of macromolecules. The concentration of macromolecules at the blood/wall interface was affected by both local flow fields and the filtration flow. It was much higher in regions of flow recirculation and stagnation with low wall shear rates than in regions of unidirectional laminar flow with higher wall shear rates. The measured results therefore were consistent with our previous numerical simulations of LDL transport in the arterial stenosis[7].

It should be mentioned that at both filtration rates when Reynolds number was 50, the mean wall macromolecule concentrations measured in the developed flow regions were approximately equal to those in the vortex regions with a higher Reynolds number (Re=100) (Figures 4 and 5). It is not clear whether these measured results are coincident or not. Our previous study[5] has shown that wall concentration of macromolecules has a strong dependence on the wall shear rate. It is possible that in the present study, the mean wall shear rate in the vortex region with Re=100 was approximately the same as that in the developed flow region with Re=50, resulting in the above-mentioned results.

In recent years, researchers have been paying more and more attention to material transport in the circulation and the interactions between blood cells and the blood vessel walls[11], proposing the concepts of 'residence time' for atherogenic agents[12,13] and deposition of atherogenic particles onto the blood vessel walls[14]. They believe that the 'residence time' and the deposition of atherogenic particles have something to do with atherosclerosis and thrombus formation. Apparently, the occurrence of concentration polarization of macromolecules in the arterial system can affect the 'residence time' and the deposition of atherogenic particles. In a recently published review article, Kleinstreuer et al.[11] especially discussed this concentration polarization phenomenon. Concentration polarization is a well-known engineering phenomenon. Because the arterial wall is a semi-permeable membrane and water can filtrate across it, we believe that concentration polarization of atherogenic lipids can occur in the arterial system. By using 19 nm fluorescent microspheres as tracer particles, Naiki and Karino[15] observed this concentration polarization phenomenon on the surface of a culture endothelial monolayer. This mass transport phenomenon was also numerically predicted in a curved segment of the aorta[16] and in arteries with a multiple bend[17].

Experimental results by Wiklund et al.[18] suggest that the flux of LDL into the arterial wall is not regulated by endothelial LDL receptors. Some cholesterol may seep into the arterial wall by infiltrating through leaky endothelial cell junctions. This lipid infiltration should depend on the concentration of lipids at the blood/arterial wall interface as the arterial wall is directly exposed to the luminal surface lipid concentration. Because of regional differences in the near-wall blood flow velocity, blood pressure, and vascular permeability, the atherogenic lipid concentration at the luminal surface may vary according to locations in the arterial tree. We therefore hypothesize that these local variations in the luminal surface lipid concentration may contribute to the localization of atherosclerosis.

In the past years, many researchers emphasized the importance of wall shear stress in atherogenesis. Although it has been widely recognized that the flow induced shear stress plays a very important role in modulating endothelial functions and is one of the most important hemodynamic factors in vascular disorders such as atherosclerosis[19], it is most probable that the mass transport phenomenon of concentration polarization at

the blood/arterial wall interface may also play an important role in the localization and development of atherosclerosis in the human circulation. In summary, the present study has provided further experimental evidence that concentration polarization can occur in the arterial system and fluid layer with highly concentrated lipids in the area of flow separation point may be responsible for the formation and development of atherosclerosis.

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