Original Research

Effect of swirling flow on the uptakes of native and oxidized LDLs in a straight segment of the rabbit thoracic aorta

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Abstract

To elucidate the physiological significance of the spiral flow in the arterial system from the viewpoint of atherogenic lipid transport, an *ex vivo* experimental comparative study was designed to investigate the effect of swirling flow on the distribution of native 3,3′-dioctadecylindocarbocyanine-low-density lipoprotiens (DiI-LDL) and DiI-ox-LDL uptakes by segments of the rabbit thoracic aorta. The experimental results showed that when compared with the normal flow, the swirling flow generated in the test arteries significantly reduced the DiI-LDL and DiI-ox-LDL uptakes by the arterial walls. The results also showed that the values of DiI-ox-LDL uptake were higher than those of DiI-LDL uptake at the same sample position in both the normal flow group and the swirling flow group. Most interestingly, the experimental results found that the percentage increase in DiI-ox-LDL uptake was much larger than that in DiI-LDL uptake when the perfusion duration increased from 3 to 24 h. In conclusion, the present study substantiated the hypothesis that the spiral flow in the arterial system plays a beneficial role in protecting the arterial wall from atherogenesis. Meanwhile, it supported the concept that the receptor-mediated bindings of LDL uptake, the barrier function of the arterial endothelial linings and the mass transport phenomenon of LDL concentration polarization are all involved in the infiltration/accumulation of atherogenic lipids within the arterial wall.

Keywords: atherosclerosis, low-density lipoproteins, swirling flow

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Introduction

Atherosclerosis is a degenerative disease that affects relatively large arteries by progressive thickening and eventual hardening of the vessel wall through the formation of atheromatous plaques rich in cholesterol content. There is evidence suggesting that in these atherosclerotic lesions, oxidative modification of low-density lipoprotein (ox-LDL) in the vessel wall is the key event in atherogenesis.[1,2] Because cellular cholesterol concentrations negatively regulate the expression of LDL receptors, exposure of vascular cells to high concentrations of LDL does not normally lead to significant LDL uptake or foam cell formation.[3] However, unlike native LDLs, ox-LDLs are not recognized by the LDL receptors, but are taken up in a non-regulated manner by the scavenger receptors in vascular cells. Moreover, in pathological conditions such as atherosclerosis, when subjected to ox-LDL, the endothelium barrier covering the blood vessel wall becomes more permeable to atherogenic lipids.[4] This process leads to the accumulation of cholesterol in the vascular cells, forming foam cells, the hallmark of the atherosclerosis lesion.

Hemodynamic studies revealed that the blood flow in the aortic arch took a form with a corkscrew-like pattern.[5,6] It was also found that the ascending aorta is not the only place in the circulation where spiral or swirling blood flow was observed. Stonebridge *et al.*[7] evidenced the existence of spiral blood flows in human infrainguinal blood vessels and found that out of the 75 arteries examined, 51 had spiral folds on their endo-luminal surfaces. This kind of flow pattern was believed to have certain beneficial effects[6,8,9] with a possible tendency of sparing the arterial wall from atherosclerotic plaques. It was found that carotid atheromatous disease was associated with a reduction in the prevalence of systolic spiral flow pattern in the aortic arch.[10]

Due to the fact that the early event leading to the genesis of atherosclerosis is the accumulation of cholesterol and other lipids within the arterial wall, we hypothesize that the spiral or swirling flow observed in the arterial system may have positive effects on the transport of atherogenic lipids in the lumen of the artery, therefore suppressing lipid infiltration/accumulation in the arterial wall. To

verify this hypothesis, in the present article, we designed a comparative study and investigated the effect of spiral or swirling flow on the spatial distribution of LDL and ox-LDL uptakes in the rabbit thoracic aorta.

Materials and methods

Spiral flow guider design

In the present study, a swirling flow guider for the test vessels was fabricated to generate a stable swirling flow in the test vessels. Figure 1 shows the geometry of the flow guider. The guider had an axial length of 6.5 mm. The internal diameters of its inlet and outlet were 2 and 3 mm, respectively. The swirling flow guider model was created using the computer-aided design (CAD) software SolidWorks 2006. In order to meet the requirement of size and precision, the guider was fabricated using a photosensitive resin with laser rapid prototyping technology.

Lipoprotein isolation, modification and labeling

Native LDLs were prepared and purified from the fresh plasma obtained from healthy volunteers by gradient ultracentrifugation according to the method of Redgrave *et al*.[11] Oxidized LDLs were prepared by incubation of ethylenediaminetetraacetic acid (EDTA)-free LDLs with 5 μmol/L $CuSO_4$ in phosphate-buffered saline (PBS) for 18 h at 37°C. Then 0.24 mmol/L EDTA was added to stop oxidation. Ox-LDLs were concentrated with Centriflo Cones (Bio-Rad, Hercules, CA, USA) (2200 rpm, 20 min, 4°C) and washed twice with PBS buffer.[12]

3,3′-Dioctadecylindocarbocyanine (DiI)-LDLs and DiI-ox-LDLs were prepared by addition of 75 μL of 3 mg/mL DiI (Biomedical Technologies Inc, Stoughton, MA, USA) to 4 mg (protein) of LDL, or ox-LDL in 8 mL of lipoprotein-deficient serum ($d > 1.21$ g/mL plasma fraction). After overnight incubation at 37°C, labeled lipoproteins were re-isolated by ultracentrifugation. The labeled LDLs were added to the Dulbecco's modified Eagle's medium (DMEM) cell culture medium that was used as the experimental perfusion solution. Then, the concentration of the labeled LDLs in the prepared perfusion solution was determined by measuring the protein concentration of the labeled LDLs by Bradford Protein Assay Kit (Sigma, St Louis, MO, USA).

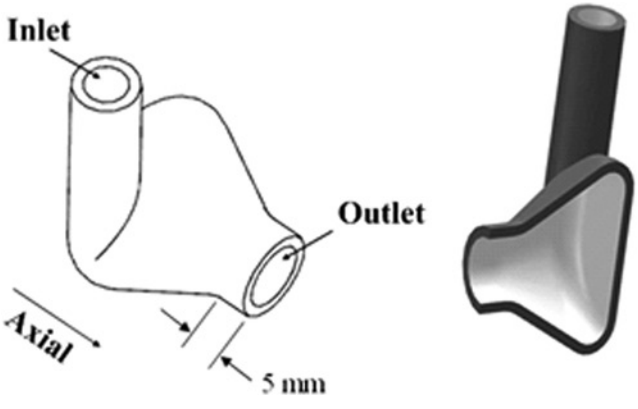

Figure 1 Model of the spiral flow guider. The geometrical parameters of the model were based on the measurements of the rabbit thoracic aorta

Arteries preparation

Male New Zealand white rabbits weighing 3.0 ± 0.1 kg were obtained from Laboratory Animal Center, Peking University (Beijing, China). The study followed a protocol approved by the institutional committee on animal use, and all animal care complied with the 'Principles of Laboratory Animal Care' and the 'Guide for the Care and Use of Laboratory Animals' (NIH Publication No. 86-23, revised 1985). Rabbits were anesthetized intravenously through the right marginal ear vein with a mixture of xylazine (2.2 mg/kg) and ketamine (22 mg/kg). Then the thoracic aorta was exposed and the surrounding tissue was gently removed. In order to minimize damage to the endothelium, we kept the vessel pressurized throughout the experiment by a reservoir placed 1.0 m 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). The aorta was thoroughly examined for leakage *in situ* by pressurization of the vessel; any points on the vessel that leaked were sealed by coagulation with an electrical fulgurator. The experimental aortic section was flushed slowly to wash out any remaining blood. The cannulae were fixed rigidly onto a metal frame to hold the thoracic aorta segment in its *in vivo* length. The cannulae were then advanced approximately 2 mm, respectively, 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 process, Krebs solution was constantly applied to the outer surface of the vessel to prevent it from drying.

Perfusion solution

For each experiment, cell culture medium DMEM containing fetal calf serum (10%), penicillin (100 IU/mL) and streptomycin (100 μg/mL) (Sigma Chemical, St Louis, MO, USA) was freshly prepared as the experimental perfusion solution, in which DiI-LDLs or DiI-ox-LDLs were added at a concentration of 10 μg/mL. The pH value of the perfusion solution was adjusted to 7.2.

Perfusion system

Figure 2 shows a schematic drawing of the experimental perfusion system. It consisted of a head tank, a downstream collecting reservoir, a peristaltic flow pump to circulate the perfusion fluid (DMEM, a cell culture medium) and a blender of air and CO_2 with a constant temperature. All of the components of the perfusion system were connected using tygon tubing. The flow rate was controlled by adjusting the height of the overflow head tank and the resistance of the needle valve so that both the desired flow rate and a perfusion pressure could be achieved simultaneously.

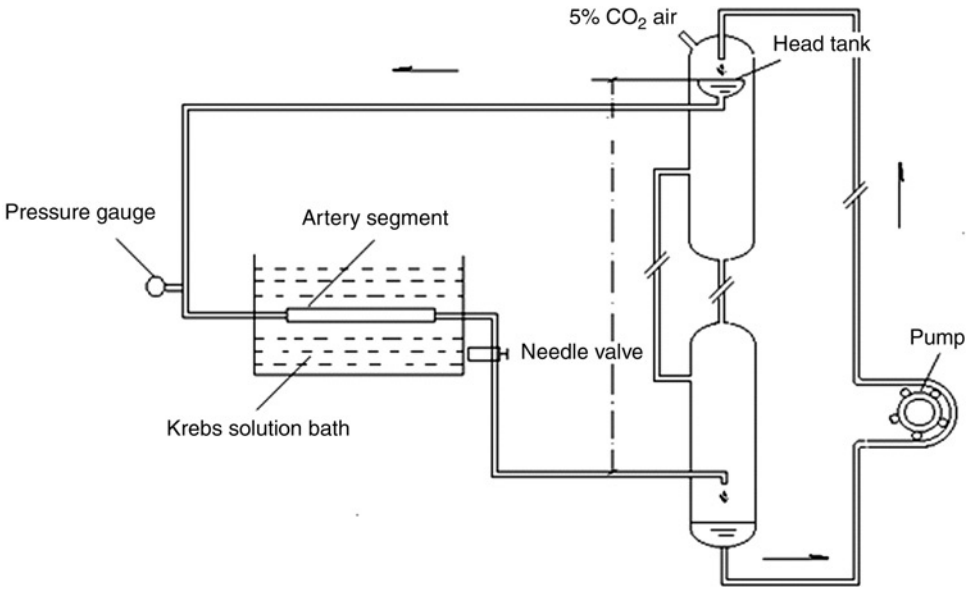

Figure 2 Schematic drawing of the experimental perfusion system. The overflow head-tank provides a steady flow to the test thoracic aorta segment

A pressure transducer and a flow meter were used to monitor the perfusion pressure and the flow rate through the artery, respectively. The harvested artery with the metal frame was immersed in Krebs solution and then connected horizontally to the overflow head tank perfusion system.

Measurement of DiI-LDL and DiI-ox-LDL uptake along the thoracic aorta

A standard procedure was followed throughout the entire experiment. For all of the experiments, the perfusion pressure, flow rate and DiI-LDL or DiI-ox-LDL concentration of the perfusion solution were kept at 90 mmHg, 40 mL/min and 10 μg/mL, respectively.

The test arteries were divided into two perfusion groups: the normal flow group and the swirling flow group. For the swirling flow group, the test vessel was connected to the spiral flow guider that was used to generate swirling flow in the test vessel. During the experiment, the flow rate and the pressure in the thoracic aorta segment were monitored constantly. The experiments were carried out for two perfusion durations (3 and 24 h) at a temperature of $37 \pm 1^{\circ}$C.

At the completion of the experimental perfusion, the thoracic aorta segment was flushed with PBS buffer to wash off the dissociative DiI-LDL or DiI-ox-LDL, then the thoracic aorta segment was fixed with a modified Karnovsky's fixative (2% paraformaldehyde, 1.5% glutaraldehyde, 2.5 mmol/L $CaCl_2$ in 0.1 mol/L cacodvlate buffer, pH 7.2) at 90 mmHg for 24 h. The thoracic aorta segment was dissected from the cannulas and given a brief rinse in PBS. The tissues around the cannulas were discarded because of the possible damage to the endothelium due to the ligatures. The test vessel was opened axially while 10 tissue sample segments were collected from the vessel (each of 2 mm in length).

Then the tissue samples were digested in a tissue solubilizer (Perkin-Elmer Life and Analytical Sciences, Boston, MA, USA) and complete lysis of the tissues was achieved by gentle pipetting of the lysate, followed by removal of cell debris by centrifugation. The fluorescence intensity of the tissue samples was measured with a spectrofluorometer (Perkin-Elmer LS-50B, Westport, CT, USA) at the excitation and emission wavelengths of 514 and 550 nm, respectively. The fluorescence intensity was then converted to the concentration of lipoproteins with the calibration curve of DiI-LDL. The amount of lipoproteins uptaken by the arterial wall was expressed as the weight of lipoproteins taken up per effective surface area per hour (ng/cm^2 h).

Characterization of the flows in the two perfusion groups

To study the effect of swirling flow on LDL and ox-LDL uptake by the wall of the thoracic aorta, the flow generated in the test vessel with the spiral flow guider was characterized numerically and compared with the flow in the normal flow model. The model used in the spiral flow study was a rigid vessel combined with the spiral flow guider. As for the comparison, the model used in the normal flow study was also a rigid vessel, but without the flow guider. The length and the inner diameter of both vessels are 20 and 3 mm, respectively. The experimental perfusion solution in the present study was DMEM, which was assumed to be a homogeneous, incompressible and Newtonian fluid. Its viscosity and density were measured as 1.3×10^{-3} and 1047 kg/m^3, respectively. In the flow simulation, the Reynolds number was set at 650 based on the flow rate in the experiment.

The boundary conditions were set as follows: (1) because flow disturbance must have occurred at the inlet of the models due to the connection, a uniform velocity profile was assumed at the inlet based on the flow rate of 40 mL/min used in the experiment; (2) the outlet condition was set to be outflow, which applied a zero diffusion flux condition like a fully developed flow; and (3) the wall of the vessels was assumed to be rigid and non-slip.

The numerical simulation was based on the three-dimensional incompressible Navier–Stokes equations as follows:

$$\rho(\vec{u}\cdot\nabla)\vec{u}+\nabla p-\mu\Delta\vec{u}=0 \quad (1)$$

$$\nabla\cdot\vec{u}=0 \quad (2)$$

where $\vec{u}$ is the fluid velocity vector; ρ and μ are the density and viscosity of the perfusion solution, respectively; and p is the pressure.

A finite volume method was used in the simulation. The computational meshes of the models created using the CAD software Gambit 2.2 (ANSYS, Inc, Canonsburg, PA, USA) were all unstructured hexahedron grid. The commercially available computational fluid dynamics (CFD) code, FLUENT 6.2 (ANSYS, Inc) was employed in the numerical simulation. Discretization of the pressure and momentum at each control volume was in a second-order scheme. The iterative process of computation was terminated when the residual of mass and velocity were all less than the convergence criterion, 1.0×10^{-5}.

Statistical analysis

Experiments were repeated at least three times under the same flow conditions. Statistical analyses were performed using the Origin 7.0 software. The results were presented as mean ± SEM.

Results

General flow patterns

Figure 3 shows the flow patterns (velocity vectors at 5 sections along the vessels) obtained numerically for the normal flow group and the swirling flow group. As evident from the figure, an apparent spiral or swirling flow was created in the test section of the swirling flow group. Meanwhile, in the normal flow group, no spiral flow occurred. The numerical simulation revealed that when compared with the normal flow group, the swirling flow created by the flow guider significantly altered the velocity profiles in the straight test section of the vessels. Different from the flow in the normal flow group, the maximums of the velocity profiles shifted away from the tube center. The simulation also revealed that the spiral flow created by the flow guider was attenuating progressively along the vessels.

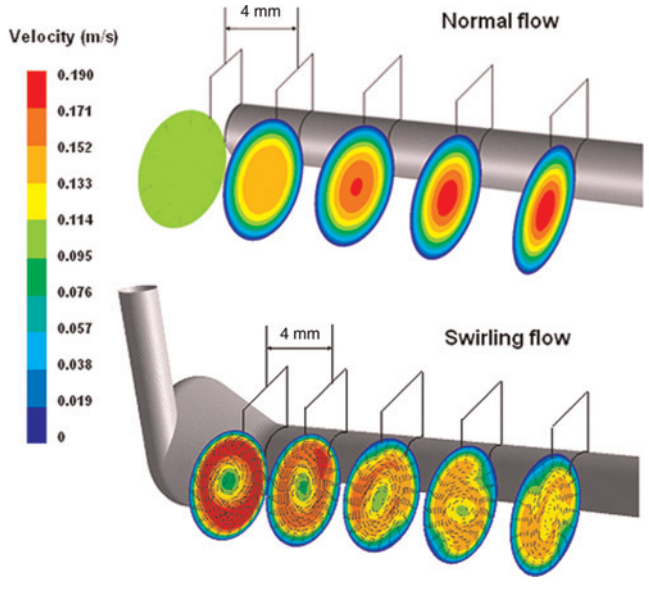

Figure 3 Velocity vector features at five cross-sections perpendicular to the tube center axis in the two groups, colored by velocity magnitude, showing an apparent helical flow in the test tube with the flow guider. Flow velocity in the tube has been redistributed due to the rotation of the flow (A color version of this figure is available in the online journal)

Helicity

In order to better characterize the flow in the swirling flow model, helicity defined by the following equation was used to evaluate the strength of the swirling flow:[9]

$$H=(\nabla\times\vec{V})\cdot\vec{V}$$

Figure 4 shows the area-weighted average of helicity of the flows at different cross-sections along the vessel. As illustrated in the figure, the helicity of the swirling flow created by the flow guider is the highest at the origin of the test vessel, decreases along the vessel and drops drastically after $L=10$ mm. At $L=20$ mm, the averaged helicity reduced to 4.2 m/s^2, which is only 60% of the helicity at the origin of the test vessel.

Distribution of DiI-LDL/DiI-ox-LDL uptake along the thoracic aorta segment

Figure 5 illustrates the distribution of DiI-LDL/DiI-ox-LDL uptake along the tested thoracic aorta segment under both normal perfusion flow and swirling flow conditions. As shown in the figures, under normal flow condition the uptakes of both DiI-LDL and DiI-ox-LDL distributed almost uniformly along the test artery segment for the two perfusion durations (Figures 5a and c). On the other hand, under swirling flow condition, the uptakes of both DiI-LDL and DiI-ox-LDL were quite low at sample position 1 when compared with the uptakes of the normal flow

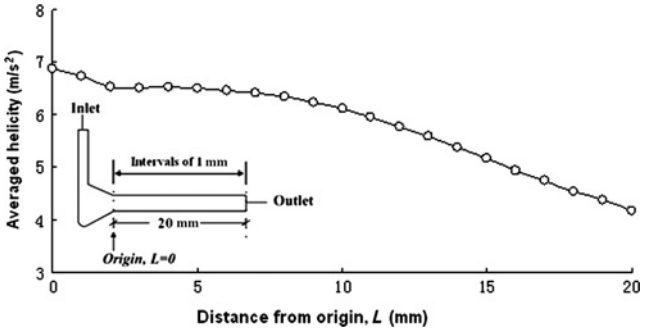

Figure 4 Plot of area-weighted average of helicity along the distance (*L*) from the origin of the spiral flow model. The origin ($L=0$) to $L=20$ mm with intervals of 1 mm

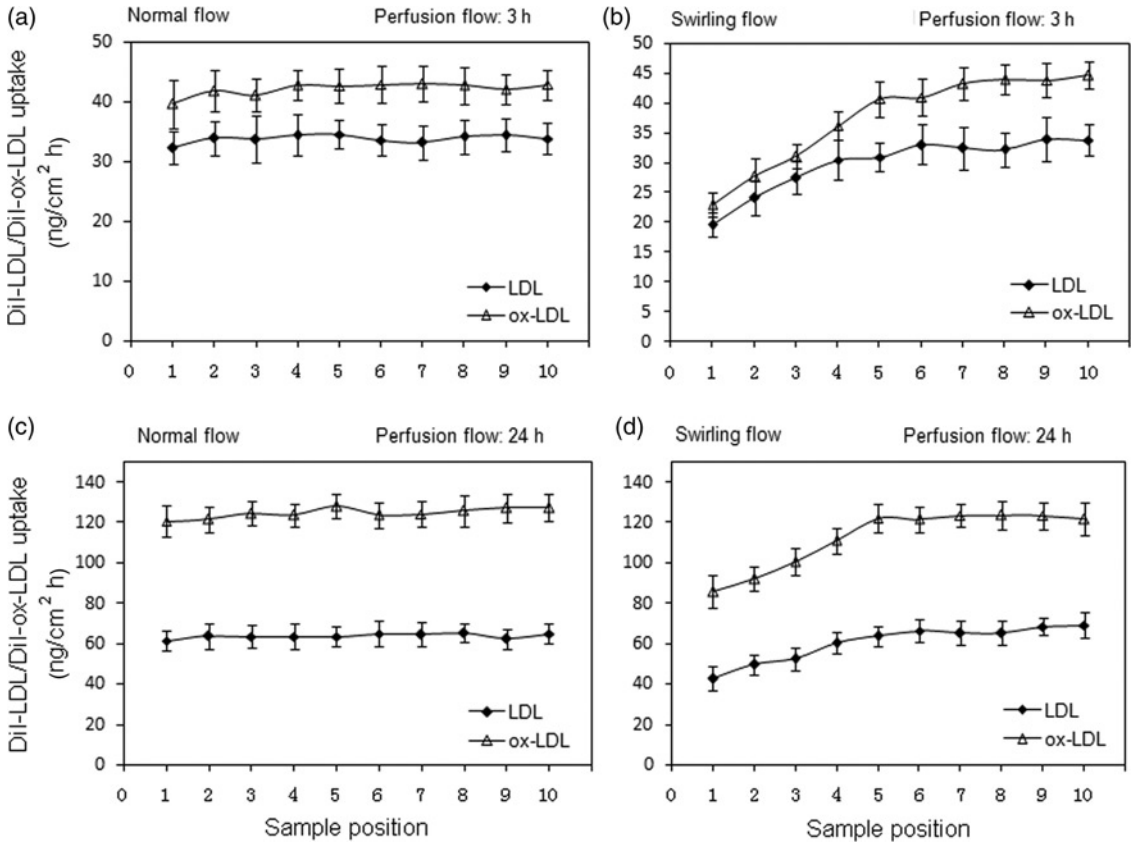

Figure 5 Distributions of 3,3′-dioctadecylindocarbocyanine (DiI)-LDL and DiI-ox-LDL uptakes along the test thoracic aorta segment for the two flow groups. (a) Normal flow group, perfusion duration of three hours; (b) Swirling flow group, perfusion duration of three hours; (c) Normal flow group, perfusion duration of 24 h; (d) Swirling flow group, perfusion duration of 24 h. Bars represent mean ± SEM from six different experiments

group, then increased gradually along the test artery segment as the swirling flow was attenuating progressively along the test vessel, and reached a constant value after sample position 6, which was the same as the corresponding value of the normal flow group.

The experimental results also showed that along the test artery segment, the value of DiI-ox-LDL uptake was higher than that of DiI-LDL uptake at the same sample position. Moreover, when perfusion duration increased from 3 to 24 h, the uptake of DiI-ox-LDL by the test artery increased very sharply for both flow groups.

Figure 6 shows the overall average values of DiI-LDL and DiI-ox-LDL uptakes in the test artery segment. As evident from the figure, the spiral or swirling flow generated in the test vessel could significantly reduce the DiI-LDL and DiI-ox-LDL uptakes compared with the normal flow at the same flow rate. Meanwhile, the overall average value of DiI-ox-LDL uptake was obviously higher than that of DiI-LDL uptake under the same condition. Most interestingly, the experimental results showed that when the perfusion duration increased from 3 to 24 h, the percentage increase in DiI-ox-LDL uptake was much larger than that in DiI-LDL uptake. Under normal flow condition, the respective percentage increase of DiI-LDL and DiI-ox-LDL uptake was 99% and 195% when the perfusion duration increased from 3 to 24 h. Under swirling flow condition, the percentage increase of DiI-LDL and DiI-ox-LDL uptake was 98% and

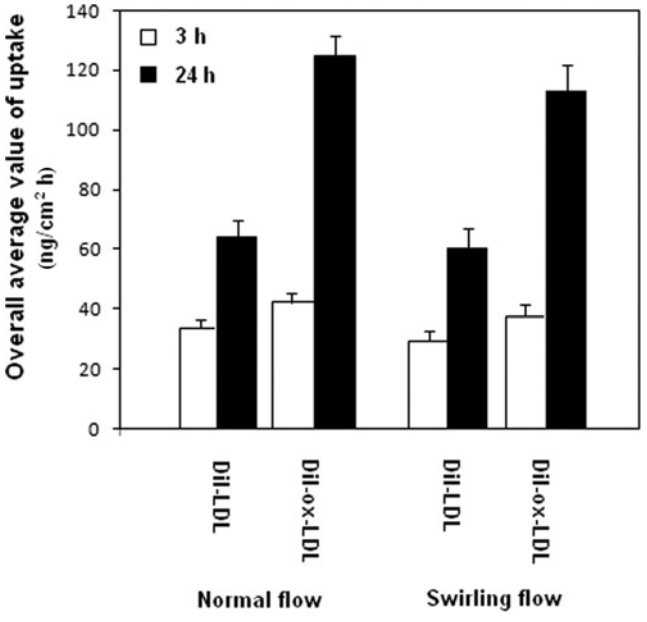

Figure 6 Overall average values of 3,3′-dioctadecylindocarbocyanine (DiI)-LDL/DiI-ox-LDL uptakes under normal flow and swirling flow conditions. Bars represent mean ± SEM from six different experiments

194%, respectively, when the perfusion duration increased from 3 to 24 h.

Discussion

It has been widely recognized that flow-induced shear stress is one of the most important hemodynamic factors in the localization of atherogenesis.[13] Nevertheless, due to the fact that the early event leading to the genesis of atherosclerosis is the accumulation of cholesterol and other lipids within the arterial wall, in recent years, researchers have been paying more and more attention to material transport in the circulation and the interactions of blood cells with the blood vessel walls.[14] From the viewpoint of mass transport, the concepts of 'residence time' for atherogenic agents[15] and deposition of atherogenic particles onto the blood vessel walls[16] were proposed to account for the localization of atherogenesis.

Deng *et al.*[17] have theoretically predicted a mass transport phenomenon of concentration polarization of atherogenic LDLs, with the LDLs increasing in concentration from bulk value towards interface within the arterial system and verified it experimentally *in vitro*.[18] Apparently, the occurrence of concentration polarization of LDLs in the arterial system can affect the 'residence time' and the deposition of atherogenic particles. They therefore have suggested that flow-dependent LDL concentration at the blood/wall interface may play an important role in the localization of atherogenesis.

The presence of spiral blood flow at several sites of the cardiovascular system has been well documented.[5–7,19] Physiological implications of spiral flow in the vascular system have been suggested.[7,20] We believe that the swirling motion of blood flow in the human aortic arch is a typical example of 'form follows function' in the vascular system and it is the spiral or swirling blood flow that provides guaranties for the inner surface of the aortic arch to get a smooth and even washing by the blood so that atherosclerotic lipids can hardly infiltrate/accumulate within the wall of the aortic arch. This may account for why the ascending aorta and the arch are relatively free of atherosclerosis.

To substantiate this hypothesis, in the present *ex vivo* study, we studied the effect of swirling flow on the distribution of DiI-LDL and DiI-ox-LDL uptakes in a straight segment of the rabbit thoracic aorta. The swirling flow in the test vessel was created by a well-designed spiral flow guider.

The measured results showed that under swirling flow condition, the values of both DiI-LDL and DiI-ox-LDL uptakes were lower when compared with those under normal flow condition. They increased gradually along the thoracic aorta from sample positions 1–5 with the attenuation of the swirling flow strength, reaching constant values after sample position 6, which were almost the same as the corresponding values for the normal group. The present study therefore indicated that the swirling action of the flow did have a beneficial effect on lipid infiltration into the arterial wall, possibly through the swirling-flow-induced in-plane mixing, which reduced concentration polarization[17] or concentration of DiI-LDLs/DiI-ox-LDLs on the luminal surface of the artery and suppressed the interaction of DiI-LDLs/DiI-ox-LDLs with the wall of the test vessel. Nevertheless, when the rotation strength of the swirling flow was lower than a threshold value, the beneficial effect of the swirling flow on lipid infiltration or uptake would be compromised.

Several lines of evidence suggest that in atherosclerotic lesions, oxidative modification of LDL in the vessel wall is the key event in atherogenesis.[1,2] Unlike the uptake of native LDL by cells of the arterial wall (such as the endothelial cells and the smooth muscle cells) which is regulated by the LDL receptor and thus cannot cause massive cellular cholesterol accumulation, the uptake of ox-LDL by the scavenger cells can lead to foam cell formation.[21] Since unmodified LDL did not appear to induce lipid loading of vascular cells, oxidized modification of LDL was tested for their ability to induce increased cellular uptake. Experiment results by Aviram[22] showed that LDL modification by cholesterol oxidase induced enhanced uptake and cholesterol accumulation in cells. Hoff and O'Neil[23] found that LDL extracted from human atherosclerotic lesions showed greater uptake, degradation and/or stimulation of cholesterol esterification than plasma LDL in primary cultures of macrophages.

Similar patterns of LDL uptake and ox-LDL uptake were observed in the present study in which the overall DiI-ox-LDL uptake was higher than the DiI-LDL uptake under the same flow condition. Most interestingly, the experimental results showed that the percentage increase in DiI-ox-LDL uptake by the test vessel was much larger than the percentage increase in DiI-LDL uptake when the perfusion duration increased from 3 to 24 h.

Vascular endothelium exposed to ox-LDL, either *in vitro* or *in vivo*, shows an increased permeability,[24] a telltale sign of endothelial dysfunction. It has been also found that ox-LDL induces cell contraction, the formation of actin stress fibers and intercellular gaps, leading to an increase in endothelial cell permeability to ox-LDL.[25,26] Recent studies revealed that toxic effects of ox-LDL could induce necrosis and/or apoptosis in a variety of cells, including the endothelial cells,[27] cultured smooth muscle cells[28] and macrophages.[29] Consequently, this may lead to damages to vascular endothelium linings. In the present study, since perfusion duration of three hours could not provide enough time for the DiI-ox-LDL to alter the endothelial linings, the rate of DiI-ox-LDL uptake by the arterial wall was relatively low. When perfusion duration increased to 24 h, the toxicity of DiI-ox-LDL would induce cell contraction and damage the endothelial linings of the test vessels,[24,25,30] which led to a much higher rate of DiI-ox-LDL uptake. Hence, the DiI-ox-LDL uptake by the test vessel increased much sharply when perfusion time increased from 3 to 24 h when compared with DiI-LDL uptake.

Most interestingly, the results from the present study also indicated that the DiI-LDL or DiI-ox-LDL infiltration/uptake was through the mass transport mechanism of concentration polarization proposed by Deng *et al.*,[17] not through a diffusion controlled mass transfer mechanism. If the infiltration or uptake were diffusion-dependent, the magnitude of the uptake should have been proportional

to $D(\partial c/\partial r)_w$ (or Dc_o), i.e. it should have been around 0.36 ng/cm^2 h at most, because the diffusion coefficient of LDLs is in the order of 10^{-8} cm^2/s.[31] However, the average value of the infiltration or uptake measured in the present study was more than 100 times of the value by the diffusion controlled mass transfer mechanism. According to Deng *et al.*[17] when concentration polarization of LDLs occurs on the luminal surface, the infiltration of LDLs into the arterial wall is convective-dependent and can be described by the equation $v_w c_w - D(\partial c/\partial n) = \dot{m}$, where $\dot{m}$ is LDL infiltration or uptake; c_w is the concentration of LDLs at the luminal surface of the artery; and v_w is the filtration rate across the vessel wall that is in the order of 10^{-6} cm/s (4.0×10^{-6} cm/s). In this case, the infiltration or uptake of LDLs is in the range of 20–150 ng/cm^2 h, which is close to our experimental results. We therefore can conclude that the infiltration or uptake of LDLs was convective-dependent, not diffusion-dependent.

Here it should be mentioned that due to the difference in the parthenogenesis of atherosclerosis between human and the rabbit,[32,33] it may not be suitable to use rabbit arteries as experimental models of atherogenesis. Nevertheless, the main purpose of the present study is to investigate the physiological significance of the spiral flow in the arterial system from the viewpoint of atherogenic lipid transport. The results evidenced apparent reduction in LDL/ox-LDL uptake in the swirling flow group. This might be the result of both the swirling-flow-enhanced wall shear stress and the swirling-flow-induced in-plane mixing, which possibly lowered the concentration of LDL/ox-LDL near the wall and suppressed the interaction of LDL/ox-LDL with the wall of the test arteries. The findings of the study therefore clearly indicate that the swirling motion of blood flow in the vascular system might indeed exert a beneficial effect to protect the arteries from excessive lipid infiltration/accumulation in the arterial wall.

Arterial bypass treatments and endovascular stents are widely used in vascular surgery to relieve arterial occlusions, but the re-occlusion problem that contributes to poor long-term patency rates remains unresolved. We believe that the beneficial function of swirling flow may be applied to vascular interventional treatments by intentionally inducing rotation flows in bypassed arteries[34] and endovascular stents.[35]

Conclusion

The present study has provided evidence to support our hypothesis that swirling or spiral flow in the arterial system has a beneficial effect on the transport of atherogenic lipids in the lumen of the artery, therefore reducing concentration polarization of atherogenic lipids and suppressing lipid infiltration/accumulation in the arterial wall. Meanwhile, the present experimental study also supports the concept that the receptor-mediated bindings of LDL uptake,[3] the barrier function of the arterial endothelial linings[4] and the mass transport phenomenon of LDL concentration polarization[17] are all involved in the infiltration/accumulation of atherogenic lipids within the arterial wall.

Author contributions: All authors participated in the design, interpretation of the studies, analysis of the data and review of the manuscript. ZFD designed the study, carried out the experiment and participated in statistical analyses and manuscript preparation; YBF was responsible for the design of the spiral flow guider and the flow simulation; XYD participated in the study design, interpretation of the results and manuscript preparation; FZ participated in the design of the spiral flow guider and the numerical simulation; HYK was responsible for statistical analyses.

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