Partial carotid ligation is a model of acutely induced disturbed flow, leading to rapid endothelial dysfunction and atherosclerosis

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Partial carotid ligation is a model of acutely induced disturbed flow, leading to rapid endothelial dysfunction and atherosclerosis. Am J Physiol Heart Circ Physiol 297: H1535–H1543, 2009. First published August 14, 2009; doi:10.1152/ajpheart.00510.2009.—Atherosclerosis is a leading cause of morbidity and mortality in developed countries and is shown to be an inflammatory disease (22, 28). Although multiple systemic factors such as hypercholesterolemia, diabetes, hypertension, and smoking are well-known risk factors, atherosclerosis occurs preferentially at particular areas of disturbed flow characterized by low and oscillatory wall shear stress (WSS) in branched or curved arteries. Using this model and method, we found that partial ligation causes upregulation of proatherogenic genes, downregulation of antiatherogenic genes, endothelial dysfunction, and rapid atherosclerosis in 2 wk in a p47-phox-dependent manner and advanced lesions by 4 wk. We found that partial ligation results in endothelial dysfunction, rapid atherosclerosis, and advanced lesion development in a physiologically relevant model of disturbed flow. It also allows for easy and rapid intimal RNA isolation. This novel model and method could be used for genome-wide studies to determine molecular mechanisms underlying flow-dependent regulation of vascular biology and diseases.

Shear stress; endothelial dysfunction; inflammation; atherosclerosis; endothelium

Atherosclerosis is a leading cause of morbidity and mortality in developed countries and is shown to be an inflammatory disease (22, 28). Although multiple systemic factors such as hypercholesterolemia, diabetes, hypertension, and smoking are well-known risk factors, atherosclerosis occurs preferentially at particular areas of disturbed flow characterized by low and oscillatory wall shear stress (WSS) in branched or curved arteries (19, 34). In contrast, straight arterial regions are exposed to high and stable shear stress and are well protected from atherosclerosis (34). Despite the close association between the two, evidence directly linking disturbed flow conditions to atherosclerosis has been lacking, and the mechanisms responsible for pro- and antiatherogenic effects of shear stress are still incompletely understood.

Shear stress is the tangential force imparted by viscous fluid flowing over endothelial cells (2, 16). Endothelial cells sense changes in shear stress and trigger mechanosensitive cell signaling events (2, 10). This in turn regulates endothelial function and structure, which affects vascular wall biology and pathophysiology (5). Endothelial cells in straight part of the arteries experience unidirectional, high-time-averaged WSS (laminar shear). Laminar shear induces acute and chronic changes in endothelial cells leading to cell alignment, vasodilation, inhibition of inflammation, and coagulation, which are atheroprotective responses. In contrast, disturbed flow stimulates proatherogenic responses, including cell turnover, inflammation, thrombosis, and oxidative stress (2, 5, 10, 16).

The differential mechanisms by which disturbed and stable flow promotes and inhibits atherogenesis, respectively, have been a subject of intense study, mostly using cultured endothelial cells (2, 10, 16). To define molecular mechanisms responsible for these changes, investigators have carried out DNA microarray studies using endothelial cells and have identified shear sensitive genes such as kruppel-like factor 2 (kif-2), endothelial nitric oxide synthase (eNOS), vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), and bone morphogenic protein 4 (BMP-4) (3, 7, 9, 11, 12, 23, 29, 31). Although these in vitro studies have provided critical insights regarding shear sensitive mechanisms in cultured endothelial cells using modeled flow conditions, it cannot be assumed whether identical mechanisms are responsible for flow-dependent changes in vessels in vivo and vascular diseases such as atherosclerosis.

Several mouse models have been used to examine the role of shear sensitive genes and proteins in atherogenesis. These models include 1) naturally occurring atheroprone regions of arterial tree, 2) complete ligation of common carotid artery, and 3) perivascular shear modifier cuff placed around common carotid artery in mice deficient in apolipoprotein E (ApoE KO) or low-density lipoprotein receptor (LDLR KO). Naturally occurring atheroprone regions, such as lesser curvature of the aortic arch and root of innominate artery, are exposed to disturbed flow (33). These flow-disturbed areas in ApoE KO and LDLR KO mice develop measurable atherosclerotic lesions upon feeding atherogenic diets for at least 2–3 mo (25). Although these studies show that flow disturbance is associated with atherogenesis, they do not provide evidence directly linking disturbed flow to atherosclerosis, since the flow disturbance is chronic from the early developmental stage and not due to an acute flow alteration. Other difficulties include small sample area of atherosclerosis, making reproducible endothelial RNA isolation from these areas in sufficient quantity and purity difficult (25). Complete ligation of common carotid artery has also been used to study atherosclerosis (17, 20). This model results in no flow through the left common carotid artery (LCA) and may be associated with endothelial denudation and...
thrombosis. It may not be a physiologically relevant model in the study of shear-mediated atherosclerosis. (36). The perivascular shear modifier cuff model was recently reported and applied to the study of shear-mediated vascular inflammation and atherogenesis. It was shown to create three distinct regions of shear stress via application of a perivascular cuff around LCA [lower shear (proximal to the cuff), high shear (inside the cuff), or oscillatory shear (distal to the cuff)] (6). It is important to note that low and oscillatory shear stress, two characteristics of disturbed flow, were predicted to be in two separate regions. The region of oscillatory shear stress (distal to cuff) had high average shear stress, and the region of low average shear stress (proximal to cuff) did not have an oscillatory shear stress component (6). This dissociation of oscillatory shear from low shear stress is a unique characteristic of the cuff model, and it is different from typical disturbed flow conditions displaying colocalized low and oscillatory shear stress.

Partial carotid ligation has previously been described as a model of flow reduction and has been used in the study of vascular remodeling (18, 32). In this model, three of the four caudal branches of LCA were ligated, resulting in a substantial flow reduction in LCA (18). Korshunov and Berk (18) showed that partial ligation of LCA in C57Bl/6 mice results in outward remodeling of LCA with intima-media-adventitial thickening. Mechanistically, the carotid remodeling by partial ligation involved macrophage infiltration, smooth muscle proliferation, and extracellular matrix reorganization (18). Sullivan and Hoying (32) used partial ligation in fibroblast growth factor 2 (FGF2) KO and wild-type mice to study the role of FGF2 in flow-dependent vascular remodeling. Although these studies have provided some insight into flow-mediated vascular remodeling, changes in shear stress levels or development of atherosclerosis in response to partial carotid ligation have not been described. We hypothesized that partial carotid ligation causes low and oscillatory shear stresses two major characteristics of disturbed flow that have been closely associated with atherogenesis (5). We further hypothesized that this disturbed flow would cause atherosclerosis in hyperlipidemic conditions. To test these hypotheses, we first measured flow velocity and direction as well as vessel dimensions by a high-resolution ultrasound system before and after partially ligating LCA. These measurements were then used for computational fluid dynamics (CFD) modeling to estimate shear stress magnitudes and directions. Next, ApoE KO mice were partially ligated and fed a high-fat diet to determine if this would result in atherosclerosis in LCA. In addition, we used a simple method of isolating intimal RNA in significant quantity and purity from carotid arteries for mechanistic studies. Here, we report that partial ligation causes disturbed flow, induces atherosclerosis rapidly within 2 wk upon feeding a high-fat diet, and that the additional simple RNA isolation method is extremely useful to determine gene expression changes occurring in carotid intima in response to changes in shear stress.

METHODS

**Animal studies with partial ligation.** All animal studies were carried out by procedures approved by the Emory University Institutional Animal Care and Use Committee (IACUC). Male and female mice were ligated between 6 and 8 wk of age. C57Bl/6 and ApoE KO mice were obtained from Jackson Laboratories, and mice deficient in p47phox in ApoE KO background (p47phox_ApoE DKO) were generated as previously described (1). All mice were fed a chow diet and water ad libitum until partial ligation. Partial ligation of LCA was carried out as previously described (32) with minor modifications. Briefly, anesthesia was induced by intraperitoneal injection of xylazine (10 mg/kg) and ketamine (80 mg/kg) mixture. Epilated area was disinfected with betadine, and a ventral midline incision (4–5 mm) was made in the neck. LCA was exposed by blunt dissection. Three of four caudal branches of LCA (left external carotid, internal carotid, and occipital artery) were ligated with 6–0 silk suture (Fig. 1A) while the superior thyroid artery was left intact. The incision was then closed with Tissue-Mend (Veterinary Product Laboratories). Mice were monitored until recovery in a chamber on a heating pad following surgery. A single subcutaneous injection of buprenorphine (0.1 mg/kg) was given 12 h after partial ligation for additional pain relief.

For atherosclerosis studies, ApoE KO and p47phox_ApoE DKO mice were fed the Paigen’s high-fat diet (25) (Science Diets) immediately following partial ligation until killed, from 2 days up to 6 wk. C57Bl/6 mice were continued on chow diet postligation.

**High-resolution ultrasound measurements.** All ultrasound measurements were taken using a VIVO 770 high-resolution in vivo microimaging ultrasound system with a 30-MHz mouse probe (Visu-alsontics). Mice were anesthetized with inhaled isoflurane, and body temperature was maintained on a heated stage for the duration of studies. Levels of anesthesia, heart rate, temperature, and respirations were continuously monitored. Pulse wave Doppler mode was used at the inlet, midpoint, and outlet of the common carotid arteries (see Fig. 1A) for measuring flow velocity, M-mode for vessel dimensions, and B-mode for vessel length. All measurements were gated to an electrocardiogram and respiration.

**CFD.** The CFD models incorporated the geometry of mouse carotid arteries as determined by the ultrasound system. LCA and RCA of mouse are similar in geometry to a straight tube with different diameters at two ends (inlet and outlet; Fig. 1A), which made a moving mesh CFD model simple. The geometric resolution of ultrasound images was 10 μm. After segmentation and measurement of the diameter waveforms, sequential diameters at three sections (inlet, outlet, and middle sections) over one cardiac cycle were obtained. A three-dimensional model was reconstructed based on the diameters at end diastole, and then a moving mesh was designed for the model according to individual diameter waveform (33). The moving model replicated the geometry and compliance characteristics of the mouse carotid artery. Computations were performed using the commercial CFD-ACE code based on a finite volume method for solving the Navier-Stokes equations as previously described (33). The velocity waveforms at three sections (inlet, outlet, and midpoint; Fig. 1A) were acquired from Doppler measurements. Two end-velocity waveforms were transformed to correspond with flow waveforms according to the diameter waveform. The flow waveforms were the inflow and outflow boundary conditions for CFD modeling. The midpoint velocity waveform was used later to compare with the CFD results at the same section. The blood was assumed to be a Newtonian and incompressible fluid, and the flowing state was assumed to be laminar flow.

**Intimal RNA isolation from carotid arteries.** Mice were killed by CO₂ inhalation according to Emory University’s IACUC protocol and pressure perfused with saline containing heparin (10 U/ml) via the left ventricle after severing the inferior vena cava. LCA and the right common carotid artery (RCA) were then isolated and carefully cleaned of periadventitial fat. The carotid lumen was quickly flushed (few seconds) with 150 μl of QIAzol lysis reagent (QIAGEN) using a 29-gauge insulin syringe in a microfuge tube. The eluate was then used for intimal RNA isolation using an miRNaseasy mini kit (QIAGEN) according to the manufacturer’s instructions. The carotid artery left over after flushing with QIAzol was used to prepare RNA from media and adventitia. Media and adventitia was snap-frozen in liquid nitrogen, pulverized by mortar and pestle, and lysed in QIAzol lysis reagent (700 μl per carotid), and RNA was isolated using the miRNaseasy mini kit as detailed in Supplemental Figs. 1–3 (Supple-
mental material for this article can be found on the American Journal of Physiology: Heart and Circulatory Physiology website).

Immunohistochemical staining studies. Mice were killed and pressure perfused with saline containing heparin as described above. LCA and RCA were collected en bloc with the trachea and esophagus. For frozen sections, tissue was embedded in Tissue-Tek optimum cutting temperature medium, frozen on dry ice, and stored at \(-80^\circ\)C until used. Frozen sections (7 μm) were fixed in acetone for 8 min, blocked with 10% goat serum for 1 hour at room temperature, and incubated with primary antibodies overnight at 4°C (3). To visualize primary antibodies, rhodamine-conjugated secondary antibody was used for 1 hour at room temperature. Nuclei were counterstained with Hoechst no. 33258. Oil red O staining was carried out using frozen sections as described (37). For pentachrome staining, fixed tissue was paraffin embedded, sectioned at 5 μm, and stained with the Russell-Movat pentachrome staining kit (American Master Tech Scientific). Micrographs were taken with a Zeiss (Jena, Germany) epifluorescence microscope. Images were analyzed with NIH Image J software to quantify lesion size in each animal as described (21).

Vascular relaxation study. The vascular relaxation study was carried out using carotid rings obtained from LCA and RCA of partially ligated ApoE KO mice as previously described (24).

Dihydroethidium staining. Thick frozen sections (30 μm) obtained from LCA and RCA of partially ligated mice were stained in 2 μM dihydroethidium (DHE) in PBS for 30 min at 37°C. Slides were mounted with DAKO mounting media and immediately imaged with a Zeiss LSM 510 META confocal microscope as described (30).

Statistical analysis. Data are presented as means ± SE. Student’s t-test for two groups and ANOVA with Games-Howell post hoc tests for comparing multiple groups were carried out using the SPSS program. P < 0.05 was considered statistically significant.

RESULTS

Partial ligation results in low and oscillatory shear stress. To determine whether partial ligation caused changes in shear stress levels and direction, we ligated the external carotid, internal carotid, and occipital arteries of LCA, while leaving
the superior thyroid artery intact in C57BL/6 and ApoE KO mice (Fig. 1A). Next, flow velocity and direction and vessel dimensions of LCA and RCA were determined by high-resolution ultrasonography. This showed a reduction in flow velocity during systole in LCA at 1 and 7 days after ligation as expected (Fig. 1B). Moreover, flow direction was reversed during diastole in LCA at 1 and 7 days after ligation (Fig. 1B). Similar flow profiles were observed at both the predefined inlet and outlet. Flow profiles as shown in Fig. 1B were obtained from the inlet. Blood flow in LCA decreased by 90% at 1 and 7 days after ligation, but blood flow in RCA did not change significantly compared with that of preligation (Fig. 1C). Blood pressure and pulse did not change by partial ligation (Supplemental Fig. 1).

Using values of flow velocity, direction, and vessel dimensions, we performed CFD modeling of LCA and RCA pre- and 1 day postligation. This showed that the WSS value remained positive without any significant difference in RCA both before and after ligation of LCA during the entire cardiac cycle (Fig. 2, A and B). In contrast, the WSS level was reduced during systole compared with that of RCA and became negative (because of flow reversal) during diastole in ligated LCA (Fig. 2, A and B). Time-averaged WSS was reduced from ~110 dyn/cm² preligation to 30 dyn/cm² postligation (Fig. 2B). These results show that partial ligation causes low and oscillatory shear stress in LCA, characteristic of disturbed flow.

**Method of isolating intimal RNA from carotid arteries.**

Lumens of LCA and RCA from sham-ligated C57 mice were flushed one time with Qiazol (Qiagen). The leftover LCA and RCA after collecting intimal RNA were also saved for analysis. RNA was then isolated with the miRNeasy kit (Qiagen), resulting in 10–20 ng total RNA from each eluate (intima), while each leftover vessel (media + adventitia) resulted in ~800 ng to 1 µg of total RNA. To test for endothelial purity of intimal RNA, we performed RT-PCR for platelet endothelial cell adhesion molecule-1 (PECAM-1) and α-smooth muscle actin (α-SMA) (Fig. 3A). Intimal eluate showed endothelial marker PECAM-1 without any evidence of smooth muscle specific α-SMA. Conversely, media + adventitial RNA contained α-SMA without any evidence of PECAM-1. Quantitative real-time PCR (qPCR) and en face protein staining further confirmed the results (Fig. 3, B and C, and Supplemental Fig. 3). This shows that intimal RNA can be obtained from carotid arteries by our simple and reproducible method in sufficient quantity and purity without significant smooth muscle RNA contamination. In addition, media + adventitial RNA can also be isolated from the arteries without significant endothelial contamination.

**Partial ligation downregulates klf-2 and eNOS while up-regulating ICAM-1, VCAM-1, and BMP4.** We tested whether intimal RNA obtained from LCA and RCA by our method could be used to study regulation of mechanosensitive genes in endothelial cells. Intimal RNA from sham and partially ligated C57BL/6 mice were collected 2 days after surgery and analyzed by qPCR. We chose this 2-day time point because we did not observe measurable accumulation of CD11b-positive leukocytes in the intima in either LCA or RCA in these mice (Supplemental Fig. 2), whereas this seems to be a sufficient duration to ensure robust gene expression changes following the partial ligation. Klf-2 and eNOS were significantly downregulated in LCA by 80 and 50%, respectively, whereas BMP4, ICAM-1, and VCAM-1 were significantly upregulated in LCA by approximately twofold compared with RCA (Fig. 3D). In sham-operated mice, mRNA levels of these genes did not differ between LCA and RCA. Protein levels for ICAM-1 and VCAM-1 were verified by immunohistochemical staining with PECAM-1 as an endothelial marker (Fig. 3, E and F). These results are consistent with previous observations in vitro and in vivo that KLF-2 and eNOS are downregulated in areas of disturbed flow while BMP4, ICAM-1, and VCAM-1 are up-

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**Fig. 2.** Computational fluid dynamics (CFD) study. Partial ligation results in low and oscillatory shear stress. A and B: CFD was carried out using the values shown in Fig. 1 (ligated ApoE KO mice). A: wall shear stress (WSS) over a cardiac cycle. B: pseudocolor images of time-averaged WSS levels over the cardiac cycle in carotid arteries before and 1 day after partial ligation.
regulated in areas of disturbed flow (3, 4, 7, 11, 12, 29, 31, 33, 38).

Partial ligation in ApoE KO mice impairs endothelium-dependent vasorelaxation. To determine if disturbed flow causes endothelial dysfunction, we examined the vasorelaxation response to acetylcholine. Carotid artery rings were obtained from LCA and RCA of partially ligated ApoE KO mice fed a high-fat diet for 2 or 7 days. At 2 days postligation, LCA and RCA showed a normal relaxation response to acetylcholine, exceeding 90% of precontracted tone (Fig. 4A). At 7 days, however, relaxation of LCA by acetylcholine was significantly inhibited, reaching only 50% of precontracted tone, whereas the RCA vasorelaxtion response remained unchanged (Fig. 4A). To determine whether the impaired response was due to endothelial defect, response to the endothelium-independent vasodilator sodium nitroprusside (SNP) was studied. At 2 and 7 days postligation, both LCA and RCA relaxed to similar degrees in response to SNP (Fig. 4B), suggesting that disturbed flow induces endothelial dysfunction in ApoE KO mice in 7 days.

Partial ligation in ApoE KO mice causes accelerated atherosclerosis in a NADPH oxidase-dependent manner. To examine whether partial ligation causes accelerated atherosclerosis, ApoE KO mice were partially ligated and fed the high-fat diet for 1, 2, or 3 wk. At 1 wk, LCA did not show any evidence of atherosclerotic lesion as determined by Oil red O staining (Fig. 5A). By 2 wk, LCA developed robust lipid lesion that increased dramatically by 3 wk after ligation (Fig. 5, A and B). However, RCA did not show any lesions for as long as 6 wk postligation (Fig. 6). Next, we examined the hypothesis that...
NADPH oxidases play a key role in atherosclerosis development in response to disturbed flow. To test this, we used p47\textsuperscript{phox}\_ApoE DKO mice. p47\textsuperscript{phox}\_ApoE DKO showed significantly less atherosclerosis at 2 wk postligation (Fig. 5B). By 3 wk, p47\textsuperscript{phox}\_ApoE DKO showed a trend toward less atherosclerotic lesion compared with ApoE KO mice, although it did not reach statistical significance (\(P = 0.08\)). This suggests that p47\textsuperscript{phox} deficiency delays but does not fully prevent development of atherosclerosis induced by disturbed flow.

Partial ligation in ApoE KO mice develops complex lesion. Next we examined whether partial ligation can induce advanced atherosclerotic lesion. Pentachrome staining of LCA of ApoE KO mice 4–6 wk postpartial ligation and high-fat diet showed robust cholesterol clefts and several remarkable in-
traplaque neovessels (Fig. 6). These results show that at least some features of advanced lesions develop quickly within 4–6 wk following partial ligation, providing additional unique advantage of our model in the study of atherosclerosis.

Partial ligation stimulates superoxide production in LCA by a p47phox NADPH oxidase-dependent manner. To determine the underlying mechanism by which p47phox deficiency delayed atherosclerosis as shown above (Fig. 5), we tested whether disturbed flow increased superoxide production in a p47phox-dependent manner. DHE staining intensity in the vessel walls of LCA obtained from ApoE KO mice was significantly higher than that of RCA (Fig. 7). This increase was significantly blunted in LCA of p47phox_ApoE DKO mice. This suggests that reactive oxygen species (ROS) was produced in response to disturbed flow in a p47phox NADPH oxidase-dependent manner. This is consistent with previous reports in vitro and in vivo (1, 15, 33a).

DISCUSSION

Here, we characterized partial carotid ligation as a model of disturbed flow with low and oscillatory shear stress that causes endothelial dysfunction and accelerated atherosclerosis. We also describe a simple method of isolating intimal RNA from mouse carotid arteries. In this mouse model, we found that partial ligation of LCA causes 1) low and oscillatory shear, 2) upregulation of proatherogenic genes, 3) downregulation of antiatherogenic genes, 4) ROS production in a p47phox-dependent manner, 5) endothelial dysfunction in ApoE KO mice in 1 wk, 6) rapid atherosclerosis within 2 wk in a p47phox-dependent manner in ApoE KO mice, and 7) advanced lesions by 4 wk.

Partial ligation causes not only low shear stress but also oscillatory shear stress, two characteristics of pathophysiologically relevant disturbed flow associated with atherogenesis (5). Previously, Cheng et al. (6) used the perivascular cuff model to create three distinct regions of shear stress: lower shear (100 dyn/cm²), higher shear (250 dyn/cm²), or oscillatory shear (140 dyn/cm²). Using 15- to 20-wk-old ApoE KO mice fed an atherogenic diet for minimum of 8 wk, they demonstrated that lower shear was more atherogenic than oscillatory shear, although flow oscillation was not directly demonstrated in their mouse model (6). It should be noted, however, that typical atheroprine regions in carotid bifurcations and aortic arches are exposed to disturbed flow characterized by colocalization of both low and oscillatory shear stress (5, 33, 34). Perhaps one reason that our partial ligation induced atherosclerosis much faster than the perivascular cuff model is that LCA is exposed to both low and oscillatory shear in our partially ligated artery while the perivascular cuff exposes LCA to either low or oscillatory shear, but not both. Complete ligation of the carotid artery (20) has also been used to induce rapid atherosclerosis (17). However, its pathophysiologically relevance is debated, since it is a model of no flow and likely does not mimic disturbed flow with low and oscillatory shear (36). We propose that the partial ligation model provides a physiologically relevant model well-suited for the study of atherosclerosis induced by disturbed flow. It has been reported that harmonic frequencies above the heart rate may be a risk factor for atherosclerosis development (14). We have not yet examined whether flow frequencies change significantly in LCA by partial ligation. If that is the case, further studies are warranted to determine whether changes in flow frequencies are an important athero-
genic component of the disturbed flow created by partial ligation.

Intraplaque neovessel formation and cholesterol clefts are important features of advanced atherosclerotic lesions (13). In our model, pentachrome staining revealed numerous large vascular structures containing red blood cells within the plaque. It is interesting to note that many of these intraplaque neovessels were found near internal elastic lamina, consistent with a previous report showing that intraplaque hemorrhage in innominate artery of 60-wk-old ApoE KO mice occurred in a similar region (27). In addition, we observed abundant needle-like cholesterol clefts within the plaque, providing further evidence of advanced lesions. This could serve as a very useful model to study the mechanisms underlying intraplaque neovascularization and its importance in atherosclerosis.

Here we describe a simple method of isolating intimal RNA from carotid arteries in sufficient quantity with virtually no appreciable contamination from underlying media and adventitia. Intimal RNA obtained by this method is nearly free of medial smooth muscle RNA contamination. Previous efforts to isolate intimal RNA from atheroprone and atheroprotected areas of vasculature have been reported using a scraping method (35). However, we found our method of simply flushing the carotid artery with lysis reagent easier and highly reproducible. Having our partial ligation model, which exposes the entire length of the common carotid artery to disturbed flow, enabled us to apply this easy method of endothelial RNA isolation. This method could easily be applied to obtain sufficient quantities of RNA for high-throughput genomewide studies, including DNA microarray and microRNA array. Although genomewide cDNA array analysis using endothelial RNAs obtained from the region of the porcine aortic arch that were chronically exposed to disturbed flow from birth was reported (26), our acute mouse model offers unique advantages over the porcine model: in the mouse model, nearly the entire length of the carotid was acutely exposed to relatively uniform disturbed flow conditions, resulting in rapid atherosclerosis development within 2 wk postligation. This accelerated atherogenesis makes it possible to reveal underlying mechanisms directly linking disturbed flow to atherogenesis. The availability of the large portion of the carotid artery allows easy and reproducible intimal RNA isolation from them, enabling RNA studies such as qPCR and genomewide analysis of cDNA and microRNA to identify mechanosensitive transcripts and pathways.

We applied this method to study mechanisms by which disturbed flow induces endothelial dysfunction and atherosclerosis. By comparing endothelial RNA from LCA and RCA, we found that partial ligation downregulated expression of antiatherogenic genes such as eNOS and Klf-2 while upregulating proatherogenic genes such as ICAM-1, VCAM-1, and BMP4. These gene expression changes are consistent with known endothelial responses to disturbed flow in vitro and in vivo (3, 7, 11, 12, 29, 31). Furthermore, using this model in p47phox−/− ApoE DKO mice, we showed that p47phox-dependent NADPH oxidases play an important role in atherogenesis induced by disturbed flow.

We believe that this is the first report demonstrating that acute induction of disturbed flow in vivo causes endothelial dysfunction. It was reported previously that endothelial dysfunction could be induced in carotid arteries of ApoE KO mice by feeding a Western diet for >26 wk (8). By comparison, our model impairs the vasorelaxation response within 1 wk, providing a rapid and useful model to study flow-dependent endothelial dysfunction.

In conclusion, we characterized partial carotid ligation as a model for acutely induced disturbed flow, which results in accelerated endothelial dysfunction and atherosclerosis and allows for a simple method of intimal RNA isolation. These could be used in ApoE, LDL receptor KO, or other transgenic mice to further study molecular mechanisms underlying flow-dependent regulation of vascular biology and disease, such as neovascularization and atherosclerosis. Moreover, this in vivo model could be used to test various therapeutic interventions targeting endothelial dysfunction and atherosclerosis in considerably reduced study duration.

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