Shear stress influences spatial variations in vascular Mn-SOD expression: implication for LDL nitration

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Submitted 4 November 2007; accepted in final form 11 April 2008

First published April 23, 2008; doi:10.1152/ajpcell.00518.2007.

Atherosclerotic lesions preferentially develop in the lateral walls of vessel bifurcations and curvatures (34). Predilection sites for atherosclerosis are modulated by flow patterns to which the endothelium is exposed (13, 40). At the lateral wall of arterial bifurcations, a complex flow profile develops; flow separation and migrating stagnation points create oscillatory shear stress (i.e., bidirectional with no net forward flow) (33). Oscillatory shear stress (OSS) increases oxidative stress, which promotes development of atherosclerotic plaque (14, 36, 46, 50, 52). In contrast, pulsatile flow downregulates adhesion molecules and reactive oxygen species in the medial wall of bifurcations or relatively straight segments.

Mitochondria are important sources of cellular superoxide anion (O2−) and nitric oxide (NO). Whether the characteristics of shear stress influence the spatial variations in mitochondrial manganese superoxide dismutase (Mn-SOD) expression in vasculatures is not well defined. We constructed a three-dimensional computational fluid dynamics model simulating spatial variations in shear stress at the arterial bifurcation. In parallel, explants of arterial bifurcations were sectioned from the human left main coronary artery and right coronary arteries for immunohistolocalization of Mn-SOD expression. We demonstrated that Mn-SOD staining was prominent in the pulsatile shear stress (PSS)-exposed and atheroprotective regions, but it was nearly absent in the oscillatory shear stress (OSS)-exposed regions and lateral wall of arterial bifurcation. In cultured bovine aortic endothelial cells, PSS upregulated Mn-SOD mRNA expression at a higher level than did OSS at τave = 0.02 dyn/cm² ± 3.0 dyn/cm² s⁻¹ and at 1 Hz (PSS by 11.3 ± 0.4-fold vs. OSS by 5.0 ± 0.5-fold vs. static condition; P < 0.05, n = 4). By liquid chromatography and tandem mass spectrometry, it was found that PSS decreased the extent of low-density lipoprotein (LDL) nitration, whereas OSS increased nitration (P < 0.05, n = 4). In the presence of LDL treatment with Mn-SOD small interfering RNA increased intracellular nitrotyrosine level (P < 0.5, n = 4), a fingerprint for nitrotyrosine formation. Our findings indicate that shear stress in the atheroprotective versus atherosclerotic regions regulates spatial variations in mitochondrial Mn-SOD expression with an implication for modulating LDL nitration.

superoxide dismutase; superoxide anion; nitric oxide; nitrotyrosine; low-density lipoprotein

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variations in Mn-SOD expression with an implication for influencing LDL nitration.

METHODS

Three-Dimensional Bifurcation Model

The novel approach in the present study is to link specific shear stress patterns in the atheroprotective versus atheroprone regions of a 3-D bifurcation model with the corresponding Mn-SOD and nitrotyrosine localization from the explants of human coronary arteries. In addition, the molecular mechanisms by which specific shear stress patterns modulate endothelial Mn-SOD mRNA expression and LDL protein nitration were elucidated by cultured BAEC and/or human aortic endothelial cells (HAEC) in a 2-D dynamic flow system.

Generation of 3-D geometries and meshes. The construction of the bifurcation model and the associated flow patterns have been described previously (5, 20, 32). Briefly, the commercial CAD software, Pro Engineer Wildfire (version 3.0, Parametric Technology; Needham, MA), was used to construct the 3-D luminal geometrical model, which was then imported into a specialized preprocessing program for mesh generation (Gambit 2.3.16, Fluent; Lebanon, NH). The mesh consisted of tetrahedral elements and was imported into the main CFD solver (Fluent 6.2.16, Fluent) for flow simulation.

Blood flow model and boundary conditions. The 2-D Navier-Stokes equations were applied to solve the blood flow. The governing equations included mass and momentum equations, which were used to solve for laminar, incompressible, and non-Newtonian flow. The arterial wall was assumed to be rigid and impermeable. Characteristics such as inlet flow waveforms, blood viscosity, and vessel diameters were controlled to achieve the physiological conditions as previously reported in human left carotid artery (5, 20, 32). The pulsatile inlet flow rate was implemented by using 12 harmonics written in a user-defined C++ code.

Two-Dimensional Flow System

Two-dimensional dynamic flow channels were used to implement PSS and OSS obtained from the 3-D bifurcation model mentioned above. The 2-D flow system provides the precise and well-defined flow profiles across the width of the parallel flow chamber at various temporal variations in shear stress (8τ/8t), frequency, and amplitude (26). BAEC were exposed to the characteristics of shear stress as obtained from the 3-D bifurcation model: 1) PSS at a time-averaged shear stress (τave) of 23 dyn/cm² with a temporal gradient (8τ/8t) of 71 dyn·cm⁻²·s⁻¹; and 2) OSS at a τave of 0.02 ± 3 dyn·cm⁻² and 8τ/8t of 0 dyn·cm⁻²·s⁻¹. The 2-D model allowed for real-time monitoring of shear stress and quantitative real-time RT-PCR and Western blots from a sufficient number of vascular endothelial cells (EC) (24, 41, 42).

Endothelial Cell Culture

Confluent BAEC between passages 4 and 7 were seeded on Cell-Tak cell adhesive and Collagen Type I (BD Bioscience, San Jose, CA) coated glass slides (5 cm²) at 3 × 10⁴ cells per slide. BAEC were grown to confluent monolayers in high glucose (4.5 g/l) DMEM supplemented with 10% heat-inactivated fetal bovine serum (HyClone), 100 U/ml l-glutamine-penicillin-streptomycin (Sigma) for 48 h in 5% CO₂ at 37°C.

Immunohistochemistry of Human Coronary Arteries

Three human coronary arteries were obtained from explanted hearts of cardiac transplant patients. The protocol was approved by the Institutional Review Board of the University of Southern California, School of Medicine, and all volunteers gave informed consent. Cross sections of the left and right coronary arteries with and without atherosclerotic lesions were analyzed. Monoclonal antibodies were used for Mn-SOD (Upstate). Immunostaining was performed with standard techniques in frozen vascular tissue using biotinylated secondary antibodies and peroxidase staining. Diaminobenzidine was used as a chromogen, and the sections were counterstained with hematoxylin for visualization of intima, media, smooth muscle cells, and adventitia.

Counterstaining was performed to distinguish EC and smooth muscle cells in the media and/or intima proximal to the point of flow separation. EC and smooth muscle cells were stained with monoclonal antibodies specific for von Willebrand factor (vWF; 1:25 dilution) and β-actin (1:4,000 dilution) (Dakocytomation, Carpinteria, CA). Negative controls were performed by omitting the primary antibody. Positive controls included brain and kidney tissues.

Separation of LDL Subspecies by High-Performance Liquid Chromatography

Venous blood samples were obtained at the Atherosclerosis Research Unit from fasting adult human volunteers under institutional review board approval. Plasma was pooled and immediately separated by centrifugation at 1500 g for 10 min at 4°C. LDL (δ = 1.019 to 1.063 g/ml) was isolated from freshly separated plasma by preparative ultracentrifugation using a Beckman L8–55 ultracentrifuge and a SW-41 rotor. The technique used for separating LDL was similar to that described previously (45).

Endothelial Cell Exposure to Shear Stress

A dynamic flow system was used to implement temporal variations in shear stress (8τ/8t); namely, PSS and OSS (24, 41, 42). Confluent BAEC were exposed to the flow conditions in the absence and presence of native LDL (50 μg/ml). After 4 h, BAEC were collected for quantitative real-time RT-PCR and Western blot analysis. In the presence of LDL, the culture medium was collected to measure apolipoprotein B (apoB)–100 nitration of the tyrosine residues.

Quantitative Real-Time RT-PCR

Confluent BAEC monolayers were exposed to PSS and OSS in a parallel plate flow system for 4 h as mentioned above. Total RNA was isolated using the RNeasy kit (Qiagen). RNA was reverse transcribed, followed by PCR amplification using the SuperScript III Platinum Two-Step qRT-PCR Kit with SYBR Green (Invitrogen, Carlsbad, CA). Oligonucleotides for Mn-SOD were 1) forward primer 5’-GGG AGC CATCAA ACG TGA CT-3’ and 2) reverse primer 3’-AGG GGG AGG ATG AGA CCT GT-5’. Fidelity of the PCR reaction was determined by melting temperature analysis. For quantification of relative gene expression, the target sequence was normalized to 18s rRNA. The difference in cycle threshold values for various flow conditions vs. control was used to determine the relative difference in the levels of Mn-SOD mRNA expression.

LDL Protein Nitration

LDL suspended in medium was collected for analysis of protein nitration. Protein-bound nitrotyrosine formation in the recovered media was determined by stable isotope dilution liquid chromatography-tandem mass spectrometry (9) on a mass spectrometer (API 4000, Applied Biosystems, Foster City, CA) interfaced with an Aria LX Series HPLC multiplexing system (Cohesive Technologies, Franklin, MA). Synthetic 13C₆-labeled nitrotyrosine internal standard was added to samples for quantification of natural abundance of analytes. Simultaneously, a universal labeled precursor amino acid, 13C₆,15N₁ tyrosine (for nitrotyrosine) was added to quantify the precursors and to assess potential intrapreparative artifactual oxidation during sample handling and analysis. Proteins were hydrolyzed under argon atmosphere in methane sulfonic acid,
and then samples passed over mini solid-phase C18 extraction columns (Supelclean LC-C18-SPE minicolumn, Supelco; Bellefonte, PA) before mass spectrometry analysis. Results were normalized to the content of the precursor amino acid, which was monitored within the same injection. Formation of $^{13}$C$_9$, $^{15}$N-labeled nitrotyrosine was routinely monitored and negligible (i.e., <5% of the level of the natural abundance product observed).

**Analysis for Nitrotyrosine**

Confluent BAEC at fourth passage were pretreated with diethyldithiocarbamic acid (DIECA), a copper chelator to inhibit CuZn-SOD (1 mM for 60 min), and/or MnTMPyP, a Mn-SOD mimetic (10 μM for 30 min) in a serum-free DMEM medium. After DIECA and/or MnTMPyP were washed with PBS, BAEC were incubated with native LDL at 50 μg/ml for 4 h. LDL suspended in medium was collected to assess LDL protein nitration. LDL in the medium was concentrated by centrifugation at 3,000 rpm in Centriprep centrifugal filter devices with YM-30 MW (Millipore). Dot blots were performed by spotting 15 μg of LDL. Membranes were soaked in methanol and washed with Tris-buffered saline plus Tween (TBS-T). Samples were blocked in BSA and incubated with a polyclonal nitrotyrosine antibody overnight (1:3,000 dilution, Upstate), followed by incubation with horseradish peroxidase-conjugated goat anti-rabbit secondary antibody (1:10,000). Blots were detected using ECL chemiluminescence kit (Pierce, Rockford, IL). Positive control was established by treating LDL (0.2 mg/ml with peroxynitrite at 100 μM).

**Silencing Mn-SOD mRNA in HAEC**

Silencer small interfering (si)RNA was custom designed for Mn-SOD by Ambion (Austin, TX). The sense sequence was GGCCU-GAUUACUAAAGCtt and the antisense sequence was GCU-UUUAGAUAAUCAGGCCtg. Confluent HAEC monolayers from passages 4 to 9 were trypsinized and resuspended in standard growth medium to 100,000 cells/ml. siPORT NeoFX transfection reagent was diluted in OPTIMEM medium and incubated at room temperature for 10 min. Mn-SOD siRNA at a final concentration of 30 nM was diluted in OPTIMEM and mixed with diluted siPORT NeoFX reagent at room temperature for an additional 10 min. The transfection solution Fig. 1. Spatial variations in shear stress. A: boundary condition. B: Carreau model for non-Newtonian blood flow (6), where $\mu$ represents viscosity, $\gamma$ represents the shear rate, $\mu_0$ represents the zero shear rate limit viscosity, $\mu_s$ represents the infinite shear rate limit viscosity, $\lambda$ represents the relaxation time constant, and $n$ is the power law index. C: reconstruction of carotid artery. D: an instantaneous velocity profile ($t = 0.244$ s). E: anterior-oblique angle of shear stress profile at an instantaneous moment with the mean Reynolds ($R_e$) number of 289. F: top view of shear stress profile. White arrow indicates the point of flow separation. PSS, pulsatile shear stress; OSS, oscillatory shear stress.
was dispensed into the six-well plates, followed by addition of HAEC in suspension. The medium was changed to standard growth medium after 24 h. The medium was replaced every other day until confluent HAEC monolayers developed. Quantitative RT-PCR and dot blot analysis were performed to validate effective silencing of Mn-SOD.

**Western Analysis of Intracellular Nitrotyrosine Level**

HAEC and siMn-SOD-treated HAEC were incubated with the CuZn-SOD chelator DIECA at 10 μM. The third HAEC sample was treated with the Mn-SOD mimetic MnTMPyP at 10 μM. After 1 h, the control sample (HAEC only) and the treated samples were incubated with LDL at 50 μg/ml. A blank sample was not treated with LDL or any other treatment. A positive control was treated with LDL, followed by ONOO− (100 μM). After 4 h, the cell lysates were collected. The Western analyses for nitrotyrosine were performed at a 1:3,000 dilution in TBS-T for primary monoclonal nitrotyrosine antibody (Upstate Cell Signaling Solutions) and 1:10,000 dilution for anti-mouse secondary antibody as previously described (27). Densitometry was performed using an NIH Scion Image Software (Scion, Frederick, MD).

**Statistical Analysis**

Data are expressed as means ± SD and were compared among separate experiments. For comparisons between two groups, two-sample independent-groups t-test was used. Comparisons of multiple values were made by one-way analysis of variance, and statistical significance among multiple groups was determined using the Tukey test (for pairwise comparisons of means between static-like and pulsatile flow conditions). P values < 0.05 were considered statistically significant.

**RESULTS**

**Characteristics of Mean Shear Stress in a 3-D Bifurcation Model**

The intention of our 3-D model was to link specific shear stress profiles with spatial variations in Mn-SOD expression. We proposed that Mn-SOD expression influenced nitrotyrosine formation. By using non-Newtonian blood flow, the dynamic 3-D CFD code demonstrated shear stress at the lateral versus medial wall of internal and external carotid arteries, and the divider of the bifurcation (6) (Fig. 1, A–C). At a given instantaneous moment (t = 0.244 s), magnitude of shear stress was the highest at the divider of bifurcation and lowest at the point of flow separation. For a Reynolds number of 289 (representative of human carotid blood flow), the time-averaged shear stress of pulsatile flow along the medial wall of internal carotid artery ranged from 20 to 26 dyn/cm² (Fig. 1, E and F), and the point of flow separation oscillated between −3 and 3 dyn/cm² in response to cardiac contraction. The shear stress valves

![Fig. 2. Mn-SOD immunostaining of a section of left coronary artery.](image)

A: a representative left coronary artery and its branches. B: von Willebrand Factor (vWF) staining for endothelial cells (EC). C: at the left main bifurcation (OSS-exposed region), Mn-SOD staining was absent in the luminal EC. D: in straight segment of left anterior descending (LAD) artery (PSS-exposed region), Mn-SOD staining was prevalent throughout the entire luminal EC. E: endothelial nitric oxide (NO) synthase (eNOS) staining was positive. This section was previously reported to be eNOS positive (27). SMC, smooth muscle cells.
derived from the 3-D CFD code provided a basis to assess spatial variations in Mn-SOD expression.

**Spatial Variations in Mn-SOD and Nitrotyrosine Immunostaining**

In the present study, Mn-SOD staining was absent in the OSS-exposed regions, but it was prominent throughout the entire endothelium in the PSS-exposed regions (Fig. 2). vWF staining and eNOS staining was positive in the entire luminal EC in the PSS-exposed region (27). In contrast, nitrotyrosine staining was prominent in the arterial bifurcation regions where eNOS and Mn-SOD staining were nearly absent (Fig. 3). The integration of CFD and immunohisto-localizations suggests that spatial variation in Mn-SOD expression was influenced by specific shear stress patterns. We have recently reported that nitrotyrosine immunohisto-tstaining was prominent in the OSS-exposed regions but absent in the PSS-exposed regions (27). Our present study...
supported the notion that Mn-SOD staining was prominent in the PSS-exposed regions where nitrotyrosine was nearly absent, but Mn-SOD was absent in the OSS-exposed regions where nitrotyrosine was prominent.

**Flow Regulation of Mn-SOD mRNA Expression and Nitrotyrosine Formation**

BAEC were exposed to shear stress in a dynamic flow system simulating flow profiles in the PSS- and OSS-exposed regions of a 3-D arterial bifurcation. Endothelial Mn-SOD mRNA was significantly upregulated in response to shear stress. Specifically, PSS induced an 11.3-fold increase, whereas OSS induced a 5-fold increase (Fig. 4B). Western blot analyses corroborated Mn-SOD protein expression (Fig. 4D). CuZn-SOD mRNA expression was increased by 2.3-fold in response to PSS (Fig. 4A). EC-SOD that was expressed in smooth muscle cells was unresponsive to shear stress (Fig. 4C). To test the pathophysiological significance of Mn-SOD expression, we assessed LDL particles as a surrogate marker for protein nitration by ONOO− (27). The LDL apoB-100 residues were analyzed by liquid chromatography/electro spray ionization/tandem mass spectrometry (LC/ESI/MS/MS). OSS increased the level of protein nitration, whereas PSS significantly decreased the level in comparison with the control under static conditions (Fig. 5).

**The Effects of Mn-SOD siRNA on Extra- and Intracellular Nitrotyrosine Levels**

To test the role of Mn-SOD on LDL nitration, we treated HAEC with Mn-SOD siRNA (siMn-SOD), followed by incuba-

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**Fig. 4.** SOD isoform expression in bovine aorta endothelial cells (BAEC) in response to OSS and PSS. Cu/Zn-, Mn-, and extracellular-SOD mRNA expression was normalized to 18s RNA (*P < 0.05 in comparison to the static samples; n = 3). A: Cu/Zn-SOD expression was upregulated by 2.3-fold in response to OSS. B: Mn-SOD mRNA expression was upregulated by 5-fold in response to OSS and by 11.4-fold to PSS (*P < 0.05 vs. static, #P < 0.05 vs. OSS, n = 3). C: EC-SOD remained relatively unresponsive to shear stress. D: Mn-SOD protein expression. The increase in Mn-SOD expression was not statistically significant in response to OSS. PSS upregulated Mn-SOD expression by 1.4-fold (*P < 0.05, n = 4).
tion with native LDL. Dot blot analysis was performed to assess extracellular LDL nitration. In the presence of MnTMPyP, a Mn-SOD mimic, the level of nitrotyrosine or LDL nitration was reduced. (Fig. 6). In siMn-SOD-treated HAEC, the level was significantly higher than that of MnTMPyP-treated HAEC. Western analysis was performed to assess intracellular nitrotyrosine levels in siMn-SOD-treated HAEC (Fig. 7). Mn-SOD siRNA significantly increased intracellular nitrotyrosine level compared with the native LDL-treated BAEC. Our observation corroborated the notion that Mn-SOD expression influenced the extent of LDL protein nitration and that PSS attenuated the extent of LDL nitration via Mn-SOD upregulation. The CFD code enabled us to link specific shear stress profiles with spatial variations in Mn-SOD expression with an implication for arterial nitrotyrosine formation.

**DISCUSSION**

In the present study, we demonstrated that shear stress influenced spatial variations in Mn-SOD expression. That OSS and PSS patterns may contribute to this phenomenon is supported by four lines of evidence: 1) the 3-D computational fluid dynamic model predicts regions of OSS and PSS in the arterial bifurcations; 2) immunohistolocalization and physiological study showed that Mn-SOD staining was prominent in the PSS-exposed regions but was absent in OSS-exposed regions; 3) nitrotyrosine staining was absent in the PSS-exposed regions but was present in the OSS-exposed regions; and 4) PSS and OSS differentially regulated LDL protein nitration via Mn-SOD mRNA expression in cultured EC.

The 3-D CFD code confirmed that the EC in medial wall or the straight segment of vessels experience PSS, whereas EC near the point of flow separation experience OSS in the atherosclerosis-prone regions (34). The mean shear stress values obtained from the 3-D model were implemented in the 2-D flow system to elucidate SOD isoform mRNA expression and LDL protein nitration. Blackman et al. (7) reported a cone-and-plate system that was capable of generating precise athero-
protective versus atherogenic waveforms from human carotid arteries. Compared with the cone-and-plate system, our 2-D microfluidic channel was designed to uniformly generate specific pulsatile versus oscillatory shear stress at various slow rates (24).

Mn-SOD is an important dismutase of reactive oxygen species acting in the mitochondria matrix. Oxidative phosphorylation in mitochondria ATP occurs as electrons are transferred to oxygen. The transfer of >98% of electrons by the electron transport chain is coupled with the production of ATP, and 1.5% to 2% of electrons leak out to form O$_2^-$, which is dismutated by Mn-SOD (39). In response to pathological conditions such as reperfusion injury, the electron transport chain may become uncoupled, leading to an increase in O$_2^-$ production (8). Bernal-Mizrachi et al. (4) reported respiratory uncoupling in smooth muscle cells caused atherosclerosis in mice. In apoE-deficient mice (apoE$^{-/-}$) we demonstrated that Mn-SOD expression is absent, and Mn-SOD staining was prominent but nitrotyrosine staining was absent in the PSS-exposed regions (27). In PSS-exposed regions, Mn-SOD staining was prominent but nitrotyrosine staining was absent, and Mn-SOD expression influenced the extent of LDL nitration (Figs. 5 and 6).

Our data also suggest that Mn-SOD and CuZn-SOD, but not EC-SOD, are responsive to shear stress in vascular EC. EC-SOD is synthesized in smooth muscle cells, and CuZn-SOD is prevalent in vascular EC. Inoue et al. (30) reported that CuZn-SOD in HAEc is upregulated in response to laminar flow. Despite different experimental designs, namely, 1) the cell type (BAEC versus HAEC), 2) duration of flow exposure (4 vs. 24 h), 3) magnitude and characteristic of shear stress profiles, and 4) flow models (cone-and-plate vs. pulsatile parallel plate), our finding in CuZn-SOD expression in response to PSS is in agreement with that of Inoue et al. (30). Moreover, we demonstrated that shear stress regulated Mn-SOD and CuZn-SOD expression in both BAEC and HAEC. Whether there exists a shear stress-responsive element in Mn-SOD gene remains undefined; however, a redox-sensitive mechanism was implicated in Mn-SOD promoter activity via a negative PI3K-akt-forkhead pathway and a positive PKC-NF-κB pathway (1).

Several studies have examined effects of SOD isoforms on endothelial function and atherogenesis. EC-SOD plays a significant protective role on arterial pressure, vascular function, or vascular levels of oxidative stress in spontaneously hypertensive rats (12). Similarly, cytosolic CuZn-SOD is atheroprotective, playing a critical role in limiting angiotensin II-induced endothelial cell dysfunction (15). The CuZn-SOD-deficient (CuZn-SOD$^{-/-}$) mice showed an increased superoxide level and altered vascular responsiveness compared with the wild-type littermates (16). The protein-encoded Mn-SOD transmigrate to mitochondria. Mn-SOD-deficient mice (Mn-SOD$^{-/-}$) were reported to develop more atherosclerosis (3). However, the relative contribution of CuZn-SOD and Mn-SOD to dismutate superoxide anion in response to shear stress requires further investigations.

Pulsatile flow significantly reduced the ratios of oxidatively modified forms of LDL relative to static conditions, whereas oscillating flow increased LDL oxidation (28), leading to upregulation of adhesion molecules and recruitment of monocytes (23, 25). In the present study, OSS increased the level of tyrosine nitration and oxidation products in LDL, whereas PSS decreased the levels of both tyrosine nitration and oxidation. Nitration of tyrosine residues by ONOO$^-$ resulted in a more hydrophilic residue, thus altering the structure of α-helices of apoB-100 of the LDL particles (2, 29).

We analyzed LDL protein nitration at both protein and amino acid levels. The former was established by dot blot analysis. Using anti-nitrotyrosine antibody, we suspended LDL in medium to assess LDL protein nitration. The latter was performed by LC/ESI/MS/MS. The proteomic approach allowed for identification of the specific apoB-100 tyrosine residue nitration in α- and β-helices as follows: α-1 (Tyr$^{3295}$), α-2 (Tyr$^{3521}$), β-2 (Tyr$^{3295}$), α-3 (Tyr$^{4110}$), and β-2 (Tyr$^{4211}$) (27). Both lipid peroxidation and protein nitration account for apoB-100 protein unfolding and consequential increase in modified LDL binding and uptake by endothelial cells.

Antioxidant gene expression is present in mouse aortic arch where disturbed flow, including OSS, develops. Passerini et al. (43) reported a host of antioxidant gene expression from adult porcine aortic arch by genomics. The authors suggested that there is an evolutionary benefit to favor a moderate level of atheroprotective gene expression in the atheroprone regions such as curvatures and bifurcations. In this context, our in vitro findings between OSS and PSS complement those of mouse genomic analysis.

In summary, our bifurcation model predicts the characteristics of shear stress in the PSS- and OSS-exposed regions of the arterial bifurcation. Explants of human coronary arteries enabled us to assess the pathophysiologial significance of this model in terms of Mn-SOD immunohistolocalization. Our findings strongly support the notion that pulsatile and oscillatory shear stress modulated spatial variations in Mn-SOD expression and that Mn-SOD plays an important role in nitrotyrosine formation.

ACKNOWLEDGMENTS

The authors are grateful for Dr. Shan-Rong Shi and Lillian L. Young for technical assistance with the coronary artery specimens in the Department of Pathology at the University of Southern California. The authors are also grateful for the native LDL provided by Dr. Mohamad Navab from the Division of Cardiology at University of California, Los Angeles David Gaffen School of Medicine. Finally, the authors express appreciation for Stanley L. Hazen from the Cleveland Clinic for biochemical analyses.

GRANTS

These studies were supported by American Heart Association (AHA) Pre-Doctoral Fellowship 0615063Y (to M. Rouhanizadeh), AHA Grant GIA 0655051Y (to T. K. Hsiai), and National Heart, Lung, and Blood Institute Grants HL-068689 and HL-083015 (to T. K. Hsiai).
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