ANTIOXIDANTS & REDOX SIGNALING
Volume 15, Number 5, 2011

DOI: 10.1089/ars.2010.3433

FORUM REVIEW ARTICLE

Anti-Atherogenic Effect of Laminar Shear Stress *via* Nrf2 Activation

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Abstract

Fluid shear stress plays a critical role in the regulation of vascular biology and its pathology, such as atherosclerosis, via modulation of redox balance. Both pro-atherogenic (either oscillatory or turbulent, nonunidirectional) shear stress and anti-atherogenic (either steady or pulsatile, unidirectional laminar) shear stress stimulate production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) that are involved in signal transduction of gene expression. Nonunidirectional shear stress induces pro-atherogenic genes encoding adhesion molecules and chemokines in a manner dependent on production of both superoxide and nitric oxide. Steady or pulsatile laminar shear stress induces expression of genes encoding cytoprotective enzymes for glutathione biosynthesis and detoxification, which are regulated by the transcription factor nuclear factor (erythroid-derived 2)-like 2 (Nrf2). We show that pulsatile laminar shear stress (PLSS)-induced expression of adhesion molecules and chemokines was enhanced in human umbilical vein endothelial cells (HUVEC) treated with Nrf2 siRNA and arterial endothelial cells isolated from Nrf2 knockout mice. Hence, we propose the hypothesis that PLSS maintains the endothelium in an anti-atherogenic state via intracellular antioxidant levels increased as a result of Nrf2 activation, thereby preventing excess ROS/RNS production required for pro-atherogenic gene expression. *Antioxid. Redox Signal.* 15, 1415–1426.

Introduction

VASCULAR ENDOTHELIAL CELLS are constantly subjected to mechanical shear stress imposed upon them by blood flow. Atherosclerotic lesions are likely to develop focally at bifurcations and branch points in the vessel (2, 26). It has been reported that the most atherosclerosis-prone regions are those exposed to nonunidirectional, disturbed, or oscillatory flow and that atherosclerosis-resistant regions are exposed to unidirectional, laminar flow (2, 66).

To investigate the response of endothelial cells upon exposure to shear stress, many studies have been performed under a variety of experimental conditions with various flow-exposing apparatuses (86a). These experiments show that both oscillatory and either steady or pulsatile laminar shear stress evoke generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) in vascular cells (33, 39, 55, 76). The mechanisms by which shear stress induces either pro-atherogenic or anti-atherogenic responses in endothelial cells have been the subject of intense studies over the past 2 decades. In this article, we will present the ROS/RNS-regulated mechanisms underlying the anti-atherogenic response of endothelial cells to pulsatile laminar shear stress (PLSS).

Materials and Methods

The details of materials and methods are shown in the Supplementary Data (available online at www.liebertonline.com/ars).

ROS and/or RNS Production by Fluid Shear Stress

Fluid shear stress caused by the dragging force generated by blood flow on endothelial cells plays a critical role in production of ROS and RNS in the vasculature. Exposure of endothelial cells to fluid shear stress activates NADPH oxidase, resulting in production of superoxide ($O_2^{\bullet -}$) (22, 29, 42, 111). Xanthine oxidoreductase also contributes to $O_2^{\bullet -}$ production in response to oscillatory shear stress (73). In addition to $O_2^{\bullet -}$, nitric oxide (NO) is generated via activation of endothelial nitric oxide synthase (eNOS) in vascular endothelial cells (12, 74, 108, 112) and inducible nitric oxide synthase (iNOS) in smooth muscle cells by either steady or pulsatile laminar shear stress (31, 91). NO plays an important role in vasodilation (36, 75, 86) and anti-inflammation (15, 27, 44). For example, inhibition of nuclear factor kappa B (NF-κB) by NO has been linked to downregulation of vascular cell adhesion molecule-1 (VCAM-1) gene expression, leading to decrease in

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monocyte binding to the endothelium (21, 68, 103). However, NO may also react with $O_2^{\bullet -}$, forming peroxynitrite ($ONOO^-$), one of the highly reactive species at a rapid diffusion-limited rate ($k = \sim 1 \times 10^{10}\, M^{-1}\, s^{-1}$) (6), which in turn modifies proteins and lipids (4, 5, 84, 85). $ONOO^-$ also induces oxidative damage and enhances adhesion molecules expression in the vasculature (77, 92). When endothelial cells were sheared in the presence of LDL, oscillatory flow caused higher levels of LDL 3-nitrotyrosine, a footprint of ONOO- formation, compared to pulsatile flow (39). The importance of $ONOO^-$ in development of atherosclerosis is also implicated by detection of 3-nitrotyrosine in human atherosclerotic lesions (3, 7, 17, 82, 96).

Several lines of evidence show that both oscillatory and either steady or pulsatile laminar shear stress produce $O_2^{\bullet -}$ and NO; however, NO production in endothelial cells by steady or pulsatile laminar shear stress is significantly higher than that by oscillatory shear stress (39, 72). On the other hand, oscillatory flow induces $O_2^{\bullet -}$ production much more than steady or pulsatile laminar flow (13, 22, 43, 73), but induces eNOS upregulation to a much lesser extent compared to steady or pulsatile laminar flow (8, 39, 93). In addition, the high level of $O_2^{\bullet -}$ generated by oscillatory shear stress reacts with NO to form $ONOO^-$, resulting in less bioavailable NO under oscillatory flow conditions. In contrast, either steady or pulsatile flow upregulated the expression of eNOS, CuZn superoxide dismutase (CuZnSOD), and MnSOD (1, 18). It is well known that reduced NO availability can lead to vascular dysfunction, including intimal hyperplasia and expression of adhesion molecules (70, 102). Based on the above, it is reasonable to propose that ROS/RNS and their reaction products can cause fluid shear stress to be either pro- or anti-atherogenic.

Activation of Transcription Factor Nrf2 by Laminar Shear Stress

The DNA microarray is a powerful tool used to reveal gene expression profiles of cells exposed to different types of shear stress. Brooks *et al.* compared gene expression of endothelial cells in response to disturbed flow and steady laminar flow (9) and showed that expression of adhesion molecules such as VCAM-1 and intercellular adhesion molecule-1 (ICAM-1) and inflammatory molecules such as monocyte chemotactic protein 1 (MCP-1) and the receptors for interleukins was selectively induced by disturbed shear stress. The recent study by Conway *et al.* (16) reported that the reversing component of disturbed flow was primarily responsible for the upregulation of endothelial receptors and monocyte adhesion. The expression of VCAM-1 and ICAM-1 at sites of the predisposed to lesion formation in rabbit and mouse was also shown (46). NF-κB and activator protein 1 (AP-1) are known as major transcription factors regulating these inflammatory genes (8, 24, 41, 48, 71, 104, 114). Our studies using DNA microarrays showed that PLSS (2 dyn/cm^2) induced antioxidant enzymes such as heme oxygenase 1 (HO-1), glutamate-cysteine ligase modifier (GCLM), glutamate-cysteine ligase catalysis (GCLC), and NADPH quinone oxidoreductase 1 (NQO1) in human umbilical vein endothelial cells (HUVEC) (106, 107; Table 1), which were regulated by stabilization of Nrf2. Similar results were shown by Chen *et al.* (14).

Nrf2 is a well-characterized transcription factor that plays an important role in the antioxidant response element (ARE)-mediated expression of a group of genes encoding phase II detoxification enzymes and antioxidant proteins, such as glutathione-S-transferase, HO-1, peroxiredoxin 1, NQO1, GCLM, and GCLC (47, 49). These enzymes are crucial for protecting cells from electrophile toxicity and oxidative stress. Under basal conditions, Nrf2 is negatively regulated by Kelch-like ECH-associated protein 1 (Keap1), which facilitates the degradation of Nrf2 through the proteasome (53). A variety of environmental stresses such as ultraviolet irradiation and exposure to cigarette smoke or heavy metals are known to induce ARE-mediated antioxidant proteins via Nrf2 activation (30, 37, 61, 62). In response to these stimuli, oxidative stress occurs with electrophile generation in cells. Electrophilic compounds are believed to attack the reactive cysteine residues in Keap1 intervening region (IVR), leading to a conformational change in the Keap1-Nrf2 association motif. The dissociation of Nrf2 from Keap1 and phosphorylation of Nrf2 prevent its proteasomal degradation, leading to accumulation of newly synthesized Nrf2 and its translocation to the nucleus (52, 65, 100, 101). Multiple sets of reactive cysteine

Table 1. Gene Expression by Pulsatile Laminar Shear Stress in HUVEC

		Gene name	*Static ave. diff.*	*PLSS ave. diff.*	*Fold changes*
○	HO-1	Heme oxygenase 1	277	4904	18.0
○	SQSTM1	Sequestosome 1	42	411	7.2
	HSPA1A	Heat shock 70 kDa 1A protein	130	962	5.7
○	SLC7A11	Solute carrier family 7A11	397	1947	4.9
	TRIM16	Tripartite motif-containing 16	71	320	4.7
	PMCH	Pro-melanin-concentrating hormone	8	235	4.2
	SLC3A2	Solute carrier family 3A2	97	443	4.2
○	TXNRD1	Thioredoxin reductase 1	727	2773	4.0
○	GCLM	Glutamate-cysteine ligase, modifier subunit	152	607	4.0
	EEF1A1	Eukaryotic translation elongation factor 1 alpha 1	2931	10384	3.8
○	NQO1	NAD(P)H dehydrogenase, quinone 1	516	2263	3.8
	PTGS2	Prostaglandin-endoperoxide synthase 2	38	202	3.8

○, Nrf2-regulated genes; PLSS, pulsatile laminar shear stress.
Microarray analysis was performed by using gene chip U133 (Affymetrix Inc., Santa Clara, CA) and calculation was performed as described in a previous article (98).

residues in Keap1 have been identified, and other signaling molecules are also reported to be involved in Nrf2 activation (11, 51, 64, 69, 78, 83, 87–90, 105, 109), suggesting that the molecular mechanisms for Nrf2 activation by various stimuli are different.

We found that in response to PLSS, Nrf2 was markedly accumulated and translocated into the nucleus (Fig. 1A) (106) and Nrf2-regulated cytoprotective genes were induced in HUVEC (Table 1) (107). Figure 1B showed increasing expression of GCLM measured by quantitative real-time PCR (qRT-PCR) which is almost completely abolished in HUVEC transfected with Nrf2 siRNA. Even more, these cytoprotective genes (Table 1) were not induced in endothelial cells isolated from Nrf2-deficient mice (Table 2). These results suggested that Nrf2 was essential for upregulation of cytoprotective genes under PLSS. We have previously revealed that steady laminar shear stress but not oscillatory shear stress enhances binding of Nrf2 to the regulatory region of NQO1 (38). Although the molecular mechanisms are not clear yet, we have presented one plausible explanation by presuming the presence of factor (X) which is induced by oscillatory flow or inhibited by laminar flow. The differential responses of Nrf2 target genes to laminar flow and oscillatory flow were shown *in vivo* by Zakker *et al.* (113). Both the accumulation of Nrf2 and the induction of *GCLM* mRNA by PLSS were significantly suppressed by N-acetylcysteine (NAC) (Fig. 2) (106). Besides our findings (106,107), several groups have reported that steady or pulsatile laminar shear stress activates Nrf2 for endothelium protection, which is blocked by ROS scavengers (14, 20, 34, 38, 40, 56). These results suggest that ROS produced by steady or pulsatile laminar shear stress plays important roles in activation of Nrf2, leading to a protective response by inducing antioxidant enzyme genes in endothelial cells. It is worthwhile to identify which ROS and/or RNS are responsible for Nrf2 activation, thereby causing laminar shear stress to be anti-atherogenic.

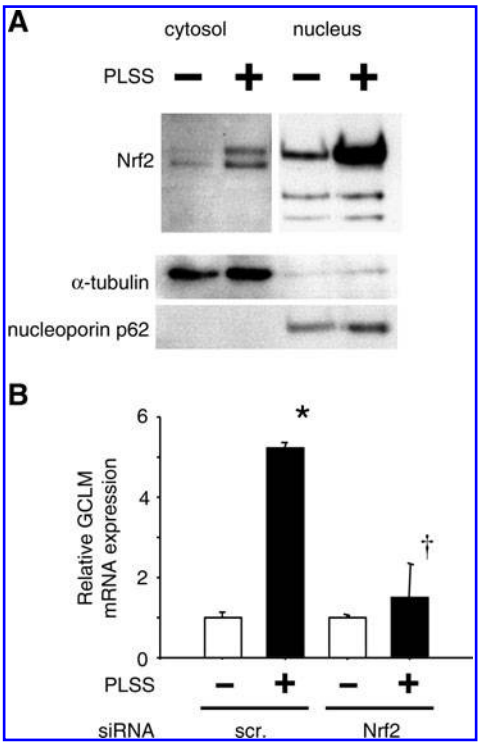

FIG. 1. Pulsatile laminar shear stress-induced Nrf2 accumulation and antioxidant gene expression. (A) HUVEC were exposed to PLSS (2 dyn/cm^2) or kept in static culture for 8 h. Western blot analysis was performed for cytosolic and nuclear fractions using polyclonal anti-Nrf2. Anti-α-tubulin and anti-nucleoporin p62 were used as a marker for fractionations. **(B)** HUVEC transfected with Nrf2 or scramble (scr.) siRNA were exposed to PLSS or kept in static culture for 8 h. The mRNA level of GCLM was analyzed by quantitative real-time PCR (qRT-PCR). The mRNA level of GCLM was normalized by those of glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The data are shown as the means from triplicate samples of three independent experiments with standard error. *$p < 0.005$ vs. static with scr., †$p < 0.005$ vs. PLSS with scr. These data have been published previously (106). PLSS; pulsatile laminar shear stress.

Another laminar shear stress-induced transcription factor, Kruppel-like factor 2 (Klf2) has been reported to act as a key role in anti-atherosclerosis (8, 25). Nrf2 is one of target molecules of Klf2 and acts in synergy with Klf2 to control approximately 70% of the genes induced by laminar shear stress (25). More details about the function of Klf2 in response to shear stress are reviewed by Nayak *et al*. (77a) and Nigro *et al*. (77b) in this Forum.

ROS/RNS Responsible for Activation of Nrf2

Several publications have shown that $O_2\bullet^-$ is produced by NADPH oxidase activation in response to oscillatory shear stress (42, 95). Our recent article (106) has shown that involvement of $O_2\bullet^-$ produced by PLSS in GCLM expression was implicated by using the XO inhibitor oxypurinol and NADPH oxidase inhibitor diphenyleneiodonium (DPI; Fig. 3A). Furthermore, knockdown of one of the components of NADPH oxidase, p22, by using siRNA in HUVEC showed the same results (Figs. 3B–3D). McNally *et al.* has reported that NADPH oxidase maintains endothelial cell XO levels in endothelial cells and that XO is responsible for increased reactive oxygen species production in response to oscillatory shear stress (73). These results suggest that PLSS induces $O_2\bullet^-$ production via activation of NADPH oxidase and XO, resulting in expression of Nrf2-regulated genes.

ROS/RNS can initiate lipid peroxidation in which lipid hydroperoxides are formed as primary products converting to electrophilic compounds such as aldehydes (54, 67). Keap1 reacts with electrophiles and dissociates Nrf2, resulting in its nuclear translocation. The hypothesis that lipid peroxidation products play a role in Nrf2-regulated gene expression is supported by using a reducing reagent of lipid hydroperoxide, diphenylpyrenylphosphine (DPPP) (79, 80). DPPP significantly attenuates PLSS-induced expression of GCLM in HUVEC (Fig. 3A) (106).

As a number of reports have shown, NO production increases with PLSS (Fig. 4A) (106); however, expression of *GCLM* was not affected by the extent of shear stress and NO production (Fig. 4B). Even more, NOS inhibitor L-NAME enhanced the expression of GCLM significantly (Fig. 4C). Furthermore, knockdown of eNOS using siRNA (Figs. 4D and 4E) did not change the expression of GCLM induced by PLSS (Fig. 4F). These results suggest that NO produced in HUVEC in response to PLSS is not involved in the expression of

Table 2. Response to Pulsatile Laminar Shear Stress in Endothelial Cells from Nrf2-Deficient Mice (Nrf2 Regulated Genes)

		WT (ICR)			Nrf2 KO		
	Gene name	static ave. diff.	PLSS ave. diff.	Fold changes	Static ave. diff.	PLSS Ave. diff.	Fold changes
HO-1	Heme oxygenase 1	893	2653	1.3	409	645	0.6
SQSTM1	Sequestosome 1	1159	1810	0.7	778	888	0.2
SLC7A11	Solute carrier family 7A11	135	279	1.2	27	25	0.2
GCLM	Glutamate-cysteine ligase, modifier subunit	488	843	0.9	111	143	0.3
GCLC	Glutamate-cysteine ligase, catalytic subunit	101	148	0.5	24	22	−0.2
NQO1	NAD(P)H dehydrogenase, quinone 1	790	613	-0.2	26	26	−0.1
Ferritin-L	Ferritin, light chain	959	1152	0.3	238	115	−1.4
Ferritin-H	Ferritin, heavy chain	2881	3707	0.3	1308	785	−0.2

PLSS, pulsatile laminar shear stress.
Microarray analysis was performed by using gene chip MOE 430A (Affymetrix Inc.) and calculation was performed as described in a previous article (98).

GCLM. There are some reports showing NO-dependent, HO-1 upregulation via Nrf2 activation in aortic endothelial cells exposed to steady laminar shear stress (34) or treated with NO donor (10, 11, 34). It is assumed that NO produced in HUVEC in response to PLSS does not work as NO produced in aortic endothelial cells exposed to steady laminar shear stress or derived from NO donor.

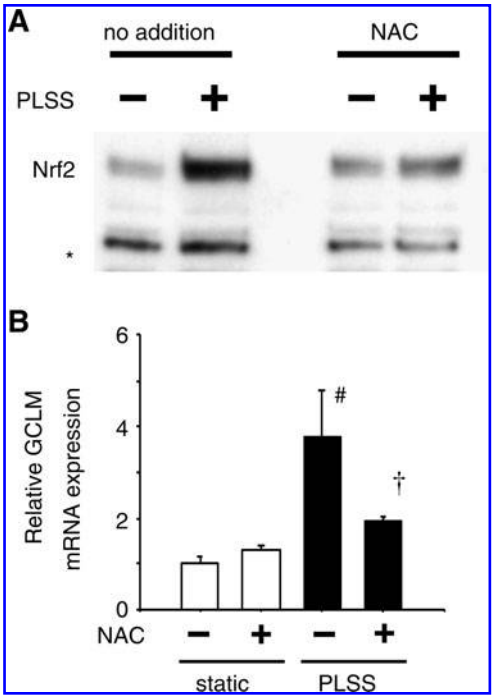

FIG. 2. Attenuation of PLSS-induced Nrf2 accumulation and antioxidant gene expression by NAC. HUVEC were pretreated with 5 m*M* of N-acetylcysteine (NAC) for 30 min and were exposed to PLSS or kept in static culture for 8 h. **(A)** Total cell lysate was analyzed for total Nrf2 by Western blot using anti-Nrf2. A nonspecific band was shown by *asterisk* *. **(B)** The mRNA level of GCLM was analyzed by qRT-PCR as mentioned in Figure 1. #$p < 0.05$ vs. static, †$p < 0.05$ vs. PLSS without NAC. These data have been published previously (106).

Regulation of Oxidative Stress by Nrf2 Activation

The ROS production by PLSS was detected by using a fluorescence dye, 2′7′-dichlorodihydrofluorescindiacetate (DCFH-DA), which is enhanced by transfecting cells with

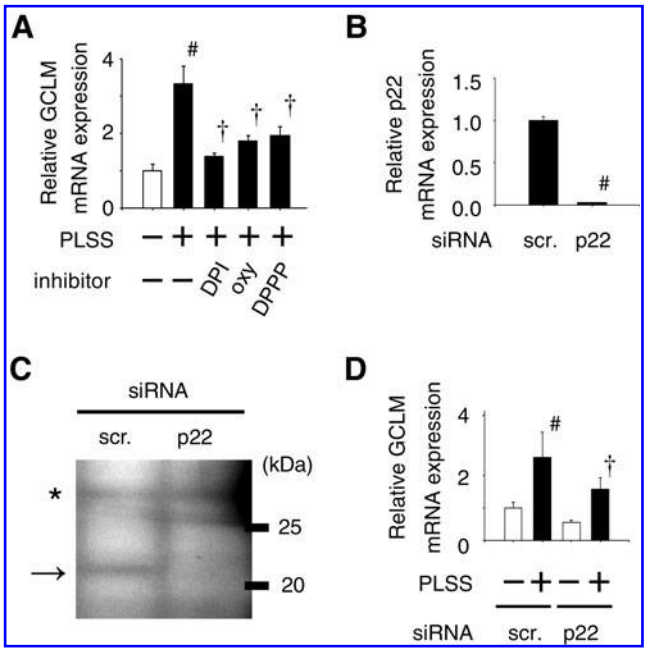

FIG. 3. Involvement of superoxide in antioxidant gene expression. (A) HUVEC were pretreated with 20 μ*M* of DPI, 100 μ*M* of oxypurinol (oxy) or 50 μ*M* of DPPP for 30 min and were exposed to PLSS or kept in static culture for 4 h. The mRNA level of GCLM was analyzed by qRT-PCR as mentioned in Figure 1. #$p < 0.005$ vs. static, †$p < 0.05$ vs. PLSS. **(B)** HUVEC were transfected with p22 or scramble (scr.) siRNA. Forty-eight hours later, the transfection efficiency of p22 siRNA was evaluated by qRT-PCR (#$p < 0.005$ vs. scr.) and **(C)** protein levels using anti-p22. The p22 band was indicated by an *arrow*. The nonspecific band was shown by *asterisk**. **(D)** HUVEC transfected with p22 or scr. siRNA were exposed to PLSS or kept in static culture for 4 h. The mRNA level of GCLM was analyzed by qRT-PCR as mentioned in Figure 1. #$p < 0.05$ vs. static with scr., †$p < 0.05$ vs. PLSS with scr. Panel **A** has been published previously (106).

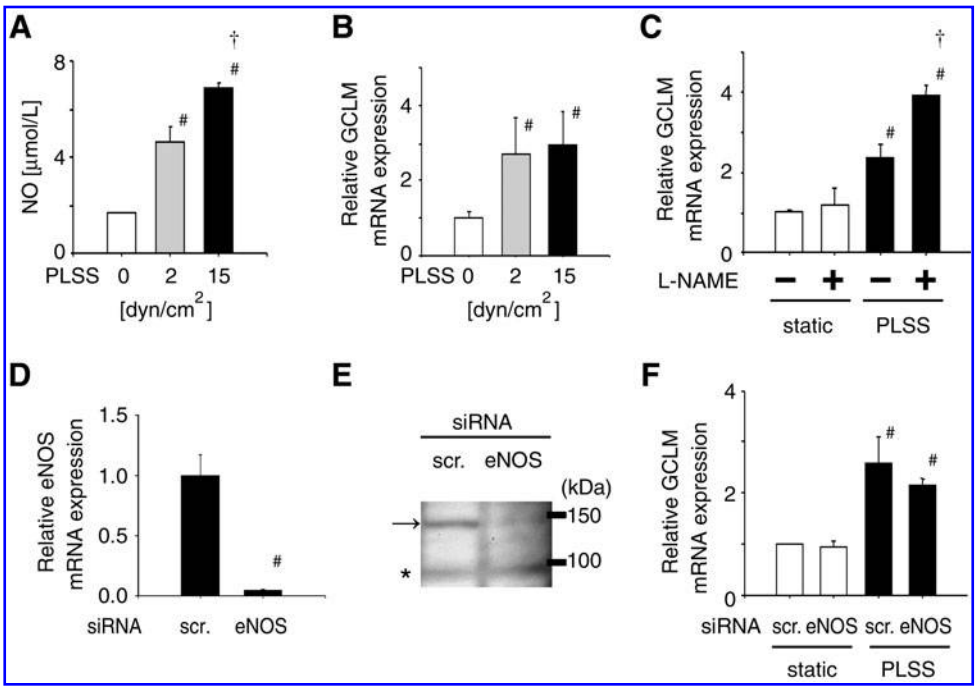

FIG. 4. Little effect of nitric oxide on antioxidant gene expression. (A) After HUVEC were exposed to 2 or 15 dyn/cm^2 of PLSS for 4 h, NOx products in medium were measured by the Griess method. #$p < 0.005$ vs. static, †$p < 0.01$ vs. 2 dyn/cm^2. **(B)** The mRNA level of GCLM at different flow rates was assessed by qRT-PCR as mentioned in Figure 1. #$p < 0.05$ vs. static. **(C)** HUVEC were pretreated with 2.5 m*M* of L-NAME for 30 min and were exposed to PLSS or kept in static culture for 4 h. The mRNA level of GCLM was analyzed by qRT-PCR as mentioned in Figure 1. #$p < 0.05$ vs. static, †$p < 0.01$ vs. PLSS without L-NAME. **(D)** HUVEC were transfected with eNOS or scramble (scr.) siRNA. Forty-eight hours later, the transfection efficiency of eNOS siRNA was measured by its mRNA levels (#$p < 0.05$ vs. scr.) and **(E)** protein levels using anti-eNOS. The eNOS band was indicated by an *arrow*. The nonspecific band was shown by *asterisk**. **(F)** HUVEC transfected with eNOS or scr. siRNA were exposed to PLSS or kept in static culture for 4 h. The mRNA levels of GCLM were analyzed by qRT-PCR. #$p < 0.05$ vs. static with scr. Panels **A–C, E,** and **F** have been published previously (106).

Nrf2 siRNA (Fig. 5), implicating that Nrf2 suppresses the extent of oxidative damage caused by PLSS via upregulation of antioxidant enzymes. For example, biosynthesis of glutathione (GSH), one of key molecules protecting cells from oxidative damage (19, 60, 99) is dependent on activity of glutamate-cysteine ligase (GCL), one of Nrf2-regulated antioxidant enzymes.

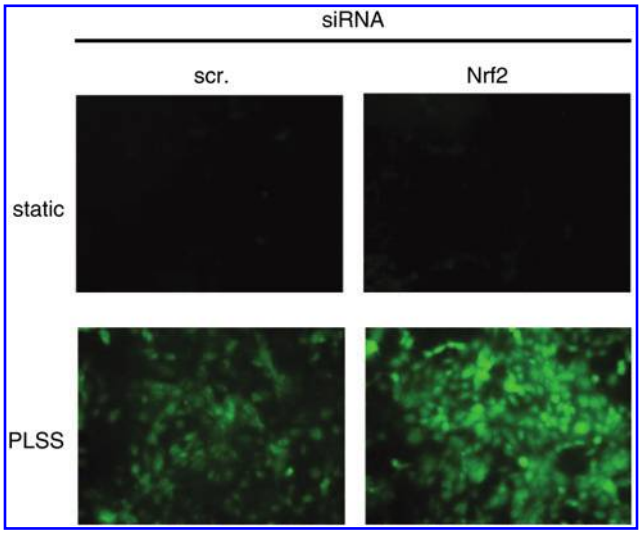

FIG. 5. Enhancement of oxidative stress in Nrf2 knocked-down cells. HUVEC transfected with Nrf2 or scramble (scr.) siRNA were incubated with 20 μ*M* of DCFH-DA for 30 min. Cells were exposed to PLSS or kept in static culture for 1 h and were imaged with an inverted fluorescence microscope.

The expression of adhesion molecules such as VCAM-1, ICAM-1, E-selectin, and monocyte chemoattractant protein-1 (MCP-1) was induced in HUVEC in response to PLSS (Fig. 6). Moreover, the expression of these genes was increased in Nrf2 knocked-down cells. It is assumed that the difference in the extent of adhesion molecule expression sustained *in vivo* may determine the fate of endothelium as either pro-atherogenic or anti-atherogenic.

The experiments using siRNA against p22 or eNOS revealed that both $O_2^{\bullet -}$ and NO were required for induction of adhesion molecules and chemokines by PLSS in Nrf2 knocked down HUVEC (Fig. 7). These results suggest that $ONOO^-$ may play an important role in signal transduction for expression of these pro-atherogenic genes. Evidence supports the contribution of $ONOO^-$ in induction of adhesion molecules and chemokines via activation of NF-κB and AP-1 (5, 35, 45, 50, 57–59, 94, 110, 115). $ONOO^-$ formation is linked to shear stress-mediated activation of a member of the mitogen-activated protein kinase family (MAPK), c-Jun N-terminal kinase (JNK) (28) and ERK (116). $ONOO^-$ easily penetrates cell membranes and modulates target lipids, proteins, and DNA via generation of highly reactive radical, nitric dioxide (NO_2) by reacting with carbon dioxide. Also peroxynitrous acid (ONOOH) derived from a reaction between $ONOO^-$ and hydrogen leads to generation of hydroxy radical (•OH) and NO_2 (97). Thus, $ONOO^-$ causes oxidative stress in vascular cells and contributes to development of atherosclerosis. Dickhout *et al.* have reported the involvement of $ONOO^-$ in atherogenesis by causing ER stress (23). It would be interesting to test whether $ONOO^-$ scavengers, such as uric acid, can attenuate the expression of pro-atherogenic genes induced by oscillatory shear stress.

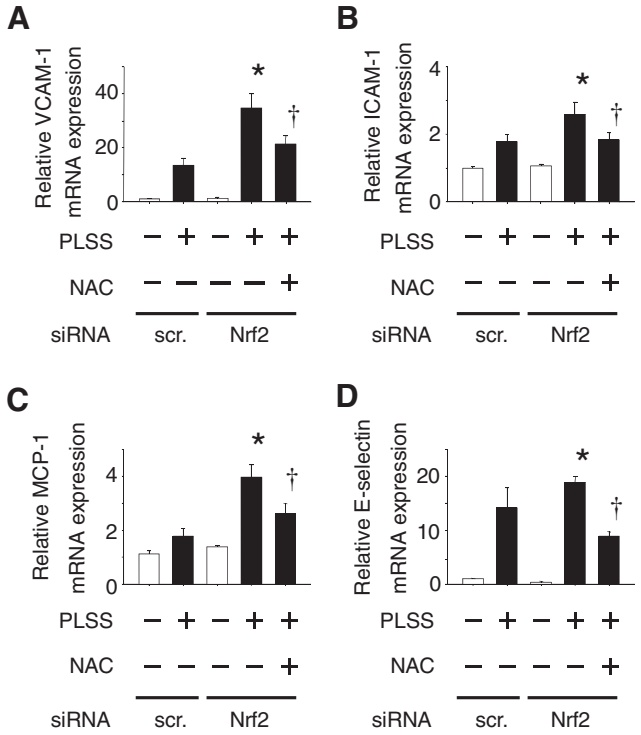

FIG. 6. Inhibitory effects of Nrf2 and NAC on PLSS-induced, atherogenic gene expression. HUVEC transfected with Nrf2 or scramble (scr.) siRNA were exposed to PLSS or kept in static culture for 2 h in the presence or absence of 5 m*M* of NAC. The mRNA levels of **(A)** VCAM-1, **(B)** ICAM-1, **(C)** MCP-1, and **(D)** E-selectin were analyzed by qRT-PCR as mentioned in Figure 1. *$p < 0.05$ vs. PLSS with scr., †$p < 0.05$ vs. PLSS with Nrf2 siRNA.

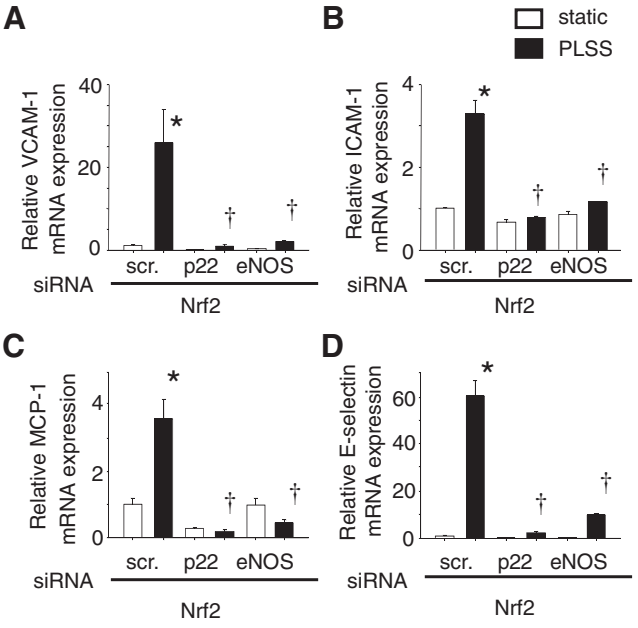

FIG. 7. Effects of ROS/RNS on PLSS-induced atherogenic gene expression. HUVEC transfected with Nrf2 siRNA were simultaneously transfected with either eNOS, p22, or scramble (scr.) siRNA. Cells were exposed to PLSS or kept in static culture for 2 h. The mRNA levels of **(A)** VCAM-1, **(B)** ICAM-1, **(C)** MCP-1, and **(D)** E-selectin were analyzed by qRT-PCR as mentioned in Figure 1. *$p < 0.05$ vs. static with scr. and Nrf2 siRNA, †$p < 0.05$ vs. PLSS with scr. and Nrf2 siRNA.

The molecular mechanisms underlying the protective effect of PLSS remain to be defined; however, inhibition of $O_2\bullet^-$ production via induction of Nrf2-regulated antioxidant enzymes, which in turn limits the formation of $ONOO^-$, may be involved. It is proposed that these antioxidant enzymes may keep oxidative stress under the threshold necessary to induce expression of pro-atherogenic genes.

Anti-Atherogenic Roles of Nrf2 *In Vivo and Ex Vivo*

Zakkar *et al.* have reported that VCAM-1 is highly induced in the aortic arch of Nrf2-deficient mice (113). We have established a primary culture system of mouse arterial endothelial cells (MAEC) (63). MAEC isolated from wild-type and Nrf2-deficient mice were exposed to PLSS for 8 h, and gene expression was analyzed using DNA microarrays. As shown in Table 2, the upregulation of antioxidant genes by PLSS (Table 1) (107) was strongly suppressed in MAEC isolated from Nrf2-deficient mice, as well as their basal levels. Interestingly, the expression levels of adhesion molecules and cytokines including VCAM-1, ICAM-1, E-selectin, and MCP-1 were markedly increased in Nrf2-deficient MAEC exposed to PLSS (Table 3). These results agree with data obtained from Nrf2 knocked down HUVEC (Fig. 6).

The global genomic analysis of adult pig inner aortic arch by Passerini *et al.* revealed the coexistence of pro- and anti-atherosclerotic transcript profiles in susceptible regions where endothelial cells are exposed to disturbed flow (81). They suggested the introduction of additional risk factors might shift this balance to favor lesion development. Hajra *et al.* also suggested that NF-κB signal transduction was primed for activation in high probability regions in mouse proximal aorta on encountering an activation stimulus such as hypercholesterolemia (32). These *in vivo* data suggest that endothelial cells possess an anti-atherosclerotic phenotype even under disturbed flow without additional pro-atherogenic factors present and support our interpretation of *in vitro* and *ex vivo* experimental data showing that Nrf2 activation by steady or pulsatile, laminar flow can induce an anti-atherogenic environment.

Conclusions

The modulation of redox balance in the vasculature by fluid shear stress regulates in the development of atherosclerotic lesions (Fig. 8). Both unidirectional and nonunidirectional shear stress produce ROS/RNS that have potential to activate signal transduction pathways leading to pro-atherogenic gene expression. The expression of pro-atherogenic genes such as adhesion molecules and chemokines requires both $O_2\bullet^-$ and NO, implying $ONOO^-$ is one of the key molecules affecting induction of pro-atherogenic genes. On the other hand, pulsatile laminar shear stress induces expression of Nrf2-regulated genes via $O_2\bullet^-$ production in HUVEC, which is not affected by NO production. The enhancement of GSH biosynthesis by induction of GCL due to Nrf2 activation can prevent generation of excess amount of ROS/RNS that

Table 3. Response to Pulsatile Laminar Shear Stress in Endothelial Cells from Nrf2-Deficient Mice (Top Rated)

		WT (ICR)			Nrf2 KO		
	Gene name	static ave. diff.	PLSS ave. diff.	Fold changes	static ave. diff.	PLSS ave. diff.	Fold changes
MCP-1	chemokine (C-X-C motif) ligand2	25	67	1.2	15	250	3.3
TSLP	thymic stromal lymphoprotein	18	20	0	27	244	3
ICAM-1	intercellular adhesion molecule 1	467	478	0.3	505	2633	2.5
COX-2	prostaglandin-endoperoxide synthetase 2	751	775	0.2	428	1331	1.8
P-selectin	selectin, platelet	1052	1187	0.2	752	2652	1.8
CX3CL1	chemokine (C-X3-C motif) ligand 1	94	56	−0.3	162	443	1.4
E-selectin	selectin, endothelial cell	89	170	1	514	817	0.9
VCAM-1	vascular cell adhesion molecule 1	764	236	−1.8	939	1615	0.6

PLSS, pulsatile laminar shear stress.

Microarray analysis was performed by using gene chip MOE 430A (Affymetrix Inc.), and calculation was performed as described in a previous article (98).

normally led to pro-atherogenic gene expression. The precise molecular mechanisms underlying pulsatile laminar shear stress specifically activating Nrf2 are unclear at this time and are interesting issues to be elucidated by further investigations.

Acknowledgments

This study was supported by the Program of Fundamental Studies in Health Sciences of the National Institute of Biomedical Innovation (NIBIO), by Focus 21 Project of New Energy and Industrial Technology Development Organization (NEDO), and by Special Coordination Fund for Science and Technology and the Academic Frontier Research Project on "New Frontier of Biomedical Engineering Research" of the Ministry of Education, Culture, Sports, Science and Technology.

We thank Dr. Ken Itoh, Hirosaki University School of Medicine, for advice based on his animal studies.

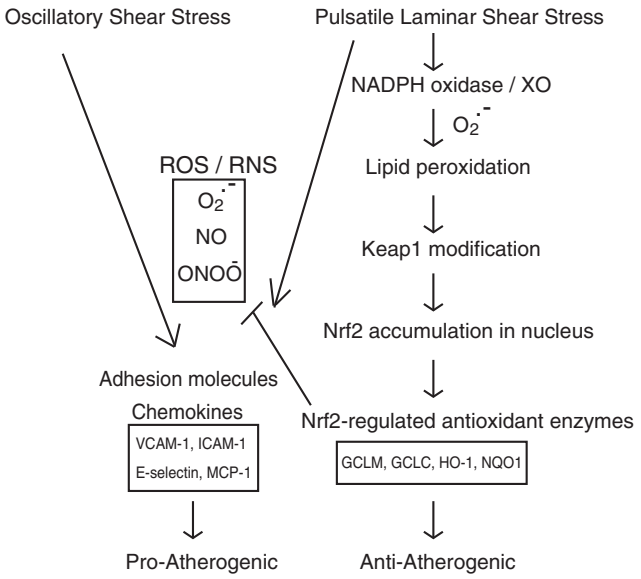

FIG. 8. Proposed mechanisms to determine the fate of shear stress as pro-atherogenic or anti-atherogenic via regulation of redox balance. Both pulsatile laminar shear stress and nonunidirectional oscillatory shear stress produce ROS/RNS that have potential to activate signal transduction pathways leading to pro-atherogenic gene expression. The pulsatile laminar shear stress induces expression of Nrf2-regulated genes via $O_2^{\bullet -}$ production, which is not affected by NO production. The enhancement of antioxidant capacity by Nrf2 activation can prevent generation of excess amount of ROS/RNS which normally led to pro-atherogenic gene expression.

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Date of first submission to ARS Central, June 27, 2010; date of final revised submission, November 24, 2010; date of acceptance, December 2, 2010.

Abbreviations

AP-1 = activator protein 1
ARE = antioxidant response element
DCFH-DA = 2′7′-dichlorodihydrofluorescindiacetate
DPI = diphenyleneiodonium
DPPP = diphenylpyrenylphosphine
eNOS = endothelial nitric oxide synthase
GCLC = glutamate-cysteine ligase catalysis
GCLM = glutamate-cysteine ligase modifier
GSH = glutathione
HO-1 = heme oxygenase 1
HUVEC = human umbilical vein endothelial cells

Abbreviations Used (Cont.)

ICAM-1 = intercellular adhesion molecule-1
iNOS = inducible nitric oxide synthase
IVR = intervening region
JNK = c-Jun N-terminal kinase
Keap1 = Kelch-like ECH-associated protein 1
MAEC = mouse arterial endothelial cells
NAC = N-acetylcysteine
NF-κB = nuclear factor kappa B
NO = nitric oxide
NO_2 = nitric dioxide
NQO1 = NADPH quinone oxidoreductase 1
Nrf2 = nuclear factor (erythroid-derived 2)-like 2
$O_2\bullet^-$ = superoxide
•OH = hydroxyl radical
$ONOO^-$ = peroxynitrite
PLSS = pulsatile, laminar shear stress
qRT-PCR = quantitative real-time PCR
RNS = reactive nitrogen species
ROS = reactive oxygen species
SOD = superoxide dismutase
VCAM-1 = vascular cell adhesion molecule-1
XO = xanthine oxidoreductase

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