Upregulation of Superoxide Dismutase and Nitric Oxide Synthase Mediates the Apoptosis-Suppressive Effects of Shear Stress on Endothelial Cells

Stefanie Dimmeler, Corinna Hermann, Jan Galle, Andreas M. Zeiher

Abstract—Physiological levels of laminar shear stress completely abrogate apoptosis of human endothelial cells in response to a variety of stimuli and might therefore importantly contribute to endothelial integrity. We show here that the apoptosis-suppressive effects of shear stress are mediated by upregulation of Cu/Zn SOD and NO synthase. Shear stress-mediated inhibition of endothelial cell apoptosis in response to exogenous oxygen radicals, oxidized LDL, and tumor necrosis factor-α was associated with complete inhibition of caspase-3-like activity, the central effector arm executing the apoptotic cell death program in endothelial cells. Shear stress-dependent upregulation of Cu/Zn SOD and NO synthase blocks activation of the caspase cascade in response to apoptosis-inducing stimuli. These findings establish the upregulation of Cu/Zn SOD and NO synthase by shear stress as a central protective cellular mechanism to preserve the integrity of the endothelium after proapoptotic stimulation. (Arterioscler Thromb Vasc Biol. 1999;19:656-664.)

Key Words: oxidative stress ■ cell death ■ hemodynamic ■ atherosclerosis ■ antioxidant

One of the most striking features of atherosclerosis is the focal nature of the disease. Atherosclerotic lesions preferentially develop in regions such as arterial bifurcations and curvatures, where disturbed flow patterns with low or fluctuating wall shear stresses occur. Importantly, the strikingly nonrandom distribution of the earliest lesions of atherosclerosis is retained even in the presence of systemic risk factors, such as elevated atherogenic plasma lipoprotein levels. These observations indicate that the local fluid mechanical environment importantly modulates the response of vascular wall cells to injurious insults, and thus contributes to the characteristic pattern of atherosclerotic lesion development.

Because of the unique anatomic location of the endothelium positioned between the flowing blood and the vascular wall tissue, disturbances of the anatomic and functional integrity of the endothelial cell monolayer have been proposed as a condition sine qua non for the initiation of atherosclerosis. Indeed, a localized increase in endothelial cell turnover and upregulation of adhesion molecules are the hallmarks of early atherosclerotic lesion development in animal models. Importantly, increased endothelial cell turnover precedes mononuclear leukocyte recruitment in lesion prone areas. Because the endothelium constitutes a single-cell-thick lining of the vasculature, the observation of a localized increased endothelial cell turnover preceding atherosclerotic lesion development is indicative of endothelial cell demise and regeneration as part of a response to injury program inciting the initiation of atherosclerosis. The demise of endothelial cells may be owing to two distinct types of cell death, apoptosis or necrosis. Apoptosis refers to the morphological alterations exhibited by “actively” dying cells that include cell shrinkage, membrane blebbing, chromatin condensation, and DNA fragmentation, whereas necrosis is characterized by cellular swelling, rupture of plasma membrane, and cell lysis. The central effector arm of the signal transduction pathway executing the apoptotic cell death program is composed of a complex array of cysteine proteases, which have been termed caspases. We have recently demonstrated that oxidized LDL (oxLDL), which plays a key role as a triggering molecule in the earliest phase of atherosclerosis, activates this suicide pathway leading to apoptosis of endothelial cells by enhancing the activity of the caspase cascade. Most importantly, physiological levels of shear stress completely abrogated apoptosis of endothelial cells in response to caspase-activating stimuli. These findings established a possible mechanistic link between local hemodynamic forces and endothelial cell integrity.

Thus, it was the aim of the present study to investigate the molecular mechanisms mediating the apoptosis-suppressive effects of physiological levels of shear stress on endothelial cells. Because accumulating evidence indicates that endothelial generation of oxygen-derived free radicals and activation of oxidant-sensitive transcriptional pathways may be a common pathophysiological mechanism for atherosclerotic disease, we investigated the effects of exogenously gener-
ated oxygen radicals by means of the xanthine/xanthine oxidase system (X/XO) on apoptosis induction in human umbilical vein endothelial cells (HUVEC). Furthermore, we determined the effects of shear stress on apoptosis induced by the proinflammatory cytokine tumor necrosis factor-α (TNF-α) as well as the proatherosclerotic oxidLDL.

Methods

Cell Culture
HUVEC were cultured in endothelial basal medium supplemented with hydrocortisone (1 μg/mL), bovine brain extract (3 μg/mL), gentamicin (50 μg/mL), amphotericin B (50 μg/mL), epidermal growth factor (10 μg/mL), and 10% fetal calf serum and used up to the third passage. Confluent monolayers of HUVEC were grown onto 6-cm wells and exposed to laminar fluid flow in a cone-and-plate apparatus as previously described.14,17 A constant shear stress of 15 dynes/cm² was used in all experiments to simulate physiological levels of shear stress.17,18

Determination of DNA Fragmentation
DNA fragmentation was determined with the cell death detection ELISA (Boehringer Mannheim).14,19 Therefore, floating cells were collected in a 15-mL Falcon tube and pooled with the attached cells, which were scrapped off the plate. Then cells were pelleted by centrifugation at 700g for 10 minutes, washed with PBS, and resuspended in incubation buffer. The histone-associated DNA fragments were linked to the antihistone antibody from mouse and the DNA part of the nucleosome to the anti–DNA-peroxidase. Then the amount of peroxidase retained in the immunocomplex was determined photometrically.

For morphological staining of nuclei, cells were centrifuged (10 minutes, 700g), fixed in 4% formaldehyde, and stained with 4',6-diamidino-phenylindole (DAPI; 0.2 μg/mL in 10 mmol/L Tris-HCl, pH 7.0, 10 mmol/L EDTA, 100 mmol/L NaCl) for 20 minutes. Five hundred cells were counted by two independent blinded investigators, and the percentage of apoptotic cells per total number of cells was determined.

Generation of O$_2^-$ by the X/XO System
The generation of O$_2^-$ by X/XO (0.1 mmol/L and 10 mU) was monitored by measuring the increase of absorbance at 560 nm after incubation in the presence of nitro blue tetrazolium (25 μmol/L) for 18 hours at 37°C. Control experiments ensured that incubation with SOD (100 mU/mL) significantly inhibited xanthine–oxidase–induced apoptosis.

Transfection With Antisense Oligonucleotides
Sense (bases 1 to 21, initiation codon at 1) or antisense oligonucleotides (bases 56 to 77) corresponding to the human SOD-1 sequence were incubated in 100 μL RPMI-medium in the presence of 5 μL lipofectamine (GIBCO RBL) for 30 minutes at room temperature. HUVEC (5×10⁶ cells in 6-cm² wells) were washed with RPMI and incubated with 2 mL RPMI before adding the lipofectamine/oligonucleotide mixture. After further incubation for 5 hours, 3 mL complete endothelial basal medium was added, and the cells again were incubated for 2 hours. Then, apoptosis was induced by the different stimuli for 18 hours. N$^6$-Monomethyl-L-arginine (LNAME) was preincubated for 1 hour before stimulation of apoptosis.

Determination of Cu/Zn SOD mRNA and Protein Levels
RNA was prepared according to Liu et al.21 and 10 μg was loaded on 0.8% formamide–agarose gels. RNA was blotted on nylon membranes, and the blots were hybridized with a radioactively labeled human Cu/Zn SOD probe and incubated for 24 hours. Then the blots were washed (0.1% SDS, 0.2% SSC) and exposed to x-ray films. HUVEC were lysed in buffer (1% Triton X-100, 0.52 mol/L sucrose, 5 mmol/L EDTA, 1 mmol/L PMSF, 2 mmol/L DTT, 10 mmol/L Tris-HCl, pH 8.0) for 15 minutes at 4°C followed by centrifugation (20 000g; 15 minutes). The amount of Cu/Zn SOD in the resulting supernatant was detected using an antibody directed against Cu/Zn SOD (Sigma; 140 μg/mL) with human SOD as standard (Sigma). The results obtained by ELISA were confirmed by Western blotting analysis with an antibody raised against human Cu/Zn SOD (1:100 in 3% BSA; Calbiochem) followed by enhanced chemiluminescence and densitometric analysis.

Preparation of oxidLDL
Human LDL was isolated by sequential ultracentrifugation and oxidized as described previously.22 Antioxidant-free LDL (0.3 mg protein/mL) was incubated with CuSO₄ (5 μmol/L) for 24 hours at 23°C. The degree of oxidation was assessed by two different methods, the increase of mobility on agarose gel (1.4 versus native LDL) and the formation of thiobarbituric acid-reactive substances (3.4±0.8 μmol/L).

Determination of Caspase-3-Like Activity and Proteolytic Cleavage
Caspase-3-like protease activity was determined as previously described19 by measuring the proteolytic cleavage of the fluorogenic substrate 7-amino-4-coumarin (AMC)-DEVD and AMC as standard using an excitation wavelength of 380 nm and an emission wavelength of 460 nm.23 Western blotting analysis was performed as previously outlined12 with caspase-3/p-20 antibody (Signal Transduction Laboratories).

Statistical Analysis
Statistical analysis was performed with ANOVA followed by modified LSD test (SPSS-Software).

Results

Effect of Shear Stress on Exogenous Oxygen Radical–Induced Apoptosis
Apoptosis was induced in HUVEC by oxygen radicals generated by the enzymatic reaction of X/XO. Incubation of HUVEC with xanthine oxidase dose- and time-dependently induced DNA fragmentation in the presence of 0.1 mmol/L xanthine as detected by an ELISA specific for histone-associated DNA fragments (Figure 1, top and bottom left). In addition, quantification of fluorescence-stained apoptotic nuclei revealed 4.2±0.4% apoptotic cells after treatment of HUVEC with 10 μM xanthine oxidase for 18 hours compared with 1.6±0.2% apoptotic cells in controls (Figure 1, bottom right). Necrosis was excluded by determination of the lactate dehydrogenase release, which was not affected by X/XO treatment for 18 hours.

To determine the effect of shear stress, HUVEC were exposed to laminar shear stress (15 dynes/cm²) in a cone-and-plate apparatus as previously described.14 As shown in Figures 1, bottom right and 3, top, X/XO-induced apoptosis was completely inhibited by shear stress. Moreover, morphological analysis of apoptotic nuclei after fluorescence staining (Figure 1, bottom right) revealed 1.5±0.4% apoptotic nuclei in the presence of shear stress and X/XO versus 4.2±0.4% apoptotic cells after treatment with X/XO without shear stress ($P<0.05$).

Because shear stress has been shown to upregulate mRNA levels and protein expression of Cu/Zn SOD,24 we used an antisense strategy with the oligonucleotide (5'-CTTCTCCCTTGTGCCTGAATTG-3') corresponding to the human Cu/Zn SOD (SOD-1) cDNA sequence to determine the influence of Cu/Zn SOD on the apoptosis-suppressive effect of shear stress. In our experimental setting, application of laminar shear stress resulted in a time-dependent increase
of Cu/Zn SOD mRNA levels (Figure 2, top). Furthermore, protein expression was significantly increased to 206±34% after 18 hours’ exposure to shear stress as determined by an ELISA specific for Cu/Zn SOD. Incubation with the antisense oligonucleotide completely prevented the shear stress–induced upregulation of mRNA (Figure 2, top). The mRNA data were confirmed by Western blotting analysis with antibodies raised against Cu/Zn SOD. As shown in Figure 2, bottom, Cu/Zn SOD antisense oligonucleotide transfection reduced basal Cu/Zn SOD protein levels about 31±11% and inhibited the shear stress–induced increase of Cu/Zn SOD protein from 189±21% to 53±10%.

Antisense oligonucleotide-mediated abrogation of Cu/Zn SOD upregulation was associated with a significant reduction of the inhibitory effect of shear stress on X/XO-induced apoptosis (Figure 3, top). In contrast, the application of sense oligonucleotides (5’-ATGGCGAGGAAGGCCGTG GCC-3’) did not significantly diminish the shear stress–mediated increase of Cu/Zn SOD mRNA and protein levels (163±10% increase in the presence of sense oligonucleotides) and had no effect on the suppression of apoptosis by shear stress (Figure 3, top). Similarly, scrambled oligonucleotides (5’-GCTGACGTTAGAGCTAGCG-3’) did not reduce the antiapoptotic effects of shear stress (data not shown). Thus, shear stress–induced protection against endothelial cell apoptosis in response to X/XO is partially mediated by the upregulation of Cu/Zn SOD.

Because shear stress also increases the expression of the endothelial NO synthase type II (eNOS) and endothelial NO production has been shown to interact with the oxidative balance of the cells and further potently inhibits endothelial cell apoptosis, we directly tested whether NO and Cu/Zn SOD synergize to mediate the apoptosis-suppressive effect of shear stress. Inhibition of NO synthesis by the competitive

Figure 1. Effect of shear stress on apoptosis induced by X/XO. Top, HUVEC were incubated for 18 hours with different concentrations of xanthine oxidase in the presence of xanthine (0.1 mmol/L), and DNA fragmentation was assessed with ELISA. Data are mean±SEM with *P<0.05 vs xanthine. Bottom left, HUVEC were incubated for the times indicated with X/XO (0.1 mmol/L and 10 mU), and DNA fragmentation was determined with ELISA. Values are mean±SEM with *P<0.05 vs 18 hours. Bottom right, HUVEC were incubated with X/XO in the presence or absence of laminar shear stress (15 dynes/cm²) for 18 hours. Then cells were stained with DAPI.
NOS inhibitor LNMA significantly reduced the protective capacity of shear stress against X/XO-induced apoptosis (Figure 3, bottom). Most importantly, the combination of Cu/Zn SOD antisense oligonucleotides and LNMA completely abolished the apoptosis-suppressive effect of shear stress (Figure 3, bottom). Control experiments ensured that the compounds did not affect X/XO-induced apoptosis (data not shown). Thus, the shear stress--induced inhibition of endothelial cell apoptosis in response to X/XO is mediated by the synergistic effects of Cu/Zn SOD and NOS upregulation.

Effect of Shear Stress on OxLDL-Induced Apoptosis

Having established a synergistic role of Cu/Zn SOD and NO for shear stress--induced protection of endothelial cells against oxygen radical--induced apoptosis, we next sought to examine the effects of shear stress on apoptosis induced by oxLDL, which is a well-established triggering molecule in the atherosclerotic process.13 OxLDL has been shown to increase the endothelial production of reduced oxygen species including superoxide anions.25 Importantly, our previous studies demonstrated complete inhibition of oxLDL-induced endothelial cell apoptosis by antioxidants, implicating oxidant stress to be involved in the oxLDL-mediated activation of the signaling pathways leading to apoptosis of endothelial cells.12 As previously described, oxLDL induced apoptosis of HUVEC in a time- and concentration-dependent manner with maximal apoptotic effects observed by incubation with 10 μg/mL oxLDL for 18 hours,12 whereas native LDL had no effect. In addition, there was no increase in lactate dehydrogenase release up to 10 μg/mL oxLDL for 18 hours, excluding the induction of necrosis.12

Simultaneous exposure to laminar shear stress (15 dynes/cm²) completely abrogated oxLDL-induced apoptosis (Figure 4, top). Antisense oligonucleotides against Cu/Zn SOD significantly reduced the apoptosis-suppressive effect of shear stress (Figure 4, top), but did not completely neutralize the effects of shear stress. Likewise, inhibition of NO production by LNMA significantly inhibited the apoptosis-suppressive effects of shear stress. However, the combined application of Cu/Zn SOD antisense oligonucleotides and LNMA completely abolished the apoptosis-suppressive effect of shear stress (Figure 4, top). Thus, shear stress-induced upregulation of Cu/Zn SOD and NOS synergistically mediate the apoptosis-suppressive effect of shear stress in endothelial cells stimulated by oxLDL.

Effect of Shear Stress on TNF-α-Induced Apoptosis

As previously reported,14 shear stress also completely abrogated apoptosis of HUVEC in response to TNF-α, a classic trigger of the apoptotic response in various cells including endothelial cells. Again, inhibition of NO production substantially reduced the apoptosis-suppressive effects of shear stress after TNF-α stimulation (Figure 4, bottom). However, antisense oligonucleotides against Cu/Zn SOD completely abrogated apoptosis suppression by shear stress (Figure 4, bottom) without any further synergistic effect of NOS inhibition. Thus, in contrast to the additive inhibitory action of Cu/Zn SOD and NOS to inhibit X/XO- and oxLDL-induced apoptosis, inhibition of shear stress--induced increase in Cu/Zn SOD appeared to be sufficient to entirely block the apoptosis-suppressive effect of shear stress when stimulating apoptosis with TNF-α.

Involvement of Caspase-3-Like Proteases

To elucidate the molecular events leading to inhibition of apoptosis by shear stress, we investigated the involvement of caspases, which represent the final common pathway of apoptosis signal transduction11 and have been shown to play a key role in TNF-α- and oxLDL-induced apoptosis of HUVEC as evidenced by complete inhibition of TNF-α- or oxLDL-induced apoptosis in the presence of caspase-3 inhibitors.12,19 Therefore, we measured caspase-3-like protease activity using the fluorogenic labeled peptide DEVD. X/XO-induced caspase-3-like protease activity was completely prevented by application of laminar shear stress (Figure 5, top left). Simultaneous coinubcation with antisense oligonucleotides directed against Cu/Zn SOD and LNMA completely abolished the shear stress--mediated...
prevention of increased caspase-3-like activity, whereas each compound alone was less effective (Figure 5, top left). Because activation of caspase-3 requires the proteolytic cleavage into its active subunits p12 and p17/p20, we also analyzed cleavage of caspase-3. Cleavage of the p32 precursor protein into the p17/p20 subunits by X/XO treatment was completely inhibited by exposure to shear stress (Figure 5, top right). Again, the combination of antisense oligonucleotides and LNMA completely abrogated the inhibitory effects of shear stress on caspase-3 cleavage. Thus, the shear stress–induced increase in NO synthesis and the simultaneous increase of the antioxidative capacity because of Cu/Zn SOD upregulation prevent activation of caspase-3-like proteases. Similar effects were demonstrated when inducing apoptosis with oxLDL (data not shown). In line with the effective neutralization of shear stress–induced protection against TNF-α–induced apoptosis, Cu/Zn SOD antisense oligonucleotides appeared to be sufficiently effective to inhibit the prevention of TNF-α–induced caspase-3-like activation by shear stress (Figure 5, bottom), whereas LNMA exhibited a minor effect (Figure 5, bottom). In addition, coinubcation of the caspase-3-like peptide inhibitor Ac-DEVD-CHO (100 μmol/L) with TNF-α and Cu/Zn SOD antisense oligonucleotides in the presence of shear stress reduced the apoptosis approximately 58±7%, demonstrating that inhibition of the apoptosis-suppressive effects of shear stress by SOD antisense oligonucleotide treatment results in caspase-3-dependent apoptosis.

Discussion

The results of the present study demonstrate for the first time that upregulation of Cu/Zn SOD and NOS mediate the apoptosis-suppressive effects of shear stress on endothelial cells in response to various reactive oxygen species–producing stimuli. Upregulation of Cu/Zn SOD and NOS by shear stress cooperate to abrogate activation of the caspase cascade, the central effector arm of the signal transduction pathway executing the apoptotic cell death program. These findings establish the regulation of Cu/Zn SOD and NOS by shear stress as an important protective cellular mechanism to preserve the integrity of the endothelium.

Enhanced production of reactive oxygen species in the vascular endothelium is a hallmark very early in the atherogenic process, even preceding atherosclerotic lesion formation.16,26 The generation of reactive oxygen species has been demonstrated to mediate apoptosis by activation of the cell death program in numerous cells27 including endothelial cells.28 Recently, an obligate role for the activation of the caspase cascade to mediate oxygen radical–induced cell death has been documented.29 Cu/Zn SOD is the primarily nonmitochondrial enzyme regulating cellular superoxide levels30,31 by dismutating O$_2^-$ to H$_2$O$_2$ and molecular oxygen.32 Previous studies have shown that downregulation of Cu/Zn SOD activity induces apoptosis of neuronal cells.29,33 The recent
finding that Cu/Zn SOD is upregulated by physiological levels of shear stress in endothelial cells suggested a physiological role of this antioxidant enzyme in endothelial cell biology, too. The results of the present study now establish the shear stress–mediated upregulation of Cu/Zn SOD as an important endothelial cellular defense mechanism contributing to the inhibition of activation of the caspase cascade by shear stress. Thus, scavenging of $O_2^-$ by the upregulation of Cu/Zn SOD by shear stress completely prevented TNF-α-induced apoptosis and increased the resistance to proapoptotic stimuli involving oxidative damage in endothelial cells. These observations considerably extend previous findings, which established activation of oxidant-sensitive transcriptional pathways by endothelial generation of oxygen-derived radicals as a common pathophysiological process involved in atherosclerosis. Moreover, the results of the present study extend our previous observation that shear stress–mediated reduction of oxidative flux interferes with $H_2O_2$-induced apoptosis by modulation of the glutathione redox cycle.

However, upregulation of Cu/Zn SOD only partially accounted for the apoptosis-suppressive effects of shear stress when apoptosis was induced by oxLDL or oxygen radicals. Complete inhibition of the shear stress–mediated antiapoptotic effects required the simultaneous blockade of Cu/Zn SOD and NOS activity. It is well established that physiological levels of shear stress enhance expression of the endothelial NOS and, ultimately, the capacity of endothelial cells to produce NO. In addition, the superoxide scavenging activity of SOD has been shown to significantly prolong the biological half-life of NO. Thus, the simultaneous upregulation of Cu/Zn SOD and NOS by shear stress will increase the bioavailability of NO. Importantly, we have recently shown that NO inhibits apoptosis of endothelial cells by inhibition of caspase activation caused by S-nitrosylation of the functionally essential cysteine in the active center of the enzymes. Taken together, the simultaneous upregulation of Cu/Zn SOD and NOS by shear stress inhibits the apoptotic signaling cascade by both scavenging of caspase-activating oxygen radicals as well as directly inhibiting caspase activity.

Figure 4. SS-mediated inhibition of TNF-α- and oxLDL-induced apoptosis. Effect of inhibition of Cu/Zn SOD expression and NOS activity. DNA fragmentation was induced by oxLDL (10 μg/mL; Top) or by TNF-α (400 U/mL, 18 hours; Bottom) in the presence or absence of SS and the effect of LNMA (1 mmol/L) and/or AS oligonucleotides (0.6 μg) was determined. Data are mean ± SEM; Top, *P<0.05 vs oxLDL + SS, #P<0.05 vs oxLDL + LNMA + AS + SS; Bottom, *P<0.05 vs TNF + SS + LNMA, #P<0.05 vs TNF + SS.
owing to NO-mediated S-nitrosylation. In addition, because the reaction of NO and O$_2^-$ leads to the formation of peroxynitrite (ONOO$^-)$, scavenging of O$_2^-$ by Cu/Zn SOD may reduce the subsequent formation of peroxynitrite, which has been shown to produce endothelial cell injury.\textsuperscript{43}

The complete inhibition of the protective effect of shear stress against TNF-\textalpha--induced apoptosis by Cu/Zn SOD antisense oligonucleotides appears—at first glance—to be surprising considering that NO synthesis is not inhibited and should still interfere with apoptosis signal transduction. However, TNF-\textalpha--potently downregulates the endothelial NOS.\textsuperscript{44} Although shear stress still enhances eNOS expression above baseline levels in the presence of TNF-\textalpha, this increase is less than 50% of the effect obtained in the absence of TNF-\textalpha.\textsuperscript{19} Thus, the biologically active amount of NO may not be sufficient to affect TNF-\textalpha--induced apoptosis in the absence of Cu/Zn SOD with increased levels of O$_2^-$ causing inactivation of NO and ONOO$^-$ formation.\textsuperscript{42,43}

OxLDL is well known to increase the endothelial production of partially reduced oxygen species including superoxide anions and hydroxyl radicals.\textsuperscript{25} TNF-\textalpha, which is locally upregulated in both experimental and human atherosclerosis,\textsuperscript{45,46} also generates reactive oxygen species in endothelial cells. The results of the present study demonstrate that shear stress not only abrogates apoptosis of endothelial cells in response to the superoxide anion--generating X/XO, but also in response to oxLDL and TNF-\textalpha, which are pathophysiologically more relevant potential mediators of endothelial injury. The demonstration that inhibition of apoptosis in response to all three stimuli was caused by the synergistic effects of shear stress--mediated upregulation of the antioxidant enzyme Cu/Zn SOD and NOS further supports the concept that oxidative stress is involved in the injurious insults of oxLDL and TNF-\textalpha on endothelial cell integrity as part of the response to injury program.

Indeed, stimulation of the integrin receptor by fibronectin has been shown to prevent apoptosis of human endothelial cells.\textsuperscript{47} Because shear stress shares similar signal transduction pathways with integrin receptors,\textsuperscript{48} one may speculate that stimulation of the mitogen-activated kinase cascade might directly or indirectly contribute to the antiapoptotic effects of shear stress. Taking into account that the apoptotic pathway allows fine-tuned regulation and possesses various checkpoints for control, the interaction of shear stress with diverse signals would make sense to inhibit apoptosis induction. Nevertheless, the results of the present study demonstrate that the combined inhibition of shear stress--induced upregulation of Cu/Zn SOD and NOS completely block the antiapoptotic

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Figure 5. Effect of SS on caspase-3-like activity and caspase-3 cleavage. Top left, Influence of SS on X/XO-induced increase of caspase-3-like activity. Data are mean±SEM with \textsuperscript{**}$P<0.05$ vs X/XO, \textsuperscript{*}$P<0.05$ vs X/XO+SS, \textsuperscript{#}$P<0.05$ vs X/XO+LNMA+AS+SS. Top right, caspase-3 cleavage demonstrated in a Western blot against caspase-3 (p32/p17-p20) and modulation by AS oligonucleotides (0.6 \textmu g) and LNMA (1 mmol/L). Bottom, Protective effect of SS on TNF-\textalpha (400 U/mL)--induced increase of caspase-3-like activity in the presence of LNMA and/or AS. Data are mean±SEM; \textsuperscript{**}$P<0.05$ vs TNF, \textsuperscript{*}$P<0.05$ vs TNF-\textalpha+SS, \textsuperscript{#}$P<0.05$ vs TNF+LNMA.
effect of shear stress, provide compelling evidence that maintaining the redox state plays a central role for the apoptosis-suppressive signaling pathways activated by shear stress.

Given the pivotal role of oxidative stress in the process of atherosclerotic lesion formation, the demonstration that physiological levels of shear stress activate a cellular defense program against oxidative damage to protect endothelial cells from being driven into apoptosis may provide important novel insights into how the local hemodynamic milieu contributes to the nonrandom localization of endothelial cell injury leading to atherosclerotic lesion development. The importance of preventing endothelial cell demise and regeneration to reduce the susceptibility of the vascular wall to atherosclerotic lesion development has very recently been highlighted by animal studies documenting that antisense oligodeoxynucleotides blocking endothelial cell cycle regulatory gene expression inhibit accelerated diet-induced atherogenesis. Thus, the results of the present study elucidating the mechanistic link between shear stress and preservation of endothelial cell integrity may not only provide a pathophysiological clue for the endothelial response to injury program preceding atherosclerotic lesion development, but may also lead to novel therapeutic strategies aiming at the inhibition of endothelial cell activation by blocking the apoptotic pathway.

Acknowledgments

We thank Christine Goebel for expert technical assistance and Dr R. Popp for the construction of the shear stress chambers. This work was supported by grants from the Deutsche Forschungsgemeinschaft Di 600/2-1 and 2-2. S.D. has a fellowship from the Deutsche Forschungsgemeinschaft and C.H. has a fellowship from Boehringer Ingelheim Fonds.

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doi: 10.1161/01.ATV.19.3.656
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1079-5642. Online ISSN: 1524-4636

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