Effects of Peroxynitrite and Superoxide Radicals on Endothelial Monolayer Permeability: Potential Role of Peroxynitrite in Preeclampsia

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OBJECTIVE: *Increased endothelial permeability is associated with increased oxidative stress in the maternal vasculature in women with preeclampsia. This study was to determine if oxidative stress elicited by peroxynitrite could lead to an increase in endothelial permeability.*

METHODS: *Endothelial oxidative stress was produced by adding 3-morpholinosydnonimine (SIN-1, a peroxynitrite generator) to the cell culture. Confluent endothelial cells (ECs) grown in cell culture inserts were treated with SIN-1 at a concentration of 0.5 mM alone or in combination with MnTMPyP (a peroxynitrite scavenger) or superoxide dismutase (SOD). EC permeability was determined by measuring EC electrical resistance (ER) and horseradish peroxide (HRP) leakage. Data are presented as means ± SE and analyzed by analysis of variance (ANOVA). Junctional protein expression and distribution for vascular endothelial (VE)-cadherin, occludin, and phosphorylated focal adhesion kinase (FAK) at tyrosine 397 [pY^{397}] were examined by fluorescent staining of ECs.*

RESULTS: *First, ER was significantly reduced and HRP leakage was significantly increased in ECs treated with SIN-1 compared to those in control cells, ER: 26.97 ± 1.41 versus 42.27 ± 0.40 $\Omega \cdot cm^2$, P <.01; HRP: 0.26 ± 0.07 versus 0.02 ± 0.01 OD 470 nm, P <.01, respectively. Second, cells treated with SIN-1 showed formation of gaps and disorganized VE-cadherin and occludin distribution at cell contact regions. FAK[pY^{397}] expression was completely lost in cells treated with SIN-1. Finally, these functional and morphologic changes in ECs induced by SIN-1 were blocked in cells pretreated with MnTMPyP and SOD.*

CONCLUSIONS: *Disorganization of junctional proteins and dephosphorylation of FAK[pY^{397}] may account for the increased endothelial permeability induced by oxidative stress associated with preeclampsia.* (*J Soc Gynecol Investig 2005;12:586–92*) *Copyright © 2005 by the Society for Gynecologic Investigation.*

KEY WORDS: Endothelial barrier function, junctional protein, oxidative stress, preeclampsia.

There is substantive evidence that increased oxidative stress plays critical roles in the pathophysiology and pathogenesis in women with preeclampsia, a hypertensive and multiple system disorder in human pregnancy. Oxidant agents such as lipid peroxides, nitrosothiols, and antibodies to oxidized low-density lipoprotein (LDL) are increased,[1–3] whereas antioxidant agents including α-tocopherol levels, superoxide dismutase activity, and antioxidant capacity are decreased[1,4] in the maternal circulation in women with preeclampsia. Therefore, it is believed that enhanced oxidative stress in the maternal vascular system may be the underlying pathophysiology of increased vasoconstriction as well as increased vascular permeability in this pregnancy disorder.

In preeclampsia, increased vascular endothelial permeability in the peripheral interstitial tissue results in edema and in the kidney glomerulus results in proteinuria. The latter, along with increased maternal blood pressure, is a diagnostic criterion for preeclampsia. Electron microscopic studies of myometrial vessels and subcutaneous arteries in women with preeclampsia have revealed disrupted and enlarged intercellular junctions in vascular endothelial cells (ECs).[5,6] These structural changes in the EC lining combined with findings of a higher rate of disappearance of intravascular albumin-bound Evans blue dye in preeclamptic women[7,8] indicate that the disturbed endothelial junctions are directly related to the increased endothelial permeability in the maternal systemic vasculature in preeclampsia. Although the direct causative agents of endothelial barrier dysfunction have not been defined, increased oxidative stress environments in the maternal circulation and intrinsic responses of vascular endothelium to the circulating stimuli, ie,

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soluble oxidants, cytokines, activated leukocytes, and platelets, are likely the candidate agents that disturb endothelial barrier function.

We recently found that endothelial monolayer permeability was increased when cells were cultured in the presence of maternal sera from women with preeclampsia, while this phenomenon was not observed in cells that were cultured in the presence of sera from non-pregnant women and from women with normal pregnancies.[9] Furthermore, preeclampsia sera–induced increase in endothelial monolayer permeability could be blocked by addition of the antioxidant superoxide dismutase (SOD) in the culture,[9] suggesting that reactive oxygen species (ROS) or oxidant stimuli may be a causative component in sera from preeclamptic pregnancies that disturb endothelial barrier function. Increased oxidative stress in the maternal vasculature was also observed by enhanced nitrotyrosine staining in the subcutaneous vascular tissue from women with preeclampsia.[10] Nitrotyrosine residues are formed when tyrosine in proteins is modified by peroxynitrite, a highly reactive oxygen species. However, the relationship between the formation of peroxynitrite and increased endothelial permeability has not been examined. Hence, the objectives of this study were to determine whether increased endothelial oxidative stress could directly induce endothelial permeability and what mechanisms are involved. 3-Morpholinosydnonimine (SIN-1), a potent peroxynitrite donor, was used to induce endothelial oxidative stress. Endothelial junctional protein vascular endothelial (VE)-cadherin, tight junction protein occludin, and focal adhesion kinase (FAK) were examined.

MATERIALS AND METHODS

Endothelial Cell Isolation and Culture

Human umbilical vein endothelial cells (HUVEC) were isolated by established procedures in our laboratory as previously described.[11] Umbilical cords were collected from normal pregnancies delivered at the Main Hospital, Louisiana State University Health Sciences Center in Shreveport (LSUHSC-Sh). Normal pregnancy is defined as a pregnancy in which the mother has normal blood pressure ≤140/90 mm Hg without medical and obstetrical complications. The study was approved by the Institutional Review Board (IRB) for Human Research at LSUHSC-Sh, Louisiana. Isolated cells were incubated with endothelial growth medium (EGM; BioWhittaker Inc, Walkersville, MD). Cells used for protein extraction were grown in 25-cm^2 culture flasks. Cells for immunostaining were grown on glass coverslips in 24-well plates and cells for permeability assay were seeded onto 8-μm pore size polycarbonate cell culture inserts (Becton Dickinson Labware, Franklin Lakes, NJ). All assays were performed when cells were confluent in culture and only the first passage cells were used in the study.

EC oxidative stress was produced by adding SIN-1 (a peroxynitrite generator) to the cell culture. Confluent ECs were treated with SIN-1 at a concentration of 0.5 mM alone or in combination with a concentration of 30 μm MnTMPyP (a peroxynitrite scavenger) or 10 μg/mL for SOD. The SIN-1 concentration at 0.5 mM used in this study was based on previous published works.[12,13] In the present study, SIN-1–induced changes in endothelial electrical resistance (ER) and horseradish peroxidase (HRP) leakage were initially tested at the concentration of 0.5 mM for 2, 4, and 8 hours (Figure 1). The duration of 4 hours treatment was selected for the subsequent experiments as a significant effective length of stimulation. This duration also allowed us to complete the staining assays in the same day after treatment.

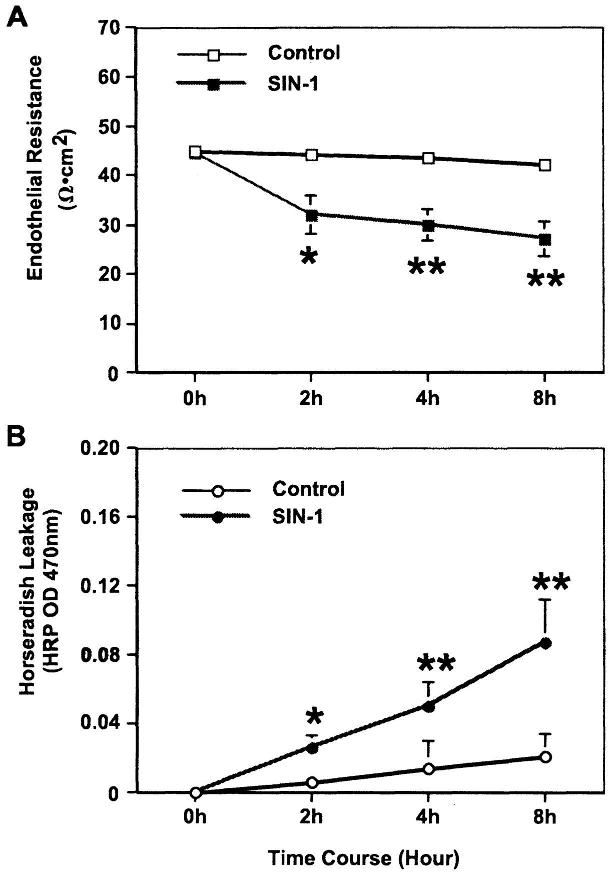

Figure 1. Changes of endothelial electrical resistance (ER) and horseradish peroxidase (HRP) leakage in endothelial cells with or without SIN-1 in culture. A concentration of 0.5 mM for SIN-1 was used. SIN-1 treated endothelial monolayer induced a time dependent decrease in ER (A) and time-dependent increase in HRP leakage (B) over 8 hours of incubation. Data are expressed as mean ± SE from eight independent experiments. *$P < .05$; ** $P < .01$.

Measurement of Endothelial Electrical Resistance

Endothelial ER across the monolayer was measured by an Endohm EVOM endothelial ohmmeter (World Precision Instruments, Sarasota, FL) as previously described.[9] The average reading from duplicated measurements was used for data calculation. The reading obtained from a blank insert was subtracted to give the net resistance, which was multiplied by the membrane area to give the resistance in area corrected units.

The value for ER was expressed as $\Omega \cdot cm^2$, taking into account the surface area of the filter (0.30 cm^2).

Endothelial Permeability Assay

Endothelial permeability was determined by measuring the passage of HRP through confluent endothelial monolayer grown on polycarbonate cell culture inserts as previously described.[9,14] Briefly, when cells were confluent, the medium in the upper and lower chambers was removed and replaced with 0.8 mL of HRP (Sigma, St Louis, MO) at a concentration of 0.5 μg/mL to the upper chamber and 1.0 mL of fresh EGM to the lower chamber, respectively. An aliquot of 50 μl medium in the lower chamber was collected and kept on ice. To measure HRP enzymatic activity, 25 μL of the culture medium was added to 860 μL of a reaction buffer (50 mM NaH_2PO_4 with 5 mM guaiacol). Each sample was measured in duplicate. The reaction was initiated by adding 500 μL H_2O_2. The samples were then read by a UV spectrophotometer at 470 nm (Ultraspec 3000; Pharmacia Biotech, Cambridge, UK). Data were calculated as OD 470-nm sample − OD 470-nm blank, and expressed as OD 470 nm for permeation of HRP across transwell filters.

Fluorescent Microscopy

EC junctional structure and protein expressions for VE-cadherin, occludin, FAK, and phosphorylated FAK ($pFAK^{397}$) were examined by fluorescent immunostaining technique. After ECs were treated with SIN-1 and inhibitors, cells were fixed with 95% ethanol at 4C for 30 minutes and then permeabilized by 80% acetone for 1 minute at room temperature. ECs were then stained with monoclonal mouse anti-human VE-cadherin antibody (Immunotech, Westbrook, ME), polyclonal rabbit anti-human occludin antibody (Zymed Laboratories, Inc, San Francisco, CA), polyclonal rabbit anti-human FAK (Upstate, Charlottesville, VA), and polyclonal rabbit anti-human phosphate FAK^{397}. Cy3 donkey anti-mouse immunoglobulin G(H+L) and anti-rabbit immunoglobulin (H+L) were used as secondary antibodies (Jackson Immunoresearch Laboratories Inc, Westgrove, PA). Coverslips were mounted with VectarShield mounting medium (Vector Laboratories Inc, Burlingame, CA). Stained cells were examined by a fluorescence microscope Olympus IX71 (Olympus, Tokyo, Japan). Fluorescent images were recorded by a digital camera linked to a computer software MicroFire (Optronics, Goleta, CA).

Western Blot Analysis

Total cellular protein was extracted from ECs with an ice-cold lysis buffer containing 50 mM/L Tris-HCL (pH 7.6), 1% triton X-100, 0.5% NP-40, 1 mM phenylmethylsulfonylfluoride (PMSF), and 5% mM dithiotheritol (DTT). All samples were stored at −70C. The total endothelial protein extract (10 μg per sample) was subject to electrophoresis on 12% polyacrylamide gels by using Mini-protein 3 gel running system (BioRad, Hercules, CA). After being transferred to nitrocellulose membranes, the membranes were probed with a primary monoclonal antibody against VE-cadherin and rabbit polyclonal antibody occludin. The secondary antibody was either horseradish-linked anti-mouse antibody or horseradish-linked anti-rabbit antibody. The bound antibodies were visualized with an enhanced chemiluminescent ECL detection kit (Amersham Corp, Arlinton Heights, IL). Nitrocellulose membranes were stripped and blocked before they were probed again with a different antibody. The density of VE-cadherin, occludin, and α-actin expression for each sample was scanned and analyzed by NIH Image 1.61 (National Institutes of Health, Bethesda, MD). The density of α-actin expression of each sample was used to normalize the relative expression for VE-cadherin and occludin expression.

Statistical Analysis

Data are presented as means ± SE. Statistical analysis was performed with analysis of variance (ANOVA) by computer software program Statview (SAS Institute Inc, Cary, NC). A probability level less than .05 was considered statistically significant.

RESULTS

SIN-1–Induced Increased in Endothelial Permeability

The functional changes of endothelial permeability were determined by measuring endothelial ER and horseradish leakage through monolayer cells grown on cell culture inserts. Figure 1A and B shows changes of endothelial ER and HRP leakage in control cells and in cells treated with SIN-1 at a concentration of 0.5 mM up to 8 hours of incubation, respectively. We found that endothelial ER was decreased and HRP leakage was increased in a time-dependent manner. To determine whether SIN-1–induced changes in endothelial ER and HRP leakage were specifically due to generation of peroxynitrite and superoxide radicals by SIN-1, endothelial monolayer was incubated with SIN-1 for 4 hours with pretreatment of cells with peroxynitrite scavenger MnTMPyP, SOD alone, or in combination for 30 minutes. MnTMPyP was used at a concentration of 30 μm and SOD at a concentration of 10 μg/mL. Our results showed that endothelial ER was significantly decreased and HRP leakage was significantly increased in SIN-1–treated cells compared to those of controls, ER: 26.97 ± 1.41 versus 42.27 ± 0.40 $\Omega \cdot cm^2$, $P < .01$; HRP: 0.26 ± 0.07 versus 0.02 ± 0.01 OD 470 nm, $P < .01$, respectively. Decreased ER induced by SIN-1 was attenuated by MnTMPyP or SOD pretreatment in separate and almost completely blocked by MnTMPyP combined with SOD compared to SIN-1–treated alone, MnT: 37.62 ± 1.02 $\Omega \cdot cm^2$; SOD: 37.65 ± 1.62 $\Omega \cdot cm^2$; and MnT + SOD: 40.25 ± 0.70 $\Omega \cdot cm^2$, respectively (Figure 2A). Consistently, SIN-1–induced increased HRP leakage was also blocked by MnT: 0.08 ± 0.02 OD 470 nm; SOD: 0.07 ± 0.02 OD 470 nm; and MnT + SOD: 0.06 ± 0.01 OD 470 nm, respectively (Figure 2B). Thus, these data indicate that the disturbed endothelial permeability resulted

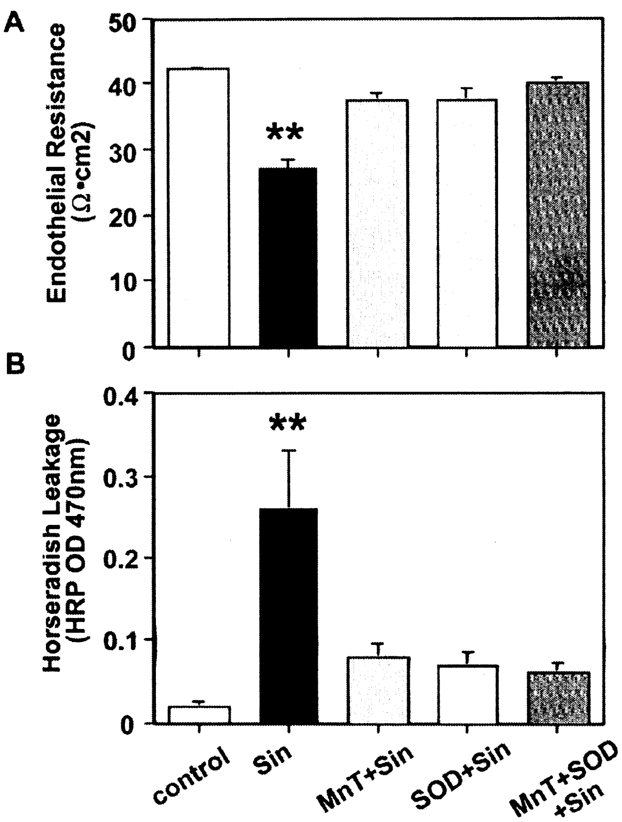

Figure 2. Effects of peroxynitrite scavenger MnTMPyP and SOD on SIN-1 induced increase in endothelial permeability. SIN-1–induced decrease in endothelial ER (A) and increase in endothelial HRP leakage (B) could be blocked by peroxynitrite scavenger MnT-MPyP and SOD in culture. Data are expressed as means ± SE from eight independent experiments. *$P < .05$; **$P < .01$.

from generation of both peroxynitrite and superoxide radicals by SIN-1 in culture.

SIN-1–Induced Endothelial Oxidative Stress

Attenuation of SIN-1–induced decreased endothelial ER and increased HRP leakage by MnTMPyP and SOD in monolayer cells indicates that oxidative reaction occurs in ECs. To confirm that the disturbed endothelial permeable function induced by SIN-1 is accompanied by increased endothelial oxidative stress, nitrotyrosine expression was examined by staining cells with fluorescent-labeled anti-nitrotyrosine antibody. Nitrotyrosine formation is considered a footprint of peroxynitrite and a marker of oxidative stress. As shown in Figure 3, the control cells displayed barely visible immunostaining for nitrotyrosine (Figure 3A), whereas intensive staining of nitrotyrosine was observed in cells treated with SIN-1 (Figure 3B). The enhanced nitrotyrosine staining was abolished by pretreatment of cells with MnTMPyP or SOD (Figure 3C and D). These results indicate that SIN-1 is capable of inducing the formation of peroxynitrite and superoxide radicals within the cells. Since the formation of peroxynitrite is a recognized marker for oxidative stress, our results confirmed that SIN-1 caused oxidative stress within the ECs through the formation of peroxynitrite and superoxide radicals.

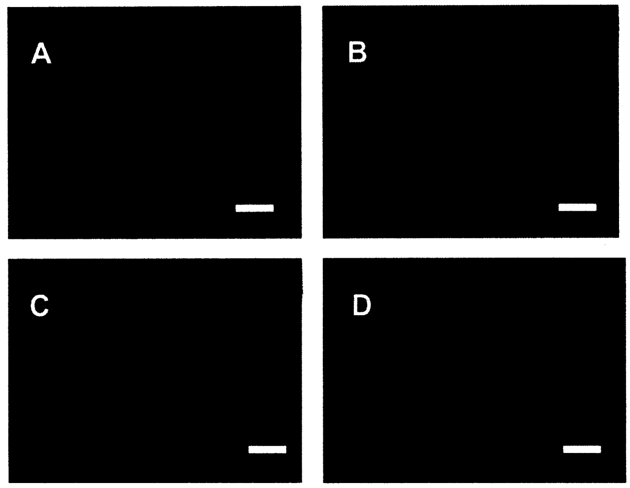

Figure 3. Nitrotyrosine expression in cells treated with or without SIN-1. Intensive nitrotyrosine staining was observed in cells treated with SIN-1 (B) compared to control cells (A). SIN-1–induced increase in nitrotyrosine expression was abolished by peroxynitrite scavenger MnTMPyP (C) and superoxide dismutase (D) in culture. Results are representatives of at least four independent experiments. Bar = 5μm.

Effects of SIN-1–Induced Intercellular Junction Disturbances

Endothelial-specific cadherin protein VE-cadherin and tight junctional protein occludin are important intercellular molecules in maintaining endothelial integrity and barrier function. To further study whether SIN-1 could disturb endothelial junctional structures and expression, distribution for VE-cadherin and occludin were examined by staining ECs with anti–VE-cadherin and anti-occludin antibodies. Results from VE-cadherin and occludin staining are shown in Figures 4 and 5, respectively. In control cells, VE-cadherin was continuously expressed around cell boundaries and only localized at cell contact regions (Figure 4A), while in SIN-1–treated cells, VE-cadherin was discontinuously expressed around cell borders, with gap and pore formation present at cell contact regions (Figure 4B). Occludin expression was remarkably changed in SIN-1–treated cells, with loss of most of its distribution at cell contact regions (Figure 5B). These SIN-1–induced disturbances in endothelial junction structure modification, as demonstrated by VE-cadherin and occludin distribution, were restored by MnTMPyP or SOD present in the monolayer culture, indicating the harmful role of peroxynitrite and superoxide radicals in interruption of endothelial junction structure. These results also point out the effects of antioxidant on protecting endothelial integrity and barrier function.

Endothelial VE-cadherin and occludin expressions were also examined by Western blot analysis. We found no significant changes in total cellular protein expressions for VE-cadherin and occludin in SIN-1–treated cells compared to that of con-

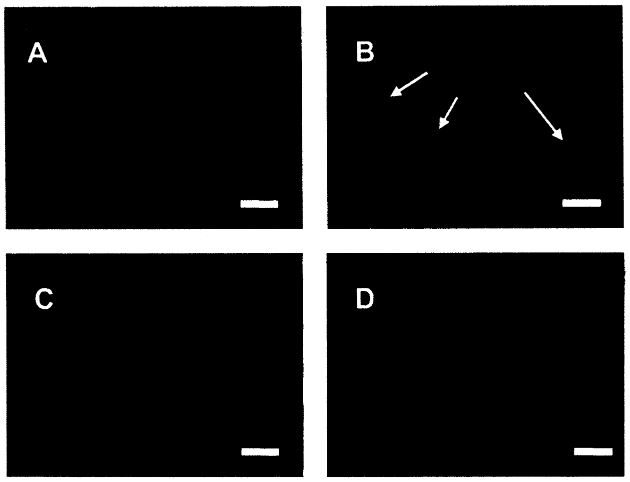

Figure 4. Effects of SIN-1on VE-cadherin expression and distribution. Disturbed VE-cadherin distribution at cell contact regions with gap formation was observed in cells treated with SIN-1 (B) compared to control cells (A). The junctional structure changes were blocked with pretreatment of cells with peroxynitrite scavenger MnTMPyP (C) and superoxide dismutase (D) in culture. Results are representatives of at least four independent experiments. Bar = 5μm.

trol cells. These results suggest that decreased VE-cadherin and occludin expression at cell contact regions induced by peroxynitrite or superoxide radicals may be due to protein structure modification without significantly affecting total protein mass (Figure 6).

Peroxynitrite-Induced Dephosphorylation of $pFAK^{397}$

FAK at the cell–matrix interface also plays a vital role in maintaining endothelial barrier function. Thus, we further examined effects of peroxynitrite and superoxide radicals on

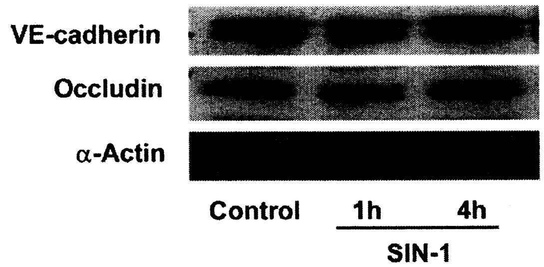

Figure 6. Western blot analysis for VE-cadherin and occludin in cells treated with or without SIN-1. ECs were treated with SIN-1 for 1 hour and 4 hours. VE-cadherin and occludin protein expression was no change in SIN-1–treated cells compared to that of control cells. These results indicate that altered junctional protein VE-cadherin and occludin distribution induced by SIN-1 without significantly affecting total protein mass. Results are representatives of three independent experiments.

FAK expression in SIN-1–treated cells. We found that $pFAK^{397}$ was intensively stained in control cells (Figure 7A), which was completely lost when cells were exposed to SIN-1 (Figure 7B). Again, both MnTMPyP and SOD restored $pFAK^{397}$ expression in SIN-1–challenged cells (Figure 7C and D). These results suggest that both peroxynitrite and superoxide radicals have effects on altering the phosphorylation-dephosphorylation of focal adhesion kinase processes in ECs, which may affect endothelial permeable function.

DISCUSSION

In this study, we found SIN-1–induced endothelial barrier dysfunction as evidenced by decreased monolayer ER and increased horseradish leakage in association with a disappearance of VE-cadherin and occludin at cell-cell contact regions. We also found dephosphorylation of FAK 397 ($pFAK^{397}$) in

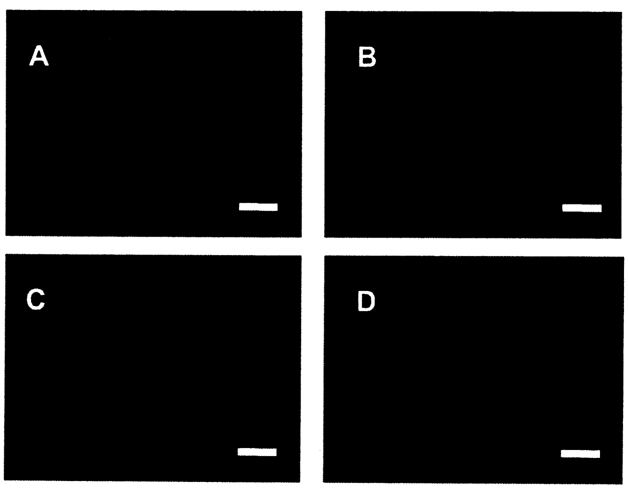

Figure 5. Effects of SIN-1 on occludin expression and distribution. Occludin expression was almost completed lost at cell contact regions in cells treated with SIN-1 (B) compared to control cells (A), which was restored with pretreatment of cells with peroxynitrite scavenger MnTMPyP (C) and superoxide dismutase (D) in culture. Results are representatives of at least four independent experiments. Bar = 5μm.

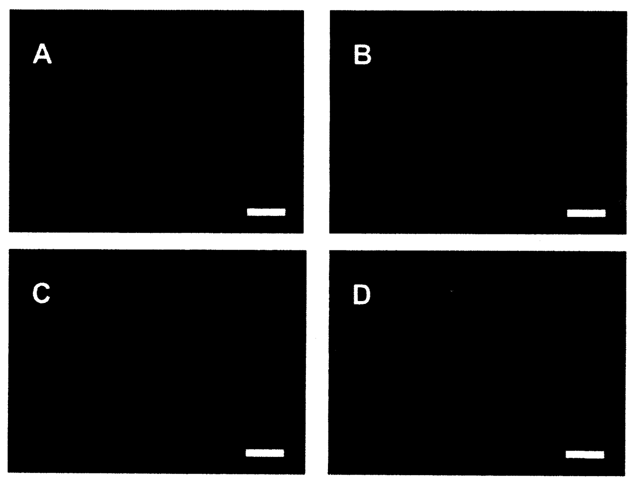

Figure 7. Effects of SIN-1on focal adhesion kinase (FAK) expression. Phosphorylated FAK 397 ($pFAK^{397}$, phosphorylation of tyrosine at 397) was intensive expressed in control cells (A), which was completely lost when cells were exposed to SIN-1 (B). When cells were pretreated with MnTMPyP and SOD, $pFAK^{397}$ expression was restored in SIN-1 challenged cells (C and D). Results are representatives of at least four independent experiments. Bar = 5μm.

SIN-1–treated cells. SIN releases both superoxide radical and nitric oxide radical, which form peroxynitrite, a highly ROS. Therefore, SIN-1 is known as an efficient generator of peroxynitrite.[12,13,15] When tyrosine in proteins is modified by peroxynitrite, nitrotyrosine residues are formed in cells. In the present study, the formation of peroxynitrite and peroxynitrite-induced endothelial oxidative stress was detected by enhanced immunostaining of nitrotyrosine in SIN-1–treated cells. Previous studies have shown that peroxynitrite could stimulate inducible nitric oxide synthase expression and decrease prostacyclin synthesis in ECs,[12] which provide the evidence of the involvement of peroxynitrite in the modulation of vascular tone. Peroxynitrite could also upregulate endothelial adhesion molecule expression.[13] The current study extends the knowledge about the role of peroxynitrite in endothelial dysfunction by showing that peroxynitrite is capable of inducing endothelial permeability, possibly through its effects on endothelial junctional protein VE-cadherin and occludin as well as cell-matrix interaction molecule FAK.

Increased endothelial permeability has been demonstrated in preeclampsia in in vivo studies evidenced by increased disappearance rate of Evans-blue dye from the maternal vasculature in women with preeclampsia compared to that in normal pregnancies.[7,8] Studies by our group revealed that altered endothelial barrier function is associated with disorganization of endothelial junction proteins in ECs from preeclampsia.[14] We also found that altered junctional protein VE-cadherin and occludin expression in ECs from preeclampsia was probably a post-translationally regulated process, since restoration of VE-cadherin and occludin at cell contact regions occurred in absence of gene and protein mass alteration.[14]

VE-cadherin and occludin are important components of adherent junction and tight junction of endothelium. They are essential for the manipulation of endothelial permeability and integrity. In an in vivo study, injection of a VE-cadherin antibody into mice results in an increase in lung and heart permeability. VE-cadherin antibody also induces redistribution of VE-cadherin from intercellular junctions in cultured ECs.[16] Endothelial junctional proteins are sensitive to microvascular environment stimulation. Groten et al showed that serum from women with preeclampsia induced subcellular redistribution of VE-cadherin in ECs.[17] We also reported that increased endothelial monolayer permeability induced by plasma from women with preeclampsia correlates with elevated levels of lipid peroxides and interleukin-8 in these samples,[9] indicating the role of oxidative stress environment and inflammatory stimulation in the regulation of cell barrier function. In the present study, we observed that peroxynitrite induced the loss of VE-cadherin and occludin in the cell contact regions in parallel with increased endothelial permeability. These results might provide the further link between the intercellular junction complex disruption and oxidative stress-induced increased endothelial permeability found in women with preeclampsia.

As to the mechanism underlying the disappearance of VE-cadherin and occludin from inter-endothelial contacts upon peroxynitrite stimulation, we further examined protein expression by Western blot analysis. Our results showed that expression of VE-cadherin and occludin were not affected after the peroxynitrite challenge. These results suggest that disappearance of VE-cadherin and occludin at cell contact regions is probably due to redistribution of these junctional proteins in ECs in response to peroxynitrite stimulation. As has been put forward by other investigators, the loss of VE-cadherin and occludin at the interendothelial contacts might be due to either internalization from the extracellular compartment or being retained within the cytoplasm[17] without reducing total protein mass, which may explain the discrepancy between the findings in VE-cadherin and occludin staining versus total protein expression.

Studies have shown that tyrosine phosphorylation regulates endothelial barrier function in a complex fashion. For example, tumor necrosis factor-α–induced endothelial barrier dysfunction was associated with tyrosine phosphorylation of VE-cadherin.[18] A relationship between tyrosine phosphorylation of occludin and barrier dysfunction was also demonstrated.[19,20] Since VE-cadherin and occludin are important components in maintaining the inter-endothelial integrity, these observations suggest that phosphorylation of tyrosine residue within junctional molecules might be a potential mechanism in altered endothelial permeability under various pathophysiologic conditions.

In this study, we also found that FAK presented as phosphorylated format in unchallenged ECs. Peroxynitrite caused this moiety of phosphorylated FAK to become dephosphorylated at tyrosine 397, which was consistent with the decreased endothelial ER and increased HRP leakage in monolayer cells treated with SIN-1. This conclusion is based on our observation that FAK expression was upregulated after SIN challenge (data not shown). In contrast, $pFAK^{397}$ was totally lost. FAK is an important tyrosine kinase in maintaining the cell-matrix interaction and phosphorylation of FAK^{397} which is coupled to the assembly of focal adhesion and is responsible for maintaining integrity and barrier function.[21] Upon activation, FAK autophosphorylates tyrosine 397 and a number of downstream events ensue, leading to the formation of focal adhesion. Inhibition of FAK tyrosine phosphorylation prevents the formation of focal adhesion[22] and is associated with cell contraction. Furthermore, tyrosine phosphatase inhibitors promote the formation of focal adhesion.[23] A recent study has also demonstrated that the site-specific tyrosine phosphorylation exerted differential effects on endothelial permeability. A study by Shikata et al, which was performed on the human pulmonary artery EC, showed both endothelial-protective sphingosine-1 phosphate (SIP) and endothelial-disruptive thrombin-activated Y576 phosphorylation.[24] Fisher's group recently reported that the expression of $pFAK^{397}$ on placental cytotrophoblast cells was closely related to its invading potential. Their study also showed that the expression of $pFAK^{397}$ was greatly reduced in trophoblast cells from preeclamptic placenta.[25] In line with the above observations, we found that FAK^{397} was in phosphorylated format in ECs under unchallenged condition, while peroxynitrite caused it to become dephosphorylated along with decreased ER and increased horseradish leakage. Taken together, these data suggest that dephosphorylation of FAK^{397} may play an important role in the endothelial pathophysiology in preeclampsia.

In summary, we found that peroxynitrite-induced oxidative stress might be involved in the pathogenesis of increased endothelial permeability found in preeclampsia through the mechanism of dephosphorylation of $pFAK^{397}$ and the disruption of VE-cadherin and occludin at inter-endothelial junction. These observations suggest that microenvironment oxidant stimuli may have a great impact on endothelial barrier function under both physiologic and pathophysiologic conditions.

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