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Nitric oxide disrupts VE-cadherin complex in murine microvascular endothelial cells

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Abstract

Vascular endothelial cadherin (VE-cadherin), which is localized at adherent junctions, is involved in the control of vascular permeability. A growing body of evidence indicates that NO modulates the movement of fluid and proteins out of the vasculature. In this paper, we investigated whether NO can disrupt the VE-cadherin complex. We found that treatment with two NO donors (SIN-1 and SNAP) markedly reduced the amount of VE-cadherin in a murine microvascular endothelial cell line (H5V) as demonstrated by immunoprecipitation analysis, cellular ELISA, and Northern blot analysis. β- and γ-Catenins were also found to be affected by the two NO donors. Moreover, the disruption of the complex, induced by NO donors, correlated with increases in vascular permeability using both in vivo and in vitro models. These results clearly demonstrate a role for NO in vascular permeability.

Keywords: VE-cadherin; Nitric oxide; Endothelial cells; Vascular permeability

Vascular endothelial cadherin (VE-cadherin) is a new member of the cadherin family that is selectively expressed by endothelial cells (EC) in all types of vessels, both in culture and in situ, at intercellular boundaries of the confluent monolayer [1]. Cadherins are able to assemble cells together and may contribute to the determination of the overall architecture of the vasculature through their signalling property [2]. In this context, VE-cadherin joins the cytoskeletal proteins through its interaction with members of the catenin family. This association is an important determinant of endothelial barrier function. VE-cadherin/catenin complex can be abolished by truncation of the cytoplasmic tail of VE-cadherin, which in turn dramatically disrupts the endothelial barrier function [3]. VE-cadherin transfectant cells display a significant decrease in permeability compared with controls [4]. Treatment of cell monolayers with antibodies directed to VE-cadherin increases permeation of macromolecules across endothelial monolayers without any obvious change in cell morphology [5]. Furthermore, in vivo administration of a monoclonal antibody against VE-cadherin induces a concentration- and time-dependent increase in vascular permeability in heart and lungs [6].

In addition, agents known to increase endothelial cell permeability such as thrombin, advanced glycation end-products, elastase, and tumor necrosis factor in combination with γ-interferon markedly alter the organization of VE-cadherin at intercellular rims [6–8].

Adherens and tight junctions in H5V cell lines show the junctional distribution of both VE-cadherin and cytoplasmic catenins. In addition, PECAM-1 (platelet endothelial cell adhesion molecule-1, CD31), which is also localized at cell–cell contacts, out of adherens or tight junctions, is presented at the intercellular contacts [9,10].

A growing body of evidence indicates that nitric oxide (NO) modulates the movement of fluid and proteins out of the vasculature. However, at the microvasculature level, the role of NO remains unresolved [11] and its mode of action is the focus of this study.

Taking into account that no studies have implicated the effect of NO on VE-cadherin expression, we

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hypothesized that NO may modulate the expression of the VE-cadherin/catenin complex.

Materials and methods

Chemicals. Two structurally unrelated NO donors, 3-morpholino sydnonimine (SIN-1) and *S*-nitroso-*N*-penicillamine (SNAP), were obtained from Sigma (St. Louis, MO, USA). SIN-1C was kindly provided by Dr. J.C. Drapier (Institute de Chimie des Substances Naturelles, CNRS-Gif-sur-Yvette, France). Throughout the study the non-releasing NO analogues, SIN-1C and/or *N*-acetyl penicillamine (NAP), were used as controls.

Antibodies. Rat mAb to murine VE-cadherin (clone BV13) and mouse mAb to PECAM-1 (clone 7.46) [10] were kindly provided by Dr. E. Dejana (Mario Negri Institute, Italy). Mouse mAb to β- (clone 14) and γ-catenins (clone 15) were from Transduction Laboratories (Kentucky, USA).

Cells. H5V, a murine microvascular endothelial cell line [12], was cultured in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal calf serum (FCS), 2 mM glutamine, penicillin (100 U/ml), and streptomycin (100 μg/ml). All studies were performed in 48 h postconfluent cultures. Cell viability and cellular protein levels were assayed by the trypan blue exclusion and Bradford methods, respectively.

In vitro assays. The methodology for immunoprecipitation of the VE-cadherin complex, Triton X-100-soluble fractions, as well as whole-cell extracts, from H5V postconfluent cells (representing $0.5–1 \times 10^6$ endothelial cells), culture supernatants or mouse tissue extracts (2 mg total proteins) was performed as described [8].

For immunoprecipitation, the entire supernatant from samples was resolved on a 7.5% SDS–polyacrylamide gel and immunoblotting using 7.46 or BV13 hybridoma culture supernatant and mAb to β-catenin or γ-catenin [8]. After washing, immunocomplexes were detected by incubation with horseradish peroxidase-conjugated secondary antibody and enhanced chemiluminescence detection system (Amersham, Little Chalfont, UK).

Cell surface expression of VE-cadherin in H5V postconfluence cells cultured in the presence and absence of NO donors for 24 h was determined as reported for other cell adhesion molecules by a cellular ELISA [8,13].

Permeability across cell monolayers incubated with medium containing NO donors was measured in Transwell units (with polycarbonate filters, 0.4-μm pores; Costar) as described previously [8].

To determine whether NO may change the VE-cadherin transcript levels, total RNA was isolated using the Chomczynski method [14]. Membranes were hybridized with a cDNA probe specific for murine VE-cadherin [15] labelled with [^{32}P]dCTP using the Rediprime kit (Amersham). Blots were exposure to an X-ray film (Hyperfilm, Amersham) for 48–72 h.

In vivo experiments. Vascular permeability assay and cutaneous VE-cadherin extraction were performed on male OF1 mice (Cenpalab, Cuba), weighing about 25 g. Plasma leakage was quantified by the extravasation of Evans blue [16]. Five minutes after the intravenous injection of 2.5% Evans blue solution (25 mg/kg), an intradermal injection of 100 μl of the following agents: saline solution, bradykinin (30 μg), and SNAP (10^{-4} M) was administered. Thirty minutes later, animals were killed by cervical dislocation and each injection site was punched out with an 18 mm diameter punch. The dye accumulation in the disc of skin was extracted with an acetone–Na_3PO_4 mixture (14:6 v/v) and the concentration was colorimetrically measured at 620 nm. The results were expressed as μg/site of dye leakage in the tested sites and compared with those of the controls.

In another set of experiments, animals were intradermically injected with SNAP (10^{-4} M) for 3, 6, and 18 h. Thirty minutes before killing, animals were i.v. injected with Evans blue solution and dye extravasation was measured as described above.

For immunoprecipitation of VE-cadherin from mouse skin, the disc of skin was homogenized in TBS containing 1 mM PMSF, 20 U/ml aprotinin, 15 μg/ml leupeptin, 2 mM each $CaCl_2$, $MgCl_2$, and $MnCl_2$, and 0.005% digitonin on ice. The homogenate was centrifuged at 400*g* for 10 min, 4 °C, and the pellet was extracted with agitation in the same buffer, but using 1% Triton X-100 and 0.5% SDS instead of digitonin, for 1 h at 4 °C. Tissue extracts were centrifuged at 14,000*g* for 30 min, 4 °C. The immunoprecipitation and immunoblotting of supernatants were done as described [8].

Statistical analysis. Data are reported as means ± standard errors of the mean (SEM). Comparisons between groups were made using Student's *t* test. Differences were considered significant at the $P \leqslant 0.05$ levels.

Results

We have evaluated the putative effects of two structurally different NO donors for potential cytotoxicity. Incubation of H5V with SNAP and SIN-1 for 24 h resulted in a significantly different toxicity profile with the maximum subtoxic concentration (concentration producing <10% decrease in either cell viability or total protein synthesis) being 10^{-4} M for both NO donors. Additionally, in preliminary experiments these treatments did not cause significant cell retraction or detachment from substratum. Subsequent experiments were therefore performed in the range of 10^{-4}–10^{-6} M. As controls, we used SIN-1C and *N*-acetyl penicillamine (NAP), which neither release nitric oxide nor cause cell toxicity.

Effects of NO donors on the VE-cadherin complex

To evaluate whether NO had any effects on the VE-cadherin complex H5V cells were exposed to NO donors (10^{-4}–10^{-6} M) for 24 h. Immunoprecipitation analysis revealed that VE-cadherin is markedly reduced in a dose-dependent manner (Fig. 1). Similarly β- and γ-catenins, two members of the "armadillo" family, which tightly associate with VE-cadherin, were also decreased to a greater extent (Fig. 1A). In contrast, PECAM-1, which is not associated directly with the VE-cadherin complex but does co-localize to the lateral endothelial cell junctions [17], remained at constant levels (Fig. 1B).

Changes in VE-cadherin protein levels were also detected when monolayers were extracted with lysis buffer containing 0.5% SDS as demonstrated in time course experiments. The presence of SDS, which completely dissociates the complex itself and from the cytoskeleton, allows the determination of the total cellular content of each component. An effect was observed as early as 9 h after exposure to both SNAP and SIN-1, and markedly decreased at 24 h. It is noteworthy that reduction of VE-cadherin by the action of NO was reverted when treated cell cultures were extensively washed, placed in fresh medium, and incubated for a further 24 h (Fig. 1C). No immunoreactive signals to VE-cadherin were observed

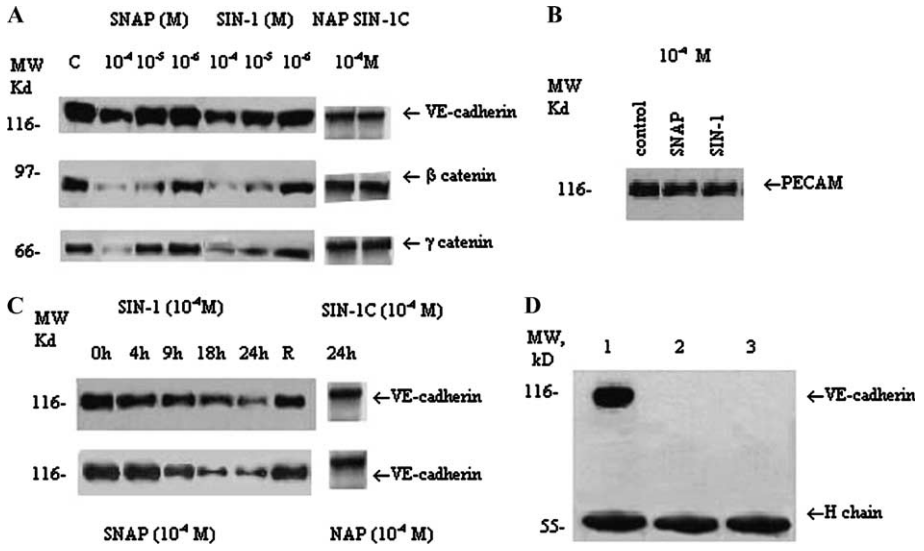


Fig. 1. Effect of NO donors on the VE-cadherin complex. Postconfluent cultures of H5V cells were incubated with NO donors (SNAP or SIN-1) for 24 h. Immunoprecipitated proteins from Triton X-100 soluble fractions were analysed by SDS–PAGE followed by immunoblotting with mAbs to VE-cadherin, β-, γ-catenins (A) and to PECAM-1 (B). Time course of changes in VE-cadherin protein levels exerted by NO donors (SNAP and SIN-1) is shown in (C). The disruption of VE-cadherin complex was reverted (lane R) when cell cultures were extensively washed, replaced with fresh medium, and further incubated for 24 h. No immunoreactive band to VE-cadherin is detectable in the supernatant from SNAP-treated cells when immunoprecipitated with anti-VE-cadherin antibody (D; lane 1: total cell extract; lane 2: supernatant of treated cultures; and lane 3: culture medium). Experiments were done in triplicate and typical results are shown. Molecular mass markers are shown on the left.

when supernatant from SNAP-treated cell cultures was immunoprecipitated, suggesting that disappearance of VE-cadherin is not due to cleavage of the extracellular domain from the cellular surface (Fig. 1D).

Effect of NO donors on VE-cadherin cell surface expression

In confluent ECs, VE-cadherin is located at cell–cell contacts. In order to directly determine whether NO donors inhibit cell surface expression of VE-cadherin, an ELISA was carried out. SNAP reduced the expression of VE-cadherin in H5V cells in a dose-dependent manner, with a maximum reduction effect observed at 10^{-4} M (63% relative to controls). Reduction effects of 37% and 8% were detected at 10^{-5} and 10^{-6} M, respectively (Fig. 2). EGTA, known to cause VE-cadherin dispersion from intercellular contacts, produced a marked reduction of the immunoreactive signal. On the other hand, NAP did not produce any effect on the cell surface expression of VE-cadherin.

NO donors modulate VE-cadherin message levels

RNA blot analysis of mRNA isolated from H5V cells treated with SNAP (10^{-4}–10^{-6} M) for 24 h revealed that VE-cadherin transcript levels were markedly reduced in a dose-dependent manner (Fig. 3). NAP, the inactive control for SNAP, did not modify the levels of VE-cadherin transcripts. Similar results were obtained when cell cultures were treated with SIN-1 (data not shown).

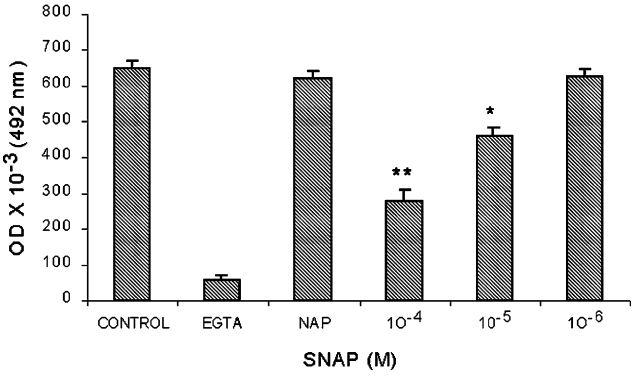


Fig. 2. NO donor induces a decrease in the cellular surface VE-cadherin content as measured by cellular ELISA. Bar graph shows the effect of NO donor on the VE-cadherin expression. VE-cadherin cell surface levels were determined by primary binding with specific mAb followed by secondary binding with conjugates as described in Materials and methods. Negative controls included wells with H5V cells incubated only with OPD, H5V cells stained with second step antibody only, and H5V cells stained with irrelevant mAb. All data points represent means of eight ELISA wells ± SEM from three independent experiments and were compared with those of untreated H5V cells. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. control.

These results support those obtained for VE-cadherin protein levels.

NO donor increases endothelial monolayer permeability

The physiological effect of NO on the observed changes in the integrity of the adherent junction was studied by measuring the permeability of confluent

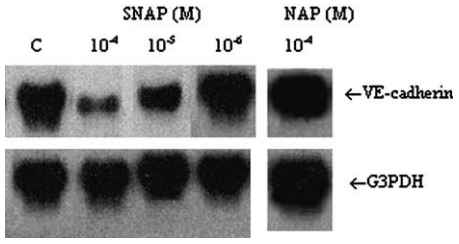


Fig. 3. NO donor reduces the message levels of VE-cadherin. Aliquots of 15 μg of total RNA from 24 h SNAP-treated cells were separated by electrophoresis, transferred to nylon membrane, and successively hybridized with cDNA probes for murine VE-cadherin and G3PDH. Results were essentially similar in three independent experiments.

endothelial monolayers. As shown in Fig. 4, SNAP treatment increased the cell permeability to an Ig-HRP conjugate, in a way comparable to that induced by 24 h TNF-α treatment. Cells incubated with SNAP showed an increasing permeability, from 28% at 10^{-5} M to 50% at 10^{-4} M (average of three independent experiments). Similar results were obtained when SIN-1 was used (data not shown). The reversible nature of VE-cadherin complex disruption was demonstrated by extensive washing and replacement with fresh medium, and is in agreement with the data shown in Fig. 1C. These data suggest that the disruption of the VE-cadherin complex induced by NO donors decreases the capacity of endothelial cells to limit the passage of soluble high molecular weight molecules.

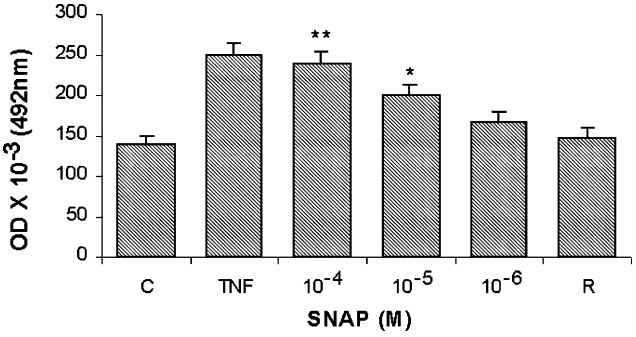
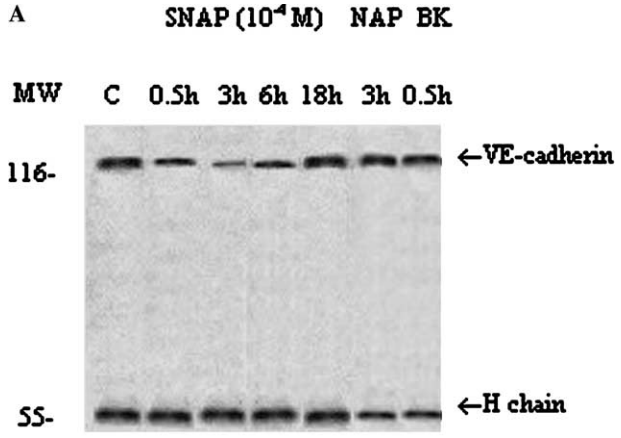


Fig. 4. NO donor perturbs the endothelial barrier function of cultured H5V monolayers. Bar graph shows the effect of nitric oxide donor (SNAP) on vascular permeability. Confluent layers of H5V cells on Transwell filter inserts were incubated for 24 h with different concentrations of SNAP. TNFα was used as positive control. Increase in vascular permeability is also reverted (bar R) by extensive washing and medium replacement. Results of one representative experiment of three performed are shown. The values are means ± SEM from triplicates. $*P < 0.05$, $**P < 0.01$.

NO increases in vivo cutaneous vascular permeability and reduces VE-cadherin immunoreactive signal

Based on the findings described above, we asked whether the effect of NO on the vascular permeability in vivo correlated with putative changes in the levels of VE-cadherin. Strikingly, the same behaviour was observed in the immmunoreactivity profile obtained by



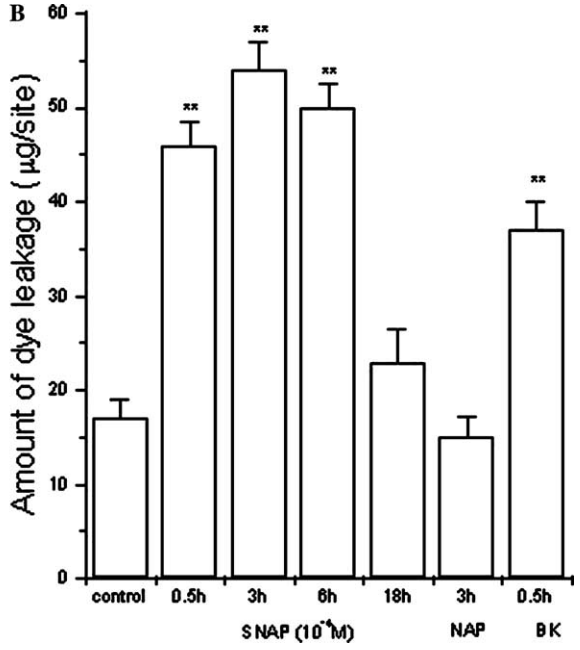


Fig. 5. Enhancement of cutaneous vascular permeability by NO donor in mice correlated with cutaneous VE-cadherin protein reduction. (A) Time course of NO-induced cutaneous VE-cadherin reduction. Animals were treated with SNAP 10^{-4} M (i.d.) for 0.5, 3, 6, and 18 h and VE-cadherin extraction was done as described in Materials and methods using buffer lysis containing 0.5% SDS. Arrows indicate bands corresponding to VE-cadherin protein and H chain of immunoglobulin to VE-cadherin. Bar graph in (B) shows the enhancement of dye leakage at the site of injection (i.d.) of SNAP (10^{-4} M), determined 30 min after Evans blue injection. Values represent means ± SEM of three experiments. $**P < 0.01$. Bradykinin (BK) was used as positive control. Experiments were done in triplicate and typical results are shown.

immunoprecipitation and Western blot analysis of VE-cadherin from mouse skin. VE-cadherin was clearly identified as a discrete band and its migration in SDS–PAGE under reducing conditions was consistent with that immunoprecipitated from H5V extracts. The levels of VE-cadherin detected were markedly reduced at 30 min. After 18 h, signals were similar to those observed in untreated animals (Fig. 5A). These results are consistent with the marked in vitro effects of NO donor on vascular permeability. It is noteworthy that bradykinin increased the amount of dye leakage at 0.5 h without degrading VE-cadherin, suggesting that at this time bradykinin-induced vascular permeability is not mediated by a disruption of the VE-cadherin complex. Increases in cutaneous vascular permeability were detected as earlier as 30 min after SNAP injection. The maximal increase was reached after 3 h, still remained at 6 h and was practically reversed at 18 h (Fig. 5B).

Discussion

VE-cadherin is a cell–cell adhesion molecule that plays a fundamental biological role in ECs, as it is essential for the assembly and integrity of the vascular structure. On the other hand, NO modulates the movement of fluid and proteins out of the vasculature. Yet at the microvasculature level, the role of nitric oxide is still unresolved [11]. We postulated that NO-induced cellular effects might be associated with structural alterations to endothelial junction organization, specifically in endothelial adherent junctions (AJ) affecting the barrier function of endothelium.

Our results indicate that NO greatly alters the organization of the VE-cadherin complex in a dose- and time-dependent manner. Exposure of postconfluent microvascular endothelial cells to NO donors induced a decrease in the amount of VE-cadherin present at the cellular surface. Furthermore a marked decrease in the cellular content of VE-cadherin and two major components of the VE-cadherin complex that are linked directly to the cytoplasmic tail of VE-cadherin, i.e., the β- and γ-catenins, was observed. Our findings suggest that these changes appear to be due to an effect on the VE-cadherin gene expression as NO produced significant reductions in transcript levels, similar in magnitude to those observed in protein levels.

The present findings may be relevant to processes in which VE-cadherin seems to play an important role. These include the control of vascular permeability and cell extravasations at sites of inflammation where the overproduction of NO either by resident or incoming inflammatory cells is a hallmark. Increased entry of immune cells into the intimal matrix, surrounding areas of neovascularization, has been associated with a down-regulation of VE-cadherin expression, which suggests that disruption of endothelial adherent junctions within neovessels is a significant step in atherogenesis. On the other hand, expression of inducible nitric oxide synthase in macrophages and smooth muscle cells in atherosclerotic lesions is thought to play an important role in atherogenesis [18].

Recently Govers et al. [19] have shown that endothelial nitric oxide synthase (NOS III) can be found at intercellular junctions and is up-regulated when cells reach confluence. In our study, loss of VE-cadherin was correlated to increases in permeability both in vitro and in vivo. Moreover, after withdrawal of NO donors the barrier properties returned to baseline with a concomitant increase in the VE-cadherin complex content. This suggests that NO must be constantly present at high levels in order to exert the observed effects. Since NOS II produces NO of the order of nanomoles in comparison to picomolar quantities produced by NOS III [20], and all experiments were performed in postconfluent cells, we think that the disruption of VE-cadherin complex by nitric oxide is much closer to pathological conditions where NOS II-mediated nitric oxide production is taking place.

Our data provide evidence that NO can induce the disruption and loss of the VE-cadherin complex, and suggests that it might be responsible, at least in part, for the weakening of intercellular contact.

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References

[1] M.G. Lampugnani, M. Resnati, M. Raiteri, R. Pigott, A. Pisacane, G. Houen, L. Ruco, E. Dejana, A novel endothelial specific membrane protein is a marker of cell–cell contacts, J. Cell Biol. 118 (1992) 1511–1522.

[2] E. Dejana, Endothelial adherens junctions: implications in the control of vascular permeability and angiogenesis, J. Clin. Invest. 98 (1996) 1949–1953.

[3] P. Navarro, L. Caveda, F. Brevario, I. Mandoteanu, M.G. Lampugnani, E. Dejana, Catenin dependent and independent functions of VE-cadherin, J. Biol. Chem. 270 (1996) 30965–30972.

[4] F. Breviario, L. Caveda, M. Corada, I. Martin-Padura, P. Navarro, J. Golay, M. Introna, D. Gulino, M.G. Lampugnani, E. Dejana, Functional properties of human vascular endothelial cadherin (7B4/cadherin-5), an endothelium-specific cadherin, Arterioscler. Thromb. Vasc. Biol. 15 (1995) 1229–1239.

[5] U. Gostsch, E. Borges, R. Bosse, E. Boggmeyer, M. Simon, H. Mossman, D. Vestweber, VE-cadherin antibody accelerates neutrophil recruitment in vivo, J. Cell Sci. 110 (1997) 583–588.
[6] M. Corada, M. Mariotti, G. Thurston, K. Smith, R. Kunkel, M. Brockhaus, M.G. Lampugnani, I. Martin-Padura, A. Stoppacciaro, L. Ruco, D.M. McDonald, P.A. Ward, E. Dejana, Vascular endothelial-cadherin is an important determinant of microvascular integrity in vivo, Proc. Natl. Acad. Sci. USA 96 (1999) 9815–9820.
[7] M.J. Rabiet, J.L. Pantier, Y. Rival, Y. Genoux, M.G. Lampugnani, E. Dejana, Thrombin-induced increase in endothelial permeability is associated with changes in cell to cell junction organization, Atheroscler. Thromb. Vasc. Biol. 16 (1996) 488–496.
[8] K. Otero, F. Martinez, A. Beltran, D. Gonzalez, B. Herrera, G. Quintero, R. Delgado, A. Rojas, Albumin-derived advanced glycation end-products trigger the disruption of the vascular endothelial cadherin complex in cultured human and murine endothelial cells, Biochem. J. 359 (2001) 567–574.
[9] I. Martin-Padura, S. Lostaglio, M. Schneemann, L. Willimas, M. Romano, P. Fruscella, C. Panzeri, A. Stopacciaro, L. Rucco, A. Villa, D. Simmons, E. Dejana, Junctional adhesion molecule a novel member of the immunoglobulin superfamily that distributes at intercellular junctions and modulates monocytes transmigration, J. Cell Biol. 142 (1998) 117–127.
[10] A. Vecchi, C. Garlanda, M.G. Lampugnani, M. Resnati, C. Mateucci, A. Stopacciaro, H. Schnurch, W. Rissau, L. Rucco, A. Mantovani, E. Dejana, Monoclonal antibodies specific for endothelial cells of mouse blood vessels. Their application in the identification of adult and embryonic endothelium, Eur. J. Cell Biol. 63 (1994) 247–254.
[11] P. Kubes, Nitric oxide affects microvascular permeability in the intact and inflamed vasculature, Microcirculation 2 (1995) 235–244.
[12] C. Garlanda, C. Parravicini, M. Sironi, M. De Rossi, R. Wainstok de Calmanovici, F. Carozzi, F. Bussolino, F. Colotta, A. Mantovani, A. Vecchi, Progressive growth in immunodeficient mice and host cell recruitment by mouse endothelial cells transformed by polyoma middle-sized T antigen: implications for the pathogenesis of opportunistic vascular tumors, Proc. Natl. Acad. Sci. USA 91 (1994) 7291–7295.
[13] B.V. Khan, D.G. Harrison, M.T. Olbrych, R.W. Alexander, R.M. Medford, Nitric oxide regulates vascular cell adhesion molecule 1 gene expression and redox-sensitive transcriptional events in human vascular endothelial cells, Proc. Natl. Acad Sci. USA 93 (1996) 9114–9119.
[14] P. Chomczynski, N. Sachi, Single-step method of RNA isolation by acid guanidium thiocyanate–phenol–chloroform extraction, Anal. Biochem. 162 (1987) 156–159.
[15] G. Breier, F. Breviario, L. Caveda, R. Berthier, H. Schnurch, U. Gotsch, D. Vestweber, W. Risau, E. Dejana, Molecular cloning and expression of murine vascular endothelial-cadherin in early stage development of cardiovascular system, Blood 87 (1996) 630–641.
[16] E. Fujii, K. Irie, K. Ohba, A. Ogawa, T. Yoshioka, M. Yamakawa, T. Muraki, Role of nitric oxide prostaglandins and tyrosine kinase in vascular permeability in mouse skins, Naunyn-Schmiedeberg's Arch. Pharmacol. 356 (1997) 475–480.
[17] O. Ayalon, H. Sabani, M.G. Lampugnani, E. Dejana, B. Geiger, Spatial and temporal relationships between cadherins and PE-CAM-1 in cell–cell junctions of human endothelial cells, J. Cell Biol. 126 (1994) 247–258.
[18] J.S. Luoma, P. Stralin, S.L. Marklund, T.P. Hiltunen, T. Sarkioja, S. Yla-Herttuala, Expression of extracellular SOD and iNOS in macrophages and smooth muscle cells in human and rabbit atherosclerotic lesions: colocalization with epitopes characteristic of oxidized LDL and peroxynitrite-modified proteins, Arterioscler. Thromb. Vasc. Biol. 2 (1998) 157–167.
[19] R. Govers, L. Bevers, P. De Bree, T. Rabelink, Endothelial nitric oxide synthase activity is linked to its presence at cell–cell contacts, Biochem. J. 361 (2002) 193–201.
[20] E. Anggard, Nitric oxide: mediator, murderer and medicine, Lancet 343 (1994) 1199–1206.