Edaravone mimics sphingosine-1-phosphate-induced endothelial barrier enhancement in human microvascular endothelial cells

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Edaravone mimics sphingosine-1-phosphate-induced endothelial barrier enhancement in human microvascular endothelial cells. Am J Physiol Cell Physiol 293: C1523–C1531, 2007. First published August 8, 2007; doi:10.1152/ajpcell.00524.2006.—Edaravone is a potent scavenger of hydroxyl radicals and is quite successful in patients with acute cerebral ischemia, and several organ-protective effects have been reported. Treatment of human microvascular endothelial cells with edaravone (1.5 μM) resulted in the enhancement of transmonolayer electrical resistance coincident with cortical actin enhancement and redistribution of focal adhesion proteins and adherens junction proteins to the cell periphery. Edaravone also induced small GTPase Rac activation and focal adhesion kinase (FAK; Tyr576) phosphorylation associated with sphingosine-1-phosphate receptor type 1 (S1P1) transactivation. S1P1 protein depletion by the short interfering RNA technique completely abolished edaravone-induced FAK (Tyr576) phosphorylation and Rac activation. This is the first report of edaravone-induced endothelial barrier enhancement coincident with focal adhesion remodeling and cytoskeletal rearrangement associated with Rac activation via S1P1 transactivation. Considering the well-established endothelial barrier-protective effect of S1P1, endothelial barrier enhancement as a consequence of S1P1 transactivation may at least partly be the potent mechanisms for the organ-protective effect of edaravone and is suggestive of edaravone as a therapeutic agent against systemic vascular barrier disorder.

THE PRIMARY PATHWAY involved in endothelial barrier permeability under pathological conditions is paracellular transport of fluid and macromolecules through intercellular gaps that form following the disruption of intercellular adherens junction (AJ) and focal adhesion (FA) complexes (27). An increase in vascular permeability is a fundamental feature of inflammation and the essential component of tumor metastasis, angiogenesis, and atherosclerosis (7).

Among several platelet-derived lipids, sphingosine 1-phosphate (S1P) is a remarkably effective endothelial cell (EC) agonist that induces proliferation, calcium mobilization, adhesion molecule expression, and suppression of apoptosis (2, 9, 14, 17, 25) by binding to the S1P1 family of receptors upon release from stimulated platelets (17, 24, 25, 54). Present in human serum (31, 34, 56), S1P exerts biophysical effects as a major endothelial barrier stabilizer via several signaling events in ECs (27, 28, 29). Measurements of transmonolayer electrical resistance (TER) have revealed that S1P not only enhances the barrier integrity of human pulmonary artery EC (HPAEC) monolayers but also protects endothelial monolayers from barrier-disruptive effect of edemagenic agents such as thrombin, indicating the pivotal role of S1P in the regulation of endothelial barrier property (19). The role of the S1P pathway in the regulation of vascular permeability in vivo has also been reported (38, 39).

The mechanisms underlying S1P-induced endothelial barrier augmentation are intriguing. We and others (12, 43, 53) have previously reported that S1P-induced pulmonary endothelial barrier enhancement is associated with FA remodeling. S1P induced the redistribution of FA proteins, paxillin and FA kinase (FAK), to the cell periphery and was associated with cortical actin ring formation (43, 44). Furthermore, S1P induced selective FAK (Tyr576) phosphorylation in the FAK catalytic domain, one of the possible mechanisms in S1P-mediated FA remodeling associated with endothelial barrier enhancement (44). Activation of the small GTPase Rac plays a pivotal role in S1P-mediated endothelial barrier enhancement (12, 43, 53). Rac regulates actin cytoskeletal remodeling and FA dynamics via ADP-ribosylation factor GTPase activation proteins (51), which interact with several signaling and cytoskeletal proteins including paxillin. We (44) have previously reported S1P-induced Rac activation associated with cortical actin ring formation and FA redistribution to the cell periphery. Among the S1P family of receptors, threonine phosphorylation of the S1P receptor type 1 (S1P1) is associated with Rac activation (26). Recent data have also indicated that the dephosphorylation of S1P1 using specific short interfering (si)RNA (S1P1 siRNA) reduced the barrier-protective effect of activated protein C (11), suggesting the critical role of S1P1 transactivation in endothelial barrier enhancement associated with Rac activation.

Edaravone (MCI-186) is a potent scavenger of hydroxyl radicals (32). Clinically, edaravone is quite successful in patients with acute cerebral ischemia via an antiedemagenic effect (8), and several organ-protective effects of edaravone have been reported in the heart (16), lung (20), and kidney (40) in addition to an anti-ischemic action in central nervous system, but the precise mechanisms underlying these effects still remain to be elucidated. Edaravone improves endothelium-dependent vasodilation in smokers partly via inhibition of nitric oxide (NO) degradation as a consequence of the hydroxyl radical scavenging activity of edaravone.
radical-scavenging action, suggesting a protective effect on the physiological function of the EC monolayer (21). In addition to the modulation of hydroxyl radical activity, edaravone induces several signaling events, i.e., inhibition of EGF receptor phosphorylation (47) and upregulation of Bcl-2 (36). These data strongly suggest that edaravone may exert an organ-protective effect via an unknown signaling pathway independently from NO effects.

In the present study, we report the novel edaravone-induced signaling events in human microvascular ECs (HMVECs). Considering several S1P-induced signaling events coincident with endothelial barrier enhancement, edaravone may modulate endothelial barrier properties, at least in part, via the activation of S1P1 and a downstream signaling pathway. Our findings provide new insights for edaravone as an effective therapeutic agent for diseases with systemic vascular endothelial disorders such as diabetes mellitus.

MATERIALS AND METHODS

Reagents and antibodies. Chemicals and reagents were obtained from Sigma Chemical (St. Louis, MO) unless otherwise noted. Edaravone was provided by Mitsubishi Welpharma (Tokyo, Japan). FBS was obtained from CanSera (ON, Canada). Cell culture medium (EBM-2) and growth supplements were obtained from Clonetics (Walkersville, MD). Alexa Fluor 488 anti-mouse IgG antibody, Alexa Fluor 488 anti-rabbit IgG antibody, and Texas red-phalloidin were purchased from Molecular Probes (Eugene, OR). Mouse monoclonal anti-FAK antibody, anti-β-catenin antibody, and anti-FAK (Tyr³⁷⁷) phospho-specific antibody were obtained from Upstate Biotechnology (Lake Placid, NY). The Rac activation assay kit, including mouse monoclonal anti-Rac1 antibody and siIMPORTER siRNA transfection reagent, were also obtained from Upstate Biotechnology. Mouse monoclonal anti-paxillin antibody was obtained from BD Biosciences-Pharmingen (San Diego, CA). Rabbit polyclonal anti-β-catenin antibody, anti-S1P1 antibody, and mouse monoclonal anti-vascular endothelial (VE)-cadherin antibody, normal mouse IgG, and normal rabbit IgG were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Rabbit polyclonal anti-FAK (Tyr³⁷⁷) phospho-specific antibody (BioSource, Camarillo, CA) and anti-FAK (Tyr⁹²⁵) phospho-specific antibody (Cell Signaling Technology, Beverly, MA) were commercially obtained. Horseradish peroxidase-linked anti-mouse and anti-rabbit IgG antibodies and the horseradish peroxidase Western blot detection kit were obtained from Amersham Biosciences (Piscataway, NJ). Protein G-Sepharose 4B conjugate was purchased from Zymed (San Francisco, CA). d-Erythro-N,N-dimethylphosphinosine (DMS) was purchased from BioMol (Plymouth Meeting, PA).

HMVEC culture. HMVECs were obtained from Clonetics and cultured in EBM-2 complete medium containing 10% FBS. EC cultures were maintained at 37°C in a humidified atmosphere and grown to contact-inhibited monolayers with a typical cobblestone morphology. Cells from each primary flask (passages 4–10) were detached with 0.05% trypsin, resuspended in fresh culture medium, and then passaged into gelatinized 6-well plates for Western blot analysis or 12-well plates with gelatinized coverslips for immunofluorescent analysis. HMVECs were grown to 95% confluence and then rendered quiescent in EBM-2 containing 0.1% FBS for 24 h before Western blot analysis or edaravone stimulation.

Western blot analysis. After a brief wash with PBS, cells prepared on six-well dishes as described in HMVEC culture and RNA interference experiments were lysed with 300 µl/well of cell lysis buffer (43, 44). Western blot analysis was then performed as previously described using appropriate primary and secondary antibodies (43, 44). Blots were visualized with the ECL Western blot detection system. The amount of detected proteins was analyzed using Image Quant software.

Rac activation assay. HMVECs were prepared and stimulated with edaravone as described in HMVEC culture and RNA interference experiments. The Rac GTPase activation assay was then performed using the Rac activation assay kit (Upstate Biotechnology) as previously described (43, 44). For total Rac detection, 10 µl of the original cell lysates were subjected to electrophoresis on EBC-2 or vehicle control, respectively, for 72 h. Cells were then rendered quiescent in EBM-2 containing 0.1% FBS for 24 h before Western blot analysis or edaravone stimulation.

Fig. 1. Edaravone induces endothelial cell barrier enhancement. Human microvascular endothelial cell (HMVEC) monolayers on gold microelectrodes were challenged with edaravone at the indicated concentrations (0–3 µM), and the change in transmonolayer electrical resistance (TER) was monitored. The arrow indicates the time point of the beginning of edaravone challenge. Values indicate the normalized resistance of HMVEC monolayers achieved from 3 independent experiments assuming the control value as 1. Values are means ± SE. *P < 0.05.
Fig. 2. Effects of edaravone on the distribution of focal adhesion kinase (FAK), paxillin, adherens junction (AJ) proteins, and F-actin. HMVEC monolayers were stimulated with 1.5 μM edaravone for 30 min and stained with anti-FAK (A), anti-paxillin (B), anti-vascular endothelial (VE)-cadherin (C), anti-α-catenin (D), and anti-β-catenin (E) antibodies (a and d) concurrently with Texas red-phalloidin to detect F-actin (b and e). c and f. Merged images. Negative control pictures using nonspecific IgG are also shown (g). Scale bars = 20 μm.
Impedance measurements with electric cell substrate impedance sensing. HMVECs were grown to confluence in polycarbonate wells containing evaporated gold microelectrodes (surface area: $10^{-3}$ cm$^2$) in series with a large gold counterelectrode (1 cm$^2$) connected to a phase-sensitive lock-in amplifier (12). Measurements of TER were performed using an electrical cell substrate impedance-sensing system (ECIS; Applied BioPhysics, Troy, NY) as described previously (12). Briefly, current was applied across the electrodes by a 4,000-Hz alternating current voltage source with an amplitude of 1 V in series with a 1-M$\Omega$ resistance to approximate a constant current source ($\sim$1 $\mu$A). The in-phase and out-of-phase voltages between the electrodes were monitored in real time with the lock-in amplifier and subsequently converted to scalar measurements of transendothelial impedance. Values of normalized resistance from each microelectrode were pooled at discrete time points and plotted versus time as means $\pm$ SE.

Statistical analysis. Results are expressed as means $\pm$ SE of independent experiments. For multiple group comparisons, one-way ANOVA followed by the post hoc Tukey-Kramer test was performed using StatView software. $P < 0.05$ was considered statistically significant.

RESULTS

Edaravone enhances EC barrier properties. HMVEC monolayers on gold microelectrodes were challenged with edaravone at the indicated concentrations, and the change in TER was monitored (Fig. 1). Values indicate the normalized resistance of HMVEC monolayers achieved from three independent experiments assuming the control value as 1 (means $\pm$ SE, $P < 0.05$).

Edaravone induced the redistribution of FAK, paxillin, and AJ proteins to the cell cortical area. The edaravone-induced redistribution of FAK and paxillin was monitored by immunofluorescent microscopy. In quiescent cells, FAK stains diffusely in the cytoplasm as well as at sites of stress fiber attachment to the randomly arranged FA (Fig. 2A). Paxillin staining was similar to FAK staining in nonstimulated cells and also associated with randomly distributed FAs (Fig. 2B). The 30-min incubation of HMVEC monolayers with 1.5 $\mu$M edaravone resulted in a dramatic redistribution of FAK and paxillin to the cell periphery and was associated with significant enhancement of cortical actin staining (Fig. 2, A and B). Staining for AJ proteins (VE-cadherin, $\alpha$-catenin, and $\beta$-catenin) was also enhanced at the cell-cell contact area after edaravone stimulation (Fig. 2, C–E).

Edaravone induced site-specific FAK tyrosine phosphorylation. To determine the major site of FAK tyrosine phosphorylation, total cell lysates were subjected to electrophoresis and blotted with site-specific anti-phospho-FAK antibodies as described in MATERIALS AND METHODS. Edaravone failed to induce an increase in FAK phosphorylation at Tyr$^{397}$ or Tyr$^{925}$ (Fig. 3, A and C), whereas a significant increase in FAK phosphorylation at Tyr$^{576}$ was observed within 30 min (Fig. 3B). Reprobing analysis with anti-FAK antibody revealed equal amounts of total FAK loadings in each lane (Fig. 3).

Edaravone induced Rac activation. To elucidate the effect of edaravone on the activity of Rac, one of the major regulators of cytoskeletal rearrangement, quiescent HMVECs were stimulated with 1.5 $\mu$M edaravone, and activated GTP-bound Rac was immunoprecipitated. Edaravone treatment induced the significant increase in the amount of activated Rac within 1 min, which was sustained for at least 60 min (Fig. 4).

Effects of siRNA-based S1P1 protein depletion. We down-regulated S1P1 protein expression using the siRNA approach. Transfection of HMVECs with S1P1 siRNA followed by Western blot analysis was performed as described in MATERIALS AND METHODS. Transfection with the S1P1 siRNA mixture (M-003655-01-0005) resulted in a remarkable S1P1 depletion without changes in the protein expression of FAK, Rac1, and S1P3 (Fig. 5). Cell treatment with siIMPORTER or transfection with control siRNA did not alter the protein expression of

Fig. 3. Site-specific FAK tyrosine phosphorylation profile induced by edaravone. HMVEC monolayers were treated with 1.5 $\mu$M edaravone for the indicated time periods. The resultant total cell lysates were probed with 3 site-specific anti-phospho-FAK antibodies and subsequently reprobed with anti-FAK antibody. A: FAK phosphorylated at Tyr$^{397}$ ([pY$^{397}$]); B: FAK phosphorylated at Tyr$^{576}$ ([pY$^{576}$]); C: FAK phosphorylated at Tyr$^{925}$ ([pY$^{925}$]). Values indicate amounts (in %) of site-specific tyrosine-phosphorylated FAK assuming the control value as 100% (means $\pm$ SE). Representative blots of 3 independent experiments are shown.
S1P₁ and other related proteins (FAK, Rac₁, and S1P₃; Fig. 5), indicating the direct and specific effect of S1P₁ siRNAs on S1P₁ depletion.

**Effect of S1P₁ protein depletion on edaravone-induced FAK tyrosine phosphorylation.** HMVECs transfected with S1P₁ siRNA were stimulated with 1.5 μM edaravone, and Rac activity was evaluated as described in MATERIALS AND METHODS. S1P₁ protein depletion did not affect the basal activity of Rac (Fig. 7), and edaravone failed to induce a significant increase in the amount of activated Rac at least within 5 min (Fig. 7).

**DISCUSSION**

Recently, emerging evidence has suggested several mechanisms underlying the regulation of endothelial barrier properties. AJ and FA proteins are active participants in the modulation of vascular permeability (29, 58). The AJ is a major component in the pathophysiological regulation of paracellular permeability of the microvascular endothelium. As a primary component of AJs, VE-cadherin connects adjacent ECs via homophilic binding of the extracellular domain (58). The intracellular domain of VE-cadherin interacts with the actin cytoskeleton through the mediation of catenins (5, 58). Several hyperpermeability factors induce tyrosine phosphorylation of β-catenin, leading to disassembly of AJs associated with intracellular gap formation (1, 6). Recent reports (26, 28) have described the S1P-induced peripheral enhancement of AJ proteins (α-catenin, β-catenin, and VE-cadherin) coincident with cortical actin enhancement, suggesting the induction of AJ assembly. The FA is also involved in the regulation of vascular permeability. The attachment of ECs to the extracellular matrix (ECM) is mediated by FAs composed of integrins and intracellular proteins that link integrins to the cytoskeleton. The cell-ECM interaction is dynamically controlled through the assembly and disassembly of FAs (13, 59). Blockade of the integrin-ECM attachment increases vascular endothelial permeability (3, 55), and several lines of evidence have suggested the participation of FA remodeling in the endothelial contractile reaction and permeability response (58).

In the present report, we evaluated effects of edaravone on the signal transduction pathway involved in the regulation of endothelial barrier properties in HMVECs. The effective concentration of edaravone was previously reported as 1.5 μM (57), and we stimulated HMVECs with several concentrations of edaravone, including 1.5 μM, to evaluate TER. ECIS experiments revealed the edaravone-induced TER augmenta-

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**Fig. 4.** Edaravone-induced Rac activation. **A:** quiescent HMVECs were stimulated with 1.5 μM edaravone for the indicated time periods and lysed as described in MATERIALS AND METHODS. Activated GTP-bound Rac was then immunoprecipitated and blotted. Total Rac contents were detected using total cell lysates. Representative blots of 5 independent experiments are shown. **B:** amounts of precipitated GTP-bound Rac were quantified and analyzed statistically. Values indicate amounts (in %) of GTP-bound Rac assuming the control value as 100% (means ± SE).

**Fig. 5.** Transfection with sphingosine-1-phosphate (S1P) receptor type 1 (S1P₁) short interfering (si)RNA does not influence the protein expression of FAK, S1P₃ receptor type 3 (S1P₃), and Rac. **A:** S1P₁ siRNA transfection into HMVECs and the following Western blot analysis with anti-S1P₁, S1P₃, FAK, and Rac were performed as described in MATERIALS AND METHODS. Representative blots of 3 independent experiments are shown. **B:** amounts of S1P₁, S1P₃, FAK, and Rac were quantified and analyzed statistically. Values indicate amounts (in %) of proteins assuming the control value as 100% (means ± SE). *P < 0.005.
min. Cell lysates were probed with anti-FAK (Tyr576) phospho-specific antibody and subsequently reprobed with anti-FAK antibody. Representative blots of 3 independent experiments are shown.

**Fig. 6.** Effect of S1P1 protein depletion on edaravone-induced FAK (Tyr576) phosphorylation. A: HMVECs were transfected with S1P1 siRNA as described in MATERIALS AND METHODS. Both transfected and nontransfected HMVECs were rendered quiescent and then stimulated with 1.5 μM edaravone for 30 min. Cell lysates were probed with anti-FAK (Tyr576) phospho-specific antibody and subsequently reprobed with anti-FAK antibody. Representative blots of 3 independent experiments are shown. B: amounts of Tyr576-phosphorylated FAK were quantified and analyzed statistically. Values indicate amounts (in %) of Tyr576-phosphorylated FAK assuming the control value as 100% (means ± SE).

Conversely, thrombin, a strong hyperpermeability factor, induced significant phosphorylation of FAK at tyrosine residues Tyr397, Tyr576, and Tyr925 through the mediation of multiple signaling pathways (44). Furthermore, S1P (0.5 μM) induced a dramatic redistribution of FAK and paxillin to the cell periphery and was associated with cortical actin ring enhancement, whereas thrombin (100 nM) stimulation resulted in the formation of massive stress fibers and intercellular gaps coincident with the redistribution of FAK and paxillin to the ends of stress fibers (43, 44). In the case of endothelial barrier property regulation by mechanical stresses, shear stress within 15 min augmented TER and was associated with the redistribution of FAK and paxillin to the cell periphery and the enhancement of the cortical actin ring and FAK (Tyr576) phosphorylation (45).

**Fig. 7.** Effect of S1P1 protein depletion on edaravone-induced Rac activation. A: HMVECs were transfected with S1P1 siRNA as described in MATERIALS AND METHODS. Both transfected and nontransfected HMVECs were rendered quiescent and then stimulated with 1.5 μM edaravone for the indicated time periods. Cells were lysed as described in MATERIALS AND METHODS, and total Rac contents were detected using total cell lysates. Representative blots of 5 independent experiments are shown. B: amounts of GTP-bound Rac were quantified and analyzed statistically. Values indicate amounts (in %) of GTP-bound Rac assuming the control value as 100% (means ± SE).
associated with AJ disassembly (23, 29) and FA formation associated with actin filament polymerization (29, 58). Furthermore, FAK may regulate Rac activity by activating G protein-coupled receptor kinase interactor-1 (GIT1), which complexes with Rac1-specific guanine nucleotide exchanging factor (35). Knockdown of GIT-1 leads to an additional increase in endothelial permeability in response to thrombin (52). In conjunction with edaravone-induced morphological changes (Fig. 2) and FAK tyrosine phosphorylation (Fig. 3), these evidences strongly suggest a pivotal role of edaravone to induce endothelial barrier enhancement via Rac activation.

To elucidate the participation of the S1P signaling pathway in edaravone-induced FAK (Tyr$^{576}$) phosphorylation and Rac activation, the role of S1P$_1$, one of the major S1P receptors involved in endothelial barrier enhancement via Rac activation (11, 12), was evaluated. S1P$_1$ protein depletion without any significant change in the amount of related proteins (FAK, Rac1, and S1P$_3$; Fig. 5) resulted in the inhibition of FAK (Tyr$^{576}$) phosphorylation (Fig. 6) and Rac activation (Fig. 7) by edaravone challenge. These data indicate the specific down-regulation of S1P$_1$ protein expression in the experiments and provide strong evidence for the edaravone-induced transactivation of S1P$_1$ and the following signaling events, compatible with S1P signaling in endothelial barrier enhancement.

S1P receptors are transactivated by several growth factors and bioactive agents. For example, differential transactivation of S1P receptors by nerve growth factor modulates neurite extension (51). APC bound to the endothelial protein C receptor (EPCR) also induces the transactivation of S1P$_1$ via the phosphorylcholinositol 3-kinase (PI3K)/Akt pathway, resulting in Rac-mediated vascular barrier enhancement (11). S1P may also bind to S1P receptor type 3 (S1P$_3$), another member of the S1P family of receptors ubiquitously expressed in humans. Activation of S1P$_3$ is associated with Rac activation, but S1P$_3$-mediated Rho activation has also been reported (19). Furthermore, depletion of S1P$_1$ by the siRNA method in HPAECs resulted in the loss of S1P-induced Rac activation and TER augmentation, whereas S1P$_1$ silencing failed to inhibit S1P-induced Rac activation and TER enhancement (46). These data strongly suggest the pivotal role of not S1P$_3$ but S1P$_1$ in the barrier-protective effect via Rac activation in an endothelial monolayer (46). The precise mechanism underlying S1P$_1$ transactivation is not well understood, but the PI3K/Akt pathway may play a key role in the interaction of S1P receptors with the EPCR, PDGF-β receptor, EGF receptor, and VEGF receptor (37, 48, 49). S1P-activated Akt induces endothelial NO synthase (eNOS) phosphorylation at Ser$^{1179}$ (18), the mechanism for S1P-induced arterial vasodilation (4). Presently, there is no certain evidence for edaravone-mediated eNOS activation, but edaravone restores reduced eNOS expression (57, 59) and induces endothelium-dependent vasodilation in smokers (21), suggesting another cross-talk between S1P and edaravone signaling pathways related to the regulation of eNOS activity. It is also intriguing whether the activity of cellular saphingosine kinases is required for barrier enhancement by edaravone. To elucidate the role of sphingosine kinases, we evaluated the effect of the saphingosine kinase inhibitor DMS on TER enhancement by edaravone. Pretreatment of HMVECs with DMS (10 μM) in advance to edaravone stimulation, as described previously (10, 15), resulted in a massive and irreversible decrease in TER (data not shown). The reason why these inhibitors affected the level of TER is unknown. The change in the basal level of sphingosine kinase activity might be responsible, but the precise mechanism(s) is unknown. Presently, the role of sphingosine kinases in barrier enhancement by edaravone is thus to be elucidated further.

In conclusion, endothelial barrier enhancement, possibly as a consequence of S1P$_1$ transactivation, may be one of the potent mechanisms for the antiedemagenic effect of edaravone. Furthermore, edaravone may serve safely as a novel therapeutic agent for a disease based on systemic vascular disorders, such as diabetes mellitus, via a novel endothelial barrier-protective effect. Considering the important role of an increase in vascular permeability as a component of angiogenesis and atherosclerosis (7), our present data suggest the potent effectiveness of a practical dose of edaravone on the early stage of diabetic microvascular complications, i.e., neuropathy, retinopathy and nephropathy, and atherosclerosis in hyperlipidemia. Future works will be forwarded to elucidate the precise mechanisms underlying the edaravone-induced S1P$_1$ transactivation and confirm the organ-protective effects of edaravone in vivo experiments.

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EDARAVONE-INDUCED ENDOTHELIAL BARRIER ENHANCEMENT


