Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Reviews

Barrier stabilizing mediators in regulation of microvascular endothelial permeability

HUANG Qiao-bing*

【**Abstract**】Increase of microvascular permeability is one of the most important pathological events in the pathogenesis of trauma and burn injury. Massive leakage of fluid from vascular space leads to lose of blood plasma and decrease of effective circulatory blood volume, resulting in formation of severe tissue edema, hypotension or even shock, especially in severe burn injury. Fluid resuscitation has been the only valid approach to sustain patient's blood volume for a long time, due to the lack of overall and profound understanding of the mechanisms of vascular hyperpermeability response. There is an emerging concept in recent years that some so-called barrier stabilizing mediators play a positive role in preventing the increase of vascular permeability. These mediators may be released in response to proinflammatory mediators and serve to restore endothelial barrier function. Some of these stabilizing mediators are important even in quiescent state because they preserve basal vascular permeability at low levels. This review introduces some of these mediators and reveals their underlying signaling mechanisms during endothelial barrier enhancing process.

Key words: ***Permeability; Endothelium, vascular; Mediator complex; Receptors, cyclic AMP; RAC1 protein, human***

Chin J Traumatol 2012;15(2):105-112

Endothelial barrier disruption and microvascular hyperpermeability contribute to the pathogenesis of various acute and chronic inflammatory diseases. The release and circulation of permeability-increasing cytokines and other mediators, such as thrombin, bradykinin, histamine, radical oxygen species, vascular endothelial growth factor (VEGF), tumor necrosis factor-α (TNF-α) and lipopolysaccharide (LPS), etc, increase vascular permeability primarily by formation of intercellular gaps between endothelial cells (ECs) of postcapillary venules.[1] Normally, ECs are tightly connected through various proteins that regulate the organization of intercellular junctional complex, such as adherens and tight junctions, then bind to cytoskeletal proteins or cytoplasmic interaction partners that allow the transfer of intracellular signals and govern the barrier function of ECs under normal or inflammatory conditions. With the stimulation of intrinsic and extrinsic proinflammatory mediators, inter-endothelial junctions are open to allow paracellular fluid passage.[2] These agonists-induced hyperpermeability is usually reversible.[3] The process of recovery of endothelial barrier function could emerge with the reannealing of previously open inter-endothelial junctions and the strengthening of adhesion of ECs to extracellular matrix, which result from the re-equilibrium of competing contractile and adhesive forces generated by the cytoskeletal proteins and the adhesive molecules. There is also an emerging concept in recent years that some so-called stabilizing mediators play a positive role in enhancing inter-endothelial junctional connections and preventing the increase of vascular permeability.[1,4] These endogenous mediators include classical anti-edema reagents such as glucocorticoid, cyclic adenosine monophosphate (cAMP), adenosine triphosphate, and re-

DOI: 10.3760/cma.j.issn.1008-1275.2012.02.008

Department of Pathophysiology, Southern Medical University, Key Lab for Shock and Microcirculation Research of Guangdong Province, Guangzhou 510515, China (Huang QB)

*Corresponding author: Tel: 86-20-61648465, Email: huangqb2000@yahoo.com, bing@fimmu.com

The study was supported by General Program from Natural Science Foundation of China (Nos. 30971201 and 81170297), Program for Changjiang Scholars and Innovative Research Team in University (No. IRT0731), and National Key Foundation for Basic Science Research of China (No. G2005CB522601).

cently found mediators such as intermedin (IMD)/adrenomedullin2 (AM2), AM, sphingosine 1-phosphate (S1P), activated protein C (APC) as well as angiopoietin-1/tyrosine kinase with immunoglobulin-like and EGF-like domains 2 (Tie2) system (Ang-1/Tie2). These mediators may be generated and released in response to pro-inflammatory mediators and serve to restore endothelial barrier function. Some exogenous reagents, such as statins, are also found to have the function of endothelial permeability stabilization. This review introduces some of these mediators and reveals their underlying signaling mechanisms during endothelial barrier enhancing process.

Barrier stabilizing mediators

Glucocorticoids Glucocorticoids are well-known anti-edema drugs in clinics, although their utilization is limited due to the adverse effect of increasing the risk of secondary infection. Glucocorticoids have been reported to reinforce the barrier via the signal transduction pathway originating from the endothelial intracellular glucocorticoid receptor, which acts as a responsive element controlling the expression of steroid responsive target genes and their corresponding proteins. Recent publications revealed that dexamethasone prevented TNF-α-induced cell permeability through glucocorticoid receptor trans-activation and nuclear factor-kappaB trans-repression in cell culture and rat retinas.[5] Dexamethasone and hydrocortisone also act to increase the endothelial expression of mRNA and protein level of tight junction molecules such as zona occludens-1, claudin-5 and occludin, in cultured cells, diabetic animals and multiple sclerosis patients.[5-7] Dexamethasone coordinately up-regulates Ang-1 expression and down-regulates VEGF expression in brain astrocytes and pericytes, leading to promotion of the integrity of ECs and the stabilization of blood-brain barrier.[8] It was also approved that dexamethasone suppressed VEGF and intercellular adhesion molecule-1 expression in diabetic rats which correlated with its effect on retinal vascular permeability and leukocyte accumulation.[9]

Glucocorticoids are well-mentioned in many clinical practice guidelines, due to the effect of steroid on stabilizing endothelial barrier function. Glucocorticoids are recommended for the treatment of various inflammatory diseases, such as sepsis shock, acute lung injury, asthma, arthritis, macular edema, etc.[10-12] During the battle with severe acute respiratory syndrome (SARS) in 2003, the proper use of corticosteroid in confirmed critical SARS resulted in lower mortality and shorter hospitalization stay.[10] But the applications of corticosteroids are restricted clinically due to the adverse effects. Surviving Sepsis Campaign guidelines recommend that intravenous hydrocortisone should be given only to adult patients with septic shock after their blood pressure is confirmed to be poorly responsive to fluid resuscitation and vasopressor therapy.[13]

Ang-1/Tie2 system The Ang/Tie2 system includes Ang-1, Ang-2 and Tie2 receptor, and regulates permeability in part by modulation of the small GTPase RhoA-mediated pathways that activate myosin-actin filament (F-actin) interaction. Ang-1/Tie2 and Ang-2/Tie2 almost play an opposite role in regulating the formation of stress fiber, the tethering forces between adjacent cells generated by junctional transmembrane proteins, in particular VE-cadherin/catenin complexes. Ang-1/Tie2 is one of best described endogenous cell signaling systems that help to maintain vascular integrity and maturation.[14,15] Ang-1, as an agonist for ECs, is a ligand for the endothelium-specific receptor tyrosine kinase Tie2. This ligand/receptor system shares many proangiogenic properties of VEGF on ECs. However, in contrast to VEGF, Ang-1 protects blood vessels from increased plasma leakage, which contributes to their stabilization. In mobile and confluent ECs, Ang-1-bound Tie2 is translocated to cell-cell contacts where it assembles a unique homotypic in trans-complex with Tie2 from the opposite cell that includes the vascular endothelial phosphotyrosine phosphatase (VE-PTP). This Tie2-VE-PTP complex preferentially activates Akt serine kinase, which phosphorylates endothelial nitric oxide synthase (eNOS).[16] While eNOS-derived nitric oxide (NO) is central to increased permeability in response to VEGF.[17,18] Inhibition of NO production by Ang-1, via phosphorylation of eNOS on 497 residue threonine by protein kinase C zeta, is responsible, at least in part, for inhibition of VEGF-stimulated endothelial permeability.[19] Report[20] also showed that Ang-1, through its receptor Tie2, activated one small G-protein, Rho, then mDia which sequestered Src, resulting in the inhibition of Src and stabilization of VE-cadherin in inter-endothelial adherens junction. Ang-1-induced Tie2 receptor phosphorylation is signaled via PI3-kinase and Rac1 to activate p190 RhoGTPase-activating protein, an enzyme that brings RhoA back to its inactive GDP-bound form. This resulted in reduced F-actin stress fi-

ber formation and endothelial permeability.[21,22] McCarter et al[23] reported that the Ang-1/Tie2 system exerted strong protective effects in a rat and transgenic mice models with LPS-induced acute lung injury. Other original experiments and studies on patients have showed that Tie-2-mediated Ang-1 attenuates the responsiveness of the endothelium to inflammation, hyperpermeability, apoptosis and vasoreactive stimuli.[24,25]

Clinically, it has been showed that level of Ang-1 is reduced in septic or non-septic critically ill patients. Circulating levels of Ang-2, Ang-1 and the Ang-2 to Ang-1 ratio have been used to predict the development of acute respiratory distress syndrome, sepsis, and other critically ill patients.[26] Numerous evidences showed that in animal models, Ang-1 over-expression or administration of exogenous Ang-1 inhibited EC activation, reduced vascular leakage, prevented the LPS-induced decrease in blood pressure and cardiac output, attenuated the LPS-induced increase in lung water, and subsequently improved the survival rate.[23,27] It is yet to be explored the effectiveness of application of Ang-1/Tie2 system in patients, but Ang-1/Tie2 surely is a potential therapeutic target.

AM and IMD/AM2 As a widely expressed peptide, AM is secreted from ECs, vascular smooth muscle cells, cardiac myocytes, as well as human leukocytes, etc. AM mediates its activities by binding to a complex receptor composed of the calcitonin receptor like-receptor (CRLR) associated with receptor activity-modifying proteins-2 and -3 (RAMP-2 and RAMP-3). The deletion of functional AM gene or its receptor gene, and the lack of G protein-coupling receptor modulator protein RAMP-2 result in formation of extreme generalized edema and the disruption of vascular integrity, suggesting the strong barrier stabilizing function of AM.[28-30] It has been demonstrated that AM increases trans-endothelial electrical resistance of ECs and improved endothelial barrier function through the activation of cAMP signal pathway and the up-regulation of tight junction protein claudin-5.[31-33] The inhibition of AM-activated cAMP/PKA pathway by cyclosporin A impairs brain endothelial barrier function.[34] The administration of AM attenuates extravasation of albumin and plasma fluid in rat with septic shock and reduces 6-h mortality.[35] IMD, a recently identified peptide targeting CRLR, is also called AM2, but it has a much stronger effect on stabilizing endothelial barrier than AM. Also by activating cAMP/PKA via CRLR/RAMP2 complex, IMD (AM2) reduced thrombin-, hydrogen peroxide- and hypoxia-induced endothelial hyperpermeability under basal conditions.[36,37] The newest report[38] shows that IMD has differential effects on macromolecule permeability of ECs in different vascular beds. It increases permeability of rat coronary microvascular ECs and reduces permeability of human umbilical vein endothelial cells (HUVECs) and rat aortic ECs.

With the potential of enhancing the endothelial barrier function and down-regulating the expression of proinflammatory cytokines, AM and IMD are considered as a novel treatment target to attenuate acute lung injury and sepsis.[39,40] It has been showed that the application of AM mitigated edema-characterized lung injury and the loss of plasma fluid in rat models with septic shock.[35,40] It still needs an intensive endeavor before AM and IMD could be translated from bench to bed.

S1P Produced by phosphorylation of sphingosine, S1P is an abundant lipid mediator in plasma and regulates numerous physiological functions of vascular and immune cells.[41] S1P is present in blood at nanomolar to micromolar concentration and is delivered by high density lipoprotein-associated apolipoprotein M to its receptors.[42] A variety of cell types from different species have different S1P receptor (S1PR) expression profiles.[43] S1PR1, R3 and a little S1PR2 are the main receptors expressed in ECs.[44] It is proposed that the balances in expression and activation of S1PR1, R2 and R3 in ECs help to maintain the physiological functions, especially the barrier function of ECs.[45] An appropriate or physiological level of S1P causes the activation of S1PR1, resulting in the strengthening of the barrier integrity of ECs by inducing Rac signaling pathway, while the lack of S1P is harmful in endothelial barrier function.[46] The administration of S1P in animal models with burn or acute lung injury attenuates the vascular hyperpermeability, resulting from the enhancement of endothelial junctional integrity and the formation of a stronger cortical actin ring.[47,48] The depression of S1P synthesis enzyme, sphingosine kinase, with inhibitor N, N-dimethylsphingosine causes obvious disorganization of junctional structure in cultured HUVECs.[48] The suppression of S1P lyase responsible for the degradation of S1P protects LPS-induced murine acute lung injury.[49] There is also an evidence showing that intracellular S1P induces barrier enhancement via similar

cellular signaling pathway(s) independent of S1P receptors.[50]

Although S1P experimentally demonstrates a strong effect on endothelial barrier protection, its usage in clinic is limited by the fact that excessive S1P will induce the activation of S1PR2/R3, leading to the disruption of inter-endothelial junctions by evoking the RhoA-ROCK pathway.[48,51] Thus, the final barrier regulation efficacy of S1P depends on the balances in expression and activation of different S1P receptors in ECs. The activation and strengthening of S1P/S1PR1 pathway are beneficial in preventing vascular leakage.

APC APC is a natural anticoagulant that plays an important role in coagulation homeostasis by inactivating the procoagulant factor Va and VIIIa. Besides, it has cytoprotective functions such as anti-inflammatory, anti-apoptotic effects and endothelial barrier protection.[52] APC dynamically augments the EC cortical actin ring and decreases formation of transcellular stress fibers, associating with decreased paracellular gaps and improved monolayer integrity that corresponds to EC barrier protection. Inactive protein C binds to a specific receptor on the surfaces of ECs, called endothelial protein C receptor, which leads to activation of PI3-kinase/Akt and trans-activation of the S1P1 receptor.[53] As mentioned before, the activation of S1P1 receptor mediates vascular endothelial barrier enhancement.[54] It is also demonstrated that APC activated Tie2 via a mechanism requiring, in sequential order, endothelial protein C receptor, protease-activated receptor-1, and EGF receptor, which selectively enhances the PI3K/Akt signaling to strengthen the junctional complexes and reduce vascular permeability.[55]

A recombinant form of human APC (rhAPC or drotrecogin alfa activated, known commercially as 'Xigris') was once approved in 2001 by the US Federal Drug Administration[56] for treatment of severe sepsis associated with a high risk of mortality because it was demonstrated that rhAPC reduced 28-day mortality in severe septic patients in the large recombinant human activated Protein C Worldwide Evaluation in Severe Sepsis (PROWESS) trial. But this rhAPC was withdrawn by European Medicines Agencies on October 25th, 2011, due to lack of efficacy for the treatment of sepsis in a reassessing PROWESS SHOCK trial since 2008.[57,58] This reminds the basic researchers and clinical physicians that the barrier enhancing effect is quite clear for APC and its adverse influence on fibrolysis may raise the risk of bleeding and hamper its clinical application.

Statins Statins are hydroxy-3-methyl-glutaryl-CoA reductase inhibitors that lower serum cholesterol. It has been noticed that statins exhibit myriad clinical benefits, including enhancement of vascular integrity.[59,60] By increasing tight junction protein claudin-5 and decreasing intercellular adhesion molecule-1 expressions, simvastatin prevented damage to cerebral endothelial tight junctions and neutrophil infiltration into the parenchyma, and exerted its anti-edematous effects of reduced neurologic deficits, cerebral edema as well as blood-brain barrier permeability 6 h after traumatic brain injury.[61,62] It is demonstrated that lovastatin attenuated RhoA activation, then induced myosin light chain (MLC) dephosphorylation under basal conditions and opposed increase in phosphorylation of MLC and myosin phosphatase targeting protein-1 in response to thrombin.[60] While the glycocalyx has key contribution to endothelial barrier function by charge-selective exclusion of plasma proteins,[1] Meuwese et al[63] reported that short-term statin therapy partially restored the endothelial glycocalyx which was reduced in patients with heterozygous familial hypercholesterolemia.

Statins are widely used prescription drugs, and the barrier enhancing effect is a desired accompanying benefit to the patients and physicians, while vascular leakage is an early pathological event during the development of related diseases such as arteriosclerosis, cardiac disease and hypertension, etc.

Except for the above-mentioned barrier stabilizing factors, lots of other mediators has barrier enhancing function, including prostaglandins (PG)E_2 and PGI_2, the essential metabolic homeostasis regulating hormone insulin,[64] and the actin-binding vasodilator-stimulated phosphoprotein,[65] etc. And there will be more and more substances to be found with stabilization function of endothelial permeability in the future.

Major signal pathways in enhancement of endothelial barrier function

Most of the above-mentioned barrier-stabilizing mediators rely on cyclic nucleotide second messengers cAMP and Rac1 pathways to realize their vascular pro-

tection function. In the process of enhancing endothelial barrier function, cAMP is a critical signal molecule utilized by various barrier-stabilizing mediators.[66,67] Various barrier-stabilizing mediators directly increase concentration of cAMP, resulting in enhancement of endothelial barrier function. Inhibition of phosphodiesterase 4 can increase endothelial cAMP, stabilize the endothelial barrier, and attenuate increased acute inflammatory in vascular permeability. On the other hand, several barrier-disruptive components, such as TNF-α, appear to increase permeability by reducing the formation of cAMP.[68,69] LPS-mediated barrier breakdown was also related to the decrease of intracellular cAMP and Rac1 activities.[70] Activation of adenylate cyclase and the subsequent increase of cAMP are the common consequences of AM receptor activation in a variety of cells. Cyclosporin A impaired brain endothelial barrier function by inhibition of AM-activated cAMP/PKA pathway.[34] The IMD (AM-2)-induced attenuation of endothelial hyperpermeability is mediated by activating cAMP/PKA via CRLR/RAMP2 complex as well.[36,37] Mediators such as PGE_2 and PGI_2 stabilize endothelial barrier function partly by increasing cAMP level and activating Rac1.[71] Some guanosine triphosphatase (GTPase) exchange factors, called as exchange protein activated by cAMP (Epac), could be directly activated by cAMP. It is also revealed that cAMP/Epac1 induces the activation of Ras-related protein1 to tighten cell-cell contacts by arrangement of F-actin in endothelial junctional area.[72] There is a report[73] demonstrating that cAMP signals are strictly compartmentalized, whereas cAMP emanating from transmembrane ACs activates barrier-enhancing targets, such as filamin, and cAMP emanating from soluble ACs activates barrier-disrupting targets, such as tau.

GTPases of Rho family regulate cell adhesion and cytoskeletal reorganization, which are also important regulators of the endothelial barrier function. While RhoA, via its effector Rho kinase (ROCK), is thought to predominantly destabilize endothelial barrier properties by promoting MLC-dependent contraction of stress fibres and reducing VE-cadherin-mediated adhesion. Rac1, as another small G-protein in the Rho family, plays an important role in regulation of actin cytoskeletal organization and is well known to be required for maintenance of endothelial barrier functions.[74] The activation of Rac1 drives cortactin translocation to the cell membrane where it colocalizes with myosin light chain kinase, a regulator of MLC phosphorylation and cytoskeletal rearrangement. Mammoto et al[75] elucidated a signaling pathway by which Ang-1 activated Rho family GTPases to organize the cytoskeleton into a junction-fortifying arrangement, which consequently decreased the permeability of the endothelium and protected against vascular leakage in vivo. Even the increased cAMP requires Rac1 to mediate its effects on endothelial function. Rac1, a downstream signal of cAMP, strengthens the inter-endothelial adherens junction.[76] Interestingly, exogenous reagent simvastatin induces the cortical arrangement of F-actin and the improvement of barrier function, also by inhibiting the geranylgeranylation, a covalent modification enabling translocation of the small GTPases Rho and Rac to the cell membrane.[77] Figure 1 gives a simplified schematic image of major signal pathways in endothelial barrier regulation, in which the enhancement pathway is emphasized.

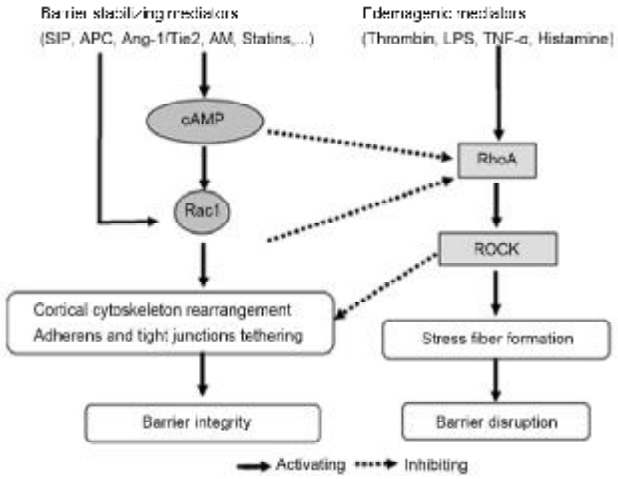

Figure 1. The simplified schematic outline of the major signal pathways in endothelial barrier regulation.

Other signals have also been revealed to participate in signal transduction of regulating the vascular barrier function with some controversial effects. Protein Kinase Czeta was found to play a role in thrombin-induced increased vascular permeability,[78] but have contribution to Ang-1-dependent inhibition of VEGF-induced endothelial permeability in vitro.[19]

When we are listing all those barrier stabilizing mediators or signals, we have to bear this notion in mind that so-called barrier enhancing mediators might have differential effects on vascular endothelial permeability, depending on the activated receptors and signaling pathways, the cellular compartment of signal and the

location of vascular beds.

Conclusion

Increased vascular leakage remains a critical event in the development of inflammatory diseases. Sometimes, it is a serious, even life-threatening clinical challenge in the setting of severe burn injury, acute lung injury and respiratory distress syndrome. Novel treatments are needed. Although the specific extent to which their endothelial barrier-protective properties has yet to be further defined, pharmacologic approaches including the use of those barrier enhancing mediators, such as AM, S1P, APC, or statins, etc, may represent novel therapeutic strategies. The possibility of reinforcing those barrier enhancing pathways also provides another train of thought to attenuate the increase of vascular permeability.

REFERENCES

1. Mehta D, Malik AB. Signaling mechanisms regulating endothelial permeability. Physiol Rev 2006;86(1):279-367.

2. Dejana E, Tournier-Lasserve E, Weinstein BM. The control of vascular integrity by endothelial cell junctions: molecular basis and pathological implications. Dev Cell 2009;16(2):209-21.

3. Komarova YA, Mehta D, Malik AB. Dual regulation of endothelial junctional permeability. Sci STKE 2007;(412):re8.

4. Van Nieuw Amerongen GP, van Hinsbergh VW. Targets for pharmacological intervention of endothelial hyperpermeability and barrier function. Vascul Pharmacol 2002;39(4-5):257-72.

5. Aveleira CA, Lin CM, Abcouwer SF, et al. TNF-α signals through PKCζ/NF-κB to alter the tight junction complex and increase retinal endothelial cell permeability. Diabetes 2010;59 (11):2872-82.

6. Blecharz KG, Haghikia A, Stasiolek M, et al. Glucocorticoid effects on endothelial barrier function in the murine brain endothelial cell line cEND incubated with sera from patients with multiple sclerosis. Mult Scler 2010;16(3):293-302.

7. Harke N, Leers J, Kietz S, et al. Glucocorticoids regulate the human occludin gene through a single imperfect palindromic glucocorticoid response element. Mol Cell Endocrinol 2008;295 (1-2):39-47.

8. Kim H, Lee JM, Park JS, et al. Dexamethasone coordinately regulates angiopoietin-1 and VEGF: a mechanism of glucocorticoid-induced stabilization of blood-brain barrier. Biochem Biophys Res Commun 2008;372(1):243-8.

9. Wang K, Wang Y, Gao L, et al. Dexamethasone inhibits leukocyte accumulation and vascular permeability in retina of streptozotocin-induced diabetic rats via reducing vascular endothelial growth factor and intercellular adhesion molecule-1 expression. Biol Pharm Bull 2008;31(8):1541-6.

10. Chen RC, Tang XP, Tan SY, et al. Treatment of severe acute respiratory syndrome with glucosteroids: the Guangzhou experience. Chest 2006;129(6):1441-52.

11. Dahlén SE, Dahlén B, Drazen JM. Asthma treatment guidelines meet the real world. N Engl J Med 2011;364(18):1769-70.

12. Haller JA, Kuppermann BD, Blumenkranz MS, et al. Randomized controlled trial of an intravitreous dexamethasone drug delivery system in patients with diabetic macular edema. Arch Ophthalmol 2010;128(3):289-96.

13. Dellinger RP, Levy MM, Carlet JM, et al. Surviving Sepsis Campaign: international guidelines for management of severe sepsis and septic shock: 2008. Crit Care Med 2008;36(1):296-327.

14. Pizurki L, Zhou Z, Glynos K, et al. Angiopoietin-1 inhibits endothelial permeability, neutrophil adherence and IL-8 production. Br J Pharmacol 2003;139(2):329-36.

15. London NR, Whitehead KJ, Li DY. Endogenous endothelial cell signaling systems maintain vascular stability. Angiogenesis 2009;12(2):149-58.

16. Saharinen P, Eklund L, Miettinen J, et al. Angiopoietins assemble distinct Tie2 signalling complexes in endothelial cell-cell and cell-matrix contacts. Nat Cell Biol 2008;10(5):527-37.

17. Wu HM, Huang Q, Yuan Y, et al. VEGF induces NO-dependent hyperpermeability in coronary venules. Am J Physiol 1996;271(6 Pt 2):H2735-9.

18. Fischer S, Clauss M, Wiesnet M, et al. Hypoxia induces permeability in brain microvessel endothelial cells via VEGF and NO. Am J Physiol 1999;276(4 Pt 1):C812-20.

19. Oubaha M, Gratton JP. Phosphorylation of endothelial nitric oxide synthase by atypical PKC zeta contributes to angiopoietin-1-dependent inhibition of VEGF-induced endothelial permeability in vitro. Blood 2009;114(15):3343-51.

20. Gavard J, Patel V, Gutkind JS. Angiopoietin-1 prevents VEGF-induced endothelial permeability by sequestering Src through mDia. Dev Cell 2008;14(1):25-36.

21. Mammoto T, Parikh SM, Mammoto A, et al. Angiopoietin-1 requires p190 RhoGAP to protect against vascular leakage in vivo. J Biol Chem 2007;282(33):23910-8.

22. Li X, Hahn CN, Parsons M, et al. Role of protein kinase Czeta in thrombin-induced endothelial permeability changes: inhibition by angiopoietin-1. Blood 2004;104(6):1716-24.

23. McCarter SD, Mei SH, Lai PF, et al. Cell-based angiopoietin-1 gene therapy for acute lung injury. Am J Respir Crit Care Med 2007;175(10):1014-26.

24. Milner CS, Hansen TM, Singh H, et al. Roles of the receptor tyrosine kinases Tie1 and Tie2 in mediating the effects of angiopoietin-1 on endothelial permeability and apoptosis.

Microvasc Res 2009;77(2):187-91.

25. Van der Heijden M, van Nieuw Amerongen GP, Koolwijk P, et al. Angiopoietin-2, permeability oedema, occurrence and severity of ALI/ARDS in septic and non-septic critically ill patients. Thorax 2008;63(10):903-9.

26. van der Heijden M, van Nieuw Amerongen GP, Chedamni S, et al. The angiopoietin-Tie2 system as a therapeutic target in sepsis and acute lung injury. Expert Opin Ther Targets 2009;13 (1):39-53.

27. Xu J, Qu J, Cao L, et al. Mesenchymal stem cell-based angiopoietin-1 gene therapy for acute lung injury induced by lipopolysaccharide in mice. J Pathol 2008;214(4):472-81.

28. Caron KM, Smithies O. Extreme hydrops fetalis and cardiovascular abnormalities in mice lacking a functional adrenomedullin gene. Proc Natl Acad Sci USA 2001;98(2):615-9.

29. Dackor RT, Fritz-SixK, Dunworth WP, et al. Hydrops fetalis, cardiovascular defects, and embryonic lethality in mice lacking the calcitonin receptor-like receptor gene. Mol Cell Biol 2006;26(7):2511-8.

30. Ichikawa-Shindo Y, Sakurai T, Kamiyoshi A, et al. The GPCR modulator protein RAMP2 is essential for angiogenesis and vascular integrity. J Clin Invest 2008;118(1):29-39.

31. Kis B, Deli MA, Kobayashi H, et al. Adrenomedullin regulates blood-brain barrier functions in vitro. Neuroreport 2001;12 (18):4139-42.

32. Hippenstiel S, Witzenrath M, Schmeck B, et al. Adrenomedullin reduces endothelial hyperpermeability. Circ Res 2002;91(7):618-25.

33. Honda M, Nakagawa S, Hayashi K, et al. Adrenomedullin improves the blood-brain barrier function through the expression of claudin-5. Cell Mol Neurobiol 2006;26(2):109-18.

34. Dohgu S, Sumi N, Nishioku T, et al. Cyclosporin A induces hyperpermeability of the blood-brain barrier by inhibiting autocrine adrenomedullin-mediated up-regulation of endothelial barrier function. Eur J Pharmacol 2010;644(1-3):5-9.

35. Temmesfeld-Wollbrück B, Brell B, Dávid I, et al. Adrenomedullin reduces vascular hyper- permeability and improves survival in rat septic shock. Intensive Care Med 2007;33 (4):703-10.

36. Aslam M, Pfeil U, Gündüz D, et al. Intermedin/ adrenomedullin2 stabilizes endothelial barrier and antagonises thrombin-induced barrier failure. Br J Pharmacol 2012;165(1):208-22.

37. Pfeil U, Aslam M, Paddenberg R, et al. Intermedin/ adrenomedullin-2 is a hypoxia-induced endothelial peptide that stabilizes pulmonary microvascular permeability. Am J Physiol Lung Cell Mol Physiol 2009;297(5):L837-45.

38. Aslam M, Gündüz D, Schuler D, et al. Intermedin induces loss of coronary microvascular endothelial barrier via derangement of actin cytoskeleton: role of RhoA and Rac1. Cardiovasc Res 2011;92(2):276-86.

39. Dwivedi AJ, Wu R, Nguyen E, et al. Adrenomedullin and adrenomedullin binding protein-1 prevent acute lung injury after gut ischemia-reperfusion. J Am Coll Surg 2007;205(2):284-93.

40. Qiang X, Wu R, Ji Y, et al. Purification and characterization of human adrenomedullin binding protein-1. Mol Med 2008; 14(7-8):443-50.

41. Hla T. Physiological and pathological actions of sphingosine 1-phosphate. Semin Cell Dev Biol 2004;15(5):513-20.

42. Singleton PA, Dudek SM, Chiang ET, et al. Regulation of sphingosine 1-phosphate-induced endothelial cytoskeletal rearrangement and barrier enhancement by S1P1 receptor, PI3 kinase, Tiam1/Rac1, and alpha-actinin. FASEB J 2005;19(12):1646-56.

43. Rosen H, Gonzalez-Cabrera PJ, Sanna MG, et al. Sphingosine 1-phosphate receptor signaling. Annu Rev Biochem 2009; 78:743-68.

44. Sanchez T, Hla T. Structural and functional characteristics of S1P receptors. J Cell Biochem 2004;92(5):913-22.

45. Christoffersen C, Obinata H, Kumaraswamy SB, et al. Endothelium-protective sphingosine-1-phosphate provided by HDL-associated apolipoprotein M. Proc Natl Acad Sci USA 2011; 108(23):9613-8.

46. Mehta D, Konstantoulaki M, Ahmmed GU, et al. Sphingosine 1-phosphate-induced mobilization of intracellular Ca2+ mediates rac activation and adherens junction assembly in endothelial cells. J Biol Chem 2005;280(17):17320-8.

47. Dudek SM, Jacobson JR, Chiang ET, et al. Pulmonary endothelial cell barrier enhancement by sphingosine 1-phosphate: Roles for cortactin and myosin light chain kinase. J Biol Chem 2004;279(23):24692-700.

48. Liu X, Wu W, Li Q, et al. Effect of sphingosine 1-phosphate on morphological and functional responses in endothelia and venules after scalding injury. Burns 2009;35(8):1171-9.

49. Zhao Y, Gorshkova IA, Berdyshev E, et al. Protection of LPS-induced murine acute lung injury by sphingosine-1-phosphate lyase suppression. Am J Respir Cell Mol Biol 2011;45(2): 426-35.

50. Usatyuk PV, He D, Bindokas V, et al. Photolysis of caged sphingosine-1-phosphate induces barrier enhancement and intracellular activation of lung endothelial cell signaling pathways. Am J Physiol Lung Cell Mol Physiol 2011;300(6):L840-50.

51. Skoura A, Hla T. Regulation of vascular physiology and pathology by the S1P2 receptor subtype. Cardiovasc Res 2009; 82(2):221-8.

52. Sarangi PP, Lee HW, Kim M. Activated protein C action in inflammation. Br J Haematol 2010;148(6):817-33.

53. Van Sluis GL, Niers TM, Esmon CT, et al. Endogenous activated protein C limits cancer cell extravasation through sphin-

gosine-1-phosphate receptor 1-mediated vascular endothelial barrier enhancement. Blood 2009;114(9):1968-73.

54. Minhas N, Xue M, Fukudome K, et al. Activated protein C utilizes the angiopoietin/Tie2 axis to promote endothelial barrier function. FASEB J 2010;24(3):873-81.

55. Xue M, Chow SO, Dervish S, et al. Activated protein C enhances human keratinocyte barrier integrity via sequential activation of epidermal growth factor receptor and Tie2. J Biol Chem 2011;286(8):6742-50.

56. Bernard GR, Vincent JL, Laterre PF, et al. Efficacy and safety of recombinant human activated protein C for severe sepsis. N Engl J Med 2001;344(10):699-709.

57. Suter PM. Xigris is withdrawn from the market. A 10 year odissey. Minerva Anestesiol 2011;77(12):1128-9.

58. Mullard A. Drug withdrawal sends critical care specialists back to basics. Lancet 2011;378(9805):1769.

59. Jacobson JR, Barnard JW, Grigoryev DN, et al. Simvastatin attenuates vascular leak and inflammation in murine inflammatory lung injury. Am J Physiol Lung Cell Mol Physiol 2005;288(6): L1026-32.

60. Shivanna M, Jalimarada SS, Srinivas SP. Lovastatin inhibits the thrombin-induced loss of barrier integrity in bovine corneal endothelium. J Ocul Pharmacol Ther 2010;26(1):1-10.

61. Béziaud T, Ru Chen X, EI Shafey N, et al. Simvastatin in traumatic brain injury: effect on brain edema mechanisms. Crit Care Med 2011;39(10):2300-7.

62. Morofuji Y, Nakagawa S, So G, et al. Pitavastatin strengthens the barrier integrity in primary cultures of rat brain endothelial cells. Cell Mol Neurobiol 2010;30(5):727-35.

63. Meuwese MC, Mooij HL, Nieuwdorp M, et al. Partial recovery of the endothelial glycocalyx upon rosuvastatin therapy in patients with heterozygous familial hypercholesterolemia. J Lipid Res 2009;50(1):148-53.

64. Gündüz D, Thom J, Hussain I, et al. Insulin stabilizes microvascular endothelial barrier function via phosphatidylinositol 3-kinase/Akt-mediated Rac1 activation. Arterioscler Thromb Vasc Biol 2010;30(6):1237-45.

65. Schlegel N, Waschke J. Vasodilator-stimulated phosphoprotein: crucial for activation of Rac1 in endothelial barrier maintenance. Cardiovasc Res 2010;87(1):1-3.

66. Vandenbroucke E, Mehta D, Minshall R, et al. Regulation of endothelial junctional permeability. Ann N Y Acad Sci 2008; 1123:134-45.

67. Surapisitchat J, Beavo JA. Regulation of endothelial barrier function by cyclic nucleotides: the role of phosphodiesterases. Handb Exp Pharmacol 2011;(204):193-210.

68. Shivanna M, Srinivas SP. Elevated cAMP opposes (TNF-alpha)-induced loss in the barrier integrity of corneal endothelium. Mol Vis 2010;16:1781-90.

69. Lin YC, Samardzic H, Adamson RH, et al. Phosphodiesterase 4 inhibition attenuates atrial natriuretic peptide-induced vascular hyperpermeability and loss of plasma volume. J Physiol 2011;589(Pt 2):341-53.

70. Schlegel N, Baumer Y, Drenckhahn D, et al. Lipopolysaccharide-induced endothelial barrier breakdown is cyclic adenosine monophosphate dependent in vivo and in vitro. Crit Care Med 2009;37(5):1735-43.

71. Birukova AA, Zagranichnaya T, Fu P, et al. Prostaglandins PGE(2) and PGI(2) promote endothelial barrier enhancement via PKA- and Epac1/Rap1-dependent Rac activation. Exp Cell Res 2007;313(11):2504-20.

72. Pannekoek WJ, van Dijk JJ, Chan OY, et al. Epac1 and PDZ-GEF cooperate in Rap1 mediated endothelial junction control. Cell Signal 2011;23(12):2056-64.

73. Sayner SL, Balczon R, Frank DW, et al. Filamin A is a phosphorylation target of membrane but not cytosolic adenylyl cyclase activity. Am J Physiol Lung Cell Mol Physiol 2011;301 (1):L117-24.

74. Spindler V, Schlegel N, Waschke J. Role of GTPases in control of microvascular permeability. Cardiovasc Res 2010;87 (2):243-53.

75. Mammoto T, Parikh SM, Mammoto A, et al. Angiopoietin-1 requires p190 RhoGAP to protect against vascular leakage in vivo. J Biol Chem 2007;282(33):23910-8.

76. Baumer Y, Spindler V, Werthmann RC, et al. Role of Rac 1 and cAMP in endothelial barrier stabilization and thrombin-induced barrier breakdown. J Cell Physiol 2009;220(3):716-26.

77. Jacobson JR. Pharmacologic therapies on the horizon for acute lung injury/acute respiratory distress syndrome. J Investig Med 2009;57(8):870-3.

78. Li X, Hahn CN, Parsons M, et al. Role of protein kinase Czeta in thrombin-induced endothelial permeability changes: inhibition by angiopoietin-1. Blood 2004;104(6):1716-24.

(Received September 15, 2011)

Edited by DONG Min and SONG Shuang-ming