Endogenous RhoA Inhibitor Protects Endothelial Barrier

Geerten P. van Nieuw Amerongen, Victor W.M. van Hinsbergh

Vascular leakage is a hallmark of many, often life-threatening, inflammatory diseases, and contributes to disease severity in disorders such as sepsis, cancer, diabetes, and atherosclerosis. Despite the tremendous medical importance of vascular leakage, few specific therapies are available today to counteract it, and current therapies often fail to do so. This is in part because the in vivo molecular targets are incompletely identified, although a wealth of data obtained from in vitro studies is available on signal transduction pathways that regulate vascular permeability. Interestingly, among various new agents that potentially reduce endothelial hyperpermeability, the cholesterol-lowering statin drugs have been proposed to reduce vascular leakage by means of their inhibitory effects on RhoA proteins.

In the current issue of Circulation Research Gorovoy et al provide an elegant proof-of-principle that increased RhoA activity results in vascular hyperpermeability in vivo. They show that elevation of RhoA activity by deletion of one of its inhibitory proteins, RhoGDI, causes a loss of endothelial junctional integrity and decrease in vascular barrier function.

Rho GTPases, particularly RhoA, Rac-1 and cdc42 have received much attention as key regulators of cell shape, movement and proliferation. In vitro studies have shown that the balance of activities of these small G proteins determines the status of endothelial barrier: Cdc42 enhancing recovery of a disturbed barrier, Rac-1 being required for establishing a tight barrier, and RhoA being involved in inducing endothelial hyperpermeability by various stimuli, such as thrombin, VEGF, angiopoietin-2 and LPA (see Figure). Similarly, these Rho GTPases are acting in other vascular cells and leukocytes, and thus influence other vascular functions as well.

Inhibition of the RhoA-target ROCK1/2 (Rho kinase) by inhibitors developed in the last decade pointed to the involvement of Rhoa/ROCK activation in the embryonic development/cytokinesis and in various vascular pathologies, such as (pulmonary) hypertension, atherosclerosis, stroke and heart failure. Recent studies provided indeed the first evidence that ROCK inhibition by the compound Y-27632 can alleviate pulmonary edema in animals after LPS challenge or reexpansion of the lung.

Given their central importance in regulation of many basal cellular functions such as migration and proliferation, it is not surprising that activity of Rho proteins is tightly controlled by regulatory proteins: guanine dissociation inhibitors or GDI’s keep Rho proteins in their inactive GDP-bound mode, guanine exchange factors or GEF’s activate Rho proteins by promoting the exchange of GDP for GTP, and GTP-ase activating proteins or GAP’s inactive Rho proteins by facilitating the conversion of Rho-bound GTP to GDP. These regulatory proteins act in close concert; dissociation of RhoA from RhoGDI is a prerequisite for its activation by RhoGEF. Regulatory proteins of all three classes (RhoGDIs, RhoGEFs, and RhoGAPs) have been implicated in regulation of thrombin-enhanced in vitro endothelial permeability. Analogous, Epac, a cAMP-activated exchange factor for Rap1 (a small GTPase not belonging to the Rho family of small GTPases) plays a pivotal role in potentiating vascular endothelial cadherin-mediated cell-cell contacts.

Gorovoy et al chose to focus on the pulmonary vasculature using a model of isolated mouse lungs. An increase in capillary permeability is the basic underlying abnormality of acute lung injury/acute respiratory distress syndrome (ALI/ARDS), which form a continuum from mild to severe lung damage. ARDS often develops in septic patients or after trauma and is a major cause of death in the Intensive Care setting. It is therefore important that the authors started with demonstrating that treatment with endotoxin, as a model for sepsis, induces activity of RhoA in the lung.

An important observation was that increased permeability in RhoGDI−/− mice was completely reversible by pharmacological inhibition of Rho kinase. First, this indicates that the hyperpermeability in RhoGDI−/− mice indeed was caused by enhanced RhoA/Rho kinase-signaling, excluding many putative side effects. Second, this brings the concept of RhoGDI/RhoA-mediated vascular leakage immediately within the clinical horizon, as Rho kinase inhibitors with a reasonable safety profile such as fasudil are available.

Surprisingly, no signs of edema were observed in the intact animals, which was explained by the authors by the presence of safety factors such as lymphatic drainage. It remains to be tested formally whether in the intact animal the vasculature is hyperpermeable, using e.g. dye extravasation tests.

While challenging, the data should be interpreted with care. It is tempting to conclude that the absence of Rho-GDI and the accompanying effect of RhoA on vascular leakage is caused by activation of RhoA in endothelial cells. Indeed the authors show that the endothelial junctions in capillaries and postcapillary venules become disturbed, but the biochemical measurements were performed in whole lung homogenates and therefore are nonconclusive in this regard. Although the
siRNA approach in cultured endothelial cells confirmed that deletion of RhoGDI by itself is sufficient for barrier dysfunction, the present study does not exclude that nonendothelial effects in RhoGDI−/− mice also contribute to the enhanced vascular permeability in the intact lung. Future studies, eg, by rescuing RhoGDI specifically in endothelial cells or leukocytes, have to exclude that the effect on vascular junctions is not indirectly evoked by a change in leukocyte influx after LPS challenge or by a change in resident leukocytes and mast cells because of the life-long depletion of RhoGDI. Nevertheless, even if such indirect effects would contribute to vascular leakage in RhoGDI−/− mice, the present study provides fuel for the suggestion that in the healthy vasculature RhoGDI is a nodal point that keeps RhoA activity low. S1P, sphingosine-1-phosphate; PAK1, p21-activated kinase; PKC, protein kinase C.

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None.

References


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