Activation of the PAR1-TRPV4 Signaling Axis Drives Endothelial Barrier Failure in COVID-19

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Study objective: Endothelial dysfunction and increased microvascular permeability are central hallmarks of severe COVID-19. At present, the exact underlying mechanisms of endothelial barrier failure in COVID-19 remain elusive. Here, we show that dysbalance of coagulation factors, characterized by a loss of antithrombin III and increased thrombin activity in plasma from severe COVID-19 patients activates endothelial protease-activated receptor (PAR1), which mediates barrier failure by triggering TRPV4-mediated Ca^{2+} influx in lung microvascular endothelial cells.

Methods: Citrate plasma was sampled as part of the Pa-COVID-19 cohort study (ethics approval EA2/066/20) from patients with severe COVID-19 (high flow O_2 or mechanically ventilated; WHO severity score: 5-7). Plasma samples were diluted to 10% (v/v) in cell culture medium without FCS and tested for their ability to disrupt barrier integrity of primary human pulmonary microvascular endothelial cell (HPMEC) monolayers by electrical cell-substrate impedance sensing (ECIS), immunofluorescence for endothelial VE-cadherin and F-actin, western blot analyses of PAR-1 cleavage, and real-time Ca^{2+} imaging. Plasma from healthy donors served as control.

Results: COVID-19 plasma had elevated thrombin activity while levels of antithrombin III, a key anti-coagulant with thromboprotective function were decreased. COVID-19 plasma caused endothelial barrier dysfunction as measured by ECIS and gap formation in HPMEC monolayers. Endothelial barrier disruption and endothelial Ca^{2+} influx in response to COVID-19 plasma could be blocked by selective antagonists targeting thrombin (Argatroban), its receptor PAR1 (SCH79797), or TRPV4 (HC-067047).

Conclusion: Here, we identify a novel signaling axis via thrombin, its receptor PAR1, and TRPV4 as mechanism for increased microvascular permeability in COVID-19. Targeting this signaling axis in endothelial barrier failure may provide a promising adjunctive therapy in patients with or at risk for severe COVID-19.

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