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Selective inhibition of matrix metalloproteinase-9 attenuates traumatic brain injury-mediated blood-brain barrier disruption in a novel dynamic in vitro model

Traumatic brain injury (TBI) is a physical disruption of brain tissue followed by a cascade of secondary inflammatory responses. The secondary responses result in a transient disruption of the blood-brain barrier (BBB). The BBB prevents crossover between the brain's microenvironment and systemic circulation. However, TBI-mediated BBB disruption initiates a chronic cycle of injury exacerbation starting with increased microvascular permeability due in part to matrix metalloproteinase (MMP) upregulation. MMPs are extracellular matrix modifying enzymes dysregulated by pathophysiological changes. MMPs increase microvascular permeability by cleaving tight junction proteins that typically seal the endothelium; this breakdown is primarily caused by a particular enzyme, MMP-9. It currently remains unknown whether or not MMP-9's role in injury exacerbation is related to previously observed TBI-induced decreased expression of Claudin-5, a tight junction protein. Here, we utilized a novel, in vitro dynamic model of the BBB that can recapitulate the pathophysiology of an injured brain with the ultimate goal of evaluating MMP-9's role in increased microvascular permeability. Contact tri-cultures of brain microvascular endothelia, astrocytes, and pericytes were exposed to exogenous tumor necrosis factor-alpha (TNF-α) for 24 hours to simulate the inflammatory injury in TBI and placed in our novel platform. They were then treated with MMP inhibitor, anti-MMP-9 antibody treatment, and MMP-9 small interfering. Microvascular barrier integrity was assessed by several real-time metrics. MMP-9 production was measured by ELISA, and Claudin-5 stability was evaluated via Western blot. qPCR was used to determine MMP-9 and MMP-2 (another CNS MMP) gene knockdown. Results showed cultures treated with the MMP inhibitor and MMP-9 siRNA resulted in significantly restored barrier function after TNF-α compared to injured controls. Additionally, small molecule treatments of MMP-9 siRNA and synthetic inhibitor resulted in significant reduction in MMP-9 gene expression. Surprisingly, treatment with the anti-MMP-9 antibody also resulted in significant reduction of MMP-9 expression. All inhibition techniques also significantly prevented Claudin-5 degradation. Importantly, the most efficacious treatments in preventing barrier dysfunction significantly knocked down the expression of both MMP-9 and MMP-2. Previous research has only investigated MMP-9 as a BBB modifier, yet our findings suggest both MMP-9 and MMP-2 play critical roles in blood-brain barrier modification. Overall, our results indicate that microvascular barrier integrity is significantly decreased after TBI through a mechanism related to MMP-9 and MMP-2 activity in conjunction with Claudin-5, providing insight into the exploration of each enzyme as a potential therapeutic target.