

EMOSTASI E TROMBOSI: ASPETTI DI BASE

FUNCTIONAL AND STRUCTURAL IMPAIRMENTS OF THE CEREBRAL MICROCIRCULATION IN A MOUSE MODEL OF HEMOPHILIA A.

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Background and Aims: Hemophilia A (HA) is an X-linked bleeding disease resulting from mutations in the coagulation factor VIII (FVIII). The main clinical manifestations are prolonged bleeding episodes, including hemarthroses and intracranial hemorrhages (ICH), which are among the most severe and life-threatening complications, especially in pediatric patients. The triggers of hemorrhagic events remain unclear, occurring spontaneously or following minimal trauma. Standard replacement therapies fail to prevent ICH, suggesting that mechanisms beyond FVIII deficiency may contribute to cerebrovascular fragility. Moreover, based on the recent findings on the role of FVIII in the maintenance of endothelial cells (ECs) functionality, within this study we aimed to evaluate both quantitative and functional differences in the cerebral microcirculation in a murine model of HA (C57BL/6-HA) compared to wild-type mice (C57BL/6-WT).

Methods: Brains were collected from HA and WT mice at 3, 8, 24, and 52 weeks of age. Quantitative analysis of the cerebral microvasculature was performed by immunofluorescence staining for mCD31, followed by image acquisition and vessel density quantification using ImageJ software. WT and HA mice at 8 weeks of age were retro-orbitally injected, with two fluorescent dextran molecules with different molecular weight (4 e 150 kDa) and different wavelength to assess vascular permeability and evaluate baseline extravasation under physiological conditions. Finally, 8 weeks WT and HA mice were retro-orbitally injected with 1% DMSO to induce vascular stress, and to evaluate the vascular leakage 5-, 10-, and 15-minutes post-injection.

Results: Quantitative analysis revealed that HA mice exhibit-

ed consistently lower microvascular density compared to age-matched WT mice across all time points, suggesting a lifelong impairment in vascular development or maintenance. In addition to a lower number of vessels, HA mice also displayed significantly shorter average vessel length relative to WT mice, indicating not only a numerical deficit but also a less complex microvascular network. Longitudinal analysis showed that WT mice exhibited significant dynamic variation in vessel number across developmental stages, by contrast HA mice showed no significant changes in microvascular parameters over time. Under physiological conditions, both HA and WT mice showed comparable levels of dextran extravasation, indicating preserved baseline vascular integrity. However, following DMSO-induced stress, HA mice displayed a significant increase in dextran leakage from cerebral vessels at all timepoints analyzed, on the contrary WT mice maintained a preserved vascular integrity comparable to baseline.

Conclusions: These findings highlight the presence of endothelial fragility of the HA cerebral microvasculature led to permeability alterations in response to injury. Our study suggests that HA mice are associated with both a reduced cerebral microvascular density and an increased susceptibility to vascular leakage under stress conditions, despite the preservation of baseline vascular integrity. This dual vulnerability—quantitative insufficiency and qualitative fragility—suggests that cerebral microcirculation in HA is profoundly impaired, potentially driving the heightened risk of intracranial hemorrhage observed in HA patients reinforcing the critical need for further investigation into therapeutic strategies aimed at strengthening vascular resilience in HA.

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