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RNS60 Substantially Reduces Early Vasogenic Edema, Mitigates Infarct Area, and Decreases Expression of HIF1α Four Days Post-Ischemic Stroke

Introduction: RNS60 is a proprietary 0.9% saline solution with an elevated oxygen concentration. Previously, we showed that 13 days of daily RNS60 treatment after transient middle cerebral artery occlusion (tMCAo) stroke reduced brain pathology (e.g., infarction, amyloid pathology, neuronal death, microglial activation, and white matter damage) while increasing microvascular perfusion and memory (Baena Caldas et al. 2024). Here, we examine earlier brain-protective effects of daily RNS60 treatment 4 days after stroke.

Methods: Male C57BL/6J mice, 3-4 months old, were randomly divided into sham surgery or unilateral 60-minute tMCAo. Each group was subdivided into three treatment arms: an experimental arm receiving daily intraperitoneal injections of 0.2 mL of stabilized RNS60, and control arms of pressurized normal saline with the same oxygen content as RNS60 (PNS60) or normal saline (NS). Injections began 2 hours after stroke, once daily for 3 days. An additional group without treatment was used as a control. Treatment group assignments were blinded throughout the study. On day 4, mice from each group were euthanized to assess infarct volume using TTC staining or were perfused with 4% PFA to perform immunohistochemistry.

Results: Four days after ischemic stroke, daily injections of RNS60 treatment significantly reduced early post-stroke vasogenic edema by 41% and infarct size by 39% compared to controls. These RNS60 brain-protective effects were related to decreased expression of hypoxia-inducible factor 1α (HIF1α), suggesting that RNS60 treatment may reduce hypoxia.

Discussion: RNS60-treated mice exhibit early and long-term significant brain protection after ischemic stroke associated with decreased expression of HIF1α, suggesting reduced hypoxia in the ischemic brain. Ongoing studies aim to identify the effect of RNS60 on blood brain barrier integrity to further elucidate the underlying molecular mechanism.