Effects of Low-dose Unfractionated Heparin on Early Brain Injury After Subarachnoid Hemorrhage in Mice

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Introduction

There is no proven effective therapy to prevent early brain injury (EBI) after subarachnoid hemorrhage (SAH) despite extensive research efforts. Sphingosine kinase (SphK) 1 has been reported as an important signaling node in anti-apoptotic signaling. Heparin is a pleiotropic drug that antagonizes many pathophysiological mechanisms. In this study, we evaluated if heparin prevents EBI after SAH by anti-apoptotic mechanisms including SphK1.

Methods

This study used 135 8-week-old male CD-1 mice. We induced SAH with endovascular perforation in mice and randomly assigned animals to sham-operated (n = 23), SAH + vehicle (n = 35), SAH+10U heparin pretreatment (n = 11), SAH+30U heparin pretreatment (n = 14), SAH+10U heparin posttreatment (n = 30), and SAH+30U heparin posttreatment (n = 22). At 24 hours post-SAH, neurological scores, brain water content and Evans blue extravasation were evaluated. Also, the expression of SphK, phosphorylated Akt, and cleaved caspase-3 was determined by Western blotting and neuronal cell death was examined by terminal deoxynucleoti¬dyl transferase–mediated uridine 5′-triphosphate-biotin nick end-labeling staining.

Results

Low-dose heparin pre- and post- treatment significantly improved neurobehavioral function and brain edema at 24 hours after SAH. Moreover, low-dose heparin posttreatment attenuated blood-brain barrier disruption and neuronal cell death in the cortex, associated with an increase in SphK1 and phosphorylated Akt, and a decrease in cleaved caspase-3. High-dose heparin had a tendency for increased SAH, which aggravated brain injury and therefore obscured the neuroprotective effects.

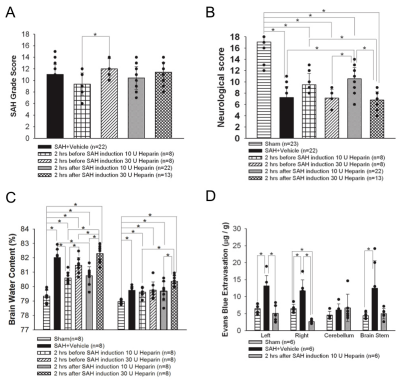
Conclusions

Low-dose heparin posttreatment may decrease the development of post-SAH EBI through anti-apoptotic mechanisms including sphingosine-related pathway activation, implying its efficacy for early prevention of brain injury after acute aneurysm rupture in a clinical setting.

Learning Objectives

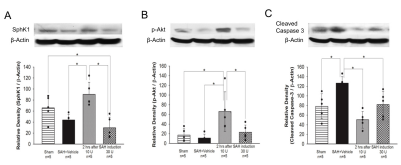
1)Early prevention of brain injury after acute aneurysm rupture is important for lifesaving in a clinical setting.
2)Low-dose heparin effect mechanism may implicate with sphingosine-related pathway activation.
3)Low-dose heparin posttreatment may decrease the development of post-SAH EBI through anti-apoptotic mechanisms including sphingosine-related pathway activation.

Heparin 1



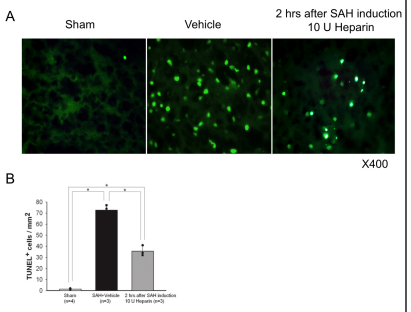
SAH grade (A), neurological score (B), brain water content (C) and Evans blue dye extravasation (D) at 24 hours post-SAH. Values, median±25th to 75th percentiles (A, B) or mean±SD (C, D); *P<0.05, Kruskal-Wallis test (A, B) or ANOVA (C, D).

Heparin 2



Representative Western blots and quantitative analysis of SphK1 (A), p-Akt (B), and cleaved caspase-3 (C) in the left cerebral hemisphere at 24 hours after SAH. The protein band density values are calculated as a ratio of that of -actin. Values are mean±SD; *P<0.05, ANOVA.

Heparin 3



TUNEL-positive cells (green) in the ipsilateral basal cortex at 24 hours after SAH. A, representative immunofluorescence images; B, quantitative analysis; values, mean±SD; *P<0.05, ANOVA