The role of glycocalyx-dependant mechanotransduction in subarachnoid hemorrhage-induced neurogenic pulmonary edema

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Introduction

Neurogenic pulmonary edema (NPE) following subarachnoid hemorrhage (SAH) has a high morbidity and mortality rate [1]. The precise mechanism(s) of NPE occurring at the lung vascular endothelium are unknown.

Our study used two animals models and a cell culture model to assess lung injury and the effect of mechanotransduction on barrier function that may be seen in SAH-induced NPE.

First we used an established model of SAH to study the role of glycocalyx breakdown on lung permeability and edema. Second, via the catecholamine storm model, we examined the effects of mechanotransduction on lung barrier function. Lastly, an in vitro study of cell culture was designed to test the effect of increased hydrostatic pressure on cell permeability.

Background

The endothelial glycocalyx (EG) is a complex layer of macromolecules that coats the luminal surface of vascular endothelium.

The EG participates in mechanotransduction by responding to changes in pressure and shear stress, resulting in the activation of the oxidative signaling pathways that involve nitric oxide, a known mediator for increased permeability [3,4].

Syndecan-1 (syn-1), a major heparan sulfate proteoglycan (HSPG) on the EG, is believed to be a primary mechanotransducer that can activate oxidative signaling. Shedding of syn-1, following mechanotransduction, may be a biomarker for endothelial activation/injury and EG degradation; the latter could be a mechanism accounting for increased vascular permeability.

During SAH-induced NPE, the hyper-adrenergic state is hypothesized to activate mechanotransduction, trigger EG breakdown, and result in lung hyper-permeability, inflammation, and edema.

Methods

Model 1: Four rats were anesthetized with isoflurane, intubated and mechanically ventilated, and maintained under general anesthesia with fentanyl, nitrous oxide and oxygen. SAH was induced by the injection of blood through the cisterna magna. Sham animals underwent identical surgery but without the blood injection (n=4). Tail arterial line was inserted for blood pressure measurement. Cerebral blood flow (CBF) and the intracranial pressure (ICP) were measured with an intracranial probe connected to a transducer and an transcranial doppler, respectively. At 12 hours post SAH, lungs were harvested for histology and western blot analysis of syn-1 and HSPG.

Model 2: Under isoflurane anesthesia, rats received a tracheotomy, jugular venous line and carotid arterial line. Baseline arterial blood gases (ABGs) were established after 30 minutes (mns) of a control state. Catecholamine storm was induced by an infusion of norepinephrine (NE) for 1 hour to increase mean arterial pressure (MAP) from 100 at baseline to 150 mm Hg. Inhibition of nitric oxide synthase, a known component of mechanotransduction, was assessed by administering a single bolus of L-NAME (final plasma concentration 200 uM) immediately prior to starting the NE infusion. ABGs were assessed every 30 mns.

Cell Culture Model: Rat lung microvascular endothelial cells were cultured to confluence on polycarbonate filters for 5 days. Monolayers were exposed to an acute increase in hydrostatic pressure to 30 cm H20 for 1 hour, to mimic Model 2. After 1 hour, monolayers were fixed and immune-stained for VE-cadherin (an adherence junction molecule).

Results

Following the induction of SAH, CBF significantly decreases as ICP and MAP increase. These physiologic changes are characteristic of SAH (Fig.1).

Model 1. SAH resulted in a 50-70% reduction in whole lung syn1 and HSPG on western blot analysis, consistent with a marked breakdown of the glycocalyx (Fig. 2). Lung histology demonstrates a moderate to severe patchy infiltrate, consistent with lung inflammation and edema in SAH. Only mild inflammation is seen in the sham group (Fig.3). Both findings are consistent with SAH-induced lung injury.

Model 2. In the Catecholamine storm model, an increase in MAP to 150 for 1 hour resulted in a 50% reduction in PaO2. A single bolus of L-NAME, which actually increased MAP slightly above 150 mm Hg, attenuated the drop in Pao2 by approximately 20-30% (Fig.4). These data suggest that mechanotransduction-induced barrier dysfunction caused a 30% reduction of PaO2 over 1 hour.

The cell culture model showed that the pressure-treated cells had a significant loss of VE-Cadherin (loss of VE-Cadherin is well described to cause hyper-permeability) (Fig. 5).

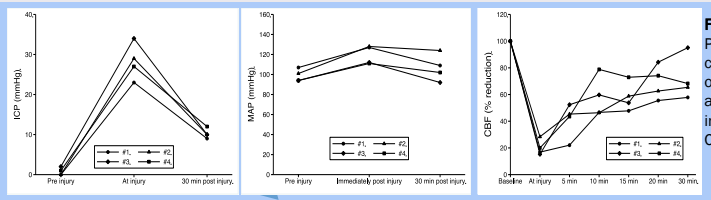
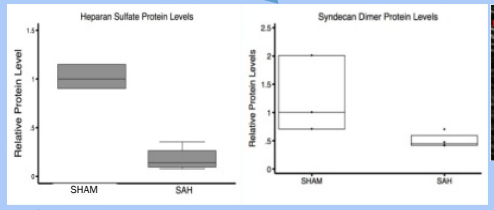


Fig 1. Physiologic characteristics of SAH. ICP and MAP increase as CBF decreases.



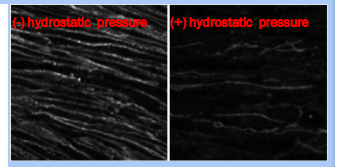


Fig 5. Hydrostatic pressure results in VE-Cadherin loss from adherence junction.

Fig 2. SAH causes significant loss of syn-1 and HSPG, indicative of EG breakdown.

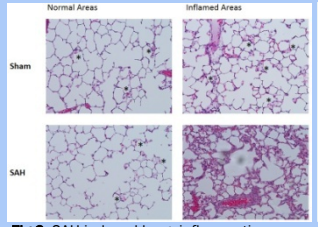


Fig 3. SAH-induced lung inflammation.

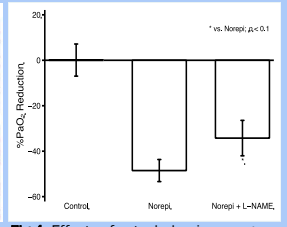


Fig 4. Effects of catacholamine surge and mechanotransduction on Pa02.

References

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Conclusion

This study provides important preliminary evidence showing the effects of SAH on endothelial glycocalyx breakdown, increased lung permeability, and inflammation while suggesting the role of mechanotransduction on barrier dysfunction in a hyper-adrenergic state.

Collectively, these results suggest important insights into SAH-induced NPE and the deleterious effects of catecholamine on the pulmonary circulation.

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