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Ultrastructural study of the permeability of *in-vitro* and *ex-vivo* human models of human arachnoid granulation CSF outflow pathway

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Background

In communicating hydrocephalus and also idiopathic intracranial hypertension, disturbed CSF dynamics may result from an increased resistance to CSF outflow at the arachnoid granulations (AGs). To better understand the mechanism of CSF egress, we modelled the outflow of CSF through human AGs using both cell culture (*in-vitro*) and whole tissue (*ex-vivo)* perfusion models. Ultrastructural studies were done using microparticles, ruthenium red, and TEM and to elucidate the mechanism of fluid flow.

Materials and methods

Human AG tissue was harvested within 24 h post-mortem and used to isolate AG cells for growth on filter inserts or fit into an Ussing perfusion chamber. Cell phenotype was identified in culture with immunocytochemical staining. Tissue was perfused at physiologic and increased pressure with serum-free media. Cells/tissue were perfused with fluorescent microparticles, or ruthenium red, and then fixed under experimental pressure. Fixed tissue was processed for TEM or cryo-sectioned and stained for visualization.

Results

In-vitro serum-free permeability results showed flow through the AG cells was uni-directional in the physiologic direction from the basal to apical (B→A) cell membrane. The average cellular hydraulic conductivity (Lp_{ave}) for AG cells perfused B→A was 93.05 ± 10.69 μl/min/mmHg/cm^2 (n = 19) with average perfusion pressure (ΔP_{ave}) across the cell layer of 2.92 ± 0.08 mmHg which was statistically higher ($p < 0.0001$) than Lp_{ave} for cells perfused A→B (non-physiologic direction), 0 μl/min/mmHg/cm^2 (n = 5) with ΔP_{ave} of 3.23 mmHg.

Ex-vivo serum-free perfusion experiments performed at 5 mmHg pressure B→A resulted in Lp_{ave} of 7.5 ± 2.2 μL/min/mmHg/cm^2 (n = 9). The Lp_{ave} of tissue perfused in the A→B direction was 0.06 ± 0.01 μL/min/mmHg/cm^2 (n = 3). The Lp_{ave} at 15.9 mmHg in the B→A direction was 6.42 ± 1.76 μL/min/mmHg/cm^2 (n = 9), which was not statistically different from the Lp_{ave} at the lower pressure.

Sections of arachnoid membrane with no visible granulations were perfused at physiologic pressure resulting in significant flow, suggesting the presence of microvilli in the membrane contributing to total CSF outflow. The CSF outflow area contributed by the microvilli must be considered in estimating total outflow capacity of the membrane.

Cells perfused physiologically showed extra-cellular cisternal spaces between overlapping AG cells suggesting a pathway for para-cellular fluid transport. Several vacuoles within the cytoplasm, which did not stain with ruthenium

red, were shown and suggest a trans-cellular pathway for fluid flow.

Conclusion

AG perfusion results in both *in-vitro* and *ex-vivo* models showed that flow was uni-directional and physiologic. TEM showed large intra-cellular vacuoles and extra-cellular cisternal spaces which represent two distinct mechanisms by which AG cells move fluid: 1: Trans-cellular transport via intra-cellular vacuoles, 2: Para-cellular transport via extra-cellular cisterns, which were traced by microparticles. *Ex-vivo* perfusion results are being studied further to understand the complex relationship between flow through the visible AGs and microvilli.