Cerebrospinal Fluid (CSF) Can Inhibit Wound Healing and Induce CSF Leaks by Inhibiting Angiogenesis

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Introduction

Mechanical pressure on dural or fascial wound edges exerted by cerebrospinal fluid (CSF) is thought to impair proper apposition of the wound borders and therefore prevent healing. Interestingly, it has been observed that the surgical tissues exposed to CSF do not bleed, exhibit smooth edges, and have no evidence of granulation tissue formation. This raises the question of whether the constituents of CSF themselves impair normal wound healing. However, the biochemical interaction of CSF with the healing process has not been investigated. Here, we hypothesize that CSF exhibits anti-angiogenic properties and therefore inhibits the healing process.

Methods

We used an in vitro model, in which human umbilical vein endothelial cells grow in a three-dimensional scaffold. Normally these cells form capillary-like structures named cords. We exposed the model to varying concentrations of CSF vs. Dulbecco's Phosphate-Buffered Saline (DPBS), used as control. We then quantified cord length. To rule out potential direct cellular toxicity or a general effect on cell migration we performed a "scratch test" on human fibroblasts exposed to the same CSF or DPBS concentrations.

Results

In all samples (n=5) of CSF at 50, 75 and 100% Volume/Volume significantly diminished cell migration and subsequent formation of capillary-like structures compared to DPBS (used to control for dilutional effect) with a dose dependent tendency. Cell migration remained unchanged in the fibroblast culture, suggesting that CSF specifically inhibits capillary formation.

Learning Objectives

By the conclusion of this session, participants should be able to

1. Describe the importance of increasing the current knowledge on the pathogenesis of CSF leaks

2. Discuss, in small groups, the relevance of identifying anti-angiogenic properties of the cerebrospinal fluid in brain development, pathology, and surgical technique.

3. Identify how addressing the pathogenesis of CSF leaks can lead to novel treatment modalities.

Figure 1

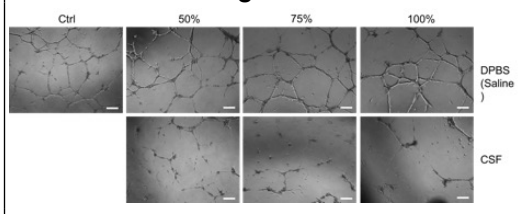
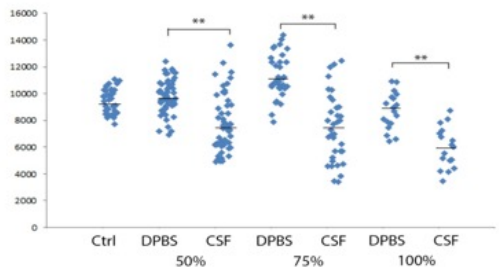


Figure 3. Human endothelial cells (HuVEC) cord formation, ramification and connectivity is inhibited in the presence of 50, 75 and 100% CSF compared to control -treated cultures (n=5) with a dose dependent tendency. DPBS added in the same concentrations did not have any effect. Scale bar = 200 m

Conclusions

CSF inhibited cord formation with a dose-dependent tendency, implying that it contains signaling molecules that preclude angiogenesis. This effect was cell specific and not observed with fibroblasts. These experiments suggest that CSF, rather than acting as an inert bystander, may actively impair healing by inhibiting capillary formation. This could impact the understanding of postoperative leaks as well as the potential physiologic role of cerebrospinal

Figure 2



Quantification of cell migration as measured by cord length in 3-D HuVECS cultures exposed to varying CSF concentrations. Each point indicates the total length of the cords for each sample (experiments were repeated 4 times for each CSF sample, n=5. The "y" axis depicts the total length of the cords (in pixels). When HuVECS were exposed to normal human CSF they exhibited a reduction in cell migration and cord formation, (7677.2+_2139.7, 7428+_4253.2 and 5908+_1488.7 pixels for CSF at 50, 75 and 100 % respectively) (n=5), p <0.05. DPBS, used as control, had no effect in the cord length and was no different than cells in normal full media (9788.9+_1276 ; 11.254.3+_1568.2 and 8712.6+_1377.1 pixels for DPBS at 50,75 and 100% respectively, p>0.1).

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