Cytokine and Growth Factor Expression in Cerebrospinal Fluid in Patients with Hydrocephalus

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Statement of Purpose: Hydrocephalus, a well known complication following subarachnoid hemorrhage, has been reported as a complication of endovascular treatment of unruptured intracranial aneurysms.[1] To better understand the pathogenesis of this type of hydrocephalus, a high-throughput method was used to identify key factors in cerebrospinal fluid (CSF) from normal patients as well as those with one of three types of hydrocephalus were used in this study. Quantification of the key factors in the CSF was then performed using ELISA kits.

Methods: CSF was collected in four groups: normal (NOR, n=15), hydrocephalus after endovascular embolization (EMB, n=4), normal pressure hydrocephalus (NPH, n=2), and hydrocephalus after subarachnoid hemorrhage (SAH, n=11). The CSF was frozen, shipped on dry ice, and stored at -80 °C until analyzed.

Custom protein arrays suitable for analysis of serum samples were purchased from Ray Biotech (Norcross, GA) to determine the expression of 40 cytokines. Cytokines related to angiogenesis that were evaluated include Ang1, Ang2, FGF-2, and VEGF. Cytokines related to inflammation that were evaluated include E-selectin, ICAM-1, IFN-γ, IGF-1, IL-1α, IL1-β, IL-1ra, IL-2, IL-4, IL-6, IL-8, IL-10, IL-13, L-selectin, and VCAM-1. Cytokines related to wound healing that were evaluated include FGF-4, FGF-6, FGF-7, FGF-9, HB-EGF, MMP-1, MMP-2, MMP-3, MMP-9, MMP-10, MMP-13, TGF-α, TGF-β1, TGF-β2, TGF-β3, TNF-α, TIMP-1, TIMP-2, TIMP-4, and PDGF-BB.

The protein arrays were processed in accordance with the manufacturer's instructions, using solutions provided in the kit. After processing, the arrays were exposed to x-ray film and developed. The films were quantified using a densitometer (GS-800, Bio-Rad, Hercules, CA).

ELISA kits (R&D Systems, Minneapolis, MN) were used to quantify the expression of IL-6, IL-8, TIMP-1, TIMP-2, TIMP-4, MMP-9, FGF-2, and TGF-β1. The ELISA kits were processed in accordance with the manufacturer's instructions, using solutions provided in the kit. The color was measured using a plate reader (Model 680, Bio-Rad). Expression was compared using ANOVA. Statistical significance was accepted at $p \leq 0.05$.

Results/Discussion: Using protein arrays, TIMP-1, TIMP-2, and TIMP-4 were observed in varying amounts in the CSF from all four patient groups. Low signals of ICAM-1 and L-selectin were observed for all four groups. MMP-9 was observed in the EMB, NPH, and SAH groups. IL-6 was observed in the EMB and SAH groups. IL-8 was observed in the EMB, SAH, NPH, and NOR groups. All other molecules were not detected above background levels on the protein arrays. ELISA results are presented in Table 1. The protein array results correlated well with the ELISA results. Statistically significant differences are marked.

Table 1. ELISA Results

	NOR	EMB	NPH	SAH
TIMP-1 (ng/mL)	67±34^	74±54	61±29	251± 161*
TIMP-2 (ng/mL)	21±22	13±14	24±27	26±24
TIMP-4 (pg/mL)	1.2±0.3^	0.5±0.1*	0.5±0.4*	0.5±0.3*
MMP-9 (ng/mL)	0±0^	0.4±0.0	0.2±0.0	183± 484*
IL-6 (pg/mL)	3.0±1.6^	39±26	3.3±1.1	1828± 2246*
IL-8 (pg/mL)	60±48^	57±11	28±31	3488± 5704*
FGF-2 (pg/mL)	0±0	0±0	0±0	0.7±2.1
TGF-β1 (pg/mL)	0±0	0±0	0±0	0±0

Although not fully understood, hydrocephalus is believed to result from decreased CSF resorption due to increased cellularity of the arachnoid membrane. The increased expression of MMP-9 and decreased expression of TIMP-4 in the hydrocephalus groups may indicate increased cellular remodeling of the arachnoid membrane.

The increased expression of IL-6 in the SAH and EMB groups imply an inflammation mediated pathogenesis of hydrocephalus that is distinct from NPH. The increased expression of IL-8 in the SAH group is probably a result of coagulating blood in the subarachnoid space. Cultures of coagulating blood produce large amounts of IL-8.[2]

No changes in the expression of TGF-β1 or FGF-2 were observed. Increased levels of TGF-β1 and FGF-2 in the intraventricular space of preclinical models is known to elicit hydrocephalus in laboratory animals.[3,4]

Conclusions: Based on the expression of growth factors, cytokines, and other molecules in the CSF, hydrocephalus after aneurysm embolization was similar to hydrocephalus resulting after SAH, with increased expression of IL-6 and MMP-9. In contrast, CSF from the NOR and NPH groups did not have inflammatory cytokines present.

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References:

[1]Meyers PM. Neurosurgery. 2004;55:1222
[2]Johnson K. Blood. 1996;87:5051-5060.
[3]Galbreath E. J Neuropathol Exp Neurol. 1995;54:339-49.
[4]Pearce RK. Synapse. 1996;23:192-200.