Cytokine Expression after Endovascular Embolization of Experimental Aneurysms

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Statement of Purpose: Reports of inflammatory complications, including edema, meningitis, and hydrocephalus, following the endovascular embolization of giant, unruptured intracranial aneurysms have recently been reported.[1] While these complications are not unexpected with ruptured intracranial aneurysms due to the presence of thrombus in the sub-arachnoid space, these reports are the first in unruptured aneurysms. As cytokines are linked to the development of hydrocephalus after sub-arachnoid hemorrhage (SAH),[2] a high-throughput method was used to follow the local and systemic expression of cytochemical factors following endovascular embolization of experimental aneurysms.

Methods: The Institutional Animal Care and Use Committee approved all procedures. Saccular aneurysms were created in 8 New Zealand White rabbits from the right common carotid artery via elastase digestion. As previous studies have recommended,[3] at least three weeks passed between creation and embolization. The aneurysms were embolized with Hydro*Coil*® (HC, MicroVention) devices (n=4) or platinum coils (Pt, n=4). Subsequently, approximately 4 mL of blood was collected on a weekly basis from each rabbit for protein array analysis. Subjects were followed for 2 wks (n=4) or 6 wks (n=4). After explanting, the aneurysm tissue and control artery tissue were lysed and the lysate was evaluated using protein arrays.

Custom protein arrays suitable for analysis of serum and tissue lysate 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, bFGF, 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). The densitometry data were inserted into Microsoft Excel for analysis. Expression was compared using ANOVA. Statistical significance was accepted at $p \leq 0.05$.

Results / Discussion: Aneurysms were successfully embolized in all 8 cases without morbidity or mortality. Statistically significant differences in the aneurysm length, aneurysm dome, aneurysm neck, aneurysm volume, device length, device number, angiographic occlusion, and angiographic durability score between the HC and Pt groups were not present.

Statistically significant differences between the serum collected at aneurysm creation and the serum collected at aneurysm embolization were not observed.

Statistically significant differences between the Pt, HC, and pre-embolization groups are reported below. At 1 wk, the expression of HB-EGF was lower in the HC group compared to pre-embolization. The expression of VCAM-1 and FGF-7 were higher in the Pt group compared to the HC group. At 2 wks, the expression of TGF-β1 was higher in the Pt group compared to the HC group and pre-embolization. The expression of IL-6 was higher in the Pt group compared to the HC group.

At 3 wks, the expression of IL-6, MMP-9, TGF-α, and TGF-β1 was higher in the Pt group compared to the HC group and the expression of TIMP-2 was higher in the Pt group compared to pre-embolization. The expression of IL-6, IL-10, IL-13, and TGF-β1 was lower in the HC group compared to pre-embolization.

At 4 wks, the expression of MMP-9, TNF-α, TIMP-2, FGF-7, FGF-9, and PDGF-BB was higher in the Pt group compared to the HC group and pre-embolization. The expression of IL-1β, IL-1α, IL-2, IL-4, TGF-α, TGF-β2, TGF-β3, FGF-6, and VEGF was higher in the Pt group compared to the HC group. At 5 wks, the expression of TGF-α was higher in the Pt group compared to the HC group. At 6 wks, the expression of TGF-α and TGF-β2 was higher in the Pt group compared to the HC and pre-embolization groups. The expression of IL-6 and TGF-β1 was higher in the Pt group compared to the HC group.

In aneurysm tissue lysate at 2 wks, the expression of TIMP-2 was higher in the HC group compared to the control artery group. At 6 wks, statistically significant differences in the cytokine expression of the HC, Pt, and control artery groups were not observed.

Conclusions: The high-throughput method displayed sufficient sensitivity to detect systemic changes in cytokine expression. Aneurysm embolization initiated changes in systemic cytokine expression, most likely resulting from the organizing thrombus. Increases in cytokine expression in serum were observed at three and four weeks in the Pt group, while minimal changes were seen in the HC group. Further studies on the development of meningitis and hydrocephalus after the embolization of unruptured, giant aneurysms may reveal the influence of cytokine expression, similar to the effects of cytokines after SAH.

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References:
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