Aneurysm wall inflammation

R. Loch Macdonald, M.D., Ph.D.

Division of Neurosurgery, St. Michael’s Hospital, Labatt Family Centre of Excellence in Brain Injury and Trauma Research, Keenan Research Centre of the Li Ka Shing Knowledge Institute of St. Michael’s Hospital, Department of Surgery, University of Toronto, Ontario, Canada

Hoh and colleagues investigate the hypothesis that stromal cell–derived factor-1 (SDF-1) mediates angiogenesis and inflammation in intracranial saccular aneurysm walls and that the latter two processes are important in aneurysm formation and rupture. Stromal cell–derived factor-1 is a chemokine known to be involved in angiogenesis and activation of inflammation. Chemokines are one subtype of cytokines. They are signaling proteins that derive their name from their ability to induce chemotaxis in cells close to them; hence, chemokines from chemotactic cytokines. Chemokines tend to be 8–10 kDa in molecular weight and contain 4 cysteine residues at characteristic locations in their structure. There are approximately 47 mammalian chemokines and they act on about 17 different G-protein coupled receptors on the different types of leucocytes.

Hoh et al. found that SDF-1 was present in the walls of mouse and human cerebral aneurysms. Circulating progenitor cells expressing CXCR4, a receptor for SDF-1, were increased in mice that were injected with elastase in order to induce aneurysms. The mouse aneurysms were created by injection of elastase around the extracranial cervical arteries or the intracranial circle of Willis. In the extracranial mouse aneurysm model, administration of an antibody to SDF-1 reduced endothelial cells, capillaries, and cell proliferation in the aneurysms. These findings have not been reported in models of cerebral aneurysms.

I would first like to commend the authors for their work. The experiments are well done and the experimental design is excellent. They incorporate many of the suggestions from the Stroke Therapy Academic Industry Round Table (STAIR), the National Institutes of Health conference on conduct of animal studies (RIGOR), and the “Animal Research: Reporting In Vivo Experiments” (ARRIVE) guidelines, including randomization, blinding, appropriate controls, power calculations, reporting of all animals used, and so forth. Some of the recommendations, especially those concerning sample size calculations, are quite difficult to implement, although they clearly indicate that they are guidelines and some components may not be appropriate for some animal studies. Sample size estimation, for example, was reported here by the authors, but it is difficult to do even in early phase human studies. Even though the mouse strains are inbred, and thus variability might be reduced, it is nevertheless interesting that we frequently estimate a sample size of 10 animals per group, yet reproducibly significant data in 10 humans is unheard of.

Regarding the findings published here, human and mouse aneurysm walls contained endothelial cells and macrophages, which is not surprising and already known. Pathology of aneurysm walls demonstrates endothelial cells and macrophages in both ruptured and unruptured aneurysms. Ferumoxytol imaging of human intracranial aneurysms in vivo also shows uptake of this agent, a marker for macrophages, in unruptured aneurysm walls. Gadolinium enhancement of the walls of ruptured but not unruptured aneurysms on MRI is supportive of wall inflammation. Hoh et al. also found capillaries, however, which appears to be a novel observation in small aneurysms, although the photomicrographs provided here are not that clear to me. Large and giant aneurysms certainly can have capillaries or vasa vasorum, although the average size of aneurysms in this series was approximately 8 mm. Does the average 7–8 mm aneurysm have capillaries in its wall? Aneurysm walls consist mainly of collagen, fibroblasts, cells variously classified as myofibroblasts or smooth muscle cells, amorphous material, and a variably continuous lining of endothelial cells. They can also have macrophages, other inflammatory cells, and thrombi of varying ages and organization. Aneurysm wall thicknesses are 16–212 μm; they are certainly thicker in some cases. The presence of capillaries is a key component of the hypothesis of Hoh et al., because the capillaries are hypothesized to be the portal of entry of inflammatory cells, that then degrade the aneurysm wall and lead to rupture. While this may be true in systemic aneurysms, it is not clear to me that these cells cannot enter the aneurysm wall through the lumen of cerebral aneurysms.

The pathway that Hoh et al. investigate, which is suggested to be SDF-1, contributes to endothelial cell and macrophage migration into the aneurysm wall, capillary tube formation, and then cell proliferation in the aneurysm wall.
wall. There is an assumption that these processes contribute to aneurysm formation, growth, and/or rupture. The experiments here are novel in identifying these processes in the aneurysm wall, but so far there is not much evidence that they cause aneurysm formation, growth, and/or rupture. Other than the anti–SDF-1 antibody experiments, most of the findings are observational only. This is not a criticism, but simply a comment, because after all, observation is the first step in any scientific endeavor. The anti–SDF-1 experiments are mechanistic but they only address the extracranial carotid aneurysms and not the intracranial ones, and I suspect that extracranial aneurysms in the neck elicit a different response than those in the subarachnoid space. Simple surgical observation suggests this; reoperation on a carotid endarterectomy patient months or years after the first operation is not the same as reoperating on an intracranial aneurysm that has recurred years later. Many of the studies of human aneurysm walls implicating inflammation compare ruptured and unruptured aneurysms, which is not the same as comparing an unruptured aneurysm to itself after it ruptures. Nevertheless, multiple studies show inflammatory cells and multiple cytokines within the walls of unruptured and ruptured aneurysms, in some cases with an association of more inflammation with rupture. Tulamo and colleagues have reviewed this data and it is clear that multiple inflammatory and remodeling pathways are activated in intracranial aneurysm walls. These multiple pathways will be a challenge for the development of methods to prevent aneurysm formation or stabilize them. Coiled ruptured aneurysms, for example, have a reduced risk of rebleeding, yet they can have enhancing walls and pathologically have abundant thrombosis and inflammation. Thus, there are other factors, including hemodynamic ones, involved in whether or not an aneurysm ruptures.

Another question is what is the relation of the mouse models to human aneurysms. The answer is to use the best model to address the question being asked, and along those lines, I would only comment that the attraction of the intracranial elastase injection model is a short time to development of aneurysms compared with the older models that took months for aneurysms to develop. Whether this tradeoff is justified is unknown. Hoh and colleagues address this, in part, by also studying human aneurysms; we are interested in human not mouse disease, so always study humans if you can.

The authors’ ultimate goal is to develop pharmacological treatment to prevent aneurysm formation or to stabilize them. This does not seem so far-fetched, especially in light of intriguing observations on the association of acetylsalicylic acid use and aneurysm rupture. Inflammation and vascular remodeling are complex, and likely mediated by multiple, interacting pathways. The challenge will be to look for common pathway components that can be modified. Also, one has to consider whether the biological process being inhibited is confined to the aneurysm wall because side effects can arise from the widespread inhibition of biological processes. At present, we use a constellation of factors to try to guess if an aneurysm will rupture. Patient age, family history, hypertensive and rheumatic symptoms, aneurysm location and size, daughter loculi or blebs, growth, and hemodynamics are a few. We balance rupture risk against treatment risk, and then make an educated guess about what the best recommendation is. At the least, the detection of stem cell antigen-1–positive cells in the mice with aneurysms in the current paper suggests the possibility that systemic biomarkers could be added into the equation. (http://thejns.org/doi/abs/10.3171/2013.5.JNS13824)

Disclosure

Dr. Macdonald receives grant support from the Physicians Services Incorporated Foundation, Brain Aneurysm Foundation, Canadian Institutes for Health Research, and the Heart and Stroke Foundation of Canada, and is a consultant for Actelion Pharmaceuticals and the Chief Scientific Officer of Edge Therapeutics, Inc.

References

Response

Brian L. Hoh, M.D.
Department of Neurological Surgery, University of Florida, Gainesville, Florida

On behalf of my colleagues, I would like to thank Prof. Macdonald for his insightful commentary. We agree with many of his astute observations.

We agree with Prof. Macdonald’s statement that capillaries are typically found in the walls of large and giant aneurysms. In our study, capillaries tended to be found more frequently in the larger aneurysms, but we did find capillaries in some small aneurysms as well (Table 1). While we agree with Prof. Macdonald that it is still not clearly defined that endothelial cell and macrophage migration and capillary tube formation in the aneurysm wall contribute to aneurysm growth and rupture, we have a strong suspicion that this is true, given the differentially increased presence of inflammatory cells in ruptured human cerebral aneurysms compared to unruptured cerebral aneurysms.²⁻⁷

We were able to demonstrate the critical role of SDF-1 in aneurysm biology using both our extracranial murine aneurysm model and our intracranial murine aneurysm model. Extracranial aneurysms from mice given anti–SDF-1 blocking antibody had a significant reduction in endothelial cells (p < 0.05), capillaries (p < 0.05), and cell proliferation (p < 0.05) in the aneurysm wall. Using our intracranial model, mice given anti–SDF-1 blocking antibody developed significantly fewer intracranial aneurysms (33% vs 89% in mice given control immunoglobulin G, p < 0.05; Fig. 7).

We agree with Prof. Macdonald that a potential criticism of our murine models is the application of elastase, which might not replicate what happens in the human clinical condition. We have attempted to address this limitation by demonstrating SDF-1, endothelial cells, inflammatory cells, and capillaries in human cerebral aneurysm specimens. Furthermore, our murine models histologically resemble true human aneurysms.⁴

Our findings regarding the role of SDF-1 in aneurysm wall biology are an initial step toward understanding the mechanisms of aneurysm formation and growth. Future studies are needed to investigate the role of infiltrating inflammatory cells in creating conditions contributing to aneurysm growth and possible rupture. Once again, we would like to thank Prof. Macdonald for his excellent comments.

References


Please include this information when citing this paper: published online October 25, 2013; DOI: 10.3171/2013.5.JNS13824.