Upregulation of Cyclooxygenase-2 (COX-2) and Microsomal Prostaglandin E\textsubscript{2} Synthase-1 (mPGES-1) in Wall of Ruptured Human Cerebral Aneurysms

Preliminary Results

David Hasan, MD; Tomoki Hashimoto, MD; David Kung, MD; R. Loch Macdonald, MD; H. Richard Winn, MD; Donald Heistad, MD

Background and Purpose—Cyclooxygenase-2 (COX-2) and Microsomal Prostaglandin E\textsubscript{2} Synthase-1 (mPGES-1) catalyze isomerization of the cyclooxygenase product PGH\textsubscript{2} into PGE\textsubscript{2}. Deletion of COX-2/mPGES-1 suppresses carotid artery atherogenesis and angiotensin II-induced aortic aneurysms formation, and attenuates neointimal hyperplasia after vascular injury in mice. The upregulation of COX-2/mPGES-1 in the wall of ruptured human cerebral aneurysms is not known.

Methods—Ten patients with intracranial aneurysms (5 ruptured and 5 nonruptured) underwent microsurgical clipping. During the procedure, a segment of the aneurysm dome was resected and immunostained with monoclonal antibodies for COX-1, COX-2, and mPGES-1. A segment of the superficial temporal artery was also removed and immunostained with monoclonal antibodies for COX-1, COX-2, and mPGES-1.

Results—All 10 aneurysm tissues stained positive for mPGES-1 monoclonal antibody. Expression of mPGES-1 was more abundant in ruptured aneurysm tissue than in nonruptured aneurysms, based on a semiquantitative grading. None of the superficial temporal artery specimens expressed mPGES-1. COX-2 was upregulated in the same distribution as was mPGES-1. COX-1 was present constitutively in all tissues.

Conclusions—COX-2/mPGES-1 are expressed in the wall of human cerebral aneurysms and more abundantly so in ruptured aneurysms than in nonruptured. We speculate that the protective effect of aspirin against rupture of cerebral aneurysms may be mediated in part by inhibition of COX-2/mPGES-1. (Stroke. 2012;43:1964-1967.)

Key Words: aneurysm ■ mPGES-1 ■ inflammation ■ COX-2 ■ COX-1

The etiology of saccular intracranial artery aneurysm is not clear. Several studies in humans and experimental animals on intracranial aneurysms support the hypothesis that chronic inflammation contributes to degeneration of intracranial aneurysms and potentially increases the risk of rupture.\textsuperscript{1-3} We recently reported that daily intake of aspirin reduces the incidence of human cerebral aneurysm rupture by 60%.\textsuperscript{4} The mechanism by which aspirin exerts this surprising effect is not clear.

Arachidonic acid is metabolized by cyclooxygenases to prostaglandin (PG) H\textsubscript{2}, which is converted to specific PGs. COX-1, COX-2, and mPGES-1 catalyze the isomerization of PGH\textsubscript{2} into PGE\textsubscript{2} and PGI\textsubscript{2}. COX-1 is responsible for baseline levels of prostaglandins, and inflammation induces expression of COX-2.\textsuperscript{5} Both COX-1 and COX-2 are inhibited by aspirin.\textsuperscript{5}

Aoki et al showed the presence of COX-2, mPGES-1, and prostaglandin E receptor 2 (EP\textsubscript{2}) in endothelial cells in the walls of unruptured cerebral aneurysms.\textsuperscript{6} They also demonstrated that inhibition or loss of COX-2 or EP\textsubscript{2} attenuated inflammation and reduced the incidence of aneurysm formation in rats and mice with cerebral aneurysm.\textsuperscript{6} Recent studies also indicate that deletion of mPGES-1, which is 1 step downstream from COX-2, suppresses carotid artery atherogenesis and angiotensin II-induced aortic aneurysm formation, and attenuates neointimal hyperplasia after vascular injury in mice.\textsuperscript{7-11}

The purpose of this study was to extend these findings to test the hypothesis that expression of COX-1, COX-2, and mPGES-1 are upregulated in ruptured human intracranial aneurysms.

Methods

The study was approved by University of Iowa Institutional Review Board. Ten consecutive patients with intracranial aneurysms who
underwent microsurgical clipping were identified during a 6-month interval. No patients were excluded, except patients who had coiling of their aneurysm. Five patients with nonruptured aneurysms and 5 patients with ruptured aneurysms were included in the study. Mean age was 55 years (range, 44–67 years; Table). Informed consent was obtained, and the patients underwent microsurgical clipping. A segment of the aneurysm dome (>1 mm) was removed and placed in formalin. A 2-mm specimen from the superficial temporal artery (STA) was removed and placed in formalin. These specimens were collected from the same 10 patients. All 20 specimens (10 aneurysms and 10 STA) were immunostained with monoclonal antibodies to COX-1 (Epitomics), COX-2 (Epitomics), and mPGES-1 (Cayman Chemical).

Semiquantitative analysis of the slides was performed based on cell count (immunostained positive cells) per high-power field (HPF; 40×): grade 0 = 0 cells per HPF, grade 1 = 0–10 cells per HPF, grade 2 = 10–20 cells per HPF, and grade 3 = >20 cells per HPF. Assessment of slides stained only with COX-1, COX-2, and mPGES-1 was made by an observer who was not aware of the source of tissues. Statistical analysis was performed using Kruskal-Wallis test, a nonparametric ANOVA test.

Results

Ten patients with 10 aneurysms were included in this study. All 10 aneurysms stained positive for expression of COX-2 and mPGES-1, using COX-2 and mPGES-1 monoclonal antibodies (Figure 1 and 2). Ruptured cerebral aneurysm stained more abundantly with COX-2 and mPGES-1 monoclonal antibodies than did nonruptured aneurysms (Figure 2). Staining of COX-2 and mPGES-1 was noted in all layers of the aneurysm tissue, but was more prominent in adventitia (Figure 1). STA tissue did not immunostain for COX-2 or mPGES-1 in any of the 10 samples (Figure 1 and 2).

All tissue samples from STA and aneurysms (ruptured and nonruptured) stained positively for COX-1 (Figure 1 and 2).

There was no difference in expression of COX-1 among STA, ruptured, and unruptured cerebral aneurysm (P = 0.574). Expression of COX-2 tended to be greater (P = 0.095) in ruptured aneurysms and was greater (P = 0.001) in ruptured and unruptured aneurysms versus STA. Expression of mPGES-1 also tended to be greater in ruptured aneurysms versus unruptured aneurysms (P = 0.071), and was greater in ruptured and unruptured aneurysms versus STA (P = 0.001).

Discussion

Inflammation in Cerebral Aneurysm

Several stresses, including hemodynamic stress, infiltration of inflammatory cells, and release of inflammatory molecules and cytokines appear to play an integral role in progression of cerebral aneurysm to being rupture-prone, and ultimately to rupture with the devastating sequela of subarachnoid hemorrhage. This concept is based on several studies in humans and animals that suggest that hemodynamic stress on endothelium leads to molecular signaling and formation of proinflammatory and proliferative pathways.12

Endothelial stress leads to activation of transcription factor nuclear factor kappa B,13 increased expression of monocyte

### Table. Characteristics of Aneurysms in Patients

<table>
<thead>
<tr>
<th>Patient #</th>
<th>Age (y)</th>
<th>Sex</th>
<th>Aneurysm Location</th>
<th>Aneurysm Size (mm)</th>
<th>Rupture (SAH)</th>
<th>COX-1 A/STA</th>
<th>COX-2 A/STA</th>
<th>mPGES-1 A/STA</th>
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<tr>
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<td>50</td>
<td>F</td>
<td>L-ICA</td>
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<td>3/3</td>
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<tr>
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<td>56</td>
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<td>R-MCA</td>
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<td>47</td>
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<td>R-Pcomm</td>
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</tbody>
</table>

Grade 0 = 0 cells per HPF, grade 1 = 0–10 cells per HPF, grade 2 = 10–20 cells per HPF, and grade 3 = >20 cells per HPF.

SAH indicates subarachnoid hemorrhage; A/STA, aneurysm/superficial temporal artery; F, female; L, left; ICA, internal carotid artery; M, male; R, right; MCA, middle cerebral artery; Pcomm, posterior communicating artery; PICA, posterior communicating artery; HPF, high-power field.
chemotactic protein-1, 14–17 and vascular cell adhesion molecule-1,17 which are highly chemotactic to inflammatory cells, macrophages, T-cells, natural killer cells, and basophils. Kanematsu et al18 reported that depletion of macrophages in mice significantly reduced incidence of cerebral aneurysm formation. They also demonstrated a reduced incidence of cerebral aneurysms in mice with deletion of monocyte chemotactic protein-1.18 This finding supports the concept that inflammation plays a major role in aneurysm formation and rupture.

Recently we reported a paradoxical effect of aspirin, at least in relation to effects of aspirin on platelets, that daily aspirin use in humans decreases the incidence of aneurysm rupture by 60%.4 Because aspirin is anti-inflammatory, we suggest that this indirect evidence supports a role of inflammation in formation and rupture of aneurysms.

COX-2, mPGES-1, and COX-1 in Cerebral Aneurysms
Aoki et al demonstrated the presence of COX-2, mPGES-1, and EP2 in endothelial cells in 5 unruptured human cerebral aneurysms and compared their findings with those of cadaver specimens.6 They also showed that inhibition or loss of COX-2 or EP2 in vivo attenuated expression of the other, suppressed nuclear factor-kB (NF-kB), mediated chronic inflammation, and reduced the incidence of cerebral aneurysm formation in rats and mice with cerebral aneurysm.6 They did not examine the presence of these enzymes in ruptured aneurysm. In the present study, we confirm the presence COX-2/mPGES-1 in unruptured cerebral aneurysms, and demonstrate that expression leads to increase in ruptured aneurysms, and these enzymes are not expressed in extracranial arteries. The role of expression of these enzymes in the pathophysiology of aneurysm rupture is not clear. Aneurysms in both humans and rodents, however, exhibit the hallmarks of inflammation, as described above. Thus, we hypothesize that this molecular complex (COX-2/mPGES-1) and prostaglandins could play major roles in the rupture of aneurysms. In our study, these enzymes (COX-2/mPGES-1) localized mainly in the adventitia, in contrast to the findings of Aoki et al.

Several studies have recently provided evidence that functional changes in the adventitia contribute to vascular remodeling during atherogenesis. Several proinflammatory molecules have been proposed to act locally to contribute to activation of the adventitia, ranging from enhanced growth factor activity and increased extracellular matrix synthesis, to generation of reactive oxygen species and accumulation of progenitor cells.19 It is not clear to us whether there is a signal from endothelium or smooth muscle cells that affects the adventitia. In addition, these enzymes maybe localized to inflammatory cells (perhaps macrophages) in adventitia.

We speculate that the presence of mPGES-1 in adventitia of cerebral aneurysm wall may contribute to the headache after subarachnoid hemorrhage, and inhibition of this enzyme may ameliorate this headache. This speculation is based on the fact that deletion of mPGES-1 in mice also has been reported to inhibit experimentally evoked pain and inflammation, and to a degree comparable with that observed from treatment with nonsteroidal anti-inflammatory drugs.20 All tissues from ruptured and nonruptured aneurysms and STA stained positively for COX-1. Expression of COX-1 was similar in ruptured and nonruptured aneurysms and in an extracranial artery. This finding is consistent with the fact that COX-1 is present constitutively in several cell lines and that expression does not change significantly with induced inflammation.

mPGES-1 and Vascular Injury
Several studies have reported that deletion or inhibition of mPGES-1 suppresses atherogenesis, angiotensin II-induced aortic aneurysm formation, and activation of aortic MMP-2; and attenuates neointimal hyperplasia after vascular injury in mice.7–11 Deletion of mPGES-1 in mice also has been reported to inhibit inflammation.20 These studies suggest a critical role of mPGES-1 in inflammation and in pathogenesis of several vascular pathologies, and suggest a potential benefit of targeting mPGES-1 in management of these diseases.

Limitations
This study is limited by the small sample size, and generalization of the results may not be appropriate. It also is difficult to determine whether increased expression of COX-2 and mPGES-1 in ruptured cerebral aneurysms (compared with nonruptured) is caused by inflammation that occurs...
following the rupture of the aneurysm, or whether there was an increase in expression of these molecules that preceded and led to rupture of the aneurysm. Our finding of localization of these 2 molecules to the aneurysm wall even in unruptured aneurysms suggests that COX-2 and mPGES-1 may contribute to formation and rupture of cerebral aneurysms.

**Conclusion**

COX-2 and mPGES-1 are expressed in human cerebral aneurysms, and expression increases in ruptured aneurysms. COX-1 is found constitutively in cerebral aneurysms and in an extracranial artery. The findings suggest that COX-2/mPGES-1 may play a role in formation and rupture of aneurysms. We also speculate that inhibition of COX-2/mPGES-1 by aspirin may contribute to protective effects of aspirin in reducing rupture of human cerebral aneurysm.

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**Disclosures**

None.

**References**

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