Differential Sex Response to Aspirin in Decreasing Aneurysm Rupture in Humans and Mice


See Editorial Commentary, pp 312–314

Abstract—We previously found that aspirin decreases the risk of cerebral aneurysm rupture in humans. We aim to assess whether a sex differential exists in the response of human cerebral aneurysms to aspirin and confirm these observations in a mouse model of cerebral aneurysm. A nested case–control analysis from the International Study of Unruptured Intracranial Aneurysms was performed to assess whether a sex differential exists in the response of human cerebral aneurysms to aspirin. A series of experiments were subsequently performed in a mouse model of cerebral aneurysms. Aneurysms were induced with hypertension and elastase injection into mice basal cisterns. We found that aspirin decreased the risk of aneurysm rupture more significantly in men than in women in the International Study of Unruptured Intracranial Aneurysms. In mice, aspirin and cyclooxygenase-2 inhibitor did not affect cerebral aneurysm formation but significantly decreased the incidence of rupture. The incidence of rupture was significantly lower in male versus female mice on aspirin. Gene expression analysis from cerebral arteries showed higher 15-hydroxyprostaglandin dehydrogenase levels in male mice. The rate of cerebral aneurysm rupture was similar in male mice receiving aspirin and 15-hydroxyprostaglandin dehydrogenase inhibitor compared with females receiving aspirin and 15-hydroxyprostaglandin dehydrogenase agonist, signaling a reversal of the sex-differential response to aspirin. Aspirin decreases aneurysm rupture in human and mice, in part through cyclooxygenase-2 pathways. Evidence from animal and human studies suggests a consistent differential effect by sex. 15-Hydroxyprostaglandin dehydrogenase activation in females reduces the incidence of rupture and eliminates the sex-differential response to aspirin. (Hypertension. 2016;68:411-417. DOI: 10.1161/HYPERTENSIONAHA.116.07515.) ● Online Data Supplement

Key Words: aneurysm ■ aspirin ■ inflammation ■ sex ■ subarachnoid hemorrhage

Inflammation in response to hemodynamic stress plays a critical role in cerebral aneurysm (CA) formation and rupture. The data derive from studies in both humans and animal models of CA and implicate several inflammatory cells and mediators.2–4

Given the central role of inflammation in the pathogenesis of CAs, several anti-inflammatory therapeutic strategies have been tested. Aspirin is a promising agent that may decrease aneurysm rupture.9,10 In a nested case–control study from a large epidemiological cohort study, the International Study of Unruptured Intracranial Aneurysms (ISUIA, published in Lancet in 2003),11 our group found that patients taking aspirin (325 mg) at least 3 times weekly had a significantly lower risk of subarachnoid hemorrhage (SAH) compared with those who never took aspirin (Figure S1 in the online-only Data Supplement).12 In this study, we further analyze the data from the ISUIA to evaluate whether a sex differential exists in the response of human CAs to aspirin. Additionally, we aim to confirm these findings in a mouse model. Specifically, we assess whether aspirin decreases the incidence of CA rupture in mice and investigate whether the underlying mechanism is through inhibition of the cyclooxygenase-2 (Cox-2) pathway. We also hypothesize that aspirin decreases the incidence of CA rupture more so in male than female mice because of a differential expression of 15-hydroxyprostaglandin dehydrogenase (15-PGDH).

Methods

Analysis of the Sex-Differential Response to Aspirin in the ISUIA

ISUIA is an epidemiological cohort study involved in a long-term follow-up of 2 prospective cohorts: untreated and treated. Prospective case ascertainment was from 1991 to 1998 (phases I and II), and prospective follow-up of the prospective cohort (phase III) was conducted from 2004 to 2007. A total of 1691
patients were managed conservatively. 1917 patients underwent surgery, and 451 patients underwent endovascular intervention. The patients analyzed in this study are selected from the 1691 patients who were initially untreated. Of patients enrolled in the conservative management group, 545 ultimately underwent a surgical or endovascular procedure to secure an aneurysm during the overall follow-up period. Patients were enrolled in 61 centers in the United States, Canada, and Europe. Details of aneurysm and patient characteristics were previously published in the original ISUIA article.11

To be eligible for the ISUIA, patients had to satisfy the following clinical and radiological inclusion and exclusion criteria: patients must have at least 1 unruptured intracranial aneurysm (symptomatic or asymptomatic). Patients who have had a ruptured aneurysm at another location that was treated must be able to care for themselves as determined at a follow-up evaluation at 30 days post-treatment (Rankin grade 0 or 2). Patients with saccular aneurysms 2 mm maximum diameter or with fusiform, traumatic, or mycotic aneurysms were not eligible for the study. Hemorrhagic events were classified by diagnostic certainty and location of aneurysmal rupture. Frequency of aspirin use (325 mg) in female and male patients was grouped as never, once a month, once a month to 2 times a week, and 3 times weekly to daily.

Subjects were selected from the prospective untreated cohort (n=1691) for a nested case–control study to determine whether there was a differential response by sex in the protective effect of aspirin against aneurysm rupture. Cases are subjects who had a primary SAH for a 5-year period after CA diagnosis. The cases and controls were matched based on aneurysm size and location. Hemorrhagic cases were adjudicated based on diagnostic criteria with no knowledge of risk factors. Analysis was done by stratified analysis by sex of the association of aspirin and case–control classification.

Mouse Model of CA

A series of 5 experiments were performed in mice. Care of the mice used in the experiments fully met the standards set forth by the National Institutes of Health (NIH) guidelines for the care and use of experimental animals. All procedures were approved by the University of Iowa Institutional Animal Care and Use Committee. CAs were induced using previously published methods as described in detail.2,13,14 Briefly, mice were anesthetized with ketamine (87.5 mg/kg) or xylazine (12.5 mg/kg), and a longitudinal incision was made in the scalp. A 1-mm hole was drilled in the skull, and elastase (20 μM in 2.5 μL) was stereotactically injected using the following coordinates: 2.7 mm posterior to the bregma, 1 mm to the right of the midline, and depth of 6.3 mm from the skull. Immediately after injection of elastase, an osmotic minipump that delivered a pressor dose of angiotensin II (1000 mg/kg/min) for 3 weeks was implanted subcutaneously. Blinded daily neurological examination was performed. Neurological symptoms were graded: 0, normal; 1, decreased drinking or eating with associated weight loss; 2, up to 2 g of body weight (10%) during 24 hours; 2, flexion of the torso and forelimbs on lifting of the animal by the tail; 3, circling to one side with a normal posture at rest; 4, leaning or falling to one side at rest; 5, no spontaneous activity. Mice were euthanized when they developed neurological symptoms (score 1–5). Mice without neurological signs were euthanized 17 to 19 days after CA induction.

Immediately after euthanasia, the chest and abdomen of each mouse were exposed and examined for major bleeding or aneurysms of the aorta. Mice were perfused transcardially at physiologic pressures with 10 to 15 mL of ice-cold physiological saline solution containing papaverine (100 μmol/L) to produce systemic vasodilation, followed by infusion of 2 mg/mL of bromphenol blue dye and 8% in gelatin saline to facilitate visualization of arteries and small vessels. The brain was then dissected and inspected for the presence of CAs and SAH. Aneurysms were defined as a localized outpouching arising from any cerebral artery with a diameter ≥1.5x the parent artery diameter as assessed by 2 independent observers blinded to the animal cohort.2,13,14 Animal cohorts were not revealed until all experimental groups had been euthanized.

Gene Expression in Mice Cerebral Arteries

Cerebral arteries (anterior and posterior cerebral arteries, anterior and posterior communicating arteries, middle cerebral arteries, and basilar arteries) were isolated from the above mice when possible and dissolved in TriZol (Life Technologies). Quantitative reverse transcription–polymerase chain reaction (qRT-PCR) was used to quantify mRNA levels as described previously.13,14 with both primers for the gene of interest (FAM fluoror, Life Technologies or Integrated DNA Technologies) and the house-keeping β-actin (VIC fluoror, Life Technologies, for normalization) in the same reaction.

Statistical Analysis

Analysis was performed using Sigma Plot 12.5 (Systat Software, Inc) and Prism 6 (Graphpad, La Jolla, CA) and SAS Version 9.2. Categorical data (incidence of aneurysms and SAH) were compared with 2-tailed Fisher exact test. Kaplan–Meier survival analysis was performed with comparison between cohorts using the log-rank (Mantel–Cox) test. Aspirin effect by sex was evaluated by Mantel–Haenszel χ2 and conditional logistic regression analysis. A P value <0.05 was considered significant.

Results

Analysis of Sex-Differential Effect of Aspirin on Risk of SAH in Humans—Results From the ISUIA

Twenty-eight of 52 men reported using aspirin, with 18 using aspirin ≥3× per week to daily. The proportion of SAH cases in males on aspirin ≥3× per week to daily (1/18, 6%) was significantly lower than in males who used aspirin less frequently (16/34, 47%; P<0.05; Table).

Seventy-six of 161 women reported using aspirin, with 49 using aspirin ≥3× per week to daily. In contrast to men, the proportion of SAH cases in women on aspirin ≥3× per week to daily (8/49, 16%) did not differ from the proportion of SAH cases in those who used less aspirin (32/112, 29%; P=0.05).

There was a significant decreasing odds ratio for hemorrhage with the increasing use of aspirin in men (odds ratio, 0.05; P=0.0024) but not in women (odds ratio, 0.46; P=0.23).

Aspirin Effect in Mice

We have recently determined in a nested case–control study from the ISUIA that aspirin confers a protective effect against aneurysm rupture in humans.12 CAs were induced in C57BL/6J mice in the following 4 groups to confirm this finding and to investigate the underlying mechanism: (1) 14 mice receiving vehicle only (dimethyl sulfoxide [DMSO]), (2) 14 mice receiving aspirin (25 mg/kg/d IP), (3) 14 mice receiving Cox-1 inhibitor (Sc-560 10 mg/kg/d IP), and (4) 14 mice receiving Cox-2 inhibitor (NS-398 20 mg/kg/d IP, dose based on the literature).15,16 Mice needed to be excluded because of death within 48 hours of surgery including 3 in the control group, none in the aspirin group, 3 in the Cox-1 group, and 2 in the Cox-2 group.

The incidence of CA formation was not different among the 4 groups (Figure 1A). However, the incidence of CA rupture was significantly lower in mice receiving aspirin (P<0.05) or Cox-2 inhibitor (P<0.05; Figure 1B). Survival was also significantly higher in mice receiving aspirin or Cox-2 inhibitor (P<0.05; Figure 1C).

To further assess the mechanisms behind alteration in aneurysm rupture, mRNA expression was assessed in
cerebral arteries using qRT-PCR. 15-PGDH was significantly higher in aspirin ($P<0.05$), Cox-1 inhibitor ($P<0.05$), and Cox-2 inhibitor ($P<0.05$) groups compared with the control group. We also examined the expression of matrix metalloproteinases-9 (MMP-9), an extracellular enzyme that plays a key role in degradation of extracellular matrix in the wall of CAs.\textsuperscript{16} MMP-9 was significantly decreased in the aspirin ($P<0.05$) and Cox-2 inhibitor ($P<0.05$) groups. There was a trend toward decreased Cox-2 ($P\geq0.05$) and monocyte chemoattractant protein-1 (MCP-1) expression ($P\geq0.05$) in the aspirin group and decreased expression of CD-68 ($P\geq0.05$), Cox-2 ($P\geq0.05$), and MCP-1 ($P\geq0.05$) in the Cox-2 inhibitor group, but this was not statistically significant.

**Cyclooxygenase-1 and Acetylsalicylic Acid**

To examine the effect of Cox-1 on CA formation and rupture, aneurysms were induced in 36 Cox-1 knockout mice (C57BL/6J genetic background; Taconic Laboratories) and divided into 3 equal groups: (1) Cox-1 knockout (KO) mice receiving vehicle (DMSO) and serving as a control, (2) Cox-1 KO mice receiving aspirin (25 mg/kg/d IP), and (3) Cox-1 KO mice receiving Cox-2 inhibitor. Three mice were excluded (death within 48 hours) in the control group; 4 mice in the aspirin group; and 4 mice in the Cox-2 inhibitor group. The incidences of CA formation and rupture were not statistically different among the 3 groups (Figure 2). However, the asymptomatic survival rate was significantly higher in Cox-1 KO mice receiving Cox-2 inhibitor ($P<0.05$).

**Inhibition of Cox-2 in Microsomal Prostaglandin E2 Synthase Type 1 KO Mice**

In a murine model of CA, we have previously shown that deficiency of microsomal prostaglandin E2 synthase type 1 (mPGES-1) is associated with a paradoxical increase in rupture of CAs, which was attenuated by low-dose aspirin.\textsuperscript{14} Aspirin also attenuated mortality in mPGES-1 KO mice.\textsuperscript{14} A potential explanation for this phenomena is that genetic deficiency of mPGES-1 in mice leads to increased production of prostaglandin D2, prostaglandin F2α, and other mediators that induce a proinflammatory state and promote aneurysm

### Table. Results From the International Study of Unruptured Intracranial Aneurysms

<table>
<thead>
<tr>
<th>Males</th>
<th>N</th>
<th>1 y</th>
<th>5 y</th>
<th>&gt;5 y</th>
<th>Proportion</th>
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<tr>
<td>Never</td>
<td>24</td>
<td>6</td>
<td>4</td>
<td>3</td>
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</tr>
<tr>
<td>&lt;1 per mo to 2 per wk</td>
<td>10</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>30.0%</td>
</tr>
<tr>
<td>3 times per week to daily</td>
<td>18</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>5.6%</td>
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<tr>
<td>$P=0.0024$</td>
<td>OR(&lt;2 wk)=0.363</td>
<td>OR(3+ week)=0.050</td>
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</table>

<table>
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<tr>
<th>Females</th>
<th>N</th>
<th>1 y</th>
<th>5 y</th>
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<th>Proportion</th>
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<td>14</td>
<td>10</td>
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<td>&lt;1 per month to 2 per week</td>
<td>27</td>
<td>4</td>
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<td>2</td>
<td>25.9%</td>
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<tr>
<td>3 times per week to daily</td>
<td>49</td>
<td>1</td>
<td>4</td>
<td>3</td>
<td>16.3%</td>
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<tr>
<td>$P=0.2369$</td>
<td>OR(&lt;2 wk)=0.84</td>
<td>OR(3+ wk)=0.468</td>
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</tbody>
</table>

OR indicates odds ratio.

Figure 1. Cerebral aneurysms (CAs) were induced in 56 C57BL/6J mice divided equally into mice receiving vehicle only (dimethyl sulfoxide [DMSO]), aspirin (25 mg/kg/d IP), cyclooxygenase (Cox-1) inhibitor (Sc-560 10 mg/kg/d IP), and Cox-2 inhibitor (NS-398 20 mg/kg/d IP). A, Aspirin and Cox-2 inhibitor did not alter the rate of CA formation in mice. B, Aspirin and Cox-2 inhibitor significantly reduced the risk of subarachnoid hemorrhage in mice. C, Aspirin and Cox-2 inhibitor significantly prolonged asymptomatic survival in mice. ASA indicates acetylsalicylic acid. *Statistically significant difference.
rupture. We had postulated that aspirin may provide a protective effect by blocking this diversion upstream through inhibition of Cox-2.

In this experiment, we examined whether a Cox-2 inhibitor has a similar effect to aspirin in mPGES-1 KO mice. CAs were induced in 16 mPGES-1 KO mice and divided into 2 groups: (1) 8 mice receiving vehicle and (2) 8 mPGES-1 KO mice receiving a Cox-2 inhibitor (NS-398 20 mg/kg/d IP, dose based on the literature\(^\text{15,16}\)). No mice were excluded. Both the incidence of CA formation and rupture did not differ between the 2 groups (Figure 3). However, similarly to aspirin, there was a trend toward increased asymptomatic survival in mPGES-1 KO mice receiving a Cox-2 inhibitor (\(P \geq 0.05\)).

**Sex-Differential Response to Aspirin in Mice**

To assess whether there is a sex-differential response to aspirin, CAs were induced in 2 groups of C57BL/6J mice: (1) 12 male mice receiving aspirin (25 mg/kg/d IP) and (2) 12 female mice receiving aspirin (25 mg/kg/d IP). Two male mice were excluded because of death within 48 hours. The incidence of CA formation was not statistically different between the 2 groups, but the incidence of CA rupture/SAH was significantly lower in male mice (\(P < 0.05\)).

Gene expression analysis from cerebral arteries using qRT-PCR showed that the expression of 15-PGDH was significantly higher in male mice (\(P < 0.05\)). Cox-2 (\(P < 0.05\)), CD-68 (\(P < 0.05\)), MMP-9 (\(P < 0.05\)), and nuclear factor-kB (\(P < 0.05\)) were significantly higher in females (Figure S2).

**15-PGDH Activation in Female Mice on Aspirin and 15-PGDH Inhibition in Male Mice on Aspirin**

15-PGDH is a critical enzyme that catabolizes prostaglandin E2 and converts it to 15-keto-prostaglandin E2, an endogenous peroxisome proliferator–activated receptor \(\gamma\) agonist. We have previously found that peroxisome proliferator–activated receptor \(\gamma\) decreases aneurysm formation and rupture in mice.\(^\text{13}\) In this experiment, we assessed whether the differential expression of 15-PGDH in male versus female mice affected the response to aspirin.

CAs were induced in 2 groups of C57BL/6J mice: (1) 12 male mice receiving aspirin and 15-PGDH inhibitor (Cay10638; 0.25 mg/d IP, dose based on the literature\(^\text{17}\)) and (2) 12 female mice receiving aspirin and 15-PGDH agonist (CDDO-Me, 250 \(\mu\)g/d IP, dose based on literature\(^\text{18}\)). Two male mice were excluded because of death within 48 hours. Aneurysm formation did not differ between the 2 groups. However, the rate of CA rupture/SAH was similar between the 2 groups, signaling a reversal of the sex-differential response to aspirin with modulation of the expression of 15-PGDH. When comparing female mice receiving aspirin alone and female mice receiving aspirin and 15-PGDH activator, the latter group had a significantly lower risk of CA rupture/SAH (75% versus 25%, respectively; \(P < 0.05\); Figure 4). Female mice receiving aspirin and 15-PGDH activator had a similar risk of CA rupture/SAH compared with male mice receiving aspirin alone (25% versus 20%, respectively; \(P < 0.05\)).

Gene expression analysis from cerebral arteries of the 2 groups described above showed that 15-PGDH was still higher (\(P \leq 0.05\)) in male mice (but no longer significantly). Expressions of Cox-2 (\(P \leq 0.05\)), CD-68 (\(P \leq 0.05\)), MCP-1 (\(P \leq 0.05\)), and nuclear factor-kB (\(P \leq 0.05\)) were no longer statistically different between male and female mice (Figure S3). Levels of Cullin-3 (\(P \leq 0.05\)) and Keap-1 (\(P < 0.05\)) were significantly higher, and Nrf2 was significantly lower in female mice (\(P < 0.05\)).

**Discussion**

The major findings of this study are that: (1) In the ISUIA, frequent aspirin use decreased the risk of aneurysm rupture more significantly in men than in women; (2) aspirin decreases the risk of aneurysm rupture (not formation) in mice and attenuates the expression of inflammatory mediators in cerebral arteries; (3) the protective effects of aspirin are likely mediated by Cox-2 (not Cox-1); (4) the risk of aneurysm rupture is lower in male mice compared with female mice receiving aspirin. Female mice on aspirin have lower levels of 15-PGDH and higher levels of inflammatory molecules than male mice on aspirin. This sex-differential response to aspirin is reversed by adding a 15-PGDH agonist to aspirin in females.

Growing evidence suggests that inflammation is a central factor in the pathogenesis of CAs. The inflammatory process is initiated by a hemodynamic insult, involves several inflammatory cells/mediators, and leads to degradation of the extracellular matrix by MMPs and apoptosis of smooth muscle cells.\(^\text{1,6}\)

![Figure 2](http://hyper.ahajournals.org/)

Figure 2. Aneurysms were induced in 36 cyclooxygenase-1 (Cox-1) knockout (KO) mice and divided into 3 equal groups: (1) Cox-1 KO mice receiving vehicle (dimethyl sulfoxide [DMSO]) and serving as a control (2) Cox-1 KO mice receiving aspirin (25 mg/kg/d IP), and (3) Cox-1 KO mice receiving Cox-1 inhibitor. Aspirin and Cox-2 inhibitor did not affect the incidences of cerebral aneurysm formation and rupture in Cox-1 KO mice. Cox-2 inhibitor increased asymptomatic survival rate. \(*\)Statistically significant difference. ASA indicates acetylsalicylic acid; and SAH, subarachnoid hemorrhage.
The protective effect of aspirin against aneurysm rupture initially noted in a nested case–control study from ISUIA was later confirmed in a study from Europe that included 1340 cases of SAH and 10,000 controls. Among the findings of this study, the authors found that long-term low-dose aspirin therapy protects against SAH and does not increase the risk of intracerebral hemorrhage. Furthermore, the trend toward increased asymptomatic survival in PGES-1 KO mice receiving a Cox-2 inhibitor.

These processes act in concert to weaken the arterial wall, resulting in dilatation, aneurysm formation, and ultimately rupture. Emerging data suggest that aspirin, through its anti-inflammatory action, may decrease the risk of CA rupture. The protective effect of aspirin against aneurysm rupture initially noted in a nested case–control study from ISUIA was later confirmed in a study from Europe that included 1340 cases of SAH and 10,000 controls. Among the findings of this study, the authors found that long-term low-dose aspirin therapy protects against SAH and does not increase the risk of intracerebral hemorrhage. Furthermore, the trend toward decreased risk of SAH was particularly pronounced in those on long-term aspirin therapy (>3 years). Along similar lines, in 747 patients with CAs, Gross et al found the rate of hemorrhagic presentation to be significantly lower in patients taking aspirin (28% versus 40%). Taken together, these studies provided the background for further research into the role of aspirin in the prevention of SAH.

This study provides evidence that, in mice like in humans, aspirin decreases the risk of CA rupture (not formation) and improves asymptomatic survival. There was also decreased expression of inflammatory molecules in cerebral arteries of mice treated with aspirin, namely MMP-9, MCP-1, and Cox-2, all of which have been shown to be involved in CA pathogenesis. The mechanism through which aspirin may exert its protective effects is likely the Cox-2 pathway as evidenced by the similarity between the effects of aspirin and a Cox-2 inhibitor on aneurysm formation (no change), rupture (decreased), asymptomatic survival (increased), and the comparable anti-inflammatory profile induced in cerebral arteries. Moreover, both aspirin and Cox-2 inhibitor improved asymptomatic survival in PGES-1 KO mice. These results are in agreement with the findings of a previously published study where we investigated the effects of aspirin on the expression of inflammatory molecules in human CAs. Decreased expression of Cox-2 (not Cox-1) and macrophages among other mediators were found in patients treated with aspirin compared with controls. This suggested that aspirin may exert its protective effects through the Cox-2 pathway. Figure S4 highlights some mechanisms through which aspirin may interact with cytokines and enzymes to protect against aneurysm rupture.

In this study, Cox-1 KO mice had a 100% rate of CA rupture, and aspirin did not affect the risk of hemorrhage or mortality in this group, suggesting that Cox-1 may not be involved in the pathogenesis of CA rupture or the pathway through which aspirin exerts its protective effects.

This report brings evidence from both human and animal studies suggesting that the protective effects of aspirin against CA rupture vary by sex. In our matched case–control analysis of ISUIA, aspirin dramatically decreased the risk of SAH in men but only minimally in women. Similarly, in mice, aspirin decreased the risk of CA rupture more significantly in males than in females. This novel observation reminds us of reports from the cardiovascular literature that aspirin decreases the risk of myocardial infarction in men but not in women. A meta-analysis by Berger et al that included 6 trials with a total of 95,456 patients showed that aspirin had no effect on myocardial infarction in women but was associated with a 32% reduction in the rate of myocardial infarction in men.

Female mice receiving aspirin in this study had lower levels of 15-PGDH in cerebral arteries and higher levels of proinflammatory mediators, namely, Cox-2, CD-68, MMP-9, MCP-1, and nuclear factor-KB compared with male mice. With the addition of 15-PGDH activator, the levels of proinflammatory mediators in cerebral arteries as well as the risk of aneurysm rupture decreased significantly in female mice equaling those in male mice. These findings suggest that females may have lower levels of 15-PGDH, an enzyme that seems to mediate the protective effects of aspirin against SAH. Restoring 15-PGDH activity in females eliminates this sex difference. Other factors may contribute to the sex variation in aspirin effect including a known difference in
aspirin metabolism with a reduced pharmacological effect of aspirin among women compared with men. Also, aspirin resistance tends to be more prevalent among women than men. There are currently no medical therapies recommended for patients with CAs. Both endovascular and microsurgical interventions may be associated with a risk of morbidity/mortality. A noninvasive medical treatment that lessens the risk of aneurysm rupture by targeting the underlying biological mechanism would be a beneficial therapy for a large number of patients, especially those who do not meet criteria for invasive treatment. Aspirin is also an inexpensive, widely available, and safe drug that has several other health benefits, including the prevention of myocardial infarction, ischemic stroke, and colorectal cancer. Clinicians are also familiar with aspirin, which facilitates its use for the prevention of SAH. Addition of 15-PGDH in women may be necessary to optimize the effects of aspirin.

Limitations
The rapidity of aneurysm formation and the use of exogenous elastase to induce CAs in mice may activate mechanisms that are distinct from those responsible for the natural progression of CAs in humans. An attempt to use Cox-2 KO mice was not successful in these experiments because of the rapid death of these mice (within first 1–10 days) secondary to cardiac effusions and other unknown conditions. The dose of aspirin that we used in mice was higher than the dose used in the ISUIA. We chose to use this high dose of aspirin to obtain the anti-inflammatory rather than the antiplatelet effect. It would have been interesting to study whether different doses of aspirin would have different effects on aneurysm formation and rupture. We did not have enough tissue from cerebral arteries to perform Western blotting or immunohistochemistry to corroborate the PCR findings. Lastly, although the number of patients evaluated from ISUIA was rather small, the data still reached statistical significance.

Perspectives
Aspirin seems to decrease the risk of SAH in men more than women. Furthermore, 15-PGDH activation in females may be necessary to optimize to prevent SAH.

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Disclosures
None.

References


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**Novelty and Significance**

**What Is New?**
- The study provides novel data from both mice and humans showing that aspirin decreases the risk of cerebral aneurysm rupture, with a consistent sex differential that can be reversed with 15-hydroxyprostaglandin dehydrogenase modulation.

**What Is Relevant?**
- Aspirin may be a potential medical therapy for prevention of cerebral aneurysm rupture.

**Summary**
Aspirin decreases the risk of cerebral aneurysm rupture more significantly in men than in women.
Differential Sex Response to Aspirin in Decreasing Aneurysm Rupture in Humans and Mice
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DIFFERENTIAL SEX RESPONSE TO ASPIRIN IN DECREASING ANEURYSM RUPTURE IN HUMANS AND MICE

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Short title: Aspirin and Cerebral aneurysms
4 Figures; 1 table; 4 supplemental figures

Word count: 5194
S1: ISUIA: Nested case-control: hemorrhages and controls (matched by site and size) frequency.

Patients taking aspirin at least three times weekly had a significantly lower risk of SAH compared with those who never took aspirin.
S2: Higher levels of 15-PGDH and lower levels of Cox-2, CD-68, MMP-9, MCP-1, and NF-kB in male mice compared with female mice treated with aspirin
S3: Similar levels of cox-2, CD-68, MMP-9 and NF-kB in male and female mice after PGDH modulation. Levels of Cullin-3 and Keap-1 were significantly higher, and Nrf2 significantly lower in female mice.
S4: Graphic summary of potential mechanisms through which aspirin exerts its protective effect in decreasing the risk of aneurysm rupture.