Aspirin Has A Gender-Dependent Impact on Antiinflammatory 15-Epi-Lipoxin A₄ Formation
A Randomized Human Trial

Nan Chiang, Shelley Hurwitz, Paul M. Ridker, Charles N. Serhan

Objective—Aspirin blocks thromboxane production that contributes to its well-appreciated antiplatelet action. Aspirin also initiates the biosynthesis of novel antiinflammatory mediators from arachidonic acid, namely aspirin-triggered 15-epi-lipoxin A₄. We recently conducted a double-blinded clinical trial with healthy subjects in whom low-dose aspirin (81 mg daily) significantly increased aspirin-triggered 15-epi-lipoxin A₄ and concomitantly inhibited thromboxane. Here, we assessed whether plasma aspirin–triggered 15-epi-lipoxin A₄ was age or gender dependent in subjects taking low-dose aspirin.

Methods and Results—A total of 128 subjects were allocated to: placebo, 81, 325, or 650 mg daily aspirin for an 8-week period. Plasma thromboxane B₂ and aspirin-triggered 15-epi-lipoxin A₄ were assessed from blood collected at baseline and the conclusion of the trial. We then performed a post-trial analysis in the group receiving low-dose aspirin. In female subjects, we found a positive correlation between age and aspirin-triggered 15-epi-lipoxin A₄ (increase of 0.37 ng/mL per decade), and a negative correlation was observed in men (decrease of 0.29 ng/mL per decade). These trends were significantly different from each other (P=0.045).

Conclusions—Low-dose aspirin has a gender-specific impact on aspirin-triggered 15-epi-lipoxin A₄ production, which may contribute to the gender-dependent clinical benefits of aspirin. Also, they may provide a molecular rationale for low-dose aspirin therapies in elderly women to reduce inflammation-related disorders. (Arterioscler Thromb Vasc Biol. 2006;26:e14-e17.)

Key Words: leukocyte traffic inflammation lipid mediators

Low-dose aspirin has beneficial effects not only in the prevention and management of occlusive vascular diseases but also in decreasing the incidence of cancers such as lung, colon, and breast, as well as possibly Alzheimer’s disease. The mechanism of the action of aspirin in these diseases remains to be completely defined. It is well appreciated that aspirin inhibits platelet cyclooxygenase (COX)-dependent (predominantly COX-1) thromboxane (TX) biosynthesis, accounting for many of its antithrombotic actions. In an aspirin-free system, platelets will initially use much of the arachidonic acid for conversion to TX in a few seconds (Figure 1). In contrast, with aspirin, TX production is inhibited via platelet COX-1, and the unconverted, unesterified arachidonic acid is more freely available to the 12-lipoxygenase with large quantities of 12-hydroxyeicosatetraenoic acid produced by isolated platelets until arachidonic acid is depleted. It is increasingly apparent that low-dose aspirin has protective actions that go beyond inhibition of prostaglandins and TX. In this regard, we identified a new class and pathway for arachidonic acid–derived mediators, namely aspirin-triggered 15-epi-lipoxin A₄ (ATL). Their biosynthesis is initiated by aspirin acetylation of vascular COX-2 and transcellular communication between COX-2–bearing cells such as human endothelial cells and leukocytes that make and release ATL from vasculature-derived precursors (Figure 1). ATL production is documented in several murine models in an aspirin-dependent fashion as well as in human aspirin-tolerant and aspirin-intolerant asthmatic subjects. First identified in 1995, this relatively new class of endogenous autacoids functions as local antiinflammatories displaying...
protective activities in several target tissues and animal disease models. These include peritonitis, dermal inflammation, reperfusion injury, asthma, angiogenesis, and periodontal disease. Thus, the protective actions of ATL are likely to underlie some of the therapeutic impact of aspirin when aberrant inflammation is a component of disease pathogenesis. For these reasons, a randomized clinical trial has been undertaken recently to establish whether aspirin administration can result in antiinflammatory levels of ATL in vivo when administered to healthy volunteers in standard clinical doses.

**Methods**

**Randomized Clinical Trial**

The randomized, double-blinded, and placebo-controlled clinical trial was conducted between May 2001 and February 2002 with 128 healthy subjects ≥40 years of age. Enrolled subjects gave informed consent and were randomized in a double-blinded format into either placebo or 1 of 3 different aspirin dose groups (ie, 81, 325, or 650 mg daily). For 8 weeks, each individual took the assigned dose or placebo once daily in the morning. Participants were ineligible if they had a previous history of diabetes or any cardiovascular, gastrointestinal, hematologic, renal, hepatic, pulmonary, or chronic inflammatory disorders. Use of aspirin, NSAIDs, aspirin-containing compounds, COX-2 inhibitors, and steroids was not allowed in the 3 weeks before enrollment, and those taking medications that may interact adversely with aspirin (eg, anticoagulants) were excluded. Randomization was prespecified by consecutive subject number and was computer generated using block randomization in groups of 4 without stratification. Blood samples were collected before and after 8 weeks of treatment. Plasma thromboxane B2 (TXB2; a stable metabolite of TX and an index of COX-1 activity in platelets) and ATL levels were measured.

**Statistical Analysis**

Change in ATL and TXB2 after the 8-week treatment period was computed. We tested for potential interrelationships between gender and age using analysis of covariance with age as the covariate and gender as the class. All probabilities were calculated using a 2-tailed α set at 0.05 with all CIs computed at the 95% level.

**Results**

In this first randomized and double-blinded human trial of ATL formation, we found that in the low-dose 81-mg group, ATL levels at 8 weeks (2.85 ± 0.79 ng/mL) were significantly greater than those before aspirin treatment (2.60 ± 0.74 ng/mL; ΔATL = 0.25 ± 0.63 ng/mL; P = 0.04). In parallel determinations with the same samples, TXB2 values significantly decreased after 8 weeks (0.39 ± 0.55 ng/mL) when compared with before aspirin treatment (1.40 ± 0.88 ng/mL; ΔTXB2 = −1.01 ± 0.99 ng/mL; P < 0.01). The values for ΔATL and ΔTXB2 were defined as the levels at the end of the 8-week trial minus the levels marked at the start of the trial. In sharp contrast, in the placebo group, neither ATL nor TXB2 levels at 8 weeks were significantly different from those before treatment. Hence, these earlier results demonstrated that low-dose aspirin (81 mg daily) has dual action, inhibiting TX as well as triggering production of the endogenous antiinflammatory ATL. The magnitude of this action did not increase further with the 325- and 650-mg aspirin dose groups. Therefore, in the results to be presented, we tested the influence of age and gender on aspirin-dependent ATL by carrying out a post-trial analysis restricted to those subjects randomized to the low-dose aspirin or placebo groups.

We tested for potential interrelationships between gender and age using analysis of covariance with age as the covariate and gender as the class. Interestingly, the age trends were significantly different from each other as the hypothesis of homogeneity of slopes was rejected (P = 0.045 for the interaction). These gave a positive correlation between age and ΔATL for women (Figure 2), with a change in ΔATL per decade of 0.37 ng/mL.
When the overall net changes in women and men (regardless of gender) were analyzed, there was no significant difference between gender. Hence, all the data points were reported (Figure 2). It appeared that there were several possible outliers. Thus, we performed the same analyses without these potential outliers and found that the age trends remained significantly different with gender. Hence, all the data points were reported (Figure 2). When the overall net changes in women and men (regardless of age) were analyzed, there was no significant difference between the 2 genders (ΔATL = (0.37)(decade) − 1.76). For men, a negative correlation was obtained, and the change in ΔATL per decade was −0.29 ng/mL (P = 0.19; ΔATL = (−0.29)(decade) + 1.85). It appeared that there were several possible outliers. Thus, we performed the same analyses without these potential outliers and found that the age trends remained significantly different with age. Hence, all the data points were reported (Figure 2). When the overall net changes in women and men (regardless of age) were analyzed, there was no significant difference between the 2 genders (ΔATL = 0.17 ± 0.60 ng/mL for women and 0.38 ± 0.65 ng/mL for men; P = 0.35). For direct comparison, aspirin inhibition of TX (ie, ΔTXB2) diminished for women (ΔTXB2 = (0.05)(decade) − 3.60) and was enhanced in men with age (ΔTXB2 = (−0.03)(decade) + 0.20). However, these slopes or rate of changes were not significantly different (P = 0.14). Also, there is no significant difference in the overall net changes between the genders (ΔTXB2 = −0.92 ± 0.94 ng/mL for women and −1.15 ± 1.02 ng/mL for men; P = 0.18). In the placebo group, the genders were similar in terms of ΔATL (P = 0.62) and ΔTXB2 (P = 0.78) across age. Together, these results suggest that ATL formation in healthy subjects taking low-dose aspirin was jointly dependent on age and gender.

**Discussion**

In several clinical trials of preventive aspirin therapy, opposite findings were reported for men and women. The earlier Physicians’ Health Study showed that aspirin in males significantly reduced the risk of myocardial infarction but not stroke. In comparison, a recent Women’s Health Study with 39,876 healthy women receiving 100 mg daily aspirin on alternate days or placebo demonstrated that in women <65 years of age, low-dose aspirin gave a 17% reduction in the risk of stroke overall (24% reduction for ischemic stroke) but has no significant effect on the risk of myocardial infarction or death from cardiovascular causes. These reports suggest that aspirin is likely to have gender-dependent actions on preventing selective events in cardiovascular diseases. The mechanisms responsible for these differential actions are currently not known. In this regard, our present results demonstrate that the ability of aspirin to trigger anti-inflammatory ATL is gender dependent. In the present analysis, the gender-specific trends for ΔATL as a function of age in low-dose aspirin were not significant. This is likely because of the relatively small population in this initial trial. Future trials with larger populations might address whether ATL formation is directly age dependent.

It is noted that inhibition of TX by aspirin was diminished in women and increased in men with age. The slopes were not significantly different, which could also be the result of the relatively small population in this trial. It is likely that estrogen may play a role in this because estrogen decreased TX production in vitro and in vivo. Also, it is possible that ATL could regulate TX generation. In this context, lipoxin A4 was shown to stimulate TX production in isolated guinea pig lung. Our results may contribute to the emerging appreciation of the gender-specific impact of aspirin. For example, the platelet inhibitory action of aspirin, inhibition of TX, may play a more dramatic role in reducing cardiovascular events in men. Or it is equally possible that aspirin-dependent ATL formation contributes to local antiinflammatory events that are beneficial in women and may thus decrease the risk of stroke.

It is important to note that in this recent Women’s Health Study, the incidence of gastrointestinal bleeding requiring transfusion was significantly higher in the aspirin group than in the placebo group. In this context, ATL exerts potent gastroprotective actions by blocking aspirin-induced hemorrhagic damage to the lining of the stomach. Also, a new ATL analog given orally once daily, 300 and 1000 μg/kg, markedly attenuated trinitrobenzenesulphonate-induced col-
tis in rodents in preventive and therapeutic regimens. Therefore, ATL-based therapeutic interventions could provide new approaches for inflammatory-related diseases that retain the antinflammatory properties of aspirin and spare the unwanted side effects.

In blood vessels, aspirin acetylates COX-2 that remains active but, instead of generating prostanoids, it converts arachidonic acid to 15R-hydroxyeicosatetraenoic acid, which, during endothelial–leukocyte interactions, switches to ATL generation (Figure 1). Given that inflammation is now appreciated to play a role in many chronic diseases including cardiovascular diseases and ATL possesses potent antinflammatory actions, it is highly likely in view of the present findings related to gender (Figure 2) that ATL contributes at least in part to the unique benefits of aspirin in elderly women, namely reducing their risk of stroke.8 In addition to arachidonic acid, recent findings indicate that the essential omega-3 fatty acids (eicosapentaenoic acid and docosahexae- noic acid) are also converted by aspirin-acetylated COX-2 to aspirin-triggered epimers of the resolvins and neuroprotectins that carry anti-inflammatory properties.14 Hence, the generation of aspirin-triggered epimeric forms of endogenous lipid mediators involves novel mechanism(s) by which essential fatty acids and aspirin may be useful in managing inflammation, neoplasia, and vascular diseases.

The action of low-dose aspirin in the primary prevention of cancer was also accessed in the recent Women’s Health Study.15 Results from this large-scale and long-term trial among healthy women suggested that low-dose aspirin (100 mg on alternate days for an average of 10 years) does not lower risk of total, breast, colorectal, or other site-specific cancers. However, in the earlier Nurses’ Health Study, an analysis of the incidence of colorectal cancer showed a statistically significant reduction in the incidence of colorectal cancer among women who took 4 to 6 aspirin tablets per week for >10 years.16 These aspirin doses were similar to those currently taken for protection against cardiovascular diseases. It could be speculated that the biosynthesis of local antiinflammatory lipid mediators such as ATL formation in women might bear a relationship to the explanation for the aspirin trial in nurses, wherein protection was obtained with aspirin in relationship to colonic polyps.17 Thus, it would be of interest to address the relationship of ATL formation and cancer prevention in women in future large-scale trials.

In summation, these are the first results demonstrating that low-dose aspirin has a gender-specific impact on ATL formation. Also, we noted that plasma ATL increased with age for women taking low-dose aspirin. However, for men, ATL levels were reduced with age. These findings may shed light on gender-dependent therapeutics of aspirin in recent reports. They also provide a molecular rationale for low-dose aspirin therapies that might be useful for elderly women to reduce local inflammation that contributes to chronic disorders of aging. Moreover, these findings may be helpful in designing new antinflammatory therapies.

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References

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