Thrombosis and Antithrombotic Therapy in Women

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Abstract—Sex-based differences in the prevalence and presentation of arterial and venous thrombosis exist, and emerging data indicate that men and women do not accrue equal benefit from antithrombotic therapy. Sex hormones alter procoagulant protein expression and the function of blood and vascular cells. Sex-based differences in platelet function have been reported, and in animal models, sex-based differences in thrombosis have been noted. Here we review plausible mechanisms that may explain how sex functions as a modifier of thrombosis and summarize clinical data on the interaction between sex and response to antithrombotic therapy. (Arterioscler Thromb Vasc Biol. 2009;29:284-288.)

Key Words: anti-thrombotic therapy ■ female ■ platelet ■ thrombosis

Thrombosis is a leading cause of death worldwide and complicates atherosclerotic disease, heart failure, cancer, surgery, and pregnancy. Antithrombotic therapy is the cornerstone in the prevention and treatment of arterial thrombosis (eg, myocardial infarction and stroke), venous thromboembolic disorders, and the complications of atrial fibrillation. Sex-based differences in the pathophysiology and treatment of thrombosis have been examined in a growing body of literature. Although women tend to be under-represented in randomized clinical trials of antithrombotic therapy, most analyses indicate that men and women accrue equal therapeutic benefit in a variety of clinical settings. However, emerging data suggest that women may respond differently to some antplatelet agents. We will review the data in support of sex-based differences in thrombosis and antithrombotic therapy and present potential biological explanations for the observed differences.

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Biological Basis for Sex Differences in Predisposition to Thrombosis

Important differences in the age of presentation of cardiovascular disease exist between men and women, which may, in part, reflect underlying differences in propensity to thrombosis. Sex hormones have complicated effects on the vessel wall, coagulation proteins, and platelets that may alter thrombosis. Cyclic patterns in coagulation proteins and circulating microparticle levels corresponding to menstrual cycle patterns have been observed in women. Liver secretion of coagulation factors is influenced by sex and growth hormones. Continuous estrogen decreases plasma levels of fibrinogen, antithrombin, protein S, and plasminogen activator inhibitor. However, while estrogen tends to lower plasma fibrinogen, women have moderately higher fibrinogen levels than their male counterparts. In the Fibrinogen Studies Collaboration, a meta-analysis of findings from 31 studies involving 154 211 apparently healthy adults, fibrinogen levels in women were on average 0.12 g/L higher than in men.

Animal models have been used to provide mechanistic insight into pathophysiology of sex-based differences in thrombosis and to avoid the confounding influences that can affect human phenotypes. While many such differences have been observed in animal models, the findings vary from species and strain, and thus extrapolating the findings from animal models to humans must be done with caution. An examination of 42 strains of mice revealed mean prothrombin times (in seconds) of 10.5±0.662 in female mice and 10.4±0.55 in male mice, with several strains (DBA/2J, C3H/HeJ, and SEA/GnJ) showing evidence for lower times in females and several (SWR/J and NZW/LacZ) in which times tended to be lower in males. A similar profile of fibrinogen levels demonstrated lower mean levels in female mice (190±31.3 mg/dL) than in males (235±36.5 mg/dL). In a mouse pulmonary embolism model, male mice were more susceptible to thrombosis and had faster clotting times ex vivo, apparently related to differences in the patterns of growth hormone secretion in males (pulsatile) and females (sustained) which in turn influence the production of proteins that regulate coagulation and thrombosis.

Sex Differences in Platelet Function

Substantial evidence points to sex differences in platelet function in animals and humans. In the case of rats, platelets isolated from male rats display greater maximal aggregation in vitro than platelets isolated from female rats. The thromboxane A2-mimetic U46619 elicits greater thrombus burden and more death in male rats. Castration reduces platelet aggregation in male rats. In contrast, platelets isolated from female mice bind more fibrinogen and have a greater maximal extent of aggregation in response to weak and low-dose agonists than do platelets isolated from male
mice. Additionally, treatment of ovariectomized mice with estrogen alters ex vivo platelet reactivity.

Many, but not all, studies of human platelet function have observed heightened responsiveness of platelets isolated from women. Although human platelets isolated from women may have less integrin αIIbβ3 [glycoprotein (GP) IIb/IIIa], they bind more fibrinogen in response to ADP and thrombin-receptor stimulation and form larger aggregates. The nature of the agonist may be important in sex difference of human platelet function, because male human platelets reportedly have greater responses to activation of α2-adrenergic and serotonin signaling pathways.

The differences in ex vivo platelet function observed in men and women could be the result of direct platelet effects of estrogens, progesterone or androgens or could be an indirect effect of sex hormones on the vasculature. Both megakaryocytes and platelets express the estrogen receptor β and the androgen receptor. A number of studies support a direct effect of estrogen on platelet function. Exposure of platelets in vitro to estrogen inhibits ADP, epinephrine, and shear-induced platelet aggregation. Differences in ex vivo platelet function during the follicular and luteal phases have been observed and may relate to alterations in plasma progesterone levels. Finally, in mice, estrogens act through the estrogen receptor α in the vessel wall to upregulate production of prostaglandin I2, lower platelet activation, and downregulate thromboxane synthesis. Estrogen-mediated platelet inhibition via prostaglandins in vivo could translate into heightened activation of isolated female platelets because of the transient nature of the platelet inhibitory effects of prostaglandins.

The androgen receptor may also regulate megakaryocyte biology and platelet production. Androgen therapy improves platelet count in myelodysplastic syndrome. Castration of mice reduces platelet count, which is restored by administration of testosterone. Indeed, a profile of platelet counts across 43 strains of mice indicated that, with a few exceptions, male mice of most strains had similar or higher platelet counts than their female counterparts.

Sex Differences in Effects of Antiplatelet Therapy as Measured by Ex Vivo Assays

Several studies have demonstrated a higher prevalence among females of the phenomena of “aspirin resistance,” defined as the failure of aspirin to inhibit platelet function as measured by a variety of assays. For example, when platelet function is assayed ex vivo by light transmission aggregometry or in the PFA-100 assay, the results are higher for women than men consuming aspirin. To determine whether aspirin fails to suppress platelet function in women, Becker et al studied platelet function in samples from 571 men and 771 women before and after aspirin use. In keeping with other reports, they found platelets from women were more responsive in 10 of 12 baseline platelet function tests. After low-dose aspirin therapy (81 mg) for 14 days, ex vivo platelet function declined to a similar or greater extent in women. However, because of the heightened baseline reactivity, the absolute extent of platelet aggregation was greater in women than in men after 14 days of low-dose aspirin. In a smaller study of 106 healthy individuals, women also had higher residual ex vivo platelet aggregation after 14 days of high-dose aspirin (325 mg) then did men. These findings may help to explain the higher prevalence of “aspirin resistance” in women; because at baseline women have greater platelet function in ex vivo assays. Thus, their values tend to remain higher after aspirin therapy.

Antithrombotic Therapy in Arterial Thrombosis (Myocardial Infarction and Stroke)

Platelets are essential to atherothrombosis and, therefore, antiplatelet therapy is pivotal in preventing and treating acute myocardial infarct (MI) and ischemic stroke (cerebral vascular accident; CVA). In the setting of acute MI, stroke or transient ischemic attack, and in other “high risk” populations, namely those with unstable angina and prior MI, the benefits of aspirin in preventing recurrent ischemic events are similar in men and women. The Second International Study of Infarct Survival (ISIS-2), the first large randomized clinical trial to evaluate the benefits of aspirin in the setting of acute MI, demonstrated that aspirin reduced the risk of vascular death at 5 weeks in both women [relative risk (RR) 0.84] and men (0.78). In the setting of acute CVA, aspirin also lowers the risk of recurrent ischemic strokes to a similar degree in women and men. Among “high-risk” patients, women receive the same benefit from aspirin therapy as do men, including a 34% decrease in nonfatal MI, a 25% decrease in nonfatal stroke, and a 15% decrease in vascular death.

Unlike studies in high-risk patients, studies of aspirin for primary prevention of cardiovascular disease in healthy individuals have revealed sex-based differences in responses to therapy. The Nurses’ Health Study, a nonrandomized prospective cohort study that followed more than 85 000 females in the United States, found that women taking 1 to 6 aspirin per week had a lower risk of MI within the first 6 years of follow-up (RR 0.75) than women who did not take aspirin. At 24 years of follow-up, a significantly lower risk of death from all causes was observed among women who used aspirin regularly versus those who never used aspirin (RR 0.75). The greatest risk reduction was in death from cardiovascular disease and stroke (RR 0.62 each). A linear relationship was seen between increasing duration of aspirin use and decreasing mortality. The observations from the Nurses’ Health Study spurred three large randomized trials to evaluate the role of aspirin as primary prevention in women (summarized in the Table). In the Hypertension Optimal Treatment (HOT) trial, men and women with hypertension were randomized to aspirin or placebo. Aspirin significantly lowered the risk of MI in men (RR 0.58), with no significant effect seen in women. There was no reduction in stroke risk seen in either men or women. The Primary Prevention Project randomized over 4400 people to aspirin or placebo with or without vitamin E. A nonsignificant increased risk of MI in women [Odds Ratio (OR) 1.37] and a decreased risk of MI in men (OR 0.50) was observed in this trial. For stroke, there was a trend toward a decreased risk in women (OR 0.68) but not in men (OR 1.16). In the largest study of aspirin in
primary prevention, the Women’s Health Study randomized over 39,000 healthy women to aspirin or placebo. Aspirin had no significant effect of the risk of MI (RR 1.02) in the overall study population, but decreased the risk of MI in women over the age of 65 (RR 0.66). Aspirin significantly lowered the risk of ischemic stroke (RR 0.76) and nonsignificantly increased the risk of hemorrhagic stroke (RR 1.24). It is important to note that the dose of aspirin used in the Women’s Health Study was low (100 mg every other day) and that higher doses might be required for adequate platelet inhibition. In a meta-analysis of primary prevention trials involving more than 95,000 patients, aspirin had no effect on risk of myocardial infarction (OR 1.01) or cardiovascular death (OR 0.99). There was an increased risk of hemorrhagic stroke noted in men taking aspirin (OR 1.69). The risk of bleeding, mostly gastrointestinal, was increased with chronic aspirin use to a similar degree in women (OR 1.68) and men (OR 1.72).

Few studies have examined responses of men and women to clopidogrel, a produg that is metabolized to a P2Y12 purinergic receptor antagonist that inhibits platelet activation by ADP. Plasma levels of the active metabolite of clopidogrel are similar in men and women. The available evidence indicates that clopidogrel is equally efficacious in women and men. In the Clopidogrel for the Reduction of Events During Observation (CREDO) trial, the benefit of a loading dose of clopidogrel prior to percutaneous revascularization was similar in men and women.

Inhibition of the major platelet integrin αIIbβ3 (GPIIb/ IIIa), which is the final common step in platelet aggregation, reduces the risk of ischemic complications in the setting of percutaneous coronary interventions. In a meta-analysis of more than 31,000 patients presenting with acute coronary syndromes, a significant interaction between sex and treatment was observed, with rates of death and MI lower in men (OR 0.81) but not women (OR 1.15) receiving GPIIb/IIIa receptor blockers. The sex and treatment interactions held after adjustment for differences in baseline characteristics. In a subset of patients for whom values of the myocardial necrosis biomarker troponin were available, GPIIb/IIIa inhibitors reduced the rates of MI and death in both troponin-positive men and women but had no benefit in troponin-negative patients of either sex. Sex differences in platelet response to GPIIb/IIIa inhibition have not been observed in vitro, thus the clinical observations may reflect fundamental differences in the presentation of acute coronary syndromes atherothrombosis in men and women.

### Atrial Fibrillation, Stroke, and Antithrombotic Therapy

Atrial fibrillation is the most common arrhythmia encountered in clinical practice and is a major risk factor for stroke. Women appear to be at higher risk for suffering a stroke in the setting of atrial fibrillation than men, and women over the age of 75 years are at the highest risk of stroke. In keeping with these observations, atrial fibrillation is more frequently noted in women presenting with a stroke than in men. In comparison to placebo, aspirin therapy reduces the incidence of stroke by 19% to 25% with equal efficacy in men and women. Warfarin therapy reduces the incidence of stroke by 64% in patients with atrial fibrillation compared to placebo. Warfarin therapy is at least equally effective in lowering the risk of thromboembolism in men and women, with some studies showing more benefit in women. Importantly, several recent trials have disclosed similar rates of major bleeding in women and men with warfarin therapy, and, in particular, there is no increased risk of intracranial bleeding. Investigational oral thrombin inhibitors are being studied in atrial fibrillation. In a randomized trial comparing the oral thrombin inhibitor ximelagatran to coumadin, the use of the oral thrombin inhibitor in women was associated with higher stroke rates, whereas there were no differences in stroke rates by treatment in men.

### Venous Thromboembolic Disease

Venous thromboembolism (VTE) occurs in 1 of every 1000 persons per year, with the incidence rising exponentially with age. Overall, the incidence of VTE appears to be the same in men and women. However, the age distribution varies by sex with slightly higher numbers of younger women and older men suffering VTE. The higher rates of VTE in younger women have been attributed largely to hormonal influences, namely oral contraceptive (OCP) use, pregnancy, and the puerperium. The absolute risk of VTE with OCP use is low, with reported rates of 1 in 2000 to 3500. However, this represents a 2- to 8-fold increase in risk. Use of combined hormone replacement therapy similarly increases the risk of VTE 2 to 4 times. Women have a lower risk of recurrent VTE after discontinuation of anticoagulation therapy as compared to men. The lower risk of recurrence may be explained in part by a significantly lower risk of recurrence in hormonally (OCP, hormone replacement, or pregnancy) related VTE (5%) versus nonhormonally related VTE (15%).
although a prothrombotic tendency in men as has been observed in animal models, cannot be excluded.

Pregnancy is associated with a 5- to 10-fold increase in the risk of thrombosis. In fact, pulmonary embolism is the most common cause of maternal death post delivery in developed countries. The risk of VTE increases with advanced maternal age, smoking, obesity, lupus, preexisting heart disease, sickle cell disease and preeclampsia, and births requiring transfusions. Of interest in discussions of thrombophilia and pregnancy is the role thrombosis plays in conditions mediated by placental insufficiency such as recurrent pregnancy loss and preeclampsia.

**Conclusions**

In summary, sex hormones alter procoagulant protein levels, platelet function, and the vessel wall in a manner that may translate into sex-based differences in thrombosis. Moreover, the male pattern of growth hormone secretion may regulate coagulation and thrombosis. The differences in platelet and coagulation function may, at least in part, explain the clinical observations that women are more likely to be aspirin-resistant, to accrue distinct benefits from aspirin therapy as primary prevention, and to present with different patterns of VTE and stroke in the setting of atrial fibrillation. Additionally, alterations in vessel wall biology between men and women may contribute to differences in thrombosis patterns and responses to antithrombotic therapy. In particular, the ability of aspirin therapy as primary prevention to lower MI in men and stroke in women and the differences in treatment benefit of GPIIb/IIIa inhibitors in women with acute coronary syndromes may reflect differences in the nature, burden, and presentation of atherosclerotic disease between women and men. All antithrombotic therapy is associated with an increased risk of bleeding. Bleeding complications are often higher in women, in part because of their smaller size and often older age at presentation than men. However, sex-based differences in vessel or blood function may exist that contribute to increased bleeding rates in women. Clearly additional studies are needed to define the mechanisms that control sex-based differences in thrombosis at the molecular, cellular, and whole organismal level. Moreover, clinical trials should rigorously examine the benefits and risks of antithrombotic therapy by sex, to ensure efficacy and safety in women and men.

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**Disclosures**

None.

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