Sex Difference in Antithrombotic Effect of Aspirin

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A number of clinical trials suggest that the antithrombotic effect of aspirin is limited to men. To test the possibility that this is due to a sex difference in the inhibitory effect of aspirin on platelet behavior, we studied whole-blood platelet aggregation in men and women and in male patients with carcinoma of the prostate receiving hormone therapy. The in vitro inhibitory effect of aspirin on so-called spontaneous platelet aggregation induced by stirring whole blood and monitored by the decrease in the number of singleton platelets was greater in men (mean±SD inhibitory ratio 1.54±0.30 in men, 1.23±0.22 in women; p<0.001). The inhibitory effect of aspirin was reduced in orchiectomized male patients and was restored by the addition of testosterone to blood samples. Estradiol had no detectable influence on the inhibitory effect of aspirin. Testosterone thus seems to influence platelet aggregation and its inhibition by aspirin as assessed by whole-blood in vitro aggregometry. Possible mechanisms for this effect of testosterone and its relevance to the choice of antithrombotic therapy are discussed. (Stroke 1989;20:34–37)

A sex difference in the efficacy of aspirin as an antithrombotic agent has been apparent in some large clinical trials involving venous thromboembolism and cerebrovascular disease. It has been suggested that this sex difference in efficacy is an artifact due to the small number of women entered into the trials and/or to a better overall prognosis in women. However, a sex difference in the antithrombotic effect of aspirin has also been proposed. We considered that the concentrations of sex hormones, particularly testosterone, might directly modify platelet aggregation and in particular might modify platelet aggregation in response to aspirin. Therefore, we studied the inhibitory effect of aspirin as measured in vitro in whole blood from male and female volunteers and from male patients with carcinoma of the prostate receiving estrogen therapy or no hormone therapy or after orchietomy.

Subjects and Methods

Blood was taken by clean venipuncture from 29 women (mean±SD age 39.2±19.34 years), 25 men (mean±SD age 47.2±16.1 years) (t=1.6, NS), and 36 older male patients with carcinoma of the prostate (mean±SD age 71.1±8.3 years) (t=7.5, p<0.01), of whom 15 underwent orchietomy (mean age 70.5 years) and six were receiving estrogen treatment (mean age 73.8 years). No subject had taken aspirin or other drugs that interfere with platelet aggregation for at least 1 and usually 2 weeks before the study. No subject was receiving vasodilators or antihypertensive treatment. No woman was pregnant or was receiving oral contraceptives. Only a small minority (three women and two men) were current smokers, but none admitted having smoked on the day of venipuncture. Alcohol consumption was not controlled, but no alcohol was taken in the hours immediately before venipuncture. Apart from the male patients chosen because of their carcinoma of the prostate, other subjects were either healthy volunteers (hospital staff) or had diverse conditions (such as epilepsy, urinary incontinence, or parkinsonism) under investigation in the hospital wards. There was no evidence of any imbalance in these diagnoses between the men and women. No subject had migraine.

The whole blood sample was anticoagulated with 3.8% wt/vol trisodium citrate (9:1 blood: citrate) and incubated with aspirin at a final concentration of 40 μg/ml for 30 minutes in a water bath at 37°C. To test the effect of testosterone on the inhibitory effect of aspirin, we added 5 μl of a solution of testosterone (Sigma Chemical Co., St. Louis, Missouri) in ethanol to the blood of a subgroup of 11 of the subgroup of 15 male patients who underwent orchietomy and to the blood of the subgroup of 11 healthy premenopausal women to give a final testosterone concentration of 25 nmol/l. We also added 5 μl of a solution of estradiol (BDH, Limited)
in ethanol to the blood of the subgroup of 11 orchiectomized male patients to give a final estradiol concentration of 500 pmol/l. Control blood samples had 5 µl of ethanol added to give a final concentration of 0.05%. In three male patients we were able to study the effect of aspirin in blood samples taken before and after orchiectomy. After incubation, 1-ml aliquots of all samples were transferred to plastic cuvettes, and each was stirred by a Teflon-coated magnetic "flea" at 200 rpm. The number of singleton platelets in whole blood before (baseline) and after 10 minutes of stirring were counted in an Ultra-Flo 100 platelet counter (Clay Adams). The number of singleton platelets decreases as platelets aggregate under the influence of stirring.

Aggregation is expressed as the percentage decrease in the number of singleton platelets. The inhibitory effect of aspirin is expressed as the ratio of the mean±SD aggregation in control blood samples divided by that in blood samples incubated with aspirin or aspirin and hormones. Probability values are calculated using Student's t test.

**Results**

Compared with the group of 29 women, aspirin had a greater inhibitory effect in the group of 25 men (Figure 1). There were no significant differences between sexes in hematocrit (35.58±5.48 for men, 35.54±3.27 for women), in baseline platelet count (243±103 x 10^9/l for men, 259±60 x 10^9/l for women), or aggregation in control samples (56.75±17% in men, 46.77±19.8% in women).

There was no significant difference in the inhibitory effect of aspirin between the subgroup of 11 premenopausal and the subgroup of 18 postmenopausal women (mean difference in the inhibitory effect of aspirin 0.71±0.8, p<0.02, and 0.35±0.23, p<0.001, respectively) (Figures 3 and 4). There was no significant difference in the inhibitory effect of aspirin after the addition of 500 pmol/l estradiol to blood samples from the subgroup of 11 male patients who had undergone orchiectomy (Figure 3).

In the three male patients whose blood was sampled both before and after orchiectomy, the inhibitory effect of aspirin was reduced after surgery but was restored by the addition of 25 nmol/l testosterone to the blood samples (Figure 5).

**Discussion**

A sex difference in the inhibitory effect of aspirin is demonstrable using an in vitro whole-blood method of measuring platelet aggregation. This is in contrast to the findings from methods that add aggregating agents to platelet-rich plasma. In the whole-

**Figure 1.** Histogram. Mean±SD ratio of number of singleton platelets in whole blood samples incubated in absence and presence of 40 µg/ml aspirin for 30 minutes at 37° C, inhibitory effect of aspirin in 25 men and 29 women (p<0.001).

**Figure 2.** Histogram. Mean±SD ratio of number of singleton platelets in whole blood samples in absence and presence of 40 µg/ml aspirin for 30 minutes at 37° C, inhibitory effect of aspirin in 15 male patients compared with six male patients receiving stilbestrol (difference not significant) and 15 male patients after orchiectomy (p<0.001).
FIGURE 3. **Histogram. Mean+SD ratio of number of singleton platelets in whole blood samples incubated in absence and presence of 40 µg/ml aspirin for 30 minutes at 37°C, inhibitory effect of aspirin in 11 male patients who underwent orchietomy.** Addition of 25 nmol/l testosterone to blood samples increased inhibitory effect (p<0.02); addition of 500 pmol/l oestradiol to blood samples did not significantly change inhibitory effect.

**FIGURE 4. Histogram. Mean+SD ratio of number of singleton platelets in whole blood samples incubated in absence and presence of 40 µg/ml aspirin for 30 minutes at 37°C, inhibitory effect of aspirin in 11 premenopausal women.** Addition of 25 nmol/l testosterone to blood samples significantly increased inhibitory effect (p<0.001).

**FIGURE 5. Graph. Ratio of numbers of singleton platelets in whole blood samples incubated in absence and presence of 40 µg/ml aspirin for 30 minutes at 37°C, inhibitory effect of aspirin in three male patients whose blood was sampled before and after orchietomy.** Addition of 25 nmol/l testosterone to postoperative blood sample restored inhibitory effect to presurgery levels.

Blood method using citrate as the anticoagulant, shear stress damage to erythrocytes, with concomitant release of adenosine diphosphate (ADP), is believed to be the principal cause of platelet aggregation. Our previous study showed a similar sex difference in the effect of aspirin on spontaneous platelet aggregation but not on collagen-induced aggregation in whole blood. Born and Wehmeir have suggested that a comparable interaction of platelets and erythrocytes may occur in vivo, although the aggregometer we used does not attempt to match in vivo shear forces.

Another factor in the lack of uniformity in research findings may lie in the dose of aspirin employed since Husted et al could not find a sex difference when high in vivo doses (1 g/day) were used. We deliberately studied a high in vitro concentration of aspirin, however, to ensure complete inhibition of cyclooxygenase and to match that concentration used in our pilot study. The results from lower concentrations approximating those achieved therapeutically have not yet been studied.

The sex difference has been claimed to be an artifact due to a difference in hematocrit, leading to different concentrations of citrate in anticoagulated blood samples from men and women. This does not seem to explain the difference we found since the hematocrit of the samples from men and women in the aggregometer, after dilution and incubation, was the same and since we demonstrated an effect of testosterone added to the samples.

The relative immunity of women to thrombotic diseases during the reproductive years has concentrated attention on the role of estrogens, which have been shown to affect coagulation factors and some aspects of platelet function. Effects on ADP-induced aggregation have not been consistently found, however, particularly in animal models, and the effects of estrogens on aggregation in whole blood in humans does not seem to have been studied in detail.

We found no evidence that estrogens affected the inhibitory effect of aspirin. Thus, we saw no difference between premenopausal and postmenopausal women, between men with carcinoma of the prostate receiving and not receiving stilbestrol, or between blood samples from orchietomized male patients before and after the addition of estradiol. Instead, our results suggest an influence of testosterone on platelet aggregation and on its inhibition by aspirin as witnessed by the effects of testosterone added to blood samples and by the changes after orchietomy.
The increased level of platelet aggregation in the male patients with carcinoma of the prostate may be related to age or to the effect of the malignancy and cannot be assumed to be a direct hormonal response. However, some preliminary findings suggest that radiolabeled testosterone binds to platelets and is displaced by dihydrotestosterone, compatible with there being a specific platelet receptor.

The mechanism of the testosterone interaction with the inhibitory effect of aspirin is not yet clear. There are three immediately obvious possibilities. First, there is some evidence that cyclooxygenase, which is inhibited by aspirin, is at a higher level of activity in men, possibly due to the presence of higher concentrations of testosterone. A second possibility is that cyclooxygenase is less readily acetylated in women. De La Cruz et al found that a higher concentration of aspirin was needed to inhibit half-maximal platelet aggregation in women. The results of Morikawa et al can be interpreted in the same way. Those authors reported that low doses of aspirin inhibited malonaldehyde and thromboxane synthesis in male rats only, a sex difference not seen at higher doses. After castration, higher doses of aspirin were needed in male rats. The high concentration of aspirin we used makes incomplete acetylation of cyclooxygenase unlikely.

A third obvious possibility is that aspirin has effects over and above its effect on cyclooxygenase and that these effects are sex-dependent. Kelton et al found that aspirin inhibited thrombus formation to a greater extent in male rats, even though the cyclooxygenase pathway was blocked completely in both sexes. Escolar et al reported the same phenomenon in human blood studied ex vivo in a Baumgartner chamber.

Clearly, this interaction between testosterone, platelet aggregation, and aspirin warrants further study. It may represent an example of a nongenomic effect of a steroid. It is also potentially important in the choice of antithrombotic treatment in patients with stroke or coronary heart disease. The number of women in the North American and United Kingdom trials of aspirin in cerebrovascular disease may not have been sufficient for statistically reliable analyses of the influence of aspirin on thrombotic clinical end points. The prognosis in women also appears to be more benign, so the number of end points reached by the women is further reduced. An overview analysis of the results of all trials involving antiplatelet agents is underway, and it will look at the collective experience of women in the many studies. If this overview analysis confirms a relative lack of aspirin response, as our data support, consideration may need to be given to using a different antiplatelet agent or a combination of drugs in women.

Acknowledgments
We are grateful to Messrs. E. Milroy, R. Turner Warwick, A. Cowie, and P. Worth for permission to study their patients and to Prof. H. Jacobs and Dr. J. Honour for help with the study.

References

Key Words: aspirin • platelet aggregation • sex hormones
Sex difference in antithrombotic effect of aspirin.
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Stroke. 1989;20:34-37
doi: 10.1161/01.STR.20.1.34

Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/20/1/34

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