

Effect of low-dose aspirin during pregnancy on fibrinolytic variables before and after parturition

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OBJECTIVE: We assessed the effects of a daily oral dose of 60 to 80 mg of aspirin from 12 weeks' gestation until delivery on fibrinolytic variables before and after parturition.

STUDY DESIGN: In a prospective controlled study labor was electively induced in 24 patients, eight receiving low-dose aspirin and 16 controls. Levels were determined in maternal and cord plasma of tissue-type plasminogen activator antigen and activity, plasminogen activator inhibitor-1 antigen, plasminogen activator inhibitor activity, and plasminogen activator inhibitor-2 antigen. We also determined metabolites of vascular prostacyclin and platelet thromboxane A₂.

RESULTS: The only maternal fibrinolytic variable affected by low-dose aspirin was plasminogen activator inhibitor activity, which showed a significant reduction before and after parturition of 40% and 70%, respectively, in low-dose aspirin users compared with controls. Concentrations of thromboxane B₂ in women using low-dose aspirin were 7% (maternal serum) and 17% (cord serum) of values in controls, but concentrations of 6-keto-prostaglandin F_{1α} were not affected.

CONCLUSIONS: Low-dose aspirin reduces plasminogen activator inhibitor activity and platelet reactivity, but not prostacyclin synthesis, before and after parturition. The reduction in plasminogen activator inhibitor activity may be caused by inhibition of platelet reactivity. (AM J OBSTET GYNECOL 1995;172:986-91.)

Key words: Fibrinolysis, low-dose aspirin, plasminogen activator, plasminogen activator inhibitor

In recent years an increasing number of pregnant women at risk of pregnancy-induced hypertensive disorders and fetal growth retardation are being prescribed low-dose aspirin,¹ although its prophylactic effect remains disputed.²⁻⁴ Aspirin in analgesic doses may have an antifibrinolytic effect, because it inhibits the endothelial release of tissue-type plasminogen activator (tPA) after venous occlusion.^{5, 6} This effect could be mediated by inhibition of prostacyclin (PGI₂) synthesis because it is reversed by a synthetic PGI₂ analog.⁷ Low-dose aspirin, 20 mg for 7 days, did not affect fibrinolytic activity after venous occlusion in healthy males.⁸ Although low-dose aspirin primarily inhibits platelet thromboxane synthesis, it may also cause a small reduction in PGI₂ synthesis in nonpregnant individuals⁹ and in pregnant women.¹⁰ For that reason an effect of long-term low-dose aspirin on the fibrinolytic

system of the pregnant woman cannot be excluded. Modulation of the fibrinolytic system by low-dose aspirin could be of particular importance after normal placental separation, when coagulation in the placental bed is balanced by a marked increase in fibrinolytic capacity.¹¹

In this study we compared variables of fibrinolysis and thromboxane and PGI₂ synthesis at the end of pregnancy before and immediately after parturition in women receiving low-dose aspirin for prevention of preeclampsia or fetal growth retardation and in women who had not taken aspirin. Because exact definition of the timing of sampling could be important in an investigation on fibrinolytic variables, we conducted this study in plasma of women in whom labor was induced, which allowed standardization of sampling times. In addition, we investigated the effect of low-dose aspirin on fibrinolytic variables in cord blood.

Material and methods

Patients. Twenty-four healthy pregnant women were enrolled in the study. Of these, eight took a daily dose of 60 to 80 mg of aspirin (1 mg/kg body weight) from 12 weeks' gestation until delivery because of a history of preeclampsia or fetal growth retardation; 16 women who did not use aspirin during pregnancy served as controls. All women opted for elective induction of

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Received for publication March 3, 1994; revised August 8, 1994; accepted August 25, 1994.

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0002-9378/95 \$3.00 + 0 6/160267

labor, an accepted procedure in the department.¹² Cervical ripeness was assessed with the Burnhill score.¹³ When the cervix was considered favorable, the membranes were ruptured, and a fluid-filled open-tip catheter was introduced transcervically into the amniotic cavity for the recording of uterine activity. An electrode was attached to the fetal scalp for fetal heart rate monitoring. Uterine contractions were induced with intravenous infusion of an incremental dose of oxytocin starting with 2 mU/min until a uterine activity of 150 to 200 Montevideo units was obtained.¹² In all cases induction of labor started at 8 AM. Low-dose aspirin users took the last tablet at 7 AM. The study protocol as approved by the University and Hospital Ethics Committee was explained, and informed consent was obtained in all cases.

Blood sampling. Maternal venous blood samples were taken 5 minutes before the start of the oxytocin infusion and within 5 minutes after delivery of the placenta. All samples were obtained from an antecubital vein without occlusion through a 19-gauge needle.

A sample of 4.5 ml was collected in tubes containing 0.5 ml of 0.11 mol/L sodium citrate, 15 mmol/L theophylline, 3.7 mmol/L adenosine, and 0.198 mmol/L dipyridamole, pH 5.5 (Becton Dickinson, Meylan, France) for determination of tPA antigen, plasminogen activator inhibitor type-1 (PAI-1) and type-2 (PAI-2) antigen and plasminogen activator inhibitor activity. Another 4.5 ml sample was collected in Stabilyte tubes (Biopool, Umea, Sweden, containing 0.5 ml of 0.45 mol/L sodium citrate buffer, pH 4.3) for determination of tPA activity. A 9 ml sample was taken in a plastic tube in 1 ml of disodium ethylenediaminetetraacetate for determination of synthesis of malondialdehyde by stimulated platelets. For determination of 6-keto-prostaglandin F_{1α} (PGF_{1α}), a stable metabolite of PGI₂, a 5 ml sample was collected in cooled plastic tubes containing 10 μl of heparin and 25 μl of indomethacin (0.1% in potassium-phosphate buffer 0.1 mol/L, pH 7.4). Finally, a 5 ml blood sample was collected in glass tubes and allowed to clot for determination of serum levels of thromboxane B₂ (TXB₂).

Samples of mixed free-flowing cord blood were collected immediately after clamping of the cord with the placenta in situ for determination of the same set of variables.

Analytic procedures. Samples for determination of variables of fibrinolysis were transported on melting ice, centrifuged for 30 minutes at 2000g at 4° C, and stored at -70° C until analysis. Samples for determination of plasma PGF_{1α} were centrifuged for 10 minutes at 1500g at 0° C and stored at -20° C until analysis. The clotted blood samples for determination of serum TXB₂ were incubated for 60 minutes at 37° C to stimulate maximum platelet TXB₂ release. After centrifugation for 10

minutes at 2500g at 20° C serum samples were kept at -20° C until analysis.

tPA antigen was determined with a commercially available enzyme immunoassay (Imulyse, Biopool) that measures both free and complexed tPA in the range of 1.5 to 30 ng/ml. The maximal sensitivity of the test was 1.5 ng/ml; intraassay variation was 8% and interassay variation 10%.

tPA activity was determined by the conversion of plasminogen to plasmin in the presence of soluble fibrinogen fragments by means of a chromogenic substrate according to Verheijen et al.¹⁴ The detection limit of this assay was 50 mIU/ml; intraassay variation was 5% to 13%, and interassay variation was 15% to 20%.

PAI-1 antigen was measured with a commercially available enzyme immunoassay that used anti-PAI-1 antibodies (TintElize PAI-1, Biopool). The test measures both free PAI-1 and PAI-1 complexed to activators. The detection limit was 2 ng/ml PAI-1; the intraassay variation was 2% at 40 ng/ml and 7% at 15 ng/ml.

PAI activity was measured by titration of diluted samples with tPA according to Verheijen et al.¹⁵ Results are expressed as a percentage of a standard normal plasma pool obtained from 40 healthy volunteers. Intraassay variation was 2% to 10%, and interassay variation was 10% to 15%.

PAI-2 antigen was determined with a commercially available enzyme immunoassay that used anti-PAI-2 antibodies (TintElize PAI-2, Biopool). The detection limit was 6 ng/ml.

The capacity of platelets to synthesize thromboxane A₂ was determined within 30 minutes after venipuncture by measuring the amount of its stable by-product malondialdehyde produced on stirring platelet-rich plasma with thrombin (1 IU/ml final concentration), as described previously.¹⁶ The concentration of malondialdehyde was measured by means of the thiobarbituric acid reaction and expressed as nanomoles of malondialdehyde produced by 10⁹ platelets.

Plasma concentrations of PGF_{1α} and serum TXB₂ levels were determined by radioimmunoassay (E.I. Du Pont de Nemours-NEN Research Products, Boston). A Sep-Pak C₁₈ cartridge (Waters, Milford, Mass.) was prewashed with 10 ml of absolute ethanol, 10 ml of distilled water, and 2 ml of air. Two milliliters of the sample was applied to the column, followed by 2 ml of distilled water and 2 ml of air. The prostaglandin metabolites were eluted with 2 ml of absolute ethanol, followed by 2 ml of air, and the eluate was brought to dryness at 40° C under a gentle stream of nitrogen. The residue was dissolved in radioimmunoassay buffer, and the assay was performed with iodine 125-labeled antigen according to the instruction manual.¹⁷

Statistical analysis. Because the results could not be expected to follow a normal distribution, data are

Table I. General characteristics of women who participated in the study (median, range)

| | Aspirin (n = 8) | Control (n = 16) |
|--|------------------|------------------|
| Age (yr) | 32 (26-38) | 30 (20-41) |
| Parity | 1 (1-4) | 1 (1-7) |
| Duration of use of low-dose aspirin (wk) | 25.5 (22-28) | — |
| Gestational age (wk) | 38.5 (37.4-40.2) | 39.4 (36.5-42) |
| Birth weight (gm) | 3305 (2780-3715) | 3235 (2670-4160) |
| Estimated blood loss at delivery (ml) | 200 (100-600) | 200 (100-1100) |

Table II. Median (interquartile range) concentrations of variables of fibrinolysis in maternal venous plasma

| Plasma | Aspirin (n = 8) | Control (n = 16) | Significance* |
|---|---------------------|---------------------|-----------------|
| tPA antigen (ng/ml) ^a | 10.8 (6.2-12.6) | 6.5 (4.2-16.4) | NS |
| tPA antigen (ng/ml) ^b | 17.6 (14.9-27.2) | 13.9 (10.5-29.3) | NS |
| Significance† | <i>p</i> = 0.01 | <i>p</i> < 0.001 | |
| tPA activity (IU/ml) ^a | 0.8 (0.6-1.1) | 1.0 (0.4-1.6) | NS |
| tPA activity (IU/ml) ^b | 2.8 (1.6-3.8) | 1.7 (1.0-2.7) | NS |
| Significance† | <i>p</i> = 0.01 | <i>p</i> = 0.003 | |
| PAI-1 antigen (ng/ml) ^a | 101.9 (52.6-127.5) | 101.5 (66.9-134) | NS |
| PAI-1 antigen (ng/ml) ^b | 44.9 (42.9-62.7) | 66.2 (38.2-80.2) | NS |
| Significance† | <i>p</i> = 0.01 | <i>p</i> < 0.001 | |
| PAI-2 antigen (ng/ml) ^a | 168 (146.5-209.8) | 189 (134.4-225.8) | NS |
| PAI-2 antigen (ng/ml) ^b | 160.5 (128.8-193.8) | 165.5 (130.8-192.8) | NS |
| Significance† | NS | <i>p</i> = 0.03 | |
| Plasminogen activator inhibitor activity (%) ^a | 326 (86-428) | 556 (431-829) | <i>p</i> = 0.02 |
| Plasminogen activator inhibitor activity (%) ^b | 108 (70-167) | 339 (100-528) | <i>p</i> = 0.02 |
| Significance† | <i>p</i> = 0.01 | <i>p</i> < 0.001 | |

NS, Not significant.

*Aspirin versus control.

†Values obtained 5 minutes before oxytocin infusion (a) versus values 5 minutes after delivery of placenta (b).

expressed as medians with 25th and 75th percentiles or range. Differences between time-related continuous variables were assessed with the Wilcoxon signed-rank test. Comparisons between maternal and cord samples and between aspirin and control groups were made with the Wilcoxon rank-sum test. Relationships between maternal and cord variables were analyzed with Spearman's rank correlation test. All *p* values are two-tailed, and a level of <0.05 is considered to represent significance.

Results

General characteristics of the women who participated in the study are summarized in Table I. None of the aspirin-treated women had a pregnancy-induced hypertensive disorder or fetal growth retardation. All deliveries were uncomplicated and resulted in live, healthy infants.

Effects of labor and delivery on fibrinolytic markers are presented in Table II. Concentrations of tPA antigen, tPA activity, PAI-1 antigen, and PAI-2 antigen before labor and after delivery of the placenta were not different between aspirin users and controls. Plasminogen activator inhibitor activity was significantly reduced in the aspirin group compared with controls, before and after parturition (*p* = 0.02).

Both tPA antigen and activity rose, whereas PAI-1

antigen and plasminogen activator inhibitor activity decreased significantly during labor and delivery. These changes were not affected by low-dose aspirin and were not related to the duration of labor. PAI-2 antigen showed a small but significant (*p* = 0.03) reduction during parturition in the control group that could not be demonstrated in low-dose aspirin users. No significant correlations were demonstrated between the levels of fibrinolytic variables in maternal and cord plasma. Comparison of fibrinolytic variables in cord blood in the aspirin and control groups showed no significant differences (Table III).

Levels of PGF_{1α} before labor in the aspirin group (median 54.7 ng/L, range 52 to 82.8 ng/L) were about 14% lower than in controls (median 63.3 ng/L, range 50.6 to 73 ng/L), but the difference was not significant. Fig. 1, A and B, shows the significant increase in PGF_{1α} in maternal plasma during parturition; no significant differences could be demonstrated between the aspirin and control groups. Cord plasma levels of PGF_{1α} were not different between groups and were about 14 times higher than maternal plasma concentrations (*p* < 0.001).

Before labor maternal malondialdehyde formation in the aspirin group (median 0.52 nmol/10⁹ platelets, range 0.48 to 1.00 nmol/10⁹ platelets) and serum TXB₂ concentrations (median 20.8 μg/L, range 12.0 to 40.2 μg/L) were significantly lower than those in controls

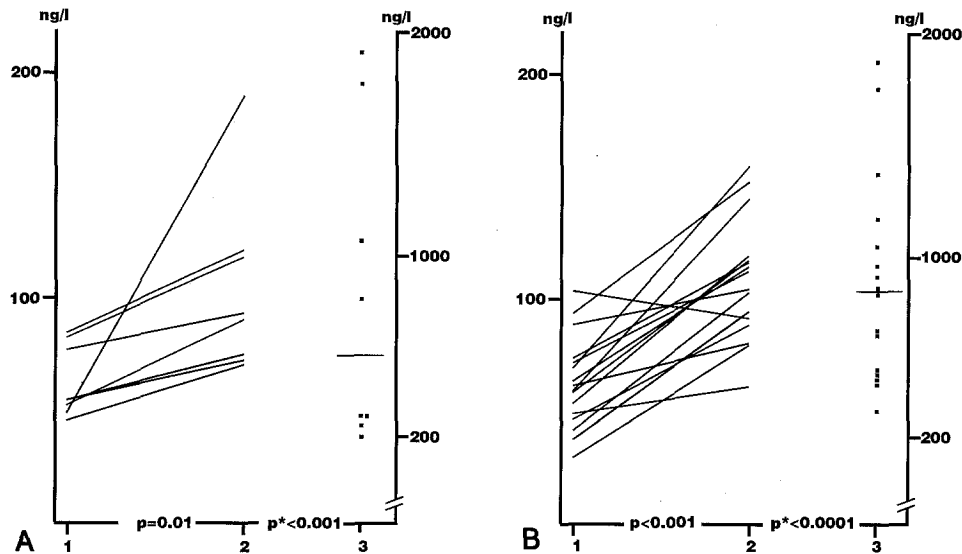


Fig. 1. A and B, Maternal venous and cord plasma levels of 6-keto-PGF_{1α} in women receiving long-term low-dose aspirin (A) compared with controls not taking aspirin (B). 1, Before parturition; 2, after parturition; 3, cord plasma. Asterisk, Compared with maternal plasma 2.

Table III. Median (interquartile range) concentrations of variables of fibrinolysis and prostanoid metabolites in cord blood

| | Aspirin (n = 8) | Control (n = 16) | Significance |
|--|-----------------|------------------|--------------|
| tPA antigen (ng/ml) | 4.5 (2.3-26) | 4.5 (3.2-7.5) | NS |
| tPA activity (IU/ml) | 7.5 (2.9-8.9) | 3.0 (1.5-11) | NS |
| PAI-1 antigen (ng/ml) | 14.9 (10.6-77) | 21.2 (17.8-50) | NS |
| Plasminogen activator inhibitor activity (%) | 43 (25-100) | 49 (23-78) | NS |
| PAI-2 antigen (ng/ml) | 3.7 (0.5-68.2) | 6.6 (3.2-27) | NS |
| 6-keto-PGF _{1α} (ng/L) | 626 (326-1164) | 862 (471-1182) | NS |
| TXB ₂ (μg/L) | 37.8 (27.7-80) | 217 (123-326) | p = 0.0002 |
| Malondialdehyde (nmol/10 ⁹ platelets) | 2.2 (1.9-2.7) | 5.2 (4.7-5.8) | p = 0.0001 |

NS, Not significant.

(malondialdehyde median 5.4 nmol/10⁹ platelets, range 4.7 to 6.4 nmol/10⁹ platelets; TXB₂ median 281 μg/L, range 205 to 411 μg/L; p < 0.0001). No significant changes were observed in platelet malondialdehyde formation and in serum TXB₂ concentrations during parturition in aspirin users and in controls.

In the control group no differences could be demonstrated between platelet malondialdehyde synthesis and serum TXB₂ concentrations in maternal and cord samples, but in low-dose aspirin users malondialdehyde synthesis and serum TXB₂ concentrations were significantly reduced in maternal compared with cord samples (p < 0.001).

Comment

Results of previous studies suggest that a daily dose of 60 to 80 mg of aspirin has no demonstrable effect on hemostasis in pregnant women, as indicated by the bleeding time,^{18, 19} but no data could be found in the accessible literature on the effect on the fibrinolytic

system. Throughout pregnancy marked alterations occur in the fibrinolytic system, characterized by increases in plasma concentrations of tPA and its inhibitors PAI-1 and PAI-2 until the end of the thirty-eighth week of pregnancy, after which plasma concentrations of all these substances decrease, except those of tPA antigen.²⁰ In a recent report on the effects of parturition on fibrinolysis a significant increase in maternal plasma concentrations of tPA antigen during labor was demonstrated, with a marked further increase in fibrinolytic potential after placental separation.²¹ In previous studies a high dose of aspirin (650 mg) ingested 18 and 2 hours before blood sampling was shown to inhibit the release of tPA antigen by vascular endothelium induced by venous occlusion.^{5, 6} A study of the effect of 20 mg of aspirin taken for 7 consecutive days showed no significant effect on fibrinolytic activity, measured by the euglobulin lysis area and the euglobulin lysis time.⁸ In our study no effect of the long-term use of low-dose aspirin on tPA activity was observed. The only fibrino-

lytic variable affected by the use of low-dose aspirin during pregnancy was plasminogen activator inhibitor activity, which was significantly reduced in the aspirin group before and after labor. This could lead to a relative dominance of tPA activity over plasminogen activator inhibitor and hence to an increased fibrinolytic potential in low-dose aspirin users. The question whether this causes an increase in effective fibrinolysis in vivo remains to be answered. The observed significant changes in levels of all fibrinolytic markers during parturition, except PAI-2, may be caused by vascular damage in the uterine placental bed²² and appear to remain unaffected by low-dose aspirin. In the nonpregnant state diurnal variations in blood fibrinolytic activity are caused by changes in the plasma levels of PAI-1, which are highest in the early morning and lowest in the late afternoon.²³ However, plasminogen activator inhibitor production is not influenced by diurnal variations in populations with high plasminogen activator inhibitor levels, as in pregnancy.²⁴ Because of the standardization of our study, all samples were obtained at approximately the same time of the day, which makes a contribution of circadian variation to the results unlikely.

We could not demonstrate an inhibitory effect of the long-term intake of 60 to 80 mg of aspirin on maternal PGI₂ synthesis, which confirms earlier observations by ourselves²⁵ and by others.¹⁸ The observed increased synthesis of PGI₂ during labor confirms earlier observations²⁶ and appears also to be unaffected by low-dose aspirin.

It is known that in normal pregnancy platelet reactivity increases, not only in association with increasing activity of the cyclooxygenase pathway but also with an elevated sensitivity of the platelet membrane.²⁷ Previous studies have shown that approximately 90% to 95% inhibition of maternal platelet reactivity can be achieved with a daily dose of 60 mg of aspirin,^{27, 28} as confirmed in the current study. Negatively charged phospholipids are known to stimulate plasminogen activator inhibitor activity,²⁹ and platelets, offering a net negative charge on activation, may provide a site for activation of plasminogen activator inhibitor.³⁰ Therefore the reduced platelet reactivity induced by low-dose aspirin could be responsible for the reduced plasminogen activator inhibitor activity in the aspirin group.

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Angiotensin-converting enzyme activity and contractile effects of angiotensin I and II in human uteroplacental arteries

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OBJECTIVE: Our purpose was to study local angiotensin-converting enzyme activity and the mechanical effects of angiotensin I and II in human uteroplacental arteries.

STUDY DESIGN: Angiotensin-converting enzyme activity was measured by a simple radioimmunoassay with tritiated benzoyl-glycyl-glycyl-glycine as substrate in isolated human intramyometrial arteries from nonpregnant ($n = 8$) and term pregnant women ($n = 8$) and placental ($n = 8$) stem villous arteries. Moreover, in these vessels the mechanical effects of angiotensin I and II were investigated in organ bath experiments. Endothelium-intact and endothelium-denuded arteries were used, and the integrity of the endothelium was examined by histologic studies.

RESULTS: The activity of angiotensin-converting enzyme ranked the intramyometrial arteries from pregnant women >> intramyometrial arteries from nonpregnant women > fetal stem villous arteries. Angiotensin-converting enzyme activity was unaffected by removal of the endothelium. Angiotensin II 10^{-5} mol/L produced contractile responses in the intramyometrial arteries without significant differences between arteries from nonpregnant and pregnant women. In fetal stem villous arteries the effects of angiotensin II 10^{-5} mol/L were less pronounced. As for angiotensin II, the contractile responses to angiotensin I 10^{-5} mol/L showed marked development of tachyphylaxis. In the endothelium-denuded preparations the contractile responses to angiotensin I 10^{-5} mol/L were significantly enhanced in intramyometrial arteries from nonpregnant women but remained unchanged in intramyometrial arteries from pregnant women and in fetal stem villous arteries. In all preparations pretreatment with captopril or perindopril (10^{-5} mol/L) markedly reduced angiotensin-converting enzyme activity, whereas no effects were observed on the contractile responses to angiotensin I. Saralasin 10^{-5} mol/L completely abolished the contractile responses to angiotensin I and II.

CONCLUSION: Local angiotensin-converting enzyme activity in human intramyometrial arteries seems to be markedly increased during pregnancy and shows marked differences between maternal and fetal uteroplacental arteries. High concentrations of angiotensin I may imply direct effects on the angiotensin II receptor independent of the local angiotensin-converting enzyme activity. (*AM J OBSTET GYNECOL* 1995;172:991-7.)

Key words: Angiotensin-converting enzyme, radioimmunoassay, uteroplacental smooth muscle

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