Effect of intravenous prostaglandin E₂ on platelet function, coagulation, and fibrinolysis

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SUMMARY Prostaglandin E₂ (PGE₂) was infused intravenously to eight women for the termination of pregnancy and tests of platelet function: coagulation and fibrinolysis were studied before and during the infusion.

Platelet adhesiveness, as measured by a cellophane membrane test-cell system, was significantly diminished by PGE₂, a change which was not noted by the glass-bead column technique. The administration of PGE₂ caused more rapid platelet disaggregation following ADP-induced aggregation but had no effect on the platelet count, collagen-induced aggregation, or platelet factor 3 activity. An increase in plasma antithrombin concentration and euglobulin lysis activity was also noted.

These results support the concept that prostaglandin E₂ might have a role in the prevention of thrombosis.

Prostaglandins have been included among the agents which may depress platelet function and so have a potential in the treatment of thrombotic disorders which may be initiated by platelets adhering to the vessel wall.

Prostaglandins have *in vitro* a powerful inhibitory effect on platelet adhesiveness and aggregation (Kloeze, 1967; Emmons, Hampton, Harrison, Honour, and Mitchell, 1967; Irion and Blombäck, 1969; Weeks, Chandra Sekhar, and Ducharme, 1969; Kinlough-Rathbone, Packham, and Mustard, 1970). Prostaglandin E₁ is the most potent inhibitor of platelet function and PGE₂ has about a fifth of its activity (Irion and Blombäck, 1969). Studies *in vivo* in animals have shown that the intravenous or topical administration of PGE₁ can inhibit platelet thrombus formation at the site of vessel injury and prolong bleeding times (Emmons *et al.*, 1967; Kinlough-Rathbone *et al.*, 1970). Such evidence suggested that prostaglandins might have a role in the prevention of thrombosis (Lancet, 1971).

In contrast to studies in experimental animals, attempts to demonstrate an effect of prostaglandin on platelets in human subjects have been disappointing as the dosage is limited by side effects (Elkeles, Hampton, Harrison, and Mitchell, 1969; Carlsson, Irion, and Orö, 1968). Although Elkeles *et al.* (1969) noted inhibition of the platelet electro-

phoretic mobility response to ADP by intravenous infusion of PGE₁ in eight male volunteers, the severity of side effects led them to conclude that PGE₁ would have no clinical value. Carlsson *et al.* (1968) found no change in ADP aggregation in three men receiving PGE₁ and Karim and Filshie (1972) found platelet adhesiveness by Hellem's method unaltered by intravenous PGE₂.

The use of intravenous PGE₂ to induce therapeutic abortion has given us an opportunity to test its effect upon platelet function, coagulation, and fibrinolysis in eight women undergoing termination of pregnancy.

Patients and Methods

The patients were all healthy women, between the 12th and 20th week of pregnancy, having an intravenous PGE₂ infusion for therapeutic abortion. Their ages ranged from 18 to 34 years. An intravenous infusion of normal saline was commenced and a venous blood sample was collated from the opposite arm; 9 ml was mixed with 1 ml of 3.8% sodium citrate for coagulation and fibrinolytic tests; 2 ml was added to edetic acid for platelet counting and 2 ml was added to a tube containing glass beads and 1 mg of the fibrinolytic inhibitor tranexamic acid for assay of fibrinolytic degradation products (FDP). The coagulation and fibrinolytic assays were performed within 45 minutes of collection of the blood.

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which was kept at 4°C. Blood for platelet function tests was collected as described by Hassanein, McNicol, and Douglas (1970).

An intravenous infusion of PGE_2 was then given at the rate of 2.5 $\mu\text{g}/\text{min}$ for the first hour and at 5 $\mu\text{g}/\text{min}$ thereafter. After three hours of PGE_2 infusion, a second blood sample was collected, and platelet function, coagulation, and fibrinolytic tests were repeated. None of the patients had aborted or shown evidence of uterine activity at the time of the second blood sample.

Platelet adhesiveness to a cellophane membrane in a test cell was measured by the method of Lindsay, Prentice, Ferguson, Muir, and McNicol (1973). In this method, 10 ml of citrated blood is passed over a cellophane membrane and platelet counts are performed on the blood before and after being passed through the cell to calculate the retention of platelets.

Platelet adhesiveness to glass was measured by the method of Hellem (1960) as modified by Hirsh, McBride, and Dacie (1966); also platelet aggregation was measured at room temperature by the turbidimetric method of Born and Cross (1963) induced by both ADP (0.05 units/ml) and collagen; platelet factor 3 availability was estimated as described by Hassanein *et al* (1970); platelets were counted by the method of Dacie and Lewis (1963).

The coagulation tests performed were thrombin clotting time (McNicol and Douglas, 1964), one-stage prothrombin time (Douglas, 1962), kaolin partial thromboplastin time (Proctor and Rapaport, 1961), recalcification time in plastic (Biggs and Macfarlane, 1962), factor V (Shanberge, Matsuoka, and Fukui, 1967), factor VIII (Breckenridge and Ratnoff, 1962), fibrinogen (Ratnoff and Menzie, 1964), and plasma antithrombin (Howie, Prentice, and McNicol, 1973). Tests of fibrinolysis were plasminogen (Remmert and Cohen, 1949), euglobulin lysis time (Nilsson and Olow, 1962), urokinase sensitivity test (McNicol, Gale, and Douglas, 1963), antiplasmin (Howie, Mallinson, Prentice, Horne, and McNicol, 1970), and serum FDP (Bonnar Davidson, Pidgeon, McNicol, and Douglas, 1969).

In respect of the ADP-induced platelet aggregation, a further five women were studied; this was done to see if there was a different response to ADP in those women who successfully aborted following infusion as compared with those in whom the PGE_2 failed to induce uterine activity.

Results

PLATELET ADHESIVENESS (FIG 1)

Platelet retention in the membrane test-cell fell significantly in seven patients from a mean of 48%

Fig 1 Platelet adhesiveness, as measured by the cellophane membrane test cell and the glass-bead column, before and during an infusion of PGE_2 .

before the PGE_2 infusion to 16% during the infusion ($p < 0.01$). In contrast, platelet adhesiveness to glass beads as measured by the Hellem technique showed no significant change during this period.

PLATELET ADP-INDUCED AGGREGATION

In this test (fig 2), performed in 13 patients, the initial rate of platelet aggregation was similar before

Fig 2 Mean ADP-induced platelet aggregation before and during infusion of PGE_2 ($n = 13$).

and during the PGE₂ infusion. The rate of platelet disaggregation was, however, more rapid during the PGE₂ infusion as compared with the pre-infusion results ($P < 0.05$).

Some patients demonstrated the effect of rapid platelet disaggregation more strikingly than others. The patients in whom intravenous PGE₂ successfully went on to induce abortion were compared with those in whom the PGE₂ failed (fig 3). Considering only the five cases in whom the PGE₂ failed to induce abortion, the PGE₂ infusion induced no change in either ADP-induced platelet aggregation or disaggregation. In contrast, in the eight patients in whom the intravenous PGE₂ caused successful abortion, the rate of platelet disaggregation was increased during the PGE₂ infusion as compared with the pre-infusion results in all cases ($P < 0.01$).

Fig 3 The effect of PGE₂ infusion on ADP-induced platelet aggregation in patients where the infusion failed or succeeded in inducing abortion.

PLASMA ANTITHROMBIN (FIG 4)

The mean antithrombin activity before PGE₂ infusion was 103% in eight patients and this rose significantly to 115% during the infusion ($P < 0.05$).

Fig 4 Antithrombin activity before and during PGE₂ infusion ($n = 8$).

EUGLOBULIN LYSIS ACTIVITY

There was a significant rise in mean euglobulin lysis activity from 1.1 units before PGE₂ infusion to 2.3 units during the infusion ($P < 0.05$).

OTHER TESTS OF PLATELET FUNCTION, COAGULATION, AND FIBRINOLYSIS

In eight patients before and during PGE₂ infusion the mean platelet count fell from 246 000/cmm to 242 000/cmm, the maximum collagen-induced platelet aggregation rose from 53 to 58%, and mean platelet factor 3 availability fell from 19.6 to 19.3 seconds. None of these changes were significant.

None of the other tests of coagulation or fibrinolysis altered significantly during the PGE₂ infusion.

SIDE EFFECTS OF PGE₂

A few patients experienced minor side effects but none of sufficient severity to stop the treatment.

Discussion

The finding that intravenous PGE₂ inhibits platelet adhesiveness to cellophane membranes indicates that PGE₂ is capable of influencing platelet function *in vivo*, in man, and is in keeping with the reports of several animal studies. Emmons *et al* (1967) showed that PGE₁ inhibited white thrombus formation in rabbits. Kinlough-Rathbone *et al* (1970) confirmed this and demonstrated that PGE₁ also prolonged

bleeding times and inhibited clot formation at the ends of transected vessels. In rats, PGE_1 inhibited platelet aggregation when given in large doses (Chandra Sekhar, 1967) and a continuous PGE_1 infusion reduced the thrombocytopenia and raised the LD 50 following intravenous ADP injections (Kloeze, 1970a).

In the human subject the effect of prostaglandins on platelets has been less clear cut. Elkeles *et al* (1969) showed that platelet electrophoretic mobility in response to ADP was abolished for up to 30 minutes after cessation of a PGE_1 infusion. Their main problem was that the prostaglandin caused side effects of flushing and constricting pains in the chest, a problem that was not encountered with PGE_2 in this study. On the other hand Karim and Filshie (1972), using Hellem's method, found no change in platelet adhesiveness during PGE_2 infusion, a finding that we confirm in this report. Elkeles *et al* (1969) used Payling Wright's rotating bulb method and, although they found diminished platelet adhesiveness in four out of eight patients given PGE_1 , the effect was not significant.

The difference in platelet adhesiveness to glass beads and to a cellophane membrane is of interest. The glass bead column method of testing platelet adhesiveness is related to the release of ADP and other substances from red cells which have been disrupted. In contrast, retention of platelets in the membrane test cell is apparently independent of haematocrit or exogenous ADP release (Lindsay *et al*, 1973). Since PGE_2 may exert its effect on platelets by inhibiting ADP release, the glass bead method may swamp the effect of PGE_2 on platelets by releasing large amounts of aggregating substances from the red cells. It would appear that, in this situation, the membrane test cell provides a more sensitive index of platelet behaviour than the glass bead technique, and may be useful in the future for the assessment of other antiplatelet drugs.

The normal platelet ADP aggregation during PGE_2 infusion was in keeping with the findings of Carlsson *et al* (1968) who, however, did not report on disaggregation. Our finding of more rapid platelet disaggregation during ADP infusion may reflect either inhibition of release of nucleotides from platelets or that the PGE_2 interferes with the stability of platelet aggregation. The difference in platelet disaggregation between the patients having successful and failed terminations of pregnancy may indicate either a difference in individual patient response or that in some cases the prostaglandin preparation had lost some of its potency.

The mechanism of platelet inhibition by prostaglandins is not clear although evidence is accumulating that they may stimulate cyclic AMP formation

and inhibit the release of ADP and calcium ions from the platelets (Marquis, Vigdahl, and Tavormina, 1969; Vigdahl, Marquis, and Tavormina, 1969; Wolfe and Shulman, 1970; Haslam and Lynham, 1972).

The reason for the rise in antithrombin activity during prostaglandin infusion is uncertain, although it is possible that a rise in antithrombin activity could contribute to an anticoagulant action of prostaglandin. Kloeze (1970b) reported that prostaglandin reduced the strength of clot formation and our finding of raised antithrombin activity could contribute to such an effect. The increase in euglobulin lysis activity was the only evidence that prostaglandin enhanced fibrinolytic activity; euglobulin lysis activity can be raised by non-specific factors such as stress or excitement which may have been responsible for the increased activity, although the prostaglandin itself could have had a direct effect on fibrinolysis.

Studies *in vitro* have shown that prostaglandins can also inhibit clot retraction (Murer, 1971), clot tensile strength (Kloeze, 1970b), and platelet aggregation induced by thrombin, collagen, noradrenaline, and serotonin (Emmons *et al*, 1967). However, the concentration of PG used in these experiments far exceeded the dosage which could be given to patients.

Our studies lend support to the evidence from animal studies *in vivo* that a continuous intravenous infusion of prostaglandin can influence platelet function and might have potential clinical benefit in conditions where platelet-initiated thrombosis is the major problem. However, the hypothesis that a reduction in platelet stickiness may cause therapeutic benefits is still not proved, and it would be important to mount controlled clinical trials to demonstrate the usefulness in the treatment of thrombosis.

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