Oestrogen-associated disease of the renal microcirculation

K. JONES,* P. F. NAISH AND G. M. ABER
Departments of Nephrology and Obstetrics and Gynaecology, North Staffordshire Hospital Centre, Stoke-on-Trent, U.K.

(Received 14 July 1976; accepted 18 August 1976)

Summary

1. The effect of oestrogen-containing compounds on platelet factor 3, platelet life span, renal platelet localization and the fibrinolytic activity of forearm venous blood has been studied in eleven women with the renal vascular abnormalities of the loin pain and haematuria syndrome. The results were compared with those obtained in twenty-nine healthy female volunteer subjects.

2. Platelet factor 3 (PF3) availability was found to be increased in patients with the loin pain and haematuria syndrome during normal menstrual cycles. Oestrogens produced an increase in PF3 availability in both patients and control subjects.

3. Platelet life span was shortened and the index of renal platelet localization increased when patients with the loin pain and haematuria syndrome received oestrogens. No change in platelet kinetics was found in the healthy control subjects receiving oestrogens.

4. The fibrinolytic activity of forearm venous blood, both at rest and after venous occlusion, was increased in the healthy control subjects in response to oestrogens. No such response was seen in the patients with the loin pain and haematuria syndrome.

5. It is suggested that the change in platelet function may be causally related to the intrarenal vascular abnormalities.

6. The possible role of oestrogens in causing these disturbances or unmasking pre-existing abnormalities of platelet function or fibrinolysis is discussed.

Key words: blood vessels, fibrinolysis, kidney, oestrogens, platelets, platelet factor 3.

Abbreviations: LPH syndrome, loin pain and haematuria syndrome; PF3, plasma factor 3.

Introduction

Intrarenal vascular changes have recently been demonstrated by selective renal angiography in adult patients with a syndrome of recurrent loin pain and haematuria (Little, Soper & de Wardener, 1967; Burden, Booth, Ockenden, Boyd, Higgins & Aber, 1975). The syndrome consists of intermittent loin pain with or without haematuria and presents most often in young woman. Intravenous urography is normal and there is no evidence of renal tract obstruction, stones or infection. Histological abnormalities, restricted to mesangial cell proliferation, are slight, although the third component of complement (C3) may be deposited in renal arterioles (Naish, Aber & Boyd, 1975). In some patients the syndrome may be aggravated by oral contraceptives. Similar angiographic appearances have also been found in patients who developed hypertension and/or impairment of renal function whilst taking oral contraceptives (Boyd, Burden & Aber, 1975).

Oestrogen-containing oral contraceptives are known to be associated with an increased...
incidence of thrombo-embolic episodes (Vessey & Doll, 1969). Whilst these predominantly affect the venous circulation, the cerebral (Vessey & Doll, 1969) and coronary arteries (Mann & Inman, 1975) have also been involved. Dugdale & Masi (1971) showed that oestrogens can produce many changes in the factors involved in coagulation, but concluded that the most important were probably related to platelet function. Renaud (1973) has since suggested that this hypercoagulability is related to changes in platelet factor 3 activity, which is known to be enhanced during oral contraceptive therapy (Renaud & Gautheron, 1973).

Although Dugdale & Masi (1971) showed oestrogens to have little effect on the mechanisms involved in fibrinolysis, Hedlin & Monkhouse (1971), Bick & Thompson (1972) and Hedlin (1975) have shown that oestrogens can increase spontaneous fibrinolytic activity. Furthermore, Åstedt, Isacson, Nilsson & Pandolfi (1973) have shown that post-occlusion fibrinolytic activity is defective in women who developed deep venous thrombosis when on oral contraceptives.

We have investigated the possibility that the intrarenal vascular abnormalities in patients with the LPH syndrome might result from disturbances of platelet or fibrinolytic function.

Subjects and methods

Subjects

Healthy women during a normal menstrual cycle. Sixteen healthy women, aged 20–42 years (mean 27 years), were studied. All were normotensive and had no history of vascular or renal disease.

Healthy women during oral contraceptive therapy. Thirteen healthy asymptomatic women, aged 19–30 years (mean 24.5 years), were studied whilst taking oestrogen-containing oral contraceptive preparations over 3 months to 8 years, and four of these women were also studied before commencing oral contraception.

Loin pain and haematuria syndrome. Eleven female patients with the LPH syndrome, aged 19–48 years (mean 32.9 years), were studied. All had typical clinical features and intrarenal vascular abnormalities characteristic of this syndrome (Burden et al., 1975; Naish et al., 1975). Nine were studied whilst not receiving oestrogens, of whom five were restudied 3 months after commencing oral contraceptive therapy. Two other patients presented whilst taking oestrogens for menstrual disturbances. No subject was receiving drugs known to affect platelet behaviour either during, or in the 14 days before, the investigations.

This study was approved by the Ethical Committee of the North Staffordshire Hospital Centre and the MRC Isotopes Advisory Panel, and all subjects gave their informed consent.

Methods

Platelet factor 3 availability. The Stypven clotting time of platelet-rich plasma was used to measure PF3 by the method of Renaud, Gautheron & Rosenstein (1973). Siliconized needles (19 G Butterfly infusion set; Abbott Laboratories) were used for venepuncture, to minimize trauma to the platelets. A platelet count was performed on an aliquot of the platelet-rich plasma. No attempt was made to standardize the platelet count by dilution with platelet-poor plasma, in order to avoid any contamination of the platelet-rich plasma with PF3 released by the high-speed centrifugation in preparation of platelet-poor plasma. Platelet-rich plasma was obtained as the supernatant from centrifuging blood anticoagulated with disodium ethylene diaminetetra-acetate (EDTA) (final concentration 0.01 mol/l) at 200 g for 8 min. Siliconized glassware or plastic was used throughout the isolation and assay.

The estimation was performed in quadruplicate, 0.1 ml of platelet-rich plasma and 0.1 ml of NaCl solution (0.15 mol/l; saline) adjusted to pH 7.3 with imidazole buffer (Spaet & Cintron, 1965) being used in plastic cuvettes. After incubation at 37°C for 10 min, 0.1 ml of CaCl2 (0.02 mol/l) and 0.1 ml of Russell's viper venom (1/100 000 in saline; Wellcome Reagents Ltd) were blown into the cuvette simultaneously, and the clotting time was recorded by the tilt-tube method.

This test was performed on days 3–5 (follicular) and days 21–24 (luteal) of normal menstrual cycles in healthy women, and on days 21–24 of oral contraceptive-treated cycles (i.e. days 17–20 of oral contraceptive therapy). The patients with the LPH syndrome were studied in days 21–24 of all cycles. All other tests were performed simultaneously unless otherwise stated.
Platelet survival. Autologous platelets were labelled in vitro with Na$_2^{51}$CrO$_4$ (Dacie & Lewis, 1968) at 20°C, with a Fenwal Triple Platelet Pack (J2A-35) containing additional citric acid, sodium citrate and glucose in one of the transfer packs to improve the platelet viability (Morrison & Baldini, 1967). Venous blood samples were obtained at 1–2 h after the infusion of the labelled platelets, and then daily for 7 days, silicon-treated needles being used, and anticoagulated with EDTA. The platelets were extracted by differential centrifugation and washing with saline (Aster & Jandl, 1964), and the radioactivity was counted in a Gamma Set 500 well-type scintillation counter (ICN Tracerlab Division).

The radioactivity of the samples was expressed as a percentage of that in the sample with the highest radioactivity. The life span of the labelled platelets was calculated from the disappearance curves (Harker & Finch, 1969), and also by using regression analysis to determine whether the disappearance of circulating platelet radioactivity was linear or exponential. Platelet survival studies were confined to the immediate post-menstrual phase, so that circulating radioactivity was minimal by the time of ovulation.

Renal platelet localization. Surface radioactivity was counted 3 h after infusion of the labelled platelets, and daily for 5 days, over the heart, spleen, liver and kidneys with a J and P Engineering MS310 counter, a 5 cm crystal, with a 16 cm collimator. It was found early in the study that interference in the kidney radioactivity counts from the liver and spleen could be minimized by positioning the centre of the aperture 3 cm below the renal angles. Renal platelet localization was calculated from the ratio: (right kidney/spleen) at half time of platelet survival/(right kidney/spleen) at day zero (Clark, Lewis, Cameron & Parsons, 1975).

Forearm fibrinolytic capacity. Venous blood was taken from an antecubital vein in each forearm, before and after venous occlusion (Robertson, 1971), into trisodium citrate (0·13 mol/l). The euglobulin fraction was precipitated (Brakman, Albrechtson & Astrup, 1966) and resuspended in sodium barbitone (0·02 mol/l) adjusted to pH 7·3 with HCl (1·0 mol/l) before determining the fibrinolytic activity on an unheated fibrin plate.

The fibrin plate (Walton 1966) was modified as follows: (a) agarose (BDH Ltd), 2 g/100 ml; (b) human fibrinogen (Kabi Pharmaceuticals Ltd); (c) thrombin (Parke Davis and Co.), 10 units/ml of CaCl$_2$ (0.025 mol/l) in NaCl (0·15 mol/l); (d) 5 ml volumes of agarose and fibrinogen were mixed and 0·5 ml of thrombin solution was added before pouring on to a 8·5 cm x 8·5 cm glass plate (wells of 4 mm diameter were cut on to the fibrin/agarose plate to contain samples (15 µl) of resuspended euglobulin or urokinase); (e) urokinase reference standard (Leo Pharmaceutical Products, Denmark), 50, 12·5 and 3·125 Ploug units/ml; (f) duplicates of the urokinase solutions and the pre- and post-occlusion euglobulin samples were applied to the wells, and the plates incubated for 18 h at 37°C. The mean diameters of the lysed zones were measured. A standard urokinase curve was prepared. The diameter of the lysed zone was found to be a linear function of the log of the urokinase concentration, and the activity of the euglobulin fraction was obtained from this relationship.

Fibrinogen and fibrinogen/fibrin-degradation products. Fibrinogen and fibrin-degradation products were measured immunonephelometrically (Farrell & Wolf, 1972). Thrombin-clottable fibrinogen was also measured by a modified method of Farrell & Wolf (1973). The fibrin clot formed from 0·5 ml of citrated plasma was harvested, washed in distilled water and acetone dried and weighed.

Haematology and biochemistry. Platelet counts on whole blood and platelet-rich plasma were performed with a Technicon electronic particle counter. Blood chylomicrons, β-lipoproteins and pre-β-lipoproteins were measured nephelometrically (Stone & Thorp, 1966). Triglycerides and cholesterol were measured enzymatically (Boehringer) as described by Wahlfield (1974) and Roeschlau, Bernt & Gruber (1974). These measurements were made after a 15 h fast, at the same time as blood was taken for PF3 and fibrinolytic activity measurements.

Statistical methods. Comparison of results was performed by the Wilcoxon rank sum test. Regression analysis was used to check possible correlation between factors within any individual group.

Results

The values obtained in patients with the LPH syndrome were obtained during the luteal phase.
of the menstrual cycle and were compared with results in normal subjects obtained during the same period.

Stypven clotting time (Fig. 1)

There was no significant difference between the mean Stypven clotting time of platelet-rich plasma found in the follicular phase (48.7 SEM 0.9 s), and that found in the luteal phase (48.2 SEM 1.1 s) of a normal menstrual cycle in the healthy control subjects. The use of oral contraception by healthy women was associated with a significant reduction of the mean Stypven clotting time to 41.3 (SEM 1.0) s $\left(P < 0.01\right)$.

The patients with the LPH syndrome had a significantly reduced Stypven clotting time (43.2 SEM 1.2 s; $P < 0.05$) during the luteal phase of a normal menstrual cycle, when compared with the luteal phase of a similar cycle in a normal subject. This value was further significantly reduced (38.7 SEM 0.7 s; $P < 0.01$) by the use of oral contraception. The values found in these patients whilst taking oral contraceptives were, however, not significantly different from those found in healthy women using oral contraception.

There were no differences in the platelet count of whole blood or platelet-rich plasma between any of the groups studied (Table 1).

![Fig. 1. Stypven clotting time of platelet-rich plasma during normal menstrual cycles (○) and during the use of oestrogen-containing oral contraceptives (●). fol. = follicular phase; lut = luteal phase.](image-url)
Oestrogens and renal vascular disease

Furthermore, the Stypven clotting time was not related to age in any of the groups.

**Platelet survival** (Fig. 2)

The mean platelet survival time in the healthy women during normal menstrual cycles (8.4 SEM 0.3 days) was similar to that reported by others. Oral contraception did not cause any significant alteration in platelet survival time (8.5 SEM 0.3 days) in healthy women. The patients with the LPH syndrome also had comparable results during normal menstrual cycles (8.8 SEM 0.4 days), but oral contraception produced a significant reduction in platelet survival in these patients (5.9 SEM 0.6 days; P<0.02).

The platelet disappearance curve was linear in all the healthy control subjects and in the patients with the LPH syndrome during normal menstrual cycles. However, four of the six patients with LPH syndrome who used oral contraception had exponential platelet disappearance curves.

**Renal platelet localization** (Fig. 3)

The ratio of renal platelet localization in healthy women during a normal menstrual cycle was found to be 0.78 (SEM 0.05). No difference in this ratio was found during oral contraception in healthy women (0.77 SEM 0.06). The mean ratio in the patients with the LPH syndrome during normal menstrual cycles (0.97 SEM 0.14) did not differ significantly from the ratio in healthy women. However, the two patients in this group with the higher ratios both had episodes of loin pain during the period of the study. The platelet localization ratio in those patients with the LPH syndrome who used oral contraception (1.27 SEM 0.16) was significantly higher than the ratio in healthy women receiving oral contraception (P<0.05).
Fibrinolytic activity (Fig. 4)

No difference in the resting fibrinolytic activity was found between the follicular phase (756 SEM 77 urokinase units/l of plasma) and the luteal phase (875 SEM 154 urokinase units/l of plasma) of a normal menstrual cycle in healthy women. The post-occlusion fibrinolytic activity of the luteal phase (2759 SEM 288 urokinase units/l of plasma) was significantly higher than that found in the follicular phase (1898 SEM 297 urokinase units/l of plasma; P < 0.02). There was no significant difference in values in patients with the LPH syndrome during the luteal phase of normal menstrual cycles (resting 783 SEM 100; post-occlusion 2957 SEM 341 units/l of plasma).

Oral contraception, however, invoked quite different responses in the two groups. Both the testing activity (1671 SEM 175 units/l of plasma) and the post-occlusion activity (4759 SEM 401 units/l of plasma) rose significantly (P < 0.01) in healthy women using oral contraception. In contrast, the patients with the LPH syndrome showed no significant rise in resting activity (1327 SEM 197 units/l of plasma), and post-occlusion activity (2836 SEM 498 units/l of plasma) was less than when they were not taking oral contraceptives. No correlation was found between age and the level of fibrinolytic response in any of the groups studied.

Blood lipids and lipoproteins (Table 2)

There were no significant differences in the plasma concentrations of chylomicrons, β-lipoproteins or cholesterol in the groups studied. The mean plasma triglyceride concentration in the healthy women taking oral contraceptives (1.03 SEM 0.14 mmol/l) was higher than that in healthy women during normal menstrual cycles (0.65 SEM 0.10 mmol/l; P < 0.05). The mean concentration in patients with the LPH syndrome during normal menstrual cycles (1.12 SEM 0.18 mmol/l) was also significantly higher than that in healthy women (P < 0.05). There was no further significant rise in response to oral contraception, although the mean concentration (1.34 SEM 0.13 mmol/l) was significantly higher than that of the healthy women taking oral contraceptives (P < 0.05).

Similar changes were found in the plasma concentrations of pre-β-lipoproteins. Oral contraception induced a significant increase in the plasma concentrations of the healthy women (0.39 SEM 0.12 g/l to 0.76 SEM 0.11 g/l; P < 0.02).
**Table 2. Serum lipids and lipoproteins**

Results are shown as mean values ± SEM with the number of measurements in parentheses.

<table>
<thead>
<tr>
<th></th>
<th>Chylomicrons (g/l)</th>
<th>Triglycerides (mmol/l)</th>
<th>Pre-β-lipoproteins (g/l)</th>
<th>β-Lipoproteins (g/l)</th>
<th>Cholesterol (mmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy women</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal menstrual</td>
<td>0.042±0.005 (9)</td>
<td>0.67±0.07 (9)</td>
<td>0.28±0.06 (9)</td>
<td>3.89±0.17 (9)</td>
<td>4.98±0.20 (9)</td>
</tr>
<tr>
<td>cycle (Follicular)</td>
<td>0.028±0.007 (14)</td>
<td>0.65±0.10 (14)</td>
<td>0.39±0.12 (14)</td>
<td>3.88±0.21 (14)</td>
<td>5.00±0.25 (14)</td>
</tr>
<tr>
<td>cycle (Luteal)</td>
<td>0.045±0.009 (10)</td>
<td>1.03±0.14 (10)</td>
<td>0.76±0.11 (10)</td>
<td>4.01±0.35 (10)</td>
<td>5.23±0.44 (10)</td>
</tr>
<tr>
<td>Oral contraception</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LPH syndrome</td>
<td>0.038±0.011 (6)</td>
<td>1.12±0.18 (6)</td>
<td>0.97±0.16 (6)</td>
<td>4.52±0.34 (6)</td>
<td>5.90±0.44 (6)</td>
</tr>
<tr>
<td>Normal menstrual</td>
<td>0.036±0.005 (5)</td>
<td>1.34±0.13 (5)</td>
<td>1.28±0.17 (5)</td>
<td>4.48±0.26 (5)</td>
<td>5.94±0.31 (5)</td>
</tr>
<tr>
<td>cycle (Luteal)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oral contraception</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
TABLE 3. Serum fibrinogen and plasma fibrin-degradation products

Patients with the LPH syndrome had a mean plasma concentration (0.97 SEM 0.16 g/l) which was significantly higher than that of the healthy women during normal menstrual cycles ($P < 0.01$). No further significant increase was found in response to oral contraception, although the mean concentration (1.28 SEM 0.17 g/l) was still higher than that of the healthy women using oral contraception ($P < 0.05$).

Variations within individual groups of women were not related to age differences, and no significant correlation was found between the concentrations of triglyceride or pre-$\beta$-lipoproteins and the Stypven clotting time of platelet-rich plasma.

Fibrinogen and fibrin-degradation products (Table 3)

No difference was noted between the concentrations of plasma fibrinogen or serum fibrin-degradation products in any of the groups studied, irrespective of whether the measurements were carried out in the follicular or luteal phase of the menstrual cycle. Likewise, oral contraceptive therapy did not alter these results in any particular group, or between groups. Furthermore, the relationship between immunoreactive and clottable fibrinogen remained comparable in all phases of the study.

Discussion

This study describes abnormalities of platelet function, platelet kinetics and the fibrinolytic response to venous occlusion in a group of patients with radiological evidence of intrarenal vascular abnormalities.
in contrast, the patients with renal vascular disease taking oestrogens also had significant shortening of their platelet life span. We can speculate that both the increase in renal platelet localization and the shortened platelet life span might be secondary to pre-existing vascular disease, but the fact that neither occurred in symptom-free patients who were not taking oestrogens, despite radiological evidence of renal vascular disease in these patients, suggests that this is unlikely to be the only explanation.

Plasma fibrinogen showed no significant differences between any of the groups and the immunoreactive fibrinogen was always comparable with the thrombin clottable fibrinogen. Although this inter-relationship is probably not as sensitive as a radioactivity-labelled fibrinogen survival, it probably indicates that there was no significant consumption of fibrinogen in any of the groups studied (Wolf, Farrell & Walton, 1972). Furthermore, the deposition of fibrin is not a common histological or immunofluorescent feature in renal biopsies from patients with the LPH syndrome (Burden et al., 1975; Naish et al., 1975). Concentrations of fibrin-degradation products in normal serum may also be too low to be measured by our method, but nonetheless there were no significant differences in these between any of the groups studied.

There was no difference between either the resting or post-occlusion fibrinolytic activity of forearm venous blood in patients with renal vascular disease, compared with healthy individuals during normal menstrual cycles. Oestrogen therapy produced a modest elevation in resting and a marked increase in post-occlusion fibrinolytic activity in healthy women, as others have found (Hedlin & Monkhouse, 1971; Bick & Thompson, 1972; Hedlin, 1975). In contrast, oestrogens failed to influence either the resting or post-occlusion fibrinolytic activity in patients with renal vascular disease. Without information on the fibrinolytic activity of the vein wall itself, or on the concentrations of inhibitors of both plasminogen activator and plasmin, these different responses to oestrogens are difficult to interpret. Further confusion in interpretation of these results arises from the observation of Åstedt (1971) that although ethynloestradiol produced a significant increase in fibrinolytic activity of forearm venous blood after venous occlusion, the fibrinolytic activity of the vein wall itself was reduced.

Impaired fibrinolytic response to a number of stimuli has been reported in a group of patients by Cash and his colleagues (Cash, 1966; Cash & Allen, 1967), who suggested that these patients might be vulnerable to occlusive vascular disease. Similar proposals have been made in relation to deep-vein thrombosis (Menon, McCollum & Gibson, 1971; Åstedt et al., 1973) and coronary artery disease (Chakraborti, Hocking, Fearnley, Mann, Attwell & Jackson, 1968). We are unaware of any reports of spontaneous elevation of PF3 availability at the same time as depression of fibrinolytic capacity in the same individuals. One must, however, exercise caution before concluding that the present findings relating to PF3 availability and disturbance in fibrinolytic activity represent the primary pathogenesis of the intrarenal vascular abnormalities. Similarly, these abnormalities in forearm blood fibrinolytic activity may not necessarily reflect any disturbance of intrarenal fibrinolysis. Likewise, our results do not separate the influence of oestrogens as a direct or indirect effect on platelets, endothelium or other clotting factors, or whether these drugs are just unmasking a group of patients who have an inherent abnormality of platelet function and fibrinolytic capacity. The fact that the present findings are common to patients who have developed vascular lesions without necessarily taking oestrogen-containing compounds lends some support to the latter view.

These findings could have important therapeutic implications in this clinical disorder, which is partly characterized by severe renal pain which hitherto has often been difficult to control, despite withdrawal of oestrogen-containing drugs. Initial experience with platelet suppressive agents such as aspirin has been encouraging, but more extended studies with such agents and possibly the use of compounds aimed at long-term stimulation of fibrinolysis are required. Serial radiological studies will be needed to find if this form of therapy can favourably influence the abnormal angiographic findings.

Acknowledgments

We are extremely grateful for the co-operation of the patients, the healthy volunteers and the staff of the Renal Unit of the North Staffordshire Royal Infirmary. We are indebted to Mrs
E. Ilderton, and the departments of Haematology, Biochemistry and Radioisotopes for technical assistance. We thank Mrs S. Cooper for secretarial help, Mr D. Thompson for statistical advice, and the Research Committee of the North Staffordshire Medical Institute and the West Midlands Regional Health Authority for financial support.

References