Changes in thrombin-stimulated platelet malondialdehyde production during the menstrual cycle

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SUMMARY Forty normal women had thrombin-stimulated platelet malondialdehyde (MDA) production measured during their menstrual cycle. Twenty women in this group were taking the combined oral contraceptive pill (OCP). Platelet MDA production was found to fall by 30% during normal menstruation and the week when the subjects were not taking a combined OCP, but it remained constant throughout the remainder of the cycle. No significant change in initial platelet aggregation response to stimulation by thrombin, change in plasma thrombin clotting time, plasma heparin neutralising activity (HNA), or plasma antithrombin III (AT-III) activity was seen when the platelet MDA production was reduced. The bleeding time results showed some variation throughout the menstrual cycle but these did not appear to be related to the variation in platelet MDA production.

MDA (malondialdehyde) is a byproduct of prostaglandin metabolism and it has been used as an indicator of the activity of the enzyme cyclo-oxygenase in platelets. The enzyme is irreversibly inhibited by aspirin and this provides a means of measuring the platelet regeneration time after aspirin ingestion.1 When MDA production in appropriately stimulated platelets after aspirin ingestion reaches the pre-aspirin level, it is assumed that the entire population of aspirin-inhibited platelets has been removed from the circulation and replaced by new platelets. The reliability of this method of measuring platelet regeneration time depends on a steady platelet MDA production over the required period.

This study was stimulated by a report that the activity of the enzymes involved in platelet prostaglandin and thromboxane synthesis might vary throughout the menstrual cycle and be influenced by the OCP (oral contraceptive pill).2 We therefore decided to investigate whether a similar variation occurred in platelet MDA production both in users and non-users of the OCP during the menstrual cycle.

Subjects and methods

Twenty “non-pill” users and 20 taking the combined OCP gave fully-informed consent to having blood taken one and two days before menstruation and then on days 1-7, 14 and 21 of the menstrual cycle, for the measurement of platelet MDA concentrations. Bleeding times were measured on the same days in 10 of the “non-pill” and 10 of the “pill” users. The platelet aggregation response to thrombin, plasma heparin neutralising activity (HNA), and plasma antithrombin III (AT III) activity were measured in these women one and two days before menstruation and on days 1-7 of the menstrual cycle.

All the “non-pill” users had a normal cycle with no history of menorrhagia or bleeding disorders. Of the 20 “pill” users, 10 were taking Eugynon-30 (ethinylestradiol 30 μg, L-norgestrel 250 μg), two were taking Eugynon-50 (ethinylestradiol 50 μg, L-norgestrel 500 μg), three were taking Ovranette (ethinylestradiol 30 μg, L-norgestrel 150 μg), three were taking Microgynon (ethinylestradiol 30 μg, L-norgestrel 150 μg), one was taking Ovulen-50 (ethinylestradiol 50 μg, ethynodiol diacetate 1 mg) and one was taking Orthonovin 1/50 (mestranol 50 μg, norethisterone 1 mg). All had been on these
preparations for more than six months and none of the women in either group was taking any other medication. All the women were between the ages of 22 and 30 yr except for one (44 yr) in the "non-pill" group (Table 1).

Table 1  Mean ages and range (yr) in women on (20) and off (20) the combined OCP

<table>
<thead>
<tr>
<th></th>
<th>&quot;Non-pill&quot; (yr)</th>
<th>&quot;Pill&quot; (yr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age</td>
<td>28·5</td>
<td>24·05</td>
</tr>
<tr>
<td>Range</td>
<td>22-44</td>
<td>22-29</td>
</tr>
</tbody>
</table>

All the blood samples were taken at approximately the same time each day. A total of 30 ml of non-fasting venous blood was removed and anticoagulated with 3·8 % sodium citrate solution (1 vol of citrate to 9 vol of blood). This was centrifuged at 200 g for 10 min at room temperature to obtain platelet-rich plasma. The supernatant was removed and the remainder of the blood centrifuged at 1400 g for 15 min to obtain platelet-poor plasma.

MEASUREMENT OF PLATELET MDA PRODUCTION

The platelet count of the platelet-rich plasma was estimated using a Coulter Thrombocounter. 50 μl thrombin (25 U/ml) and 50 μl streptokinase (2500 U/ml) were added to Eppendorf tubes placed in a water bath at 37°C. 400 μl of platelet-rich plasma was added and the tubes mixed at three-minute intervals. The reaction was stopped after 15 min by the addition of 500 μl of 10% solution of trichloroacetic acid. The tubes were centrifuged at 9000 g for two minutes in an Eppendorf 3200 centrifuge. A 400 μl aliquot of the supernatant was placed for 30 min in a water bath at 70°C with 400 μl of 0·53% solution of thiobarbituric acid in water. Deionised water (1·2 ml) was then added to each sample and the fluorescence of the thiobarbituric acid-malondialdehyde complex was read in a spectrofluorimeter at an excitation wavelength of 510 nm and emission wavelength of 553 nm. The results were read off a standard curve which was obtained by using MDA solutions in the range 0-1000 pmol/ml instead of platelet-rich plasma, but omitting the thrombin and streptokinase incubation step. The sample fluorescence was then converted into pmol MDA/10^9 platelets.

Antithrombin III activity was estimated by a chromogenic substrate method.

Heparin neutralising activity of plasma was estimated as described by Donati et al.

Platelet aggregation to thrombin and thrombin clotting time. A Payton dual channel aggregometer at 37°C was used. The platelet count of the platelet-rich plasma was adjusted to 200 000-250 000 mm^3 (200-250 × 10^9/l) and the platelets were then aggregated with thrombin using a final concentration 0·2 U/ml. The rate and maximum of aggregation was measured. The thrombin clotting time was estimated from the time lapse between the addition of thrombin to platelet-rich plasma and the appearance of fibrin in the cuvette causing a sharp increase in optical density of the plasma.

Bleeding time. This was measured by the Template method using a Simplate bleeding time device (General Diagnostics).

Results

PLATELET MDA PRODUCTION

Table 2 shows that the amount of MDA produced by platelets stimulated with thrombin fell by approximately 30% during menstruation in both "non-pill" users and those on the combined OCP. These values had returned to premenstrual concentrations by the seventh day of the menstrual cycle and no further fluctuations were seen during the rest of the cycle. This table also shows that the absolute amount of

Table 2  Percentage change in MDA production by thrombin-stimulated platelets during menstrual cycle (Mean ± SEM)

<table>
<thead>
<tr>
<th></th>
<th>Pre</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 5</th>
<th>Day 6</th>
<th>Day 7</th>
<th>Day 8</th>
<th>Day 9</th>
<th>Day 10</th>
<th>Day 11</th>
<th>Day 12</th>
<th>Day 13</th>
<th>Day 14</th>
<th>Day 15</th>
</tr>
</thead>
<tbody>
<tr>
<td>&quot;Non-pill&quot; group</td>
<td>n</td>
<td>20</td>
<td>18</td>
<td>19</td>
<td>15</td>
<td>11</td>
<td>16</td>
<td>17</td>
<td>14</td>
<td>16</td>
<td>15</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>mean</td>
<td>78·1</td>
<td>69·8</td>
<td>70·4</td>
<td>82·8</td>
<td>91·1</td>
<td>98·9</td>
<td>99·7</td>
<td>101·9</td>
<td>100·8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(%)</td>
<td>(257·1)*</td>
<td>±28·5</td>
<td>±3·5</td>
<td>±4·0</td>
<td>±4·8</td>
<td>±3·7</td>
<td>±0·6</td>
<td>±0·4</td>
<td>±1·3</td>
<td>±0·73</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>20</td>
<td>15</td>
<td>15</td>
<td>17</td>
<td>15</td>
<td>17</td>
<td>10</td>
<td>18</td>
<td>18</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>mean</td>
<td>82·1</td>
<td>73·3</td>
<td>68·7</td>
<td>79·0</td>
<td>92·7</td>
<td>99·3</td>
<td>99·9</td>
<td>100·0</td>
<td>102·1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(%)</td>
<td>(251·3)*</td>
<td>±21·3</td>
<td>±3·6</td>
<td>±4·4</td>
<td>±5·6</td>
<td>±2·6</td>
<td>±0·9</td>
<td>±0·2</td>
<td>±1·45</td>
<td>±1·6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

n = number of women.

*Mean total amount of MDA premenstrually in pmol/10^9 platelets.
MDA produced by thrombin-stimulated platelets did not differ between the two groups.

Table 3 shows that the results for both HNA and AT III varied very little during menstruation. Platelet aggregation responses to thrombin and the thrombin clotting time also showed no significant change. The values of AT III are shown for each group separately since it has been reported that these values are influenced by the OCP. However, there was no significant difference between the two groups. This was also true for the other results shown in this table and therefore these have not been separated.

The Figure illustrates the bleeding time throughout the menstrual cycle in the “non-pill” users. All the results were within the normal range for the Template method (3-9 min). A variation throughout the cycle was noted but the results could not be correlated with the change in platelet MDA concentrations. The bleeding times in those on the combined OCP showed less variation and were generally slightly longer than in the other group but still within the normal range.

Discussion

Our study shows that the amount of platelet MDA produced after stimulation with thrombin decreases during the first three days of menstruation, then gradually returns to premenstrual values by day 7 and remains fairly constant for the rest of the cycle. A remarkably similar pattern is observed in the “pill” users suggesting that the decrease in the first week of the cycle may not be a direct result of hormonal influences, but may be related to uterine bleeding.

Survival of \(^{51}\)chromium-labelled platelets in

“non-pill” users during menstruation was found to be normal and no excess radioactivity could be demonstrated in the menstrual fluid. This suggests that if any platelets were taking part in haemostatic events in the menstruating uterus, they might have been reabsorbed from the uterus into the general circulation without destruction. Alternatively, the number of platelets taking part in uterine haemostasis might be insignificant. The role of platelets in uterine haemostasis is not doubted as menstruation is a common problem in both quantitative and qualitative platelet disorders. The scarcity of platelets in the histological examination of the menstruating uterus might be due to the reversibility of platelet involvement in uterine haemostasis. That

Table 3  Results of measurements on plasma heparin neutralising activity (HNA), antithrombin III activity (AT III), platelet aggregation response to thrombin and thrombin clotting time in twenty women (“pill” group = 10, “non-pill” group = 10)

<table>
<thead>
<tr>
<th></th>
<th>Pre</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 5</th>
<th>Day 6</th>
<th>Day 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>*HNA n = 20</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean</td>
<td>0-12</td>
<td>0-12</td>
<td>0-14</td>
<td>0-13</td>
<td>0-13</td>
<td>0-16</td>
<td>0-13</td>
<td>0-10</td>
</tr>
<tr>
<td>SEM</td>
<td>±0-01</td>
<td>±0-02</td>
<td>±0-03</td>
<td>±0-01</td>
<td>±0-01</td>
<td>±0-02</td>
<td>±0-01</td>
<td>±0-01</td>
</tr>
<tr>
<td>†AT III</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&quot;Non Pill” n = 10</td>
<td>mean</td>
<td>100-8</td>
<td>113-0</td>
<td>105-8</td>
<td>102-7</td>
<td>105-3</td>
<td>104-5</td>
<td>105-0</td>
</tr>
<tr>
<td>&quot;Pill” n = 10</td>
<td>mean</td>
<td>106-4</td>
<td>93-6</td>
<td>113-5</td>
<td>104-0</td>
<td>105-3</td>
<td>111-8</td>
<td>110-5</td>
</tr>
<tr>
<td>SEM</td>
<td>±7-5</td>
<td>±8-9</td>
<td>±14-5</td>
<td>±10-7</td>
<td>±6-4</td>
<td>±7-7</td>
<td>±7-5</td>
<td>±14-8</td>
</tr>
<tr>
<td>‡Thrombin clotting time</td>
<td>mean</td>
<td>87-4</td>
<td>80-4</td>
<td>83-56</td>
<td>89-4</td>
<td>94-8</td>
<td>87-5</td>
<td>84-6</td>
</tr>
<tr>
<td>n = 20</td>
<td>SEM</td>
<td>±4-28</td>
<td>±2-83</td>
<td>±7-79</td>
<td>±11-1</td>
<td>±13-2</td>
<td>±11-6</td>
<td>±11-9</td>
</tr>
<tr>
<td>‡Aggregation to thrombin rate</td>
<td>mean</td>
<td>186-7</td>
<td>203-8</td>
<td>180-4</td>
<td>213-0</td>
<td>197-4</td>
<td>201-9</td>
<td>188-8</td>
</tr>
<tr>
<td>n = 20</td>
<td>SEM</td>
<td>±6-84</td>
<td>±12-05</td>
<td>±12-3</td>
<td>±10-14</td>
<td>±19-54</td>
<td>±3-25</td>
<td>±25-25</td>
</tr>
<tr>
<td>Tmax</td>
<td>mean</td>
<td>40-4</td>
<td>37-8</td>
<td>32-4</td>
<td>38-7</td>
<td>33-9</td>
<td>38-3</td>
<td>35-5</td>
</tr>
<tr>
<td>SEM</td>
<td>±2-84</td>
<td>±4-37</td>
<td>±4-77</td>
<td>±5-28</td>
<td>±7-33</td>
<td>±4-27</td>
<td>±13-0</td>
<td>±3-99</td>
</tr>
</tbody>
</table>

n = number of women.
*Units of heparin neutralised by 1 ml of platelet-free plasma.
†Percentage of normal activity.
‡In seconds after addition of 0-2 U/ml thrombin (final concentration).
§Rate in mm/min. Tmax relative increase in optical density using 0-2 U/ml thrombin (final concentration).
this involvement includes platelet granular secretion is suggested by a high concentration of \( \beta \)-thromboglobulin in the menstrual fluid (Foley ME, unpublished observations). Experiments on animals suggest that platelets depleted of their granular stores through secretion might continue to circulate and survive normally.9

It has also been suggested that once platelets have been stimulated by thrombin (being the most likely candidate to stimulate platelets in the menstruating uterus), their receptors for thrombin are irreversibly altered.9 However, in our experiments platelets aggregated normally with low concentrations of thrombin, and it is unlikely that the decrease of platelet MDA production on stimulation by thrombin was due to alteration in the relevant surface receptors. Normal plasma antithrombin III concentrations and normal plasma thrombin clotting time during menstruation suggest that the plasma had no significantly increased capacity to inhibit thrombin.

The reason for the decrease of MDA production during menstruation is at present unresolved. However it is of practical importance to note that the aspirin method for platelet survival measurements in women can only be used between menstrual periods.

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References


Requests for reprints to: Dr H Tindall, Department of Medicine, The Martin Wing, Leeds General Infirmary, Great George Street, Leeds LS1 3EX, England.
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