Platelet derived malonyldialdehyde production in patients with thalassaemia major

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SUMMARY  Malonyldialdehyde, a product of membrane lipid peroxidation, was measured in the platelets of 16 normal subjects after stimulation with a variety of aggregating and stimulating agents. Nethylnmaleimide and hydrogen peroxide generated the largest amounts of malonyldialdehyde. These agents were used to stimulate platelets from 11 patients with thalassaemia major suffering from iron overload due to repeated transfusion. Mean malonyldialdehyde concentrations were the same in normal subjects as in thalassaemic patients, but high concentrations were recorded in patients with severe iron overload. There was a highly significant correlation between malonyldialdehyde and serum ferritin concentrations in all thalassaemic patients. Platelet derived malonyldialdehyde may be a useful test of continuing membrane damage in patients with iron overload.

Lipid peroxidation of cell membranes is associated with a number of pathological conditions, such as radiation damage to the lungs, liver injury, atherosclerosis, and thrombosis and many inflammatory processes. Malonyldialdehyde, a stable secondary product of lipid peroxidation, can be measured after oxidant stress. With this technique red cells have been shown to be more susceptible to autoxidation in autoimmune haemolytic anaemia and thalassaemia major. Iron salts are among the most potent catalysts of lipid peroxidation, and high red cell malonyldialdehyde concentrations found in patients who have had multiple transfusions may reflect the damage to other organs caused by excess iron. In patients with thalassaemia major it is difficult to correlate red cell malonyldialdehyde concentrations with membrane damage in other tissues as many of these cells are transfused, producing inappropriately low values, and thalassaemic red cell membranes contain large amounts of lipid susceptible to autoxidation compared with normal cells.

Malonyldialdehyde is also formed in platelets during the non-enzymatic peroxidation of polyunsaturated fatty acids and enzymatically during prostaglandin production. There has been one previous report of abnormal platelet function in thalassaemia major. We have therefore investigated malonyldialdehyde generation in the platelets of normal subjects and repeatedly transfused patients with beta-thalassaemia major on exposure to oxidant stress with hydrogen peroxide and on activation with nethylnmaleimide, an agent used to assess platelet activation in vitro. We have also correlated platelet malonyldialdehyde production with the degree of iron overload assessed by serum ferritin.

Material and method

Twenty millilitres of venous blood was collected into 3-13% sodium citrate and centrifuged at 150 g for 15 min at 20°C. The supernatant platelet poor plasma was then inverted and centrifuged at 2000 g for 15 min at 4°C. The supernatant was read at 532 nm against the appropriate blank, and the concentration of malonyldialdehyde was calculated as follows:

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nmol malondialdehyde per 10⁶ platelets = \frac{A \cdot Vf}{N \cdot Vi \cdot E} \times 10^P

where A = optical density at 532 nm, Vf = final volume (1 ml), Vi = initial volume (0-9 ml), N = platelet count (platelet rich – platelet poor plasma), and E = molar extinction coefficient of malondialdehyde (1.55 × 10⁵).

PATIENTS
Malondialdehyde concentrations were measured in 16 healthy volunteer subjects (age range 18–30 years) and 11 patients with transfusion dependent thalassaemia major (age range 16–25 years) who were attending the thalassaemia clinic at University College Hospital. Serum ferritin concentrations were measured in samples taken on the same day as the malondialdehyde measurements were performed.

Results
Malondialdehyde generation from platelets was measured in 16 normal volunteers after stimulation with one of the above agents. These induced different concentrations in normal subjects: adenosine diphosphate, adrenaline and collagen induced low concentrations; ristocetin gave higher values; and hydrogen peroxide and nethylmaleimide induced the highest values (Table 1).

Nethylmaleimide and hydrogen peroxide were then used to generate malondialdehyde in 11 patients with \( \beta \)-thalassaemia major who had had multiple transfusions. Both agents stimulated malondialdehyde production in all patients and there was no significant difference between mean malondialdehyde concentrations derived from thalassaemic and normal platelets, but the range of values for the patients with thalassaemia was increased (Table 2).

The level of nethylmaleimide and hydrogen peroxide stimulated malondialdehyde production was then compared with serum ferritin concentrations in 10 patients (Figure). There was a highly significant correlation between malondialdehyde concentrations and serum ferritin concentrations when either stimulant was used: for nethylmaleimide \( r = 0.84 \) (\( p < 0.001 \)) and for hydrogen peroxide \( r = 0.90 \) (\( p < 0.01 \)). Three patients with substantially raised serum ferritin concentrations had malondialdehyde values outside the normal range (> ±2 SD for 16 normal subjects). These patients were all heavily iron overloaded and complied poorly with desferrioxamine. All had strikingly abnormal liver function tests, were heavily pigmented, and two had signs of compromised cardiac function.

<table>
<thead>
<tr>
<th>Stimulating agent (concentration)</th>
<th>Malondialdehyde concentration (nmol/10⁶ platelets) ±2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nethylmaleimide (1 mM)</td>
<td>1.05 ± 0.25*</td>
</tr>
<tr>
<td>Hydrogen peroxide (10 mM)</td>
<td>0.85 ± 0.33</td>
</tr>
<tr>
<td>Ristocetin (12 mg/ml)</td>
<td>0.46 ± 0.04</td>
</tr>
<tr>
<td>ADP (10 μM)</td>
<td>0.14 ± 0.02</td>
</tr>
<tr>
<td>Adrenaline (1 mg/ml)</td>
<td>0.08 ± 0.02</td>
</tr>
<tr>
<td>Collagen (1 μg/ml)</td>
<td>0.05 ± 0.01</td>
</tr>
</tbody>
</table>

*Values given as mean ± 2 SD

Table 2. Malondialdehyde (nmol/10⁶ platelets) generated from platelets from patients with \( \beta \)-thalassaemia major

<table>
<thead>
<tr>
<th>Patient no</th>
<th>Nethylmaleimide (1-0 mM)</th>
<th>Hydrogen peroxide (10 mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.09 ± 0.06*</td>
<td>0.78 ± 0.15</td>
</tr>
<tr>
<td>3</td>
<td>1.78 ± 0.11</td>
<td>1.10 ± 0.23</td>
</tr>
<tr>
<td>4</td>
<td>2.61 ± 0.02</td>
<td>1.75 ± 0.11</td>
</tr>
<tr>
<td>5</td>
<td>0.66 ± 0.05</td>
<td>ND</td>
</tr>
<tr>
<td>6</td>
<td>1.15 ± 0.13</td>
<td>ND</td>
</tr>
<tr>
<td>7</td>
<td>0.44 ± 0.04</td>
<td>0.62 ± 0.32</td>
</tr>
<tr>
<td>8</td>
<td>0.25 ± 0.03</td>
<td>0.10 ± 0.07</td>
</tr>
<tr>
<td>9</td>
<td>1.77 ± 0.04</td>
<td>ND</td>
</tr>
<tr>
<td>10</td>
<td>ND</td>
<td>0.23 ± 0.09</td>
</tr>
<tr>
<td>11</td>
<td>0.40 ± 0.02</td>
<td>0.34 ± 0.17</td>
</tr>
<tr>
<td>Normal subjects (mean)</td>
<td>1.05 ± 0.25</td>
<td>0.85 ± 0.33</td>
</tr>
<tr>
<td>Thalassaemia major (mean)</td>
<td>1.07 ± 0.73</td>
<td>0.70 ± 0.53</td>
</tr>
</tbody>
</table>

*Values given as mean ± 2 SD.
ND = not done.

Discussion
In this study we assessed the ability of several platelet stimulating agents to induce malondialdehyde release from the platelets of normal subjects and of patients suffering from thalassaemia major. In platelets from patients with \( \beta \)-thalassaemia, mean malondialdehyde concentrations were normal, but there was a close correlation between platelet

Concentrations of platelet derived malondialdehyde and serum ferritin in patients with thalassaemia major.
malondialdehyde values and serum ferritin concentrations whether hydrogen peroxide or nethylmaleimide was used as the stimulating agent. The highest malondialdehyde concentrations were recorded in patients who not only had considerably raised serum ferritin concentrations but also had other signs of iron overload. As platelet associated malondialdehyde provides a measure of lipid peroxidation of cell membranes, the raised concentrations found in the most heavily iron overloaded patients may therefore reflect increased iron mediated cell membrane damage; such high values may be associated with increased quantities of free iron or that loosely bound in the postulated toxic iron pool.

Serum ferritin concentrations closely correlate with total and hepatic iron stores, and in the well chelated, iron overloaded patient much of the transfused iron is sequestered in a relatively inert form and plays a comparatively small part in continuous iron mediated membrane damage. In grossly iron overloaded patients, however, or in those patients who comply poorly with treatment, serum ferritin concentrations but also iron and other signs of iron toxicity may be sufficient iron in an active form to cause increased membrane lipid peroxidation and consequent tissue damage. High concentrations of platelet malondialdehyde may be recorded only in these patients, and our data suggest that values outside the normal range are indeed obtained in patients who show signs of iron toxicity in addition to raised serum ferritin concentrations.

Clinical improvement in iron overloaded thalassaemic patients may be brought about by increasing the dosage of desferrioxamine and deterioration may follow administration of large amounts of vitamin C, with comparatively minor changes in serum ferritin; this suggests that the serum ferritin concentration is not an adequate measure of continuing organ damage.

We conclude that the use of stimulated platelet malondialdehyde concentrations provides a useful, simple measure of lipid peroxidation and consequent cell membrane damage in chronically iron overloaded patients and that serial measurements may be a useful guide to clinical progress.

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References


Requests for reprints to: Dr RE Marcus, MRC Leukaemia Unit, Royal Postgraduate Medical School, DuCane Road, London W12 OHS.
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