Thrombokinetics in patients with rheumatoid arthritis treated with D-penicillamine

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SUMMARY The mechanism of D-penicillamine induced thrombocytopenia in rheumatoid arthritis was investigated by measuring platelet life-span and platelet production rate in 2 groups of rheumatoid arthritis patients treated with 250-750 mg/day D-penicillamine, 14 with a normal platelet count and 9 with thrombocytopenia (platelet count 50-130 x 10^9/l). Age matched control patients not treated with D-penicillamine included 14 with rheumatoid arthritis and 9 with osteoarthritis. The platelet life-span was normal, but platelet production rate was significantly reduced in the thrombocytopenic patients, suggesting that D-penicillamine causes thrombocytopenia through bone marrow suppression.

The haematological side effects of D-penicillamine treatment are well recognised and potentially serious. They include marrow aplasia, which has contributed significantly to reported fatalities, neutropenia, an isolated case of haemolytic anaemia, and thrombocytopenia. Thrombocytopenia is the commonest haematological side effect, with a reported incidence of 12-27% in patients treated with D-penicillamine for rheumatoid arthritis. The mechanism is unknown, but 2 major processes may be involved. One is drug-induced failure of platelet production, and the other is accelerated peripheral destruction through a drug-dependent immune-mediated process. There is some clinical evidence which suggests that thrombocytopenia during D-penicillamine treatment may be an early manifestation of bone marrow failure. Thus Kay found that a fall in platelet count or neutrophil count had been present for some months before the development of fatal bone marrow aplasia in 7 of 10 patients who developed this complication. Marrow aplasia caused 10 of the 18 deaths in her review. We are not aware of any reports which demonstrate the presence of drug-dependent anti-platelet antibodies during thrombocytopenia caused by D-penicillamine.

The standard, simple, haematological techniques of bone marrow aspiration and biopsy are insensitive to early marrow failure or mildly increased platelet consumption. Thrombokinetic studies are more sensitive and have been used in rheumatology to demonstrate increased platelet production which returned to normal during steroid therapy in patients with giant cell arthritis. We have measured platelet survival in patients with rheumatoid arthritis in order to investigate the mechanism of D-penicillamine induced thrombocytopenia.

Materials and methods

Thirty-two platelet survival studies were performed on 27 patients with definite or classical rheumatoid arthritis, the patients falling into 3 groups (Table 1):

Group A. Fourteen studies on patients not treated with D-penicillamine who had a normal platelet count.

Group B. Nine studies on patients treated with D-penicillamine who had a normal platelet count (4 were repeat studies on patients originally studied in Group A).

Group C. Nine studies on patients who developed a platelet count below 150 x 10^9/l during treatment with D-penicillamine (one was a repeat study of a patient previously studied in Group B).
Table 1. Platelet classification

<table>
<thead>
<tr>
<th>Group</th>
<th>No.</th>
<th>Study</th>
<th>Age (mean, range)</th>
<th>Duration of disease (mean, range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>15</td>
<td>Patients with RA not taking D-penicillamine, non-ribonucleoprotein</td>
<td>58 (40-69)</td>
<td>13-1 (0-25-35)</td>
</tr>
<tr>
<td>B</td>
<td>9</td>
<td>Patients with RA taking D-penicillamine, non-ribonucleoprotein</td>
<td>63 (46-75)</td>
<td>24 (16-60)</td>
</tr>
<tr>
<td>C</td>
<td>9</td>
<td>RA patients using D-penicillamine with platelet count below 150 x 10^9/L</td>
<td>65 (52-76)</td>
<td>9-4 (1-20)</td>
</tr>
<tr>
<td>D</td>
<td>9</td>
<td>Hospital controls with normal platelet count</td>
<td>54 (27-77)</td>
<td>—</td>
</tr>
<tr>
<td>E</td>
<td>3</td>
<td>Two patients with ITP, one with Felty’s syndrome</td>
<td>70 (65-75)</td>
<td>—</td>
</tr>
</tbody>
</table>

*Four patients were also studied in Group A.
†One patient was also studied in Group B.
‡ITP = idiopathic thrombocytopenia purpura.

The mean age of these patients and the known duration of rheumatoid arthritis are shown in Table 1.

In addition platelet survival was measured in 9 age-matched hospital controls without rheumatoid arthritis who had a normal platelet count and did not have any illness known to influence platelet kinetics. These patients had osteoarthritis and had been in hospital for a variety of minor orthopaedic procedures. Lastly, platelet survival studies were carried out on 3 patients with non-drug related thrombocytopenia: 2 with idiopathic thrombocytopenia purpura, and one with Felty’s syndrome (Table 1).

The platelet count was measured with Hema-gnost 8/90 (Technicon) at 2- to 4-week intervals in all patients treated with D-penicillamine in the rheumatoid outpatient clinic, which allowed the selection of patients for study in group C as soon as possible after they developed thrombocytopenia.

Table 2 shows the D-penicillamine dose and platelet count in the group C patients during the 3 months before thrombocytopenia was detected, the minimum platelet count and D-penicillamine dose taken when this was observed, the D-penicillamine dose and platelet count during platelet survival study, the time for the platelet count to return to within the normal range after dose-reduction or stopping D-penicillamine, and the D-penicillamine dose and average platelet count during the 3 months after the thrombocytopenic episode.

It will be stated that 7 of the group C patients continued to take D-penicillamine (2 at a reduced dose) despite thrombocytopenia throughout the platelet survival study, while D-penicillamine was temporarily stopped before the study began in 2 patients (8 and 9).

D-penicillamine was permanently discontinued before or after platelet survival study in 3 group C patients (4, 7, and 9) with return of the platelet count to normal (4 and 7) or just below the normal range (9). It is of interest that the platelet count also returned to within the normal range despite continued D-penicillamine treatment in the other 6 group C patients, 2 of whom resumed their previous dose (1 and 5), while the others were kept at a reduced maintenance dose of D-penicillamine.

Platelet survival was measured by the 51Cr-sodium chromate labelling technique recommended by the International Committee for Standardization in Haematology after the patients had had the procedure explained to them and had signed the appropriate consent form. The study was approved by the institutional ethics committee. Autologous platelets were used in all studies except on the 2 patients with idiopathic thrombocytopenic purpura, for whom ABO and Rh-compatible HCl antigen-negative platelets were obtained from volunteer Red Cross blood donors.

51Cr-labelled platelets were prepared as follows. Approximately 350 ml of blood was collected by gravity into the first bag of a triple collection pack containing 47.5 ml of acid citrate dextrose (ACD) (Fenwall-Travenol). After centrifugation at 370 g for 15 minutes in a Sorvall GP RC-3 automatic refrigerated centrifuge, platelet-rich plasma (PRP) was transferred by gravity to the scioloid bag containing 25 ml of ACD. PRP was then centrifuged at 1,700 g for 15 minutes to prepare a platelet pellet, which was resuspended in 5 ml of platelet-poor plasma (PPP). The resulting platelet concentration was incubated with 250 μCi (9.25 x 10^6 Becquerel) preservative-free 51Cr-sodium chromate solution obtained at a specific activity of 1 μCi/ml from the Radiochemical Centre, Amersham, England, then washed once with PPP to remove unbound 51Cr-sodium chromate, and finally centrifuged at 170 g for 10 minutes to remove any contaminants by 51Cr-labelled red cells.

The packed red cells were then returned to the
The $^{31}$Cr-labelled platelets were injected into a volunteer's arm via a 22 G butterfly needle. The labelled platelets were washed three times with normal saline and then resuspended in 20 ml of blood collected from the volunteer. The $^{31}$Cr-labelling of the platelets was done using a method similar to that described by others.

The percentage of radioactivity bound to red cells was measured by counting the platelet-rich plasma and the platelet-poor plasma. The percentage of radioactivity bound to red cells was in every case less than 5%. The time of injection of radio-labelled platelets was recorded, and blood samples were collected for 24 hours after injection of the labelled platelets, and then daily for 3 days. For each sample, 0.5 ml of blood was collected into ethylenediamine-tetra-acetic acid (EDTA), the platelet count was measured, and 2 aliquots of 2 ml whole blood were prepared by mixing with sodium dodecyl sulphate for counting in a scintillation counter (Markle 1/195). Labeled samples and standards were counted together at the end of each platelet survival study.

The following calculations were made: linear estimate of platelet survival (days), obtained by fitting a linear decay function to the observed level of circulating radioactivity in each blood sample and extrapolating this to zero activity on the time axis; platelet recovery, representing the proportion of injected platelet-bound radioactivity measured from circulating blood when the platelet survival curve was extrapolated back to the time of injection; and platelet production rate, the number of platelets produced per litre of blood per day, calculated from platelet life-span, platelet recovery, and the radioactive platelet count. The calculation of platelet recovery, which was assumed to be 70 ml/kg.

Similar calculations were also made after fitting the platelet survival data to an exponential decay model, but they are not reported here as they led to identical conclusions.

**Data Analysis**

Standard statistical methods were used. Mean values ± standard deviation of means (SD) are reported. Mean values for platelet count, platelet recovery, platelet life-span, and platelet production rate were compared between patient groups by the unpaired Student's t-test after logarithmic transformation to normalise the data (for platelet count and platelet production rate). Linear regressions were computed on a hand-held programmed HP-67 calculator.

**Results**

The mean platelet count, platelet recovery, and platelet survival, and the calculated daily platelet production rate found in the 4 study groups are summarized in Table 3. The mean platelet count during the platelet survival study was significantly lower in group C patients (107 ± 25 × 10⁹/l) than in groups A, B, and D.
Thrombocytopenia in patients with rheumatoid arthritis

<table>
<thead>
<tr>
<th>Group</th>
<th>Platelet count (&lt;10^9/L)</th>
<th>Platelet recovery (%)</th>
<th>Platelet survival (days)</th>
<th>Platelet production rate (μL/μg/10^9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>18 355 ± 106</td>
<td>67 ± 16</td>
<td>67 ± 13</td>
<td>6.7 ± 0.9</td>
</tr>
<tr>
<td>B</td>
<td>9 249 ± 31</td>
<td>71 ± 14</td>
<td>6 ± 13</td>
<td>6.7 ± 1.5</td>
</tr>
<tr>
<td>C</td>
<td>9 197 ± 27</td>
<td>73 ± 13</td>
<td>6 ± 12</td>
<td>6.0 ± 1.2</td>
</tr>
<tr>
<td>D</td>
<td>9 258 ± 58</td>
<td>67 ± 12</td>
<td>8 ± 13</td>
<td>24 ± 6</td>
</tr>
</tbody>
</table>

A, B, or D (p < 0.001 for all 3 comparisons). In addition, the mean platelet count was significantly higher in group A than in group B and D (p < 0.05). Repeat platelet counts done at the end of the platelet survival study were available in 7 of 9 group C patients and showed persisting thrombocytopenia (coefficient of variation = 6%), implying a steady state of platelet production and destruction in these patients throughout the measurement period.

Platelet recovery and platelet survival were similar in the 4 study groups (Table 3). The calculated daily platelet production rate was significantly lower in group C than in groups A, B, or D (p < 0.005 for all 3 comparisons) (Table 3). Platelet production rate in D-phenylalanine treated patients without thrombocytopenia (group B) was similar to that in group D controls, but significantly less than that of untreated patients with rheumatoid arthritis (group A) (p < 0.02). Platelet survival in the 2 patients with idiopathic thrombocytopenia purpura was markedly reduced, and both platelet recovery and platelet survival were reduced in the patient with Felty's syndrome, as expected for these conditions.

Discussion

These data suggest that thrombocytopenia associated with D-phenylalanine treatment was due to a reduced platelet production rate in the platelet studies. Thus the marked platelet survival time measured during the thrombocytopenia episode in group C patients was not significantly different from that in control patients with osteoarthritis or in patients with rheumatoid arthritis and a normal platelet count but without D-phenylalanine treatment. Furthermore, platelet survival was within the normal range reported from previous studies. In the other hand the calculated daily platelet production rate was markedly reduced when D-phenylalanine treatment was completed.

Drug-dependent antibody-mediated platelet destruction which had ceased by the time of platelet survival study seems to have been ruled out as a cause of the thrombocytopenia, as 7 of the 9 thrombocytopenic patients were studied while they were still taking D-phenylalanine. The predicted results in the 3 group E patients (with immune thrombocytopenia or Felty's syndrome) showed that our platelet survival method could detect increased platelet destruction.

The reduced platelet production rate during thrombocytopenia implies that D-phenylalanine caused this complication through bone marrow suppression. It is therefore of great interest that treatment could be resumed without recurrence of thrombocytopenia in most of these patients, suggesting that some other, transient, unknown aetiological factor may also have been involved. Whether the patients who developed thrombocytopenia will now be at high risk of further D-phenylalanine induced marrow suppression is not known.

The observation that D-phenylalanine treatment uncomplicated by thrombocytopenia (group B) was associated with a significant reduction in platelet count and platelet production rate from that seen in untreated patients with rheumatoid arthritis (group A) probably reflects suppression of disease activity by D-phenylalanine, as results in the group B patients were similar to those seen in group D controls. Increased platelet production is commonly seen in inflammatory disorders, and active rheumatoid arthritis may be associated with thrombocytosis. Although the mechanism remains unclear, this study strongly suggests that D-phenylalanine causes thrombocytopenia by suppressing platelet production. The platelet production rate in a sensitive indicator of bone marrow suppression, and is often the key haematological parameter to return to normal after an episode of marrow hyperplasia. D-phenylalanine can cause marrow hyperplasia or splenomegaly, and it is possible that the thrombocytopenia associated with D-phenylalanine treatment reflects general marrow activity rather than a specific effect on platelet precursors. It would therefore seem wise to monitor patients who continue to take D-phenylalanine after an episode of thrombocytopenia.
References


