Drug-Induced Thrombocytopenia is Associated With Increased Binding of IgG to Platelets Both In Vivo and In Vitro


Thrombocytopenia is a common serious adverse effect of drug treatment. A variety of in vitro diagnostic techniques to confirm the diagnosis are available, but the majority lack sufficient sensitivity to detect all cases of drug-induced thrombocytopenia. We studied 19 patients with suspected drug-induced thrombocytopenia and demonstrated that platelet-associated IgG (PAIgG) was elevated in all at the time of thrombocytopenia, and PAIgG returned to normal levels as the thrombocytopenia resolved. In the majority of patients, the platelet count rapidly returned to normal after the drug was discontinued; however, in six patients, the thrombocytopenia persisted well beyond the period of time that the offending drug would be expected to be cleared from the blood. In 13 patients, serum obtained after recovery was used to identify the drug responsible for the thrombocytopenia in an in vitro assay. In all cases, the addition of the drug historically associated with the thrombocytopenic episode was associated with an increased binding of IgG to control platelets. For uncertain reasons, the concentration of drug required to increase the in vitro binding of IgG to test platelets was often more than the concentration usually achieved in vivo. Wider application of these techniques may provide better understanding of the clinical characteristics and mechanisms responsible for drug-induced thrombocytopenia.

Thrombocytopenia is a common serious adverse effect of drugs. Often, the temporal relationship between drug ingestion and onset of thrombocytopenia suggests a causal relationship. On other occasions a relationship between thrombocytopenia and a specific drug cannot be assumed from clinical history either because the patient is taking multiple drugs or an associated disorder is present, which is known to be complicated by thrombocytopenia. Rechallenging the patient with the suspected drug has been used in the past to identify the causal agent, but there are potential risks to this approach that may make it ethically unacceptable. Furthermore, this diagnostic trial does not provide insight into the basic mechanism responsible for the thrombocytopenia.

A number of in vitro assays have been used to investigate drug-induced thrombocytopenia. These tests infer the occurrence of an immune drug-platelet interaction by measuring an effect of the test drug and patient serum on target platelets. Endpoints such as platelet aggregation, platelet factor 3 release, platelet lysis, or 51Cr release have been measured giving positive results in some but not all cases of suspected drug-induced thrombocytopenia. Therefore, a negative result with one of these tests implies that either the drug did not cause the thrombocytopenia, or alternatively, the in vitro test lacked the sensitivity to detect the drug-platelet interaction.

Recent developments in the quantitative assays for platelet-associated IgG (PAIgG) and serum platelet-bindable IgG (S-PBiG) suggest that these techniques might be applicable to the investigation of patients with drug-induced thrombocytopenia. We have used these techniques to study a number of patients with suspected drug-induced thrombocytopenia during the thrombocytopenic episode and after recovery. In all instances, the thrombocytopenia was associated with elevated levels of platelet-associated IgG (PAIgG). We then performed an in vitro “rechallenge” using the patients’ convalescent serum in the indirect assay (S-PBiG) to identify the drug responsible for the thrombocytopenia.

MATERIALS AND METHODS

Patients Studied

Patients were considered to have drug-induced thrombocytopenia if they fulfilled all of the following criteria: (A) they were not known to have previously had thrombocytopenia; (B) thrombocytopenia developed after the ingestion of the drug, and the platelet count returned to normal when the drug was discontinued; (C) thrombocytopenia did not recur after treatment was discontinued; (D) there was no clinical or serologic evidence of another cause of thrombocytopenia.

Direct Assay for PAIgG

Platelet-associated IgG (PAIgG) was assayed on platelets obtained from the patients at the time of thrombocytopenia. PAIgG was also quantitated during convalescence and after the return of normal platelet counts in the majority of patients. PAIgG was quantitated using a modification of the antiglobulin consumption assay of Dixon and Rosse that has been previously described in detail. Sheep erythrocytes coated with human IgG are lysed by the
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addition of anti-human IgG and complement. This lysis can be inhibited by the prior addition of known concentrations of IgG (standard curve) or dilutions of patient platelets (test curve).

Since our previous report, the method for the calculation of results has been improved. The relationship between both the percent inhibition of lysis of IgG-coated sheep erythrocytes and the final concentration of IgG in the standard curve, and the percent inhibition of lysis and the platelet dilution in the test curve are each graphed. The number of platelets that causes 50\% inhibition of lysis is related to that concentration of IgG that also produces 50\% inhibition. This allows the utilization of most precise portion of each curve.

Application of the Indirect Assay for S-PBIgG to Identify the Causative Agent

The amount of IgG that bound to target platelets following the addition of convalescent patient serum was determined using the indirect assay for S-PBIgG.\textsuperscript{16} Test serum was heat-inactivated (56°C, 30 min) prior to testing. Test or control serum (0.3 ml) was incubated with varying concentrations of 0.3 ml of the test drug, diluted in veronal-buffered saline (VBS) for 30 min at 37°C. Blood group O platelets were collected into heparin (1.5 U/ml) plus 2% serum and drug mixture for 90 min at 37°C. The platelets were washed three times in VBS. The final platelet suspension was 200,000/µl, and 0.3 ml of this suspension was incubated with the serum and drug mixture for 90 min at 37°C. The platelets were then washed three times in VBS. The amount of S-PBIgG determined from the standard curve. The microtiter modification of the anti-globulin consumption assay was used to reduce the requirements for test serum.\textsuperscript{16} Serum to be tested was obtained from all patients (except patient 12) after they had discontinued all medications and the thrombocytopenia had resolved. In all cases (except patient 12), convalescent serum was obtained within 4 wk of the return of the platelet count to normal. Patient 12 was tested while he still had moderate thrombocytopenia. The following drugs were tested in vitro: quinidine, solvent, propylene glycol: no diluent, (Sterilab Damon Co., Downsview, Ontario); gold, solvent, phenyl-mercuric nitrate; diluent water (Myochrysine; Poulenc, Montreal, Quebec); trimethoprim-sulfamethoxazole, solvent propylene glycol, and ethyl alcohol, (Septara; Burroughs Wellcome Ltd., LaSalle, Quebec); ampicillin, diluent, sodium chloride, (Penbritin; Ayerst Laboratories, Montreal, Quebec); cimetidine, solvent, 0.5\% phenol; diluent water (Tagamet; Smith Kline and French, Mississauga, Ontario); gentamicin, solvent, methyl peribin; diluent water, (Roussel Canada Ltd., Montreal, Quebec). All corresponded to the agent the patient had received that was suspected to have caused the thrombocytopenia, but when available the injectable preparation was tested.

Determination of Cross-Reactivity With Other Agents

To investigate the specificity of the drug-antibody relationship, a number of sera obtained after recovery were tested against a panel of drugs over a wide range of dilutions.

RESULTS

Nineteen patients fulfilled the criteria for drug-induced thrombocytopenia (Table 1). In particular, no patient had splenomegaly or evidence of other clinical disorders that could cause thrombocytopenia. The antinuclear antibody was measured in many of the patients and in all six patients who took longer than 1 mo to recover and was negative in all. Fourteen of the patients had severe thrombocytopenia (platelet counts less than 15,000/µl), and all of these patients had purpura. Two patients with extensive purpura had platelet counts of 20,000/µl and 26,000/µl at time of presentation. The 19 patients had received drugs for a variable interval ranging from 4 days to 6 mo. This was not a prospective study, and therefore comments cannot be made concerning the incidence of thrombocytopenia.

| Patient Number | Age | Sex | Drug | Interval Between Initiation of Drug Treatment and Thrombocytopenia | Platelet Count When PAIgG Assayed (µl) | PAIgG IgG/Patelet (µl) | Time to Recovery (Days) | Recovery | Treatment | In Vitro Rechallenge (Normal < 100) |
|----------------|-----|-----|------|---------------------------------------------------------------|----------------------------------------|-------------------------------|--------------------------|---------|----------------|-----------------------------------|-----------------------------------------------|
| 1              | 59  | F   | Guanidine | 2 - 3 mo                                                      | 100,000                                | 33                           | 3                        | S       | S              | Nil                               | 23                                            |
| 2              | 74  | F   | Guanidine | 6 wk                                                        | 100,000                                | 33                           | 3                        | S       | S              | Nil                               | 23                                            |
| 3              | 63  | F   | Guanidine | 6 yr                                                       | 100,000                                | 33                           | 3                        | S       | S              | Nil                               | 23                                            |
| 4              | 36  | M   | Guanidine | 2 mo                                                        | 100,000                                | 33                           | 3                        | S       | S              | Nil                               | 23                                            |
| 5              | 56  | M   | Guanidine | 1 mo                                                        | 100,000                                | 33                           | 3                        | S       | S              | Nil                               | 23                                            |
| 6              | 63  | M   | Guanidine | 1 mo                                                        | 100,000                                | 33                           | 3                        | S       | S              | Nil                               | 23                                            |
| 7              | 55  | M   | Guanidine | 6 mo                                                        | 100,000                                | 33                           | 3                        | S       | S              | Nil                               | 23                                            |
| 8              | 53  | F   | Guanidine | 1 day                                                       | 100,000                                | 33                           | 3                        | S       | S              | Nil                               | 23                                            |
| 9              | 44  | F   | Gold     | 11 wk                                                       | 100,000                                | 33                           | 3                        | S       | S              | Nil                               | 23                                            |
| 10             | 63  | M   | Gold     | 22 wk                                                       | 100,000                                | 33                           | 3                        | S       | S              | Nil                               | 23                                            |
| 11             | 70  | F   | Gold     | 12 wk                                                       | 100,000                                | 33                           | 3                        | S       | S              | Nil                               | 23                                            |
| 12             | 30  | M   | Gent     | 4 wk                                                        | 80,000                                 | 33                           | 3                        | S       | S              | Nil                               | 23                                            |
| 13             | 6   | F   | Trimethoprim-sulfamethoxazole | 1 mo                                      | 100,000                                | 33                           | 3                        | S       | S              | Nil                               | 23                                            |
| 14             | 48  | F   | Trimethoprim-sulfamethoxazole | 1 mo                                      | 100,000                                | 33                           | 3                        | S       | S              | Nil                               | 23                                            |
| 15             | 20  | F   | Trimethoprim-sulfamethoxazole | 7 days                                     | 100,000                                | 33                           | 3                        | S       | S              | Nil                               | 23                                            |
| 16             | 24  | F   | Sulfasoxazole | 4 days                                                   | 100,000                                | 33                           | 3                        | S       | S              | Nil                               | 23                                            |
| 17             | 78  | M   | Amoxicillin | 9 days                                                   | 100,000                                | 33                           | 3                        | S       | S              | Nil                               | 23                                            |
| 18             | 69  | M   | Cimetidine | 2 mo                                                        | 100,000                                | 33                           | 3                        | S       | S              | Nil                               | 23                                            |
| 19             | 61  | F   | Penicillin | 4 days                                                      | 100,000                                | 33                           | 3                        | S       | S              | Nil                               | 23                                            |

S, indicates corticosteroids; P, indicates platelet transfusions; ND, indicates not done.
cytopenia associated with a particular drug; however, in this series, quinidine, gold, and trimethoprim-sulfamethoxazole accounted for most cases of drug-induced thrombocytopenia.

PAIgG was determined at the time of the thrombocytopenic episode in 18 of the 19 patients and was elevated in all. The normal range for PAIgG for our laboratory is less than 5 fg IgG/platelet. There was an inverse relationship between the level of PAIgG and the platelet count \((r = 0.65, n = 18, p < 0.01, \text{logarithmic transformation})\), with the most severely thrombocytopenic patients having the highest level of PAIgG. In 15 patients, platelets were tested following recovery when the patient was no longer receiving medications, and in all cases, the level of PAIgG had returned to normal (less than 5 fg IgG/platelet).

Following discontinuation of the drug, the platelet count returned to normal in 12 of the 18 patients within 10 days, but in 6 patients the thrombocytopenia continued for over 4 wk even though the offending drug was no longer being taken. Fifteen patients were treated with corticosteroids and one received platelet transfusions.

Three cases of drug-induced thrombocytopenia are described since each illustrates a different aspect of this syndrome in Figs. 1, 2, and 3.

Results of In Vitro Tests

The final concentration of the drugs tested in vitro ranged from below to above the therapeutic level. The patient’s convalescent serum and four or more different control sera were tested at each drug concentration. The patient’s convalescent serum alone or control serum plus the test drug with the one exception of quinidine did not produce increased binding of IgG to control platelets. High concentrations of quinidine plus control serum caused a slight increase in the binding of IgG to target platelets (Fig. 4). In contrast, the incubation of convalescent patient serum plus the appropriate drug resulted in a significant rise in the level of S-PBIgG. The patient with quinine-induced thrombocytopenia reacted in vitro to both quinine and quinidine and the level of S-PBIgG was 15 and 22 fg IgG/platelet, respectively.

Two patterns were observed when the level of platelet bindable IgG was related to the concentration of the test drug. The first showed a dose-dependent increase in the binding of S-PBIgG to target platelets and was observed with quinidine, penicillin, ampicillin, trimethoprim-sulfamethoxazole, and sulfamethox-
Fig. 3. The clinical course of patient 17, a 78-yr-old man who developed a urinary tract infection and was treated with trimethoprim-sulfamethoxazole (TS); ampicillin (AMP); and gentamicin (GEN). Repeated blood cultures had no growth and 1 wk later, the platelet count was less than 10,000/µl. Bone marrow examination was normal without megaloblastic changes. The patient was treated with a brief course of prednisone (PRED), and the platelet count (- - -) rapidly returned to normal and the level of PAIgG (O—O) also returned to normal. Only ampicillin caused increased binding of S-PBIgG to target platelets.

azone (Fig. 4). The second pattern was a dose-dependent biphasic relationship between the level of S-PBIgG and the drug concentration. This was seen with gold and cimetidine. The relationship between the level of S-PBIgG and gold concentration is shown in Fig. 5. One patient (patient 12) was tested at month 3, (Fig. 2) while he was still moderately thrombocytopenic, had a measurable serum gold level, and his PAIgG was slightly elevated.

To exclude the possibility of nonspecific reactions, convalescent sera or control sera were tested against a panel of test drugs (Table 2). The increased binding of S-PBIgG only occurred when convalescent serum was incubated with the drug historically associated with the development of the thrombocytopenia. The specificity of the drug–antibody reaction is exemplified by the testing of the recovery serum from patient no. 17 (Fig. 3) against all of the agents he was on at the time of the thrombocytopenia. Only ampicillin produced increased binding of S-PBIgG to target platelets.

DISCUSSION

The diagnosis of drug-induced thrombocytopenia is usually a diagnosis of exclusion. It can be difficult to implicate a specific drug on clinical grounds because patients may be treated with several drugs at the time of thrombocytopenia or may have an underlying condition, such as septicemia, which may be complicated by thrombocytopenia. The responsible agent can be identified by rechallenging the patient, but this is potentially hazardous.

Therefore, a variety of in vitro techniques have been developed to confirm the diagnosis of drug-induced thrombocytopenia, but these assays are not sufficiently sensitive to diagnose all cases of drug-induced thrombocytopenia. In this report, using techniques for quantitating bound and unbound platelet-associated IgG, we were able to confirm the diagnosis of drug-induced thrombocytopenia in 13 of 13 patients tested. We did not perform comparative studies, and therefore we do not know whether these techniques will prove more sensitive in the diagnosis of drug-induced thrombocytopenia than the previously reported methods.

Elevated levels of PAIgG were found in all patients with suspected drug-induced thrombocytopenia when they were tested at the time of thrombocytopenia. Patient no. 8 was not tested during the acute episode of thrombocytopenia. In those patients followed with serial platelet counts and PAIgG determinations during recovery, the PAIgG returned to normal levels either at the same time or before the platelet count had become normal.

The majority of our patients with drug-induced
thrombocytopenia had severe thrombocytopenia and extensive purpura. Two patients had only mild thrombocytopenia (platelet count of 100,000/\(\mu\)l), which was discovered by routine surveillance. One of these patients (patient no. 3) had been on quinidine therapy for 6 yr, and it is possible that the mild thrombocytopenia had been present for some time.

The clinical course of some of our patients differs from the “typical” description of drug-induced thrombocytopenia.\(^1,2,7,11,13\) Several of the patients, including two with gold-induced thrombocytopenia, two with quinidine-induced thrombocytopenia, one with cimetidine-induced thrombocytopenia, and one with penicillin-induced thrombocytopenia, had thrombocytopenia that persisted for 1 mo or more after the drug was discontinued. All of these patients had an increased number of megakaryocytes in their bone marrow. Persistent thrombocytopenia is not unexpected with gold, since this drug is slowly cleared from the body (as illustrated by patient no. 12); however, in the other cases, thrombocytopenia persisted longer than the anticipated clearance time for the drug. Five of these six patients had serial platelet counts and PAIgG determinations performed and in all, the PAIgG remained elevated while the platelet count was depressed. The clinical course of one of these patients (patient no. 5) is illustrated in Fig. 1. It is possible that the prolonged thrombocytopenia was mediated by a protein-bound drug metabolite with a long half-life. Alternatively, in these patients a transient autoantibody directed against the protein component of the drug–hapten complex may have developed.

Four patients developed gold-induced thrombocytopenia and in all patients the thrombocytopenia was associated with elevated levels of PAIgG. One patient had an autologous platelet survival performed, and this was significantly shortened despite relatively mild thrombocytopenia (patient no. 12, Fig. 2). Previous reports in the literature have indicated that gold-induced thrombocytopenia is associated with a shortened platelet survival.\(^13\) In patient no. 12, the platelet count gradually returned to normal despite the presence of measurable gold in his serum. The reason for this is uncertain, but similar cases have been previously described.\(^14\) It is possible that the thrombocytopenia in this patient was caused by a dose-dependent immunogenic metabolite.\(^15\) Alternatively, it is possible that a critical concentration of the drug is required either to adsorb to the platelet or to form immune complexes, and concentrations of gold below this level do not cause thrombocytopenia. This seems unlikely since the concentration of gold in his serum was well above the minimal level that produced interaction in vitro (Fig. 5). Finally, the apparent recovery of this patient may in fact represent a compensated thrombocytolytic state.\(^16\)

Rechallenge with the suspected drug in vitro produced an increase in serum platelet bindable IgG (S-PBlG) in all convalescent patient sera tested. The elevated S-PBlG was specific for the drug historically related to the thrombocytopenia and did not occur when other nonrelated drugs were tested (Table

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**Table 2.**

<table>
<thead>
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<th>Sera From Patients DIT</th>
<th>Test Agent</th>
<th>Quinidine</th>
<th>Ampicillin</th>
<th>Sulfamethoxazole</th>
<th>Cimetidine</th>
</tr>
</thead>
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<tr>
<td>Quinidine</td>
<td>2</td>
<td>+</td>
<td></td>
<td>ND</td>
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</tr>
<tr>
<td>Ampicillin</td>
<td>1</td>
<td>-</td>
<td>+</td>
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<tr>
<td>Cimetidine</td>
<td>1</td>
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</tr>
<tr>
<td>Sulfamethoxazole</td>
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<td>-</td>
<td>ND</td>
<td>+</td>
<td>ND</td>
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<tr>
<td>Controls</td>
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</table>

Assessment of cross-reactivity of sera from patients with drug-induced thrombocytopenia (DIT) caused by the drug listed in the first column with other nonrelated drugs. A plus sign indicates the level of S-PBlG was elevated. ND indicates the test was not performed.
2). One patient with quinine-induced thrombocytopenia also reacted to quinidine. For unknown reasons, the optimal concentration of drug that resulted in increased binding of IgG to control platelets was as much as ten times higher than the concentration that would be expected to occur in the patient. It is also uncertain why the amount of IgG that bound to normal platelets following addition of the test drug and patient serum was usually lower than the amount of IgG on the platelets after in vivo sensitization.

Recently, questions have been raised concerning the specificity of elevated levels of IgG on the platelet surface. Two groups of investigators have demonstrated that PAIgG can be increased in hypergammaglobulinemic patients who were either only marginally thrombocytopenic, or in whom it was unlikely that the thrombocytopenia was caused by immune mechanisms. Certain drugs may damage precursor cells and cause thrombocytopenia with elevated PAIgG, yet the drug does not interact with the platelets themselves. For these reasons, we suggest that it is important to confirm the clinical relevance of the elevated PAIgG by performing in vitro studies using convalescent serum. In the patients described in this report, the in vitro tests were highly sensitive, giving positive results in all tests. One approach to the patient with suspected drug-induced thrombocytopenia is to test the serum with the suspected drug in vitro. If the test is negative, the clinician may elect to perform a careful in vivo rechallenge.

These studies indicate that most cases of drug-induced thrombocytopenia are associated with increased levels of PAIgG at the time of thrombocytopenia. A drug-induced immune mechanism is supported by the demonstration that recovery sera causes increased binding of IgG to control platelets only in the presence of the suspected drug. Our studies do not indicate whether the mechanism of increased binding of IgG to platelets is mediated through binding of drug–antibody immune complexes to platelets or through binding of antibody to a drug–platelet complex. The two different patterns of IgG binding to platelets associated with the different drugs (Figs. 4 and 5) suggest that there may be either more than one mechanism of drug-IgG interaction with the platelets, or alternatively, certain drugs exhibit a marked prozone effect. These results could also be explained by the non-specific adsorption of IgG to platelets that have been damaged by loosely bound complexes of drug and antibody that might have been eluted during washing.

Wider application of these techniques may provide both a better understanding of the mechanism responsible for drug-induced thrombocytopenia and help in the appropriate selection of subsequent medication for patients with previous thrombocytopenia.

REFERENCES

Drug-induced thrombocytopenia is associated with increased binding of IgG to platelets both in vivo and in vitro

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