Observations on the Thrombocytopenia Due to Hypersensitivity to Quinidine

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ALTHOUGH a number of instances of thrombocytopenia due to drugs have been reported, relatively little evidence is available to establish the precise role of the drug in the process and the mechanism involved. The development of a severe thrombocytopenia with hemorrhagic manifestations in a patient who had been taking quinidine, afforded an opportunity to study the causal relationship of the drug to the platelet disturbance and perhaps to elucidate further the nature of the reaction. These observations partially confirm and extend those recently reported by Larson¹ in the study of a similar case.

CASE REPORT

E. L. (unit history no. A-916), an American born white female, aged 53, was admitted to the Pennsylvania Hospital on February 4, 1953, complaining of the presence of spontaneously appearing bruises and purple spots on her skin, and the passage of bloody urine. Apart from the associated emotional disturbance caused by these signs, she felt well. The hemorrhages were first noticed about nine days before admission. For the previous five months she had taken, intermittently, tablets of quinidine prescribed for a “jumping heart”. There was no tendency to bleed or bruise before this present episode. Two previous pregnancies were normal and there was no family history of a hemorrhagic diathesis. No allergy had ever been noticed. Except for the previous few months she had never taken quinidine before. In addition to the quinidine she had been taking “Premarin”, an estrogen-containing preparation.

The patient was moderately obese and relatively comfortable. There were subconjunctival hemorrhages in both eyes. There was a purple mark about 5 mm. in diameter on the outer aspect of the upper lip and several dark blue ruptured bullous lesions about 5 mm. in diameter were seen on the gums. Examination of the chest and abdomen was negative. There were numerous petechial hemorrhages in the skin over the lower abdomen and lower extremities, increasing in number peripherally. The urine was grossly bloody.

The peripheral blood on admission contained 44,000 platelets per cu.mm., hemoglobin 11.6 Gm., erythrocytes 4,000,000 per cu.mm., leukocytes 7000 per cu.mm., and a normal differential count. The skin bleeding time (forearm) was greater than 15 minutes. Venous blood coagulation times in both glass and silicone tubes were normal (15 minutes and 23 minutes respectively). Normal acceleration of clotting was shown on dilution of the 1:100(1 to 60 and 30 per cent with physiologic saline. There was no retraction of the clot even in the diluted blood specimens. The one-stage prothrombin time test was 14.1 seconds, corresponding to a prothrombin activity of 86 per cent of normal. Sternal aspiration disclosed normocellular marrow with slight reduction in the number of megakaryocytes.

Since it was thought that the thrombocytopenia was most likely due to the drugs that the patient had been taking, probably quinidine, no specific therapy was instituted. The platelet count gradually returned to normal, concomitant with a gradual disappearance of the hemorrhagic signs; seven days after admission the count was 222,000 per cu.mm.

Experiments were then carried out to confirm our diagnosis and study the mechanism of quinidine induced thrombocytopenia.

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WORKING MATERIALS AND METHODS

Syringes and all glassware with which the blood was brought into contact were silicconized. Sodium citrate, 3.8 per cent (1 part of citrate to 9 parts of blood), was used as anticoagulant.

Pure quinidine sulfate* and quinine sulfate† as well as the medicinal products containing these drugs were used in concentrations of 50 mg. dissolved in 100 ml. of physiologic saline. Sedormid‡ (allyl-isopropyl-acetyl-carbamide) was dissolved in physiologic saline in a concentration of 50 mg. per 100 ml. Premarin, the pharmaceutical estrogen preparation, was dissolved in physiologic saline using a tablet (containing 1 mg. of the active principle) dissolved in 10 ml. of physiologic saline.

The test for platelet agglutinins in the patient's serum was performed according to the method of Stefanini and co-workers.1

Wet preparations for studying platelet changes microscopically were made by mixing four drops of platelet-rich plasma with one drop of the test solution on a siliconized slide, and then overlaying with a siliconized cover slip, ringed with vaseline. The observations were made at room temperature (25°C).

The degree of clot retraction was estimated in duplicate by placing 1 ml. of freshly drawn whole blood in a siliconized tube containing 0.25 ml. of the material to be tested. Control tubes containing 1.0 ml. blood and 0.25 ml. of 0.85 per cent NaCl were prepared at the same time. Three hours after clotting, the serum, if any, was poured off and its volume measured.

\[
\text{Volume of serum obtained} = \frac{\text{Original volume of blood}}{100} \times 100.
\]

By this method, 55 to 65 per cent of the volume of a three hour old normal clot is usually expressed as serum. During the hypersensitive period, the patient's blood, after clotting in the presence of quinidine, showed no retraction from the wall of the tube, thus no serum could be poured off. After the patient's platelet count had become normal, it was found that 0.1 ml. of the added quinidine solution was as effective as 0.25 ml. in completely inhibiting retraction of a clot formed from 1 ml. of blood.

For the in vitro study of the effect of quinidine on platelet levels, one solution was made of 3.8 per cent sodium citrate in physiologic saline and another of 3.8 per cent sodium citrate in physiologic saline containing 50 mg. quinidine sulfate per 100 ml. One-tenth ml. of the anticoagulant solution was used for each ml. of whole blood. Immediately after adding the blood to the tubes containing the anticoagulant, these were gently inverted three times. Serial platelet counts were then performed on the two samples at about the same time, the first count being performed within 3 minutes of drawing the blood and subsequent ones at 15 minute intervals for 90 minutes. Platelet counts were performed directly, using Rees-Ecker diluting fluid. The counts were corrected for the slight dilution effect of the added quinidine solution.

Quinidine concentrations in the patient's plasma and urine were estimated by a slight modification of the method of Brodie, Undenfriend, and Dill.4

RESULTS

1. Effect of Adding Quinidine in Vitro to the Patient's Blood after Recovery

The test was done at room temperature (25°C). Within 30 minutes the platelet count dropped to a minimum of 63,000 per cu. mm. from an initial level of 248,000. No significant change in the platelet level of the control was found (fig. 1). A duplicate set of tubes was left undisturbed. Here, it was noted that the red cells sedimented much more rapidly in the tube containing the quinidine than in the 0.85 per cent NaCl control. Moreover, the supernatant plasma in the quinidine sample cleared slowly, while that in the control remained opaque. The difference could be seen clearly by viewing the plasma against a

* Kindly supplied by Merck & Company, Inc., Rahway, N. J.
† Kindly supplied by Hoffmann-LaRoche, Inc., Nutley, N. J.
Fig. 1.—Addition of quinidine (5 mg./100 ml.) or saline to patient’s citrated blood.

Fig. 2.—Patient’s citrated blood without (tube on left) and with quinidine (tube on the right), after standing for two hours.

White background crossed by two black lines, the latter being easily visualized through the quinidised plasma but not through the control plasma (fig. 2). This effect was maximal within two hours. No clumping of platelets was noticed in the counting chamber. The coagulation times of the patient’s blood in silicone tubes was accelerated in the presence of quinidine (15 minutes, as compared with 30 minutes in the control containing 0.85 per cent NaCl).

2. Effect of Quinidine on Retraction of Patient’s Blood Clots

Addition of quinidine to the patient’s blood before clotting, completely inhibited clot retraction (table 1). Quinine, the optical isomer of quinidine, was
TABLE 1.—Abolition of the Clot Retraction of the Patient's Blood after Addition of Quinidine Sulfate but not after Quinine Sulfate, Sedormid, and Premarin (1.0 ml. Blood Plus 0.25 ml. Testing Solution)

<table>
<thead>
<tr>
<th>Substance added:</th>
<th>Per cent clot retraction at 3 hours after clotting</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quinidine sulfate</td>
<td>0</td>
</tr>
<tr>
<td>Quinine sulfate</td>
<td>60</td>
</tr>
<tr>
<td>Sedormid</td>
<td>65</td>
</tr>
<tr>
<td>Premarin</td>
<td>60</td>
</tr>
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</table>

inert in this respect and so were Premarin and Sedormid (allyl-isopropyl-acetyl-carbamide). Sedormid was used because of its well known thrombocytopenic effects in hypersensitive individuals, and to determine whether patients sensitive to quinidine might also be sensitive to Sedormid. The patient's serum alone (0.1 ml.), and also in combination with an equal volume of the standard quinidine solution, when added to four specimens of group compatible blood (1.0 ml.) from four normal individuals, did not impair the retraction of the resulting clots.

3. Changes in the Patient's Platelet-Rich Plasma after the Addition of Quinidine

Both the quinidine and the control preparations were placed at opposite ends of the same slide to enable differences to be easily observed by merely making use of the movable stage of the microscope. At a magnification of 400×, platelets in the quinidinised preparation were seen to undergo obvious changes within a few minutes after the mixture was made. There was loss of refractility followed shortly by swelling, the changes progressing until, after 30 minutes, there were many platelet “ghost” forms. Fine projections were seen to arise from the pale swollen platelets; the degenerating forms gradually coalesced to form clusters of a fused amorphous type. The interesting observation in this experiment was that lysis of the individual platelet appeared to precede agglutination. Examination of the control disclosed no significant alteration in the morphology of the platelets or in their discrete distribution during the same period. This experiment was repeated with a suspension of the patient's platelets washed three times in 0.85 per cent NaCl. To one preparation, quinidine was added as in the previous experiment. After two hours no significant change was noted in either the preparation containing quinidine or that containing 0.85 per cent NaCl.

Platelets from a blood group compatible normal donor were washed with 0.85 per cent NaCl and a suspension of these platelets was mixed with equal volumes of the patient's platelet-poor plasma and the standard quinidine solution. After two hours standing at room temperature, no morphologic changes could be observed in the platelets as compared with the control (platelets and quinidine solution alone). Thus, neither with this test nor with that in which clot retraction was shown to be unaffected by a combination of the patient's serum, quinidine, and normal blood, could a lytic effect be demonstrated against normal platelets.

Platelet panagglutinins could not be demonstrated in the patient's decalcified and deprothrombinized plasma, when the latter was tested against the platelet-
rich plasma of four normal subjects of the same sex and blood group. Sera not adequately treated to remove prothrombin and calcium would, however, invariably induce platelet agglutination and fibrin formation when mixed with platelet-rich normal plasma.

4. Effect of Hastening Coagulation of the Patient's Blood on the Inhibition of Clot Retraction by Quinidine

In a tube containing 0.1 ml. quinidine sulfate in saline (50 mg. per 100 ml.) and 0.02 ml. commercial thrombin (Upjohn 100 units per ml.), was placed 1 ml. of the patient’s freshly drawn blood. The tubes were rotated gently a few times to mix the contents. Clotting occurred after three minutes. Complete inhibition of clot retraction was still present at the end of twelve hours. Ackroyd’s observation in Sedormid thrombocytopenia indicated that the clot retraction inhibiting effect of the Sedormid could be reduced by hastening coagulation. Such was not found to be the case in our studies.

5. Effect of Increasing the Concentration of Sodium Citrate on the Thrombocytolytic Action of Quinidine

We have been able to confirm, in connection with our studies, the observation of Ackroyd in his experiments on Sedormid thrombocytopenia, that an excess of anticoagulant had a retarding influence on the in vitro platelet lytic action of the drug. Using 0.1 ml. of sodium citrate of 3.8, 19 and 38 Gm. per cent concentration respectively, placed in tubes containing a constant amount of quinidine sulfate in physiologic saline (50 mg. per 100 ml.) and then adding 1 ml. of the patient’s freshly drawn blood (taken during the phase of complete recovery), a definite increase in the number of recovered platelets was found when the citrate concentration was increased, this being the greatest with the highest concentration (fig. 3). The tubes were stoppered and inverted gently a few times to mix the contents. Platelet counts were done after allowing the tubes to stand at room temperature for 2 hours.

6. Confirmation of the in Vitro Thrombocytopenic Effect of the Quinidine by Administering a Test Dose to the Patient

Having obtained an unequivocal indication that quinidine caused the destruction of the patient’s platelets suspended in her own plasma “in vitro”, it was now desirable to reproduce the phenomena “in vivo”. Accordingly, with the patient’s consent, a test dose of 0.2 Gm. of quinidine sulfate was administered orally to her while fasting. She was readmitted to the Hospital so that close watch could be maintained and the necessary countermeasures taken should an untoward reaction result. For the previous five weeks, the patient had been well and her platelet count had remained normal. Bleeding and clotting time and platelet determinations were made just prior to and at 15 minute intervals after the administration of the drug. Normal values were obtained at the beginning of the test, but at 75 minutes there was a profound decrease in the platelet count (fig. 4) associated with a prolongation of the bleeding time and absence of clot retraction (table 2). During this time there was no clinical evidence of distress and at no time did the patient complain of any discomfort.
The only noticeable bleeding was a slight ooze of blood from a previous venepuncture site, when a skin petechiae reaction test using positive pressure was applied. This test was negative otherwise. The subsequent course of the platelet count, the bleeding time, and quinidine levels of plasma and urine are shown in Table 2. Four days after the ingestion of the drug, the platelet count had reached
TABLE 2.—The Changes in the Platelet Count, Bleeding Time, Clot Retraction, and the Quinidine Levels in the Plasma and Urine Following the Administration of the Test Dose of Quinidine to the Patient

<table>
<thead>
<tr>
<th>Time (hrs.)</th>
<th>Platelets per cu. mm.</th>
<th>Bleeding time (mins.)</th>
<th>Clot retraction (%)</th>
<th>Quinidine concentration (µg. per 100 ml.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>plasma</td>
</tr>
<tr>
<td>0</td>
<td>222,000</td>
<td>14</td>
<td>60</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>28,000</td>
<td>13½</td>
<td>0</td>
<td>88</td>
</tr>
<tr>
<td>28</td>
<td>26,000</td>
<td>4½</td>
<td>0</td>
<td>40</td>
</tr>
</tbody>
</table>

90,000 and nine days after, it was normal. Urine specimens collected during the first 48 hours of the test showed no macroscopic or microscopic evidence of blood.

7. Patch Tests for Skin Sensitivity

A paste of quinidine sulfate on a piece of gauze moistened with physiologic saline was applied to the inner aspect of the skin of the patient's upper arm, and held in place with adhesive tape. A control with physiologic saline alone was placed on the opposite arm. These tests were done within a few days after the first attack of thrombocytopenia. Inspection of the areas of the skin after twenty-four hours showed no visible reaction. No petechiae could be induced in the area that had been in contact with quinidine by applying a pressure of 40 mm. Hg for 5 minutes to the arm above the test site.

8. Disappearance of Hypersensitivity to the Quinidine

Following recovery from the inadvertently induced thrombocytopenia, the patient was seen at weekly intervals, during which time the foregoing observations were made. About six weeks after her recovery from the purpura and eleven days after she had been given the test dose of quinidine which resulted in thrombocytopenia, it was found that the presence of quinidine no longer impaired clot retraction or depressed the platelet levels of her blood as detected by the usual in vitro methods. At this time, addition of quinidine and her four week old stored (frozen) serum failed to show any destructive effects on the patient's platelets when observed microscopically.

DISCUSSION

Both the in vitro and the in vivo observations obtained in this study indicate that the fundamental effect of quinidine on the platelets was one resulting in the direct destruction of the platelets, with agglutination being of lesser prominence. The rapidity of the fall in the platelet level following contact of the quinidine with the blood would likewise make it unlikely that inhibition of platelet formation by megakaryocytes in the bone marrow is an important factor in the thrombocytopenia. While such an effect cannot be excluded and probably plays some part, it does not appear to be of primary importance. Larson has pointed out some of the experimental evidence which renders such a hypothesis improbable. However, in the studies reported by him in a similar
case he failed to find evidence of in vitro destruction of platelets, and accordingly suggested that agglutination might have been the method of destruction rather than lysis. The in vitro tests as carried out by Larson involved placing the tubes in an ice-bath. At the low temperature employed the lytic reaction was probably inhibited; failure to demonstrate a fall in the platelet level under those conditions would, therefore, not exclude a lytic mechanism. The other possibility is that the test might have been done at a time when the patient had lost hypersensitivity to the drug. The fact that platelet lysis preceded the appearance of agglutination, raises the question as to whether or not the current concept of the importance of platelet agglutinins as a cause of idiopathic thrombocytopenia is not being overemphasized.

In our experiments no effect of the patient’s serum, in combination with quinidine, on the clot retraction of normal blood could be shown, the technic being similar to that used by Larson. Moreover, the patient’s washed platelets alone underwent no morphologic changes in the presence of quinidine; this suggests that a plasma factor was of importance in the reaction. However, the stored plasma collected at a time when the patient was sensitive to the drug did not affect clot retraction in the patient’s blood when tested together with quinidine at a time when hypersensitivity to the drug had apparently disappeared. This might simply mean that the factor deteriorated during storage. But it may also mean that some alteration in the platelets themselves existed during the state of hypersensitivity and that this, along with the factor in the plasma, was necessary for the manifestation of the thrombocytolytic effect of quinidine.

Quinine, the optical isomer of quinidine, had no effect on the patient’s blood, a finding also reported by Larson. This indicates the highly specific nature of the reaction.

In his studies on Sedormid thrombocytopenia, Ackroyd demonstrated that lysis of platelets did occur in vitro but that this reaction required an overnight period to be most clearly demonstrated on a quantitative basis. Our observations show a rapid in vitro lysis in the case of quinidine. Ackroyd was also able to show that accelerating the coagulation of the blood with thrombin reduced the clot retraction retarding effect of Sedormid. This observation could not be duplicated in these studies, possibly because of the faster rate of destruction of the platelets by the quinidine in the blood of our patient, in vitro. Ackroyd’s further finding that increasing the amount of anticoagulant beyond the conventional concentration preserved the platelets in the presence of Sedormid, was found to apply to quinidine also. His work indicates that the antagonistic effect of the anticoagulant is related to its inactivating effect on complement which is required in many immune lytic reactions.

The emergence and duration of the hypersensitive state is a problem of great interest. It would appear that a period of repeated exposure to the drug is necessary for the development of such a state. Evidence derived from in vitro studies suggest that hypersensitivity to the drug may disappear. Bigelow and Desforges, in a study of platelet agglutination by an abnormal factor present in the plasma of two individuals who developed thrombocytopenia following quinidine therapy, found that the factor disappeared from the plasma of the
first patient at six months, and from the second at thirty-nine days, after the onset of purpura. In the patient reported here, no effect of the quinidine on the platelets in vitro could be demonstrated after six weeks, despite the finding of marked thrombocytopenia following ingestion of the drug eleven days previously. Whether or not a corresponding change in vivo had occurred, could not be ascertained. The patient was reluctant to take another test dose of quinidine.

SUMMARY

Studies are reported on a patient who developed thrombocytopenic purpura following quinidine therapy. Quinidine caused lysis of the patient's platelets in vitro within 40 minutes and complete inhibition of clot retraction. Platelet lysis preceded platelet agglutination. Increasing the concentration of the sodium citrate used as anticoagulant inhibited the in vitro action of the quinidine. No thrombocytolytic effect of the patient's serum with quinidine could be shown when tested against normal blood.

A test dose of quinidine administered orally after the patient had recovered from the thrombocytopenia produced a profound decrease in the number of platelets within 90 minutes, associated with a prolongation of the bleeding time and disappearance of clot retraction.

The in vitro hypersensitivity to quinidine persisted for at least four weeks after recovery from the purpura; after six weeks it could no longer be demonstrated.

SUMMARIO IN INTERLINGUA

Es reportate studios super un patiente qui disveloppava purpura thrombo-cytopenic post therapia a quinidina. Quinidina in vitro causava lyse del plachettas sanguinee del patiente intra 40 minutas e etiam complete inhibition del retraction de grumo. Lyse de plachettas precedeva agglutination de plachettas. Un augmentate concentracion del citrato de sodium usate como anticoagulante inhibiva le action in vitro del quinidina. Nulle effecto thrombocytolytic del sero del patiente super sanguines normal poteva esser demonstrate—con a sin quinidina addite.

Un dose experimental de quinidina, administrate oralmente post que le patiente se habeva restablite del thrombocytopenia, produceva un forte diminution del numero de plachettas intra 90 minutas, conjunctemente con prolongation del tempore de sanguination e disparition de retraction de grumo.

Le hypersensitivitate in vitro a quinidina persisteva al minus quatro septimanas post que le patiente esseva restablite del purpura; post sex septimanas illo non plus esseva demonstrabile.

REFERENCES

3. Brodie, B. B., Undenfriend, S., and Dill, W.: The estimation of basic organic com-


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