The In Vitro Sensitivity of Erythrocyte Aggregation to Quinine: Assessment by a Serial Blood Sedimentation Procedure

R. C. Robbins and James E. Harbin, Jr.

A serial erythrocyte sedimentation procedure is based on maintaining blood in vitro in thermal (body temperature) and flow equilibrium, as changes in sedimentation rate are evaluated at 20-min intervals. Each measurement was made 1 h after the determination was begun. We thus confirmed in vitro the clinically observed effects of quinine on blood cell aggregation. A delayed effect of quinine on aggregation corresponded to in vivo observations that aggregation is first affected 3 to 12 h after the drug is administered. Quinine's greatest effect (p < 0.01) on erythrocyte sedimentation in vitro was at 200 min with 2 mg of quinine per 100 ml of blood, 220 min with 1 mg/100 ml, and 240 min with 0.5 mg/100 ml.

Additional Keyphrases  horse · in vivo results compared

Intravascular aggregation of blood cells and their rate of settling in vitro are not always correlated (1, 2), although both phenomena depend on rouleau formation or clumping of blood cells (3, 4). This discrepancy has provoked controversy in the literature concerning the effect of quinine on intravascular aggregation of blood cells and the erythrocyte sedimentation rate (ESR).

Knisely et al. (5) reported that, in monkeys infected with malaria (P. knowlesi), administration of quinine decreased blood cell aggregation ("sludging") that accompanies the disease. This effect of quinine does not appear to be secondary to its action against the parasite, since quinine also reportedly decreases blood sludging in patients with rheumatoid arthritis (6, 7).

Attempts to demonstrate an in vitro effect on blood cell aggregation of quinine in nontoxic concentrations have been unsuccessful. Groth et al. (8) reported that 2 mg of quinine per 100 ml of blood, the maximum nontoxic dose, had no effect on ESR. This discrepancy between in vivo and in vitro effects of quinine on blood cell aggregation is not readily explained. Quinine might exert effects in vivo not exhibited when it is added to blood in vitro. However, Knisely et al. (5) reported that the disaggregating effect of antimalarials on blood cells in malaria-infected monkeys was not apparent until 3 to 12 h after the drugs were administered. This suggests that the in vitro effect of quinine on the ESR might be similarly delayed.

We accordingly undertook to devise a procedure that would test this suggestion.

Materials and Methods

Rationale for the Serial ESR Procedure

For the usual ESR measurement, it is recommended that the determination be done within 2 h after the blood is sampled (9). However, cells in drawn blood retain their aggregating and settling tendency for several hours, the rate and duration being influenced by temperature and stasis (4, 10). If quinine is to be incubated in blood to determine whether the effect on the ESR is time-dependent, standardization of conditions for time, temperature, and stasis effects is necessary. Agglutination and settling of erythrocytes depend on properties of both the erythrocytes and plasma. The agglutinating effect of plasma or serum on erythrocytes is gradually lost if drawn blood is maintained at body temperature, but this loss can be partially negated if blood is kept in motion (4). Agglutinability of erythrocytes is also lost in incubated blood, but in this case the effect is not negated by motion of the blood (4). Thus, if drawn blood is maintained at body temperature and kept in motion, the erythrocytes gradually lose their settling tendency and in 4 or 5 h fail to settle.

The principle of our serial ESR procedure is that drawn blood, treated or untreated, is both maintained at the body temperature of the donor animal and kept in motion from time of drawing until the ESR determinations. Aliquots of blood are taken from the incubating bath at 20-min intervals, and the ESR determined by use of the Wintrobe sedimentation tube. Each determination is read 1 h after it was begun. Determinations are continued...
at 20-min intervals until the cells in the incubation bath lose their settling tendency.

**Procedure: Effect of Quinine on the ESR**

We used horse blood because its strong tendency to aggregate (rouleau formation) results in a normally high ESR. Horses free of any apparent disease were used.

Two grams of quinine sulfate (Merck & Co., Rahway, N.J.) was dissolved in 1 liter of saline solution (9 g/liter). Caffeine (Eastman Organic Chemicals, Rochester, N.Y. 14650), similarly prepared, was at the same concentration. The caffeine was used as a control, to demonstrate that the effect of quinine on the ESR was a specific attribute of the quinine, not a nonspecific effect of alkaloids generally, or of any foreign substance in the blood. Quinine and caffeine (2, 1, or 0.5 mg/100 ml of blood) was added to the 125-ml Erlenmeyer flasks that were to receive the blood. The flasks contained 30.2 mg of potassium oxalate and 38.5 mg of ammonium oxalate as anticoagulants. Control blood was added to flasks containing only the oxalates and a volume of saline (9 g/liter) equivalent to that used for test drugs.

For blood collection, the Erlenmeyer flasks, prepared as described above, were transported from the laboratory to the horse in an 8-liter Styrofoam vessel containing water at 37° to 38°C, the body temperature of the horse (11). At each bleeding, 75 ml of blood was collected from the jugular vein of a horse, and 25 ml transferred from the syringe (needle removed) into each of three Erlenmeyer flask (controls and quinine-treated), the time being recorded as zero time at this point. The Styrofoam container was swirled manually to keep the blood moving during its transfer to the laboratory.

On arriving at the laboratory we placed the flasks containing the blood in a water bath (Model S5-060, Acme Laboratory Equipment Co., New York, N.Y.) at 37° to 38°C, mounted on a linear shaker (Eberbach, no. 6000, Scientific Glass Apparatus Co., Inc., Bloomfield, N.J. 07003). The flasks were held upright by inserting them into holes in a Styrofoam block 4.5-cm thick, secured in the water bath. (The flasks were not removed from the bath until the end of the determinations.) The shaker was set at 88 excursions per min.

Each day, the first ESR determinations were started 20 min after time zero and continued at 20-min intervals for 11 serial determinations (the determinations were discontinued at this point, since at the higher quinine concentrations the blood cells had lost their settling tendency). The transfer pipets were filled with blood from the flasks with the shaker in motion and the blood transferred to a Wintrobe disposable ESR tube, 3-mm i.d. × 115-mm long (Clay–Adams, Inc., Parsippany, N.J. 07054). All ESR determinations were run in duplicate. At the end of the sedimen-
tation determinations, packed-cell volume was determined by centrifuging at 15 g for 30 min.

The hematocrits were not adjusted to a constant value before the ESR determinations because of the loss of sensitivity due to the manipulations. Instead, a paired t test (12) was used to determine whether a significant difference existed between the treated and untreated aliquots of a blood sample in ESR and hematocrits.

**Results and Discussion**

Quinine at therapeutic blood levels (2, 1, or 0.5 mg/100 ml), incubated in blood under conditions standardized as to time, temperature, and motion, significantly (p < 0.01) decreased the ESR. In contrast, the ESR for cells in untreated and caffeine-treated blood was not significantly (p > 0.80) different. The data (Table 1) substantiate the hypothesis that the effect of quinine on blood-cell aggregation is time-dependent. The effect of quinine on the ESR was delayed in proportion to concentration of the drug. Although the mean ESR of control blood was greater than that of quinine in all instances, the differences were initially relatively small and constant. After 140 min of exposure to quinine, the ESR of blood cells with the higher quinine concentrations was markedly slower than that for untreated blood. This effect of quinine was greatest at 200 min at the 2 mg/100 ml concentration, 220 min at the 1 mg/100 ml concentration, and 220 to 240 min at the 0.5 mg/100 ml concentration. Subsequent differences between ESR of cells of treated and control blood narrowed, since in both cases the ESR was decreasing rapidly. The ESR for blood cells in quinine-treated blood was not only slower but the cells lost their settling tendency earlier than did cells in untreated blood.

Our findings that quinine has a delayed effect on the ESR in therapeutic blood concentrations is consistent with clinical observations that the effect of quinine on blood cell aggregation in vivo does not appear until at least 3 h after the drug is orally administered. Also, the disaggregating effect of quinine on rouleau formation of normal blood cells (Table 1), as well as blood cell aggregation occurring in disease (7, 8), lend support to Fahraeus' contention (4) that the factors responsible for aggregation in normal blood are intensified in stress and disease and are the cause of the clinically observed increase in blood cell aggregation in many disease states.

The mean packed-cell volumes (11 determinations) for each treatment and its control, respectively, were as follows (in %): caffeine, 2 mg/100 ml, 37.6, 37.6; quinine, 2 mg/100 ml, 42.4, 40.9; quinine, 1 mg/100 ml, 41.5, 40.9; and quinine, 0.5 mg/100 ml, 39.8, 39.7. A statistically significant (p < 0.05) effect on the packed-cell volume of quinine-treated blood had appeared by 160 min, and persisted through 280 min at the 2 mg/100 ml concentration, appeared by 200 min and persisted through
280 min at the 1 mg/100 ml level, and appeared only at 280 min at the 0.5 mg/100 ml level. No increase in packed-cell volume was observed with caffeine-treated control blood. The alteration of the packed-erythrocyte volume affected by quinine is consistent with the observations of Groth et al. (8) that high concentrations of quinine cause pathological alteration of erythrocytes. However, quinine at nontoxic dose levels has been noted to effectively disaggregate blood cells in vivo in patients with various pathological conditions (6, 7). At the lowest quinine concentration, we saw significant effects on blood cell aggregation before significant effects on the packed-cell volume appeared. Whether the factors associated with quinine action on blood cells that result in an increased packed-cell volume or pathologic alteration of erythrocytes are also involved in its disaggregating action on blood cells, or in the toxic effects of quinine, remains to be determined.

Our data show that the discrepancy between in vivo and in vitro observations of the effect of quinine on blood cell aggregation resulted from methods used to measure the phenomenon. While the usefulness of the serial ESR procedure must be evaluated in other areas of discrepancies between in vivo and in vitro observations on blood cell aggregation, it does appear that the serial procedure offers promise as a sensitive and perhaps generally useful technique for testing in vitro the effect of drugs on blood cell aggregation. The need for such procedures is indicated, for instance, by recent evidence of the adverse effects of intravascular aggregation of blood cells (13-16) and the relatively few drugs available to combat the phenomenon when it occurs.

### Table 1. Effect on ESR of Quinine and Caffeine Added to Horse Blood

<table>
<thead>
<tr>
<th>ESR determinations, min</th>
<th>Caffeine control, mg/100 ml of blood</th>
<th>Quinine, mg/100 ml of blood</th>
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</thead>
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<tr>
<td></td>
<td>C T SD</td>
<td>2 C T SD</td>
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<tr>
<td>Began</td>
<td>48.2 43.1 2.2</td>
<td>48.4 45.7 2.6</td>
</tr>
<tr>
<td>Ended</td>
<td>49.6 45.6 1.8</td>
<td>49.8 47.6 0.9</td>
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<td>20</td>
<td>53.6 53.9 1.2</td>
<td>53.3 58.8 3.2</td>
</tr>
<tr>
<td>40</td>
<td>53.6 53.8 3.2</td>
<td>53.8 63.4 5.6</td>
</tr>
<tr>
<td>60</td>
<td>54.3 54.3 1.6</td>
<td>59.6 45.0 9.8</td>
</tr>
<tr>
<td>80</td>
<td>53.7 55.0 1.9</td>
<td>50.5 42.3 6.8</td>
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<tr>
<td>100</td>
<td>54.5 54.7 4.0</td>
<td>50.9 35.4 10.7</td>
</tr>
<tr>
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<td>53.6 53.5 31.</td>
<td>50.5 20.3 8.8</td>
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<td>52.4 52.2 4.4</td>
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<td>38.1 1.8 18.5</td>
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<td>29.2 0.4 15.3</td>
</tr>
<tr>
<td>200</td>
<td>15.3 19.1 2.3</td>
<td>20.7 0.0 11.7</td>
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<tr>
<td>220</td>
<td>6.8 6.9 2.3</td>
<td>6.0 0.0 6.9</td>
</tr>
</tbody>
</table>

* Table based on 880 determinations: 440 control, 440 treated. The drugs were incubated in blood at the body temperature of the horse and the blood was kept in motion. ESR determinations were begun at 20 min intervals; each determination was read after 1 h.

C, control; T, treated; SD, standard deviation between control and treated. The data were analyzed statistically using a paired t test.

[1] The quinine and caffeine were placed in the receiving flasks and were mixed with blood as soon as it was drawn from the animal. The incubating period started at this point and 20 min lapsed (transport of blood to lab) before the first ESR determination was started. Successive determinations were started at 20-min intervals.

[2] ESR's significantly different (p < 0.01).

### References