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THE EFFECT OF DIPYRIDAMOLE ON PLATELET FUNCTION: CORRELATION WITH BLOOD LEVELS IN MAN

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1 The effect on platelet functions of dipyridamole (a pyrimido-pyrimidine compound) was compared with a control group of patients taking warfarin.

2 Adhesion, aggregation and platelet factor 4 availability showed a significant decrease in the dipyridamole group.

3 Aggregation and platelet factor 4 showed a significant correlation with blood dipyridamole level.

4 Adhesion, aggregation and platelet factor 4 were reduced below the lower limit of normal at blood dipyridamole levels above 3.5 μmol/l.

Introduction

Platelets initiate thrombosis in the arterial circulation and the clot formed is a 'white thrombus' composed chiefly of platelets (Mustard, Jorgensen, Hoving Glynn & Rowsell, 1966). Patients with thrombotic complication after prosthetic heart valve replacement, exhibit definite 'white thrombus' on the valves (personal observation). This may explain the failure of warfarin to prevent thromboembolism in this group of patients and the success of the combination of warfarin and dipyridamole in eliminating this complication (Sullivan, Harken & Gorlin 1969). The effect on platelets of dipyridamole (a pyrimido-pyrimidine compound) has been extensively investigated in man and animals (Emmons, Harrison, Hondor & Mitchell, 1965; Didishiem & Owen, 1970; Gray, Wilson & Douglas, 1968). It is claimed to have little consistent effect on human platelet function, though its action in reducing platelet turnover in man has been demonstrated (Harker & Slichter, 1972). The failure to demonstrate a consistent alteration in platelet functions may be due to inadequate blood levels of the drug. It is with this in mind, that we monitored blood dipyridamole level which was correlated with its *in vivo* effect on platelet functions.

Methods

Twenty-three patients after isolated mitral valve replacement were studied for platelet functions. Twelve of these patients were on warfarin and eleven were on dipyridamole. The age and sex distribution of the two groups were similar. All patients had undergone isolated mitral valve replacement with the Bjork-Shilley prosthesis. Informed consent was obtained from all patients and those on dipyridamole were continually monitored for any evidence of thrombosis or side-effects.

Warfarin dose was adjusted according to the prothrombin ratio (therapeutic range 1.9-3). Dipyridamole (200 mg) was given initially then gradually increased to 400 mg/day in four divided doses. The 400 mg daily dose was exceeded if blood levels did not reach the therapeutic range. The maximum dose was achieved in 3-4 days. The drugs were given as soon as the patients were able to swallow tablets and always within 48 h post-operatively. Blood dipyridamole level was estimated on all patients 2 h after the morning dose of the drug. Following their return home, samples were collected in the out-patient clinic for blood dipyridamole estimation between 1 and 3 h after the morning dose.

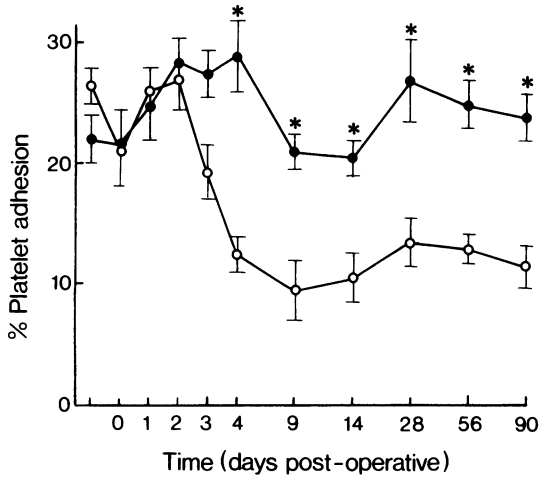

Figure 1 Platelet adhesion in the warfarin group (•) and in the dipyridamole group (○). Day 0 refers to immediate post-operative values. Each point on the curves represents a mean and s.e. mean of the values of all patients on the day indicated. * $P < 0.001$ between treatments on the days indicated.

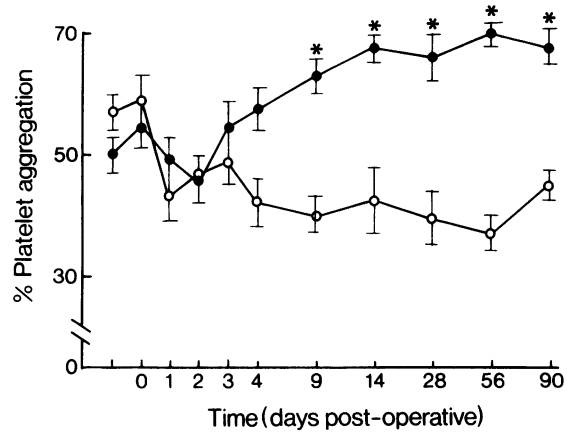

Figure 2 Platelet aggregation in the warfarin group (•) and the dipridamole group (○). Day 0 refers to immediate post-operative values. Each point on the curves represents a mean and s.e. mean of the values of all patients on the day indicated. * $P < 0.01$ between treatments on the days indicated.

Haemoglobin, haematocrit, platelet count, fibrinogen and euglobulin clot lysis time (ECLT) were determined by standard methods (Dacie & Lewis, 1968). Fibrinogen degradation products (FDPs) were determined using the tanned red cell haemagglutination inhibition immunoassay method (Merskey, Kliener & Johnson, 1966). Blood collected into 3.8% sodium citrate (9 : 1) was used for the determination of platelet functions within 2 h of venepuncture. Clot retraction and platelet factor 3 availability were measured using standard methods (Dacie & Lewis, 1968). Platelet factor 4 availability was determined by using the method of Niewiarowski & Thomas (1969) the result being expressed as percentage platelet factor 4 available. Platelet aggregation was determined using a Medicon Aggregometer (Model No. MA2) based on Born's principal of optical density variation (Born, 1962). Collagen and ADP were used as aggregating agents. Platelet adhesion was obtained using a modification of Hellem's method (Hellem, 1960). The column contained 1.8 g of glass beads and the transit time for 1 ml of blood was 45 s, controlled with a syringe pump.

Blood dipyridamole levels were determined as follows (Boehringer Ingleheim, personal communication): Serum (2.0 ml) and 1 mole/l tris buffer pH 8.6 (1.0 ml) were extracted into water saturated ether (40 ml), 30 ml of ether phase was re-extracted into 1.0 mol/l HCl (5.0 ml). The acid phase was removed, freed of dissolved ether and 4.0 ml mixed with 1.8 mol/l tris buffer pH 9.0 (1.0 ml). The fluorescence was measured at 500 nm (excitation wavelength 400 nm). A water blank and standard were run through the entire extraction with each batch of tests.

The patients on warfarin gave zero reading for serum dipyridamole level indicating, as far as we are aware, that none of the other drugs used in the treatment of these patients interfere in the assay of dipyridamole. The major metabolite dipyridamole glucuronide is not extracted by ether and so is not included in the assay of the parent compound.

Standard deviation and coefficient of variation for the methods used were determined from the difference between duplicate analyses. The Student's *t*-test was used to determine the significance of the difference between the warfarin and dipyridamole groups on each of the days indicated.

The significance of the relationship between blood dipyridamole level and platelet function values was determined by applying a *t*-test to the coefficient of linear correlation.

Results

Comparison of warfarin and dipyridamole groups

The warfarin and dipyridamole groups of patients showed no difference in haemoglobin, haematocrit, platelet count, fibrinogen, FDP's and ECLT. Platelet adhesion results are shown in

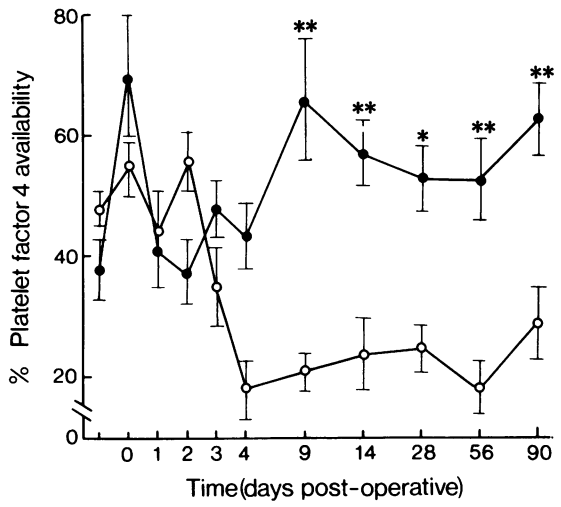

Figure 3 Platelet factor 4 in the warfarin group (•) and in the dipyridamole group (○). Day 0 refers to immediate post-operative value. Each point on the curve represents the mean and s.e. mean of the values for all patients on the day indicated. * $P < 0.01$, ** $P < 0.001$ between treatments on the days indicated.

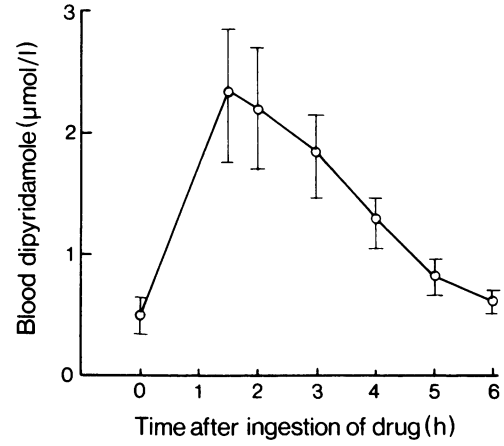

Figure 4 The rate of clearance of dipyridamole. Each point represents the mean and s.e. mean for eight normal volunteers.

Figure 1, the mean and standard error for both groups on each day are indicated and the difference between the groups is statistically highly significant ($P < 0.001$) from day 4 onwards. Platelet aggregation with collagen is quantitated as percentage maximum change in optical density (% aggregation). This was significantly lower ($P < 0.01$) in the dipyridamole group than in the warfarin group from day 9 onwards (Figure 2). Availability of platelet factor 4 is shown in Figure 3, a highly significant difference between the two groups is evident from day 9 onwards ($P < 0.001$). Availability of platelet factor 3 estimation gave no difference between the two groups, except the clotting times for the warfarin group were usually longer. Clot reaction values were within the normal range and no difference bwtween the groups was detectable. Normal values of platelet function and coefficients of variation for the methods are shown in Table 1.

Pharmacodynamics of dipyridamole

Two experiments were carried out to determine the normal absorption and clearance rate of dipyridamole. Six volunteers took a single dose of dipyridamole (50 mg) and their blood levels were measured at half hour intervals. Blood dipyridamole was always at a maximum within 2 h (average time 1.5 h) but there was individual variation in the level attained. Eight normal volunteers were given dipyridamole in the same dosage as the patients, i.e. 200 mg on the first day, increasing to 400 mg by day 3. After the morning dose on day 4 serial blood samples were collected for dipyridamole analysis. The rate of clearance of dipyridamole is shown in Figure 4, the mean level at 2 h was not significantly different from that at 1.5 h. The patients blood was collected as near the peak as possible, usually within 2 h and always within 3 h after the morning dose. The coefficient of variation for the dipyridamole assay was 4.6%.

Correlation of platelet function with blood dipyridamole level

Platelet adhesion in patients on dipyridamole was reduced below normal. However, no significant correlation with blood dipyridamole levels was evident. Platelet aggregation gave a very significant negative correlation ($r = -0.64$, $P < 0.01$) with blood dipyridamole level (Figure 5). All vlaues for platelet aggregation were below normal at a blood

Table 1 Normal values and coefficient of variation (CV) for platelet function tests

Normal values for platelet function		*CV for each method*
Adhesion	21–35%	9.5%
Factor 4 availability	45–90%	8.9%
Factor 3 activity	1.40–1.62	3.8%
Clot retraction	48–70%	
% aggregation	> 50%	10%

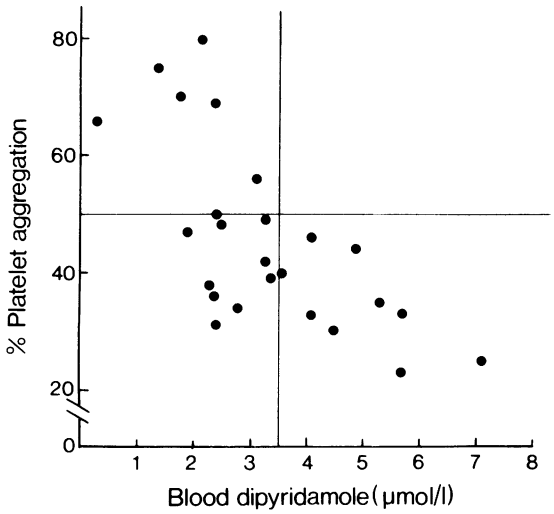

Figure 5 Platelet aggregation shows significant inverse ($P < 0.01$) correlation ($r = -0.64$) with increasing dipyridamole blood level. The horizontal line represents the lower limit of normal. The vertical line shows the suggested minimum therapeutic level of dipyridamole.

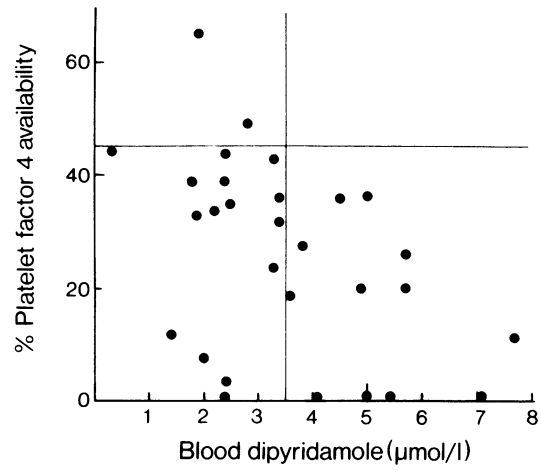

Figure 6 Platelet factor 4 availability shows significant inverse ($P < 0.05$) correlation ($r = -0.38$) with increasing dipyridamole blood level. The horizontal line represents the lower limit of normal. The vertical line shows the suggested minimum therapeutic level of dipyridamole.

dipyridamole level of 3.5 μmol/l. Platelet factor 4 availability gave statistically significant correlation with blood dipyridamole level ($r = -0.38$, $P < 0.05$), all values of platelet factor 4 being below the lower limits of normal at a blood dipyridamole level of 3.5 μmol/l (Figure 6) The platelet factor 3 values were equally reduced in both warfarin and dipyridamole groups, therefore no correlation analysis was performed for this function with dipyridamole level.

Discussion

Numerous studies using dipyridamole, *in vitro* and *in vivo* in animals and man (Emmons *et al.*, 1965; Didishiem & Owen, 1970) have shown depression of platelet functions, though the results have not been consistently reproducible. Harker & Slichter (1972) have shown shortened platelet survival in patients after prosthetic heart valve replacement, which became normal after treatment with dipyridamole. This has been substantiated by us in this unit (Manohitharajah, Rahman, Donnelly, Deverall & Watson, 1974; Rajah, 1976).

In this study platelet aggregation and adhesion both showed a significant lessening of activity in the dipyridamole group as compared with the warfarin group of patients. The percentage of platelet factor 4 available was also reduced in the dipyridamole group of patients as compared to the warfarin group. The other platelet functions did not show any significant difference between the two groups of patients.

Platelet aggregation and platelet factor 4 availability showed a very significant inverse correlation with increasing blood dipyridamole level. The other platelet functions showed less significant correlation. Some patients at the beginning of treatment had low values for blood dipyridamole which increased on raising the dose. When the blood level reaches 3.5 μmol/l platelet functions demonstrated a significant alteration. It is clear that any level below this value may not be effective in suppressing platelet function and may therefore indicate the minimum therapeutic level. The upper level is only limited by any side-effects the drug may cause, such as persistent headache, dyspepsia, muscular weakness and dizziness due to hypotension.

The drug dose was not empirical, it was regulated by the blood level. One patient developed transient hepatic insufficiency post operation, his dipyridamole level rose to 22 μmol/l hence the dose was reduced to 100 mg/day and the dipyridamole level came down to within the range of the other patients. The dose was later increased to 400 mg/day without a recurrence of high blood levels. The significant aspect of our study is that the drug dose, blood level and platelet functions were all monitored regularly. We have demonstrated significant alteration in platelet function when the blood level reached the

suggested therapeutic level of 3.5 μmol/l. This drug has been used to prevent the initiation of thrombosis but not the extension, hence timing of administration post-operative may be very important.

The trial is still in progress and the clinical results are under evaluation.

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