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Effect of Dipyridamol on the Electrokinetic Potential of Platelets

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The effect of different doses of dipyridamol on the electrokinetic potential of platelets has been studied in plasma from 31 normal subjects. As increase of the platelet migratory velocity was found, being statistically significant at 4 μg/ml final concentration.

The possible implications of this increase as related to changes in the platelet membrane an the antiaggregating effect of dipyridamol are discussed.

Dipyridamol in addition to effects on the coronary circulation (12, 13, 20, 21) inhibits platelet aggregation, which has been demonstrated both *in vivo* (5, 6) and *in vitro* (7). Although the mechanism has not been completely elucidated, it appears that the antiaggregations property is due to the ability of dipyridamol to maintain an elevated level of adenosine, either in the plasma or in the platelet (4, 10, 11, 14, 16).

Whatever the mechanism of action of adenosine is, it can be influenced by dipyridamol, and it is possible that the antiaggregating effect of dipyridamol is a morphological-biophysical alteration of the membrane.

This hypotetical alteration of the membrane can be studied by various techniques, such as electron microscopy, by chemical and immunological techniques, evaluation of functional aspects of the membrane, as e.g. studies of the possible alterations of the platelet electrokinetic potential. This last aspect has been evaluated in the present work.

Materials and Methods

Blood was collected in plastic tubes, using 0.11 M sodium citrate as anticoagulant in the proportion 9:1. After sedimentation at room-temperature for two hours, the supernatant, platelet rich plasma was collected. The measurements were performed between 2-4 hours after collection.

The plasma supernatant was divided into four aliquots of 0.5 ml. To three of them 10 μl of dipyridamol solution was

added, the concentration of which was adjusted to give 4, 8 and 12 μg/ml of platelet rich plasma respectively as final concentration. The fourth aliquot was used as control, 10 μl of isotonic saline solution being added.

In order not to modify the physical properties of the suspending medium the various aggregating substances were added in proportions smaller than 3 % of the whole volume (19).

The samples were incubated for 10 minutes at room temperature, diluted with 9.5 ml of isotonic saline, and the electrophoretic migratory velocity of platelets determined immediately at room temperature. This was done by recording the time needed for each of 20 different platelets to travel 64 μ. After moving 32 μ the polarity of the electric field was reversed, to avoid phenomena of polarisation of the electrodes. The sum of the times of these readings was considered to be the time required to travel 1,280 μ and the platelet electrophoretic mobility (P.e.m.) in $cm^2 \times v^{-1} \times sec^{-1}$ was calculated.

The estimations were done utilizing a cytopherometer (Zeiss), essentially following the technical instructions by the manufacturer. These have been described in detail previously (1).

Statistical calculation was performed according to the paired samples technique and students t-test.

Results

With the above described methods, the platelet electrophoretic mobility was studied in 31 different plasmas using the concentrations of dipyridamol given in table I.

The results show there is a statistically highly significant increase in migratory velocity already with 4 μg/ml dipyridamol, a concentration similar to that used clinically. (Control vs. 4 μg, $t^2 = 14.67$, $P < 0.001$). However, the dose-response relationship seems very weak (Table I). In general, the increase in migratory velocity did not exceed the limits of normal variation without any drug added.

Table I. *Effect of dipyridamol on platelet electrophoretic mobility (p.e.m.).*
The figures indicate the time in seconds for each platelet to travel 1,280 μ in an electric field of 8 mA and 150 vol. Number of samples, 31 for each group.

Dipyridamol (μg/ml)	$\bar{X} \pm SD$	p : e : m ($cm^2 \times v^{-1}$ sec^{-1})
—	226.64±21.05	1.004×10^{-4}
4	218.83±23.98	1.020×10^{-4}
8	217.06±22.16	1.500×10^{-4}
12	214.64±21.67	1.600×10^{-4}

Discussion

As has been shown, dipyridamol inhibits platelet aggregation, both *in vivo* and *in vitro* (5, 6). Adenosine likewise strongly inhibits platelet aggegration initiated by ADP (2). This antiaggregating effect if potentiated by dipyridamol, which may be the mechanism of action of the postulated antithrombotic property of dipyridamol (10).

Adenosine inhibits platelet aggregation, an inhibition which is potentiated (3) by substances that block cyclic phosphodiesterase, such as dipyridamol (15). These substances also block adenosine uptake by platelets (3, 17). The mechanism of adenosine induced inhibition of aggregation has been explained by numerous hypotheses:

1. Competition between ADP and adenosine for receptor sites in the membrane (2); 2. Increase of ATP intracellulary making more ATP available to keep platelets non-sticky (18); 3. Removal of ATP during adenosine uptake making less ATP available for aggregation (18); 4. Inhibition of adenosine transport carrier complex (15); and 5. Increase of cAMP by stimulation of adenylcyclase (15). Only the last two hypotheses offer an explana-

tion of the potentiating effect of dipyridamol.

None of these possibilities have been clearly substantiated or eliminated. The fourth hypotesis suggests a membrane alteration as the cause of inhibition. Such an alteration could lead to biophysical changes in the membrane resulting in charge changes.

Our results indicate that dipyridamol induces such a change, butwhether these are great enough to fully explain the antiaggregating effect of this drug cannot with certainly be concluded from our results. They are, however, in line with the fact that the strong aggregation inducer, ADP, leads to decreased platelet electrophoretic mobility (9).

The main groups responsible for the electrical charge of the platelet membrane are carboxyl groups of acetyl neuraminic acid, phosphate groups, amino groups, and sulfhydryl groups (8). Alterations in these could therefore modify the platelet electrophoretic mobility. We are at present studying the influence of dipyridamol on the phospholipid protein ratio of platelets and preliminary experiments tend to show that there is a decrease in this quotient after incubation with the drug.

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