DIPYRIDAMOLE INCREASES RED CELL DEFORMABILITY

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- 1 The effects of dipyridamole on red cell filterability both *in vitro* and *ex vivo* were measured.
- 2 In a balanced, randomised and double-blind trial, six healthy male and female volunteers (22–37 years) were given dipyridamole 400 mg/day or matching placebo in four divided doses for 3 days, and heparinised venous blood samples were taken 1 h after the ingestion of the last dose.
- 3 Filterability of red cells was increased significantly ($P < 0.05$ paired *t*-test) when the subjects were on dipyridamole compared with placebo.
- 4 In separate experiments, 15 min incubation with 2 or 20 $\mu\text{g/ml}$ dipyridamole *in vitro* was found to have no effect on the filterability of freshly prepared red cell suspensions.
- 5 After 24 h storage at 4°C, the filterability of red cells was significantly decreased ($P < 0.01$) and this could be partially prevented by adding dipyridamole to the stored cells ($P < 0.05$).
- 6 These results suggest that dipyridamole has an effect on the behaviour of the red cell membrane to increase the deformability of the cells. This may contribute to its therapeutic effect.

Keywords dipyridamole red cell deformability

Introduction

Dipyridamole is used in the treatment of angina and thromboembolic disorders. The therapeutic effect of dipyridamole has been attributed to its action on platelets. Dipyridamole is known to inhibit phosphodiesterase (Mills & Smith, 1971) leading to elevation of cyclic AMP levels and inhibition of platelet aggregation. It has been suggested that dipyridamole may require endogenous epoprostenol (PGI_2) to be fully effective (Moncada & Korb, 1978; Masotti *et al.*, 1979) and PGI_2 is itself a potent stimulator of adenylcyclase (Gorman *et al.*, 1977). As well as being important in platelet aggregation, cyclic nucleotides may also be involved in the regulation of red cell shape and flexibility (Yawata *et al.*, 1976) and PGI_2 also increases filterability of red cells (Kovacs & O'Grady 1984). As decreased deformability of red cells is a critical factor in causing diminished peripheral blood flow, we have studied the effect of dipyridamole on red cell filterability both *in vitro* and *ex vivo*.

Methods

Nine healthy volunteers (three males and six females) aged between 22–37 years participated in the study

after giving informed consent. Three withdrew during the study (see results) leaving six who completed. The subjects received dipyridamole 400 mg/day or placebo orally each day for 3 days prior to the study. An interval of at least 1 week separated the two treatment periods. The last 100 mg dose of dipyridamole/placebo was taken 1 h before the blood sample. The order of treatments was balanced, randomised and double-blind. A 25 ml venous blood sample was taken from each subject, 10 ml being placed into a plain plastic bottle for analysis of plasma dipyridamole levels using an h.p.l.c. method (Davies, to be published) and the remaining 15 ml blood into heparinised tubes for the red blood cell (RBC) filterability study. Filterability of RBCs was measured by the technique described in detail by Kovacs & O'Grady (1984). Briefly, washed RBCs were suspended (10% v/v) in Dulbecco's phosphate buffered saline containing 0.25% human albumin (Immuno Ltd, Kent, England) and passed through polycarbonate filters with pore diameter of 3 μm (Nuclepore Corporation, Pleasanton, CA94566, U.S.A.), with a negative pressure of -100 mmHg . The results are expressed as relative filterability which is the ratio of the flow rate of RBC suspension to the flow rate of the suspending medium in accordance with the recom-

mentation of the Working Group Report on the Measurements of RBC Filterability (1981). Triplicate determinations were performed on each sample of venous blood, the filterability measurements being performed in all cases within 2 h of venepuncture at $26 \pm 1^\circ \text{C}$.

In the *in vitro* studies, the washed RBCs were suspended (10% v/v) in saline or saline with dipyridamole at two different concentrations (2 and 20 $\mu\text{g/ml}$). After 15 min incubation the filterability of the RBC suspensions were measured. In another experiment the RBCs were stored at 4°C in phosphate buffered saline containing 0.10% glucose with dipyridamole at 2 or 20 $\mu\text{g/ml}$ final concentration for 24 h. The filterability of the stored RBCs was measured at $26 \pm 1^\circ \text{C}$, with 5 μm pore diameter filters.

Results

Complete results were obtained from six subjects. Three experienced extreme headache, palpitation and facial flushing while on dipyridamole and had to be withdrawn from the study before completion. Results were not obtained from them and three further volunteers were recruited to take their place.

Filterability of RBCs from normal healthy volunteers was significantly increased by dipyridamole at the plasma concentration shown (Table 1). Employing a paired *t*-test, this improvement was significant at the $P < 0.05$ level. No significant change in RBC filterability was observed with 2 or 20 $\mu\text{g/ml}$ dipyridamole after 15 min incubation (Table 2). Storage of RBCs caused a significant reduction in filterability (Table 3) which was partially prevented by dipyridamole (Table 3). The degree of improvement of filterability was dependent on the concentration of dipyridamole used.

Discussion

Although until now the rationale for the use of dipyridamole has been its effect on platelets, the

present findings show that dipyridamole also has effects on red cells and these may contribute to its therapeutic action. We found that the greatest effect of dipyridamole was on stored cells where deformability is greatly reduced; a small effect was also demonstrated in normal subjects *ex vivo*. The conditions for which dipyridamole is presently prescribed include those, such as peripheral vascular diseases, where RBC deformability is known to be abnormal, but we do not know if dipyridamole therapy is able to reverse these changes. If this effect of dipyridamole is confirmed in patients, the indications for the drug may warrant extension.

The improvement in filterability of RBCs of normal subjects treated with dipyridamole could be due to an enhancement of PGI_2 production (Masotti *et al.*, 1979) and potentiation of the actions of endogenous PGI_2 (Moncada & Korbust, 1978). Dipyridamole was efficient in reversing the decreased filterability of stored RBCs; it did not, however, increase the filterability of freshly prepared RBC suspension after 15 min incubation. The reason why dipyridamole is effective *ex vivo* and also on stored RBCs but failed to increase filterability of fresh RBCs *in vitro* is not completely understood. It seems that the decreased deformability of RBCs during either the preparation of the cell suspension or ageing of the cell is prevented by the drug. However, the applied concentrations and the 15 min incubation period were inefficient in preventing the decreased filterability of freshly prepared RBCs suspension, caused by the artificial conditions of the preparation.

Storage is associated with accumulation of calcium ions (Ca^{++}) and depletion of adenosine triphosphate (ATP) in RBCs resulting in decreased filterability. The improvement in filterability of stored RBCs could be due to the delay of the decline of RBC ATP levels by inhibition of adenosine deaminase (Gibson & Lionetti, 1966), by enhancing intracellular glycolysis and by increasing glucose uptake (Gibson & Lionetti, 1966; Zachara, 1972). High intracellular ATP is necessary to maintain the adenine nucleotide levels which has been shown to be involved in the regulation of red blood cell shape and deformability (Yawata *et al.*, 1976).

Table 1 Effect of dipyridamole (400 mg daily) on RBC filterability *ex vivo*

Number of subjects	Pore diameter of filters (μm)	Treatment	Filterability* Mean $\pm$ s.e. mean	Plasma concentration ($\mu\text{g/ml}$) Mean $\pm$ s.e. mean
6	3	Placebo	0.070 $\pm$ 0.02	
6	3	Dipyridamole	0.075 $\pm$ 0.02**	2.432 $\pm$ 0.21

* Relative filterability = $\frac{\text{Flow rate of RBC suspension}}{\text{Flow rate of suspending medium}}$

** Difference from placebo is significant ($P < 0.05$)

Table 2 Effect of dipyridamole on RBC filterability *in vitro*

Number of samples	Pore diameter of filters (μm)	Dipyridamole ($\mu\text{g/ml}$)	Filterability* Mean $\pm$ s.e. mean
6	3	0	0.067 $\pm$ 0.02
6	3	2	0.066 $\pm$ 0.02
6	3	20	0.065 $\pm$ 0.02

* For explanation, see Table 1

Recent work on stored RBCs showed that there was an increased influx and accumulation of intracellular Ca^{++} when RBCs were stored at 4°C and then brought out to stand at 37°C (Wiley *et al.*, 1982). In the light of these findings, the efficiency of dipyridamole in preventing the loss of deformability of RBCs during storage could be explained by greater availability of cellular ATP, ensuring a more efficient pump system to maintain low Ca^{++} level in the cells. Reduced flexibility of stored RBCs and decrease in their capacity for oxygen uptake and release is a

potential disadvantage in using stored blood for transfusion into patients and dipyridamole may prove to be clinically useful in this respect to improve the haemorheological properties of stored blood.

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Table 3 Filterability of stored and freshly prepared RBCs *in vitro* and effect of dipyridamole on filterability of stored RBC.

Number of samples	Pore diameter of filters (μm)	RBC suspension	Dipyridamole ($\mu\text{g/ml}$)	Filterability* Mean $\pm$ s.e. mean
6	5	Freshly prepared		0.663 $\pm$ 0.07
6	5	24 h storage		0.268 $\pm$ 0.03**
6	5	24 h storage	2	0.326 $\pm$ 0.02***
6	5	24 h storage	20	0.432 $\pm$ 0.04***

* For explanation; see Table 1

Difference from freshly prepared RBC is significant ** $P < 0.01$ Difference from stored RBC is significant *** $P < 0.05$

References

- GIBSON, J.G. & LIONETTI, F.J. (1966). The effect of dipyridamole on the adenosine triphosphate level of stored human blood. *Transfusion Philad.*, **6**, 427–437.
- GORMAN, R., BUNTING, S. & MILLER, O.V. (1977). Modulation of human platelet adenocyclase by prostacyclin (PGX). *Prostaglandins*, **13**, 377–388.
- KOVACS, IREN B. & O'GRADY, J. (1984). Prostacyclin PGI_2 increases red cell deformability. *Agents & Action*, **14**, (in press).
- MASOTTI, G., POGGESI, L., GALANTI, G. & NERI SERNERI, G.G. (1979). Stimulation of prostacyclin by dipyridamole. *Lancet*, **i**, 1412.
- MILLS, D.C.B. & SMITH, T.N. (1971). The influence on platelet aggregation of drugs that affect the accumulation of adenosine 3,5 cyclic monophosphate in platelets. *Biochem. J.*, **121**, 185–196.
- MONCADA, S. & KORBUT, R. (1978). Dipyridamole and other phosphodiesterase inhibitors act as antithrombotic agents by potentiating endogenous prostacyclin. *Lancet*, **i**, 1286–1289.
- WILEY, JAMES S., McCULLOCH, KATHLEEN E. & BOWDEN, DAVID S. (1982). Increased calcium permeability of cold-stored erythrocytes. *Blood*, **60**, 92–98.
- WORKING GROUP REPORT ON RED CELL DEFORMABILITY (1981). *Methods and Terminology*. A meeting held in conjunction with the Second European Conference on Clinical Haemorheology. Royal Society of Medicine, London. 29th September, 1981. London: Russell Wilks Associates.
- YAWATA, Y., JACOB, H.S., MATSUMOTO, N. & WHITE, J. (1976). A possible role for cyclic nucleotides in the regulation of erythrocyte shape and permeability. *J. lab. clin. Med.*, **88**, 555–562.
- ZACHARA, B. (1972). The effect of Persantin on the phosphate compounds in erythrocytes during blood conservation. *Acta Haemat.*, **48**, 164–175.

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