The acute effects of nifedipine on red cell deformability in angina pectoris

D. G. WALLER¹, H. P. NICHOLSON² & S. ROATH²

¹Departments of Clinical Pharmacology and ²Haematology, University of Southampton, Southampton General Hospital, Southampton SO9 3TU

- 1 In a randomised double-blind study, the effects on red cell deformability of a single sublingual dose of nifedipine were compared with placebo in eight patients with stable angina pectoris.
- 2 Red cell deformability, measured by filtration and centrifugation techniques, was significantly increased at rest in all eight patients 1 h after nifedipine, while no change occurred after placebo.
- 3 The improvement in deformability after nifedipine was maintained at the end of a period of exercise and unchanged from resting values after placebo.
- 4 The results suggest that the increased deformability of red cells after nifedipine could contribute to the therapeutic effects of the drug in myocardial ischaemia.

Keywords nifedipine red cell deformability angina

Introduction

The resistance to arterial blood flow is highly dependent on the radius of the vessel, and to a lesser extent on its length. As a result of the high driving pressure (= shear stress) in healthy vessels, the viscosity of the blood is low and, according to the Poiseuille-Hagen equation, contributes little to flow properties (Baekstrom *et al.*, 1971; Gaeghtens & Uekerman, 1973). In the presence of a significant vascular stenosis, the fall in shear stress distal to the narrowing causes the intrinsic properties of the blood to assume a greater importance (Schmid-Schonbein *et al.*, 1971). In the heart, the flow in perfusing vessels is further reduced by the high intra-myocardial pressure generated during systole (Tillmans *et al.*, 1981; Nellis *et al.*, 1981).

Viscosity is influenced by the individual red cells' ability to deform in response to shear stresses (Taylor *et al.*, 1965). In addition, flow through nutritive capillaries depends on the ability of the red cell, with a normal diameter of approximately 7 μ , to flex sufficiently to pass through vessels as small as 3 μ diameter (Kiesewetter *et al.*, 1981). Erythrocytes may be in-

herently less deformable in some patients with vascular disease (Reid *et al.*, 1976b) and coupled with the reduced shear stress in the ischaemic circulation, this may be important in impairment of tissue oxygen transport.

It is known that intracellular calcium loading reduces the ability of red cells to deform (Palek & Liu, 1979); calcium translocation blockers are also effective in the therapy of angina (Dargie *et al.*, 1981). It is therefore possible that the anti-anginal actions of these drugs may in part relate to their effects on red cell deformability. We therefore studied the acute effects of one of these drugs, nifedipine, on red cell deformability in patients with angina pectoris.

Methods

Eight patients participated in the study, seven male and one female, with a mean age ($\pm$ s.e. mean) of 59.5 ± 2.7 years (range 49–71 years). All had stable angina pectoris controlled by β -

adrenoceptor antagonists alone (atenolol in four patients, propranolol in three, timolol in one); three had treated hypertension and one had retroperitoneal fibrosis with mild impairment of renal function. The study was approved by the Joint Ethical Sub-Committee of the Southampton and South West Hampshire Health District.

The patients attended the laboratory between 09.00 and 10.00 h after a light breakfast with no methylxanthine-containing drinks. All took their regular medication on the morning of the study but no glyceryl trinitrate tablets. A cannula was inserted into a forearm vein, and patency maintained by flushing with 5 ml of 0.9% saline solution after blood sampling. The patients rested lying semi-recumbent for 1 h and blood was withdrawn without venous stasis for rheological measurements as detailed below. Following this, a sublingual dose of either 10 mg of nifedipine or matching placebo was administered in double-blind random order. A further blood sample was withdrawn 60 min later and the patients were immediately exercised on a treadmill. Exercise was conducted at the pre-determined maximal comfortable walking speed (between 2 and 4 kph) at 5% elevation. The exercise was terminated at 10 min or earlier if the patient experienced chest pain, if the systolic blood pressure fell or if ischaemic depression of the ST-segments developed on the ECG. A further blood sample was withdrawn at the conclusion of the exercise.

Whole blood was anticoagulated with EDTA for plasma viscosity estimation, and heparin sodium 40 iu/ml for determination of red cell deformability. Samples were transferred to a light proof box immediately after collection and deformability studies were carried out under a sodium lamp to prevent degradation of nifedipine. Red cell deformability was measured by two techniques.

The filtration technique relies on the ability of red cells to pass through membrane pores smaller than the diameter of the erythrocyte. The method adopted was modified from Reid *et al.* (1976a). All filtration studies were carried out on white cell reduced blood (buffy coat removed) at 37°C and the time taken for passage of 0.6 ml of blood through a 5 μ polycarbonate nucleopore filter in response to a negative pressure induced by a 20 cm head of water, was measured on triplicate samples. The passage of the first 0.1 ml of blood was ignored to allow acceleration to a constant flow rate, and timing stopped after the passage of a total of 0.7 ml, subsequent to which flow becomes non-linear. The results were expressed in ml blood filtered min^{-1} and corrected to a haematocrit of 45% according to the formula: flow rate $\times 45/\text{haematocrit}$. Previous studies in

our laboratory demonstrated a linear relationship between flow rate and haematocrit, with a similar regression for samples from different individuals. Filtration studies were completed within 2 h of venepuncture.

The centrifugation method assesses the packing behaviour of red cells subjected to a centrifugal force, using the apparatus and technique described by Sirs (1970) with minor modifications. The centrifuge consists of a boxed Hawksley microhaematocrit head separated from the drive motor to avoid overheating. The head was maintained at 37°C by a thermostatically controlled air blower. The rate of cell packing in response to a constant centrifugal force of 200 g was recorded photographically at 30 s intervals for 6 min. The initial packing rate was expressed as $\% \text{ min}^{-1}$ and corrected to a haematocrit of 45% from a standard curve calibrated for the instrument. Centrifugation studies were completed within 3 h of venepuncture.

Plasma viscosity was measured at 37°C using a Harkness type linear viscometer. Haematocrits were obtained on a Coulter 'S' automatic cell counter.

All results are expressed as mean $\pm$ s.e. mean. Results were analysed by the method of paired comparisons between observations on nifedipine and placebo treatment days and significance assessed by Student's *t*-test for paired observations.

Results

All patients completed the study; the only unwanted effect reported was facial flushing experienced by the female patient on the final study day. Exercise duration was the same for each subject on both study days and was terminated after 3 and 4 min respectively in two subjects, on both occasions, the first experiencing light headedness, the second shortness of breath.

Plasma viscosity

The mean pre-treatment plasma viscosity was 1.28 ± 0.03 centipoise before placebo, and 1.34 ± 0.05 cP before nifedipine ($t = 1.38$, NS). There was no significant difference between the viscosity at rest after nifedipine (1.35 ± 0.03) and placebo (1.34 ± 0.03) and the expected rise on exercise to 1.42 ± 0.04 cP after nifedipine did not differ significantly from the rise after placebo to 1.38 ± 0.04 cP ($t = 1.49$, NS).

Haematocrit

The mean haematocrits of 41.0 prior to placebo and 40.3 prior to nifedipine were not significantly different ($t = 2.33$) and there was no change in haematocrit at rest after placebo (41.0) or nifedipine (41.0). The haematocrit increased on exercise to 43.7 after placebo and to 43.6 after nifedipine ($t = 1.80$).

Red cell deformability by filtration (Figure 1)

The coefficient of variation for triplicate samples was 7%. The mean deformability index pre-treatment was $2.65 \pm 0.11 \text{ ml min}^{-1}$ before placebo compared with $2.75 \pm 0.13 \text{ ml min}^{-1}$ before nifedipine ($t = 2.01$, NS). There was no change at rest after placebo ($2.62 \pm 0.14 \text{ ml min}^{-1}$) but filterability was significantly increased after nifedipine in all eight subjects to $3.09 \pm 0.13 \text{ ml min}^{-1}$ ($t = 5.15$, $P < 0.005$). At the end of exercise, filterability was unchanged after placebo at $2.57 \pm 0.11 \text{ ml min}^{-1}$ but still significantly greater after nifedipine at $3.02 \pm 0.15 \text{ ml min}^{-1}$ ($t = 3.97$, $P < 0.025$).

Red cell deformability by centrifugation (Figure 2)

The coefficient of variation for triplicate samples was 4%. The mean deformability index prior to treatment was $6.98 \pm 0.65 \% \text{ min}^{-1}$ before placebo and $6.78 \pm 0.67 \% \text{ min}^{-1}$ before nifedipine ($t = -1.58$, NS). The deformability index was unchanged at rest after placebo at $7.00 \pm$

$0.65 \% \text{ min}^{-1}$, while a rise was observed after nifedipine in all eight patients to a mean of $7.80 \pm 0.66 \% \text{ min}^{-1}$ ($t = 5.98$, $P < 0.001$). At the end of the exercise, the index after placebo was unchanged at $7.02 \pm 0.64 \% \text{ min}^{-1}$ while a further significant increase was seen on active treatment to a mean of 8.34 ± 0.71 ($t = 4.89$, $P < 0.005$).

Discussion

Both the filterability and packing rate on centrifugation of the red cells were enhanced in all eight patients 1 h after a single sublingual dose of nifedipine, a time chosen to coincide with the peak plasma concentrations of the drug (Ramsch, 1981). It is necessary to further evaluate this response, since the properties of individual red cells are not the only factors to influence the tests currently used to measure red cell deformability.

The filterability of whole blood is impaired by the presence of white cells which block the membrane pores (Kiesewetter *et al.*, 1981). In the current study, between 70 and 90% of white cells were removed by buffy coat aspiration, and previous studies in our laboratory have shown that the improvement in filterability achieved by this method is reproducible (most remaining white cells being the more flexible mononuclears). There is some evidence that increasing fibrinogen concentrations may reduce filterability (Marcel, 1981), but removing the protein by washing and resuspending red cells in the fibrinogen-free buffer risks altering cell characteristics and the sample may no longer be in a physiologically relevant state.

Figure 1 Red cell deformability by filtration (a) after nifedipine and (b) after placebo in eight patients.

Figure 2 Red cell deformability by centrifugation (a) after nifedipine and (b) after placebo in eight patients.

White cells do not affect sedimentation of blood during centrifugation but fibrinogen concentrations are directly related to the packing rate (Sirs, 1970). It is unlikely that the effect of nifedipine in the current study was solely related to an alteration of erythrocyte-fibrinogen interaction. A drug-related enhancement of cell-cell interaction would be expected to increase the rate of packing in the centrifuge, but impair or have no effect on the ability of cells to pass through membrane pores. An increase in deformability on exercise after nifedipine was demonstrated by the centrifugation technique but not after placebo, suggesting that haemoconcentration with a consequent increase in plasma viscosity is not the explanation for these results either. It therefore appears that the improvement in deformability after nifedipine is mediated by its direct action on the red cell membrane.

A recent conference report (Slonim *et al.*, 1981) also showed that a single dose of nifedipine enhanced filterability of red cells in eight out of 12 patients with angina or peripheral vascular disease, an effect which persisted for about 4 h. The variability in the response to nifedipine in the study of Slonim *et al.* (1981) may either reflect the selection of patients or methodological variations between laboratories.

There is some evidence that the maximum therapeutic benefit of nifedipine in angina may not be seen until 4 to 6 weeks after treatment commences (Cocco *et al.*, 1979). An evaluation of the effects of nifedipine on red cell deformability during long-term therapy is necessary to determine whether the improvement observed in the current study is thereby maintained or even enhanced.

Most of the deformability of red cells is due to the physical properties of the membrane, the fluid interior making little contribution (Skalak, 1981) and intracellular calcium loading increases red cell viscosity and reduces membrane deformability (Weed *et al.*, 1969; Palek & Liu, 1979). Normal intracellular calcium concentrations are low in erythrocytes, probably due to a low membrane permeability to the ion (Porzig, 1972) and active extrusion of the ion by a high capacity ATP-dependent membrane pump (Schatzman & Vincenzi, 1969). A small influx of calcium, however, rapidly depletes intracellular ATP (Plishker & Gitelman, 1977) and local increases in shear stress such as may occur in the microcirculation or in association with vessel stenoses may greatly increase calcium permeability (Larsen *et al.*, 1981). Accumulation of calcium in the cell membrane alters the characteristics of the spectrin component of the membrane cytoskeleton (Quist, 1980), increasing the rigidity of the cell.

Nifedipine exerts many of its clinical effects by blocking the slow inward transmembrane calcium flux in the target cell (Nayler & Poole-Wilson, 1981), and the 1,4 dihydropyridine derivatives, of which nifedipine was the first to be used therapeutically, bind to erythrocyte membranes (Glossman *et al.*, 1982). Other inhibitors of calcium flux such as cinnarizine and flunarizine (De Cree *et al.*, 1979) have been reported to improve both red cell deformability and tissue perfusion in ischaemic vascular disease, although the mechanism of action of the many drugs with 'calcium antagonist' properties may differ (Kauffman *et al.*, 1982). There are few *ex vivo* studies reported of the effects of other vasoactive drugs on red cell deformability.

Although the concept is not yet fully substantiated, it has been suggested (Van Neuten & Vanhoute, 1980) that hypoxia-induced calcium accumulation and increased rigidity of the red cell membrane is an important mechanism in the impairment of tissue perfusion. The results of the present study suggest that improvement by

nifedipine of the deformability of red cells could contribute to the therapeutic effects of the drug in myocardial ischaemia.

We thank Dr K. D. Young and Bayer UK Limited for support of this study. We are grateful to Professor C. F. George and Dr J. R. Dathan for permission to study patients under their care.

References

- Baekstrom, P., Folkow, B., Kendrick, E., Lofving, B. & Oberg, B. (1971). Effects of vasoconstriction on blood viscosity *in vivo*. *Acta Physiol. Scand.*, **81**, 376–384.
- Cocco, G., Strozzi, C., Chu, D., Amrein, R. & Castagnoli, E. (1979). Therapeutic effects of pindolol and nifedipine in patients with stable angina pectoris and asymptomatic resting ischaemia. *Eur. J. Cardiol.*, **10**, 59–69.
- Dargie, H., Rowland, E. & Krikler, D. (1981). Role of calcium antagonists in cardiovascular therapy. *Br. Heart J.*, **46**, 8–16.
- De Cree, J., De Cock, W., Geukens, H., De Clerck, F., Beerens, M. & Verhaegen, H. (1979). The rheological effects of cinnarizine and flunarizine in normal and pathological conditions. *Angiology*, **30**, 505–515.
- Gaeghtens, P. & Uekermann, U. (1973). The apparent viscosity of blood in different vascular compartments of the autoperfused canine foreleg, and its variation with haematocrit. *Bibliotheca Anatomica*, **11**, 76–82.
- Glossman, H., Ferry, D. R., Lubbecke, F., Mewes, R. & Hofmann, F. (1982). Calcium channels: Direct identification with radioligand binding studies. *Trends pharmac. Sci.*, **3**, 431–437.
- Kiesewetter, H., Schmid-Schonbein, H., Seiffge, D. & Teitel, P. (1981). Problems of measurement of red cell deformability. In *Clinical aspects of blood viscosity and cell deformability*, eds. Lowe, G. D. O., Barbenel, J. C. & Forbes, C. D., pp. 3–8. Berlin, Heidelberg, New York: Springer-Verlag.
- Kauffman, R., Bayer, R., Rodenkirchen, R. & Mannhold, R. (1982). Differentiation of calcium-antagonist drugs with respect to their myocardial effects. In *Hypertrophic cardiomyopathy*, eds. Kaltenbach, M. & Epstein, S. E., pp. 276–284. Berlin: Springer-Verlag.
- Larsen, F. L., Katz, S., Roufogalis, B. D. & Brooks, D. E. (1981). Physiological shear stresses enhance the Ca^{2+} permeability of human erythrocytes. *Nature*, **294**, 667–668.
- Marcel, G. A. (1981). Pharmacological improvement of altered red cell deformability. In *Symposium on recent developments in microcirculation research*, eds. Davis, E. & Marcel, G. A., pp. 4–14. Amsterdam, Oxford, Princeton: Excerpta Medica.
- Naylor, W. G. & Poole-Wilson, Ph. (1981). Calcium antagonists: definition and mode of action. *Basic Research in Cardiology*, **76**, 1–15.
- Nellis, S. H., Liedtke, A. J. & Whitesell, L. (1981). Small coronary vessel pressure and diameters in intact beating rabbit heart using fixed position and free motion techniques. *Circulation Res.*, **49**, 342–353.
- Palek, J. & Liu, S. (1979). Dependence of spectrin organisation in red blood cell membranes on cell metabolism: Implications for control of red cell shape, deformability and surface area. *Seminars in Haematology*, **16**, 75–93.
- Plishker, G. A. & Gitelman, H. J. (1977). Calcium dependent ATP losses in intact red blood cells without cellular accumulations of calcium. *J. membrane Biol.*, **35**, 309–318.
- Porzig, H. (1972). ATP-independent calcium net movements in human red cell ghosts. *J. membrane Biol.*, **8**, 237–258.
- Quist, E. E. (1980). Regulation of erythrocyte membrane shape by calcium. *Biochem. Biophys. Res. Comm.*, **92**, 631–637.
- Ramsch, K. (1981). Zur Pharmakokinetik von Nifedipin. *Schwerpunkt Medizin*, **4**, 55–61.
- Reid, H. L., Barnes, A. J., Lock, P. J., Dormandy, J. A. & Dormandy, T. L. (1976a). A simple method for measuring erythrocyte deformability. *J. clin. Pharmac.*, **29**, 855–878.
- Reid, H. L., Dormandy, J. A., Barnes, A. J., Dormandy, T. L. & Lock, P. J. (1976b). Impaired red cell deformability in peripheral vascular disease. *Lancet*, **i**, 666–668.
- Schatzman, H. J. & Vincenzi, R. (1969). Calcium movements across the membranes of human red cells. *J. Physiol.*, **201**, 369–395.
- Schmid-Schonbein, H. & Wells, R. E. (1971). Rheological properties of human erythrocytes and their influence upon the 'anomalous' viscosity of blood. *Ergebnisse der Physiologie, Biologischen Chemie und Experimentellen Pharmakologie*, **63**, 147–219.
- Sirs, J. A. (1970). Automatic recording of the rate of packing of erythrocytes in blood by a centrifuge. *Physics Med. Biol.*, **15**, 9–14.
- Slonim, A., Cristal, N., Erez, R. & Shainkin-Kestenbaum, R. (1981). The effect of nifedipine, a calcium antagonist on red blood cell filterability. Abstract, *Second European Conference on Clinical Haemorheology*, London.
- Skalak, R. (1981). What is red cell deformability? In *Symposium on recent developments in microcirculation research*, ed. Davis, E. & Marcel, G. A., pp. 31–44. Amsterdam, Oxford, Princeton: Excerpta Medica.
- Taylor, H. M., Chien, S., Gregerson, M. I. & Lundberg, J. L. (1965). Comparison of viscometric behaviour of suspensions of polystyrene latex and human blood cells. *Nature*, **207**, 77–78.

- Tillmans, H., Steinhausen, M., Leinberger, H., Thederan, H. & Kubler, W. (1981). Pressure measurements in the terminal vascular bed of the epimyocardium of rats and cats. *Circulation Res.*, **49**, 1202–1211.
- Van Neuten, J. M. & Vanhoutte, P. M. (1980). Improvement of tissue perfusion with inhibitors of calcium ion flux. *Biochem. Pharmac.*, **29**, 479–481.
- Weed, R. I., Lacelle, P. L., Merrill, E. W., Craib, G., Gregory, A., Karch, F. & Pickens, E. (1969). Metabolic dependence of red cell deformability. *J. clin. Invest.*, **48**, 795–809.

(Received June 6, 1983,
accepted October 13, 1983)