Ex vivo effects of nifedipine, nisoldipine and nitrendipine on filterability of red blood cells from healthy volunteers

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In a balanced, randomised and double-blind trial, the effects of single oral dose of nifedipine, nitrendipine and nisoldipine were compared with placebo in eight healthy volunteers. Red cell filterability, measured with a gravity driven filtration technique, was not significantly altered by any of the three calcium antagonists compared with placebo, when RBCs were filtered within 2 h of venepuncture. Storage of RBCs for 24 h at 25°C, however, significantly reduced RBC filterability compared with 2 h ($P < 0.05$), but the reduction after nifedipine and nitrendipine was significantly ($P < 0.05$) less than after placebo. The above results demonstrate an effect of calcium antagonists on filterability of stored RBCs.

Keywords nifedipine nisoldipine nitrendipine red cell filterability

Introduction

Nifedipine is a member of the 1, 4 dihydropyridine family of calcium antagonists now being widely used in the treatment of hypertension as well as ischaemic heart disease. It inhibits calcium influx through slow channels in the cell membrane of vascular smooth muscle and this is believed to be the basis of its action as a peripheral vasodilator. Red blood cells (RBCs) of patients with hypertension (Isles *et al.*, 1981), ischaemic heart disease (Dormandy *et al.*, 1981) and other vascular diseases (Reid *et al.*, 1976) are less deformable than normal, and calcium antagonists such as verapamil have been shown to improve filterability of RBCs from patients with progressive systemic sclerosis who are known to have reduced RBC filterability (Sowemimo-Coker *et al.*, 1985). Waller *et al.* (1984) found increased deformability after nifedipine treatment of patients with angina. This study was therefore designed to investigate the *ex vivo* effects of nifedipine and other members of the 1,4 dihydropyridine family such as nisoldipine and nitrendipine on filterability of RBCs from healthy volunteers.

Methods

Subjects

Eight subjects (five males and three females), aged 22 to 36 years participated in the study. Each subject received the following oral treatment in a double-blind random order with at least 4 days between treatments:

- (a) placebo,
- (b) 20 mg nitrendipine,
- (c) 10 mg nisoldipine
- and (d) 10 mg nifedipine.

Blood samples (35 ml) were obtained from each volunteer by venepuncture in dark conditions 1.5 h after oral administration for analysis of drug level and filterability studies. This time was chosen so as to coincide with the peak plasma concentrations of the drug (Raemisch & Sommer, 1983). All the subjects were asked to abstain from food containing methyl-xanthines at least 3 days prior to the study. The study was approved by the Hospital Ethics Committee.

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Methodology

RBCs were separated from heparinised venous blood (heparin sodium, without preservatives, 20 u/ml, Weddel Pharm, UK) by centrifugation. The buffy coat was carefully removed and the red cells washed three times in Dulbecco's phosphate buffered saline, pH 7.4 containing 0.25% (v/v) human albumin (PBS-A), (Immuno Ltd, Sevenoaks, UK). Packed cell volume was determined from a concentrated suspension (about 50%) with a microhaematocrit (Hawksley, UK) and adjusted to 5% (v/v) with PBS-A. The white cell contamination in the final RBCs suspension was less than $0.1 \times 10^9 \text{ l}^{-1}$. During the preparation of the RBC suspensions, the samples were covered with aluminium foil to prevent degradation of the drugs, and filterability studies were carried out under reduced light using the gravity driven filtration described by Sowemimo-Coker *et al.* (1984). All filterability studies were performed within 2 h of venepuncture except in the second part of the study where RBC suspensions were prepared from whole blood stored in the dark at 25°C for 24 h.

Plasma levels of the calcium antagonists were analysed by gas liquid-mass spectrometry, the limit of detection of which was 0.1 ng ml^{-1} for each of the drugs. The coefficient of variation (CV), measured at 3.64 and $36.4 \mu\text{g l}^{-1}$ was < 5% for nifedipine and nitrendipine and < 8% for nisoldipine (Bayer UK, in file and Ryman & Beck, 1984). The results were analysed with Wilcoxon's matched pairs signed rank test.

Table 1 Mean relative filterability of red blood cells (RBC) from eight volunteers following administration of nifedipine, nisoldipine and nitrendipine.

RBC storage time (h)	Treatment	Relative filterability (mean $\pm$ s.e. mean)
2 h	Placebo	0.645 ± 0.016
	Nifedipine	0.650 ± 0.017
	Nisoldipine	0.657 ± 0.016
	Nitrendipine	0.677 ± 0.011
24 h	Placebo	$0.493 \pm 0.016^*$
	Nifedipine	$0.592 \pm 0.014^{**}$
	Nisoldipine	0.587 ± 0.025
	Nitrendipine	$0.627 \pm 0.014^{**}$

* $P < 0.05$ compared with placebo treated RBCs filtered within 2 h of venepuncture

** $P < 0.05$ compared with placebo at 24 h

Results

No significant difference (Table 1) was found between red cell filterabilities after any of the drugs compared with placebo ($P > 0.05$) when RBCs were filtered within 2 h of venepuncture. Filterability of stored RBCs was significantly ($P < 0.05$) reduced compared with fresh RBCs. The reduction in filterability after nifedipine and nitrendipine at 24 h was significantly ($P < 0.05$) less than after placebo. The effect of nisoldipine did not reach statistical significance.

Mean ($\pm$ s.e. mean) plasma levels of nifedipine, nisoldipine and nitrendipine at 1.5 h after ingestion were 52.6 ± 7.3 , 2.1 ± 0.7 and $12.2 \pm 6.0 \text{ ng ml}^{-1}$ respectively.

Discussion

The maintenance of normal shape and deformability of RBCs requires the preservation of cellular adenosine triphosphate (ATP) and a low content of calcium ions. Storage of RBCs for 24 h or longer can result in metabolic depletion, conversion of the cells to echinocytes, increased influx and cellular content of calcium (Weed *et al.*, 1969). Our results show that nifedipine and nitrendipine improve the filterability of stored RBCs. This may be due to inhibition of calcium influx into the cells delaying a decline in ATP levels and preventing a decrease in RBC filterability (Weed *et al.*, 1969). These calcium antagonists, however, had no significant effect on filterability of freshly prepared RBCs. It may not be possible to increase further the filterability of normal fresh RBCs by drugs of this type (Perret *et al.*, 1984). On the other hand, there is some evidence that the maximum therapeutic benefit of nifedipine in angina may not be seen until 4–6 weeks after treatment commences (Cocco *et al.*, 1979). It is therefore possible that a longer treatment period may be required before an increase in filterability of fresh RBCs can be observed. Our results suggest that calcium antagonists such as nifedipine and nitrendipine may be useful in improving haemorheological properties of RBCs with abnormal calcium metabolism such as that seen in stored RBCs and sickle cell anaemia.

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