Prolonged reduction in polymorphonuclear adhesion following oral colchicine

J. N. FORDHAM, J. KIRWAN, J. CASON, AND H. L. F. CURREY

From the Bone and Joint Research Unit, the London Hospital Medical College, and the Department of Rheumatology, the London Hospital

SUMMARY Polymorphonuclear leucocyte (PMN) function was studied in 7 healthy subjects before and after 5 mg of colchicine taken in divided doses over 24 hours. Mean adherence to nylon fibre colums fell from a pretreatment level of 51 % to 33·6 % by day 1, remained low (31·8 %) at day 5, and returned to pretreatment levels only by about day 9. Random motility (agarose plate method) fell from 932 μm to 688 μm by day 1, but had returned to normal by day 5. Directed migration and the phagocytic index (opsonised *Candida albicans* method) were not affected by colchicine treatment. In view of the fact that the plasma half life of colchicine is 19 minutes and that of PMN only 7 hours, this marked and prolonged reduction in PMN adhesion implies an influence on maturing PMN precursors in bone marrow. Adherence to nylon fibres may reflect in-vivo properties of PMN which are important in the pathogenesis of acute gouty arthritis.

Although colchicine has been used for over a century in the prophylaxis and treatment of acute gout, the mode of action of this drug has not been clearly defined. Following the work of McCarty[1] it is generally accepted that the polymorphonuclear leucocyte (PMN) is the chief cell concerned in the inflammatory response mounted against urate crystals. Most work on colchicine has centred on its effect on this cell. Such studies have generally employed in-vitro systems to test the effect of the drug on various aspects of PMN function. These often involved such high concentrations of the drug as to make the clinical relevance of any findings dubious.[2] Because of this we have followed 3 aspects of PMN function in volunteers given colchicine within the dosage range used in acute gout.

SUBJECTS AND METHODS
Healthy male laboratory and medical staff took 5 mg of colchicine orally in divided doses over 24 hours. Blood samples were taken into heparinised containers (10 IU heparin/ml) on the morning prior to the first dose and subsequently each morning.

Cell preparation. Heparinised whole blood was used for the adherence studies. For the other studies

purified PMN were prepared by a Hypaque-Ficoll separation procedure.[3] Cells were washed 3 times in 199 culture medium with 1 % human serum albumin (AB KABI Stockholm) with $NaHCO_3$ 350 mg/ml, 500 units penicillin/ml, and 500 μg streptomycin/ml.

PMN adhesion was measured by a modification of Stecher and Chinea's method.[4] Plungers were removed from 1 ml disposable syringes, which were then packed with 20 mg of scrubbed nylon fibre (Fenwal Labs, USA) to the 0·1 ml mark and clamped in the vertical position. A 25 G disposable needle was fitted and the syringe warmed to 37°C prior to introducing 300 μl of heparinised whole blood into the open end of the syringe. The plunger was then reintroduced and advanced until the bottom of the column of blood reached the bottom of the nylon fibre column. After incubation at 37°C for 10 minutes the blood was expelled by gentle pressure on the plunger, leaving adherent cells on the nylon column. White cell counts were carried out on the original and effluent samples with a Coulter electronic particle counter and differential white cell counts on a Leishman stain of blood smears. Adherence was expressed as a percentage of PMN retained in the column. All tests were performed in triplicate.

Motility studies were carried out by the agarose plate method described by Repo.[5] Agarose (Marine Colloids Inc, USA) was dissolved in distilled water

heated in a water bath at 60°C. The agarose solution was allowed to cool to 45°C and added to an equal volume of diluted 199 medium containing human serum albumin, penicillin, and streptomycin. Sodium bicarbonate was then added to give the following concentrations: 1% albumin, 350 mg/ml $NaHCO_3$, 500 units penicillin/ml, 500 μg streptomycin/ml. 8·5 ml of this solution was decanted into tissue culture plates (85 × 10 mm; Sterilin Products, Teddington, UK) and allowed to harden by refrigeration at 4°C for 30 minutes. Wells were cut 2 mm apart in the agarose film with a 3 mm skin punch biopsy punch (Steifel Laboratories, UK). The chemoattractant used was zymosan-activated pooled human serum prepared by adding 100 μl of zymosan suspension (10 mg/ml in saline) to 500 μl of pooled human serum. 7 μl of zymosan-activated serum was placed in one well. One hour later 2·5 × 10⁵ purified PMN was added to the opposite well and the plates incubated in a humidified incubator with 4% CO_2 for 2½ hours. The cells were then fixed by pouring 100% methanol on to the plates and leaving for at least 1 hour. Next the agarose gel was peeled off leaving the cells adherent to the plates. After drying, the cells were stained with toluidine blue. Migration distances were measured to the leading cell by an electronic micrometer attached to the microscope stage. Random motility was similarly measured on separate tissue culture plates from which chemotaxins were excluded. All experiments were carried out in quadruplicate.

Phagocytosis was measured by a modification of Lehrer and Cline's method.[6] Heat killed candida blastospores were washed 3 times, then opsonised by incubation with 20% pooled AB serum for 30 minutes. 500 μl of purified PMN was added to an equal volume of candida suspension and incubated for 1 hour. The final concentration of PMN was 10 000 μl and candida 90 000/μl. Phagocytosis was stopped by adding 0·1 M iodoacetate. One hundred consecutive PMN were examined on smears stained with methylene blue. Phagocytosis was assessed as the mean number of candida per PMN (phagocytic index). Each experiment was carried out in duplicate.

Statistical evaluation was by a paired *t* test.

Results

ADHERENCE (Fig. 1, Table 1)
There was a reduction in the percentage adherent PMN in all subjects following colchicine. Mean values fell from 51% precolchicine to 33·6% on day 1 (p<0·005) and this reduction was maintained at day 5 (mean 31·8% p<0·005). The time taken to return to precolchicine values ranged from 5 to 16 days, with a mean of 9 days.

MOTILITY STUDIES (Fig. 2, Table 2)
Directed migration did not alter significantly after

Table 1   *Change in PMN adherence following colchicine (mean % ± SD). Values of p calculated from paired* t *test*

Before colchicine	Days after colchicine		
	1	*5*	*8*
51·0 (8·7)	33·6 (13·2)*	31·8 (15·3)*	42·8 (19·3)‡

*Significantly different from precolchicine value p<0·005.
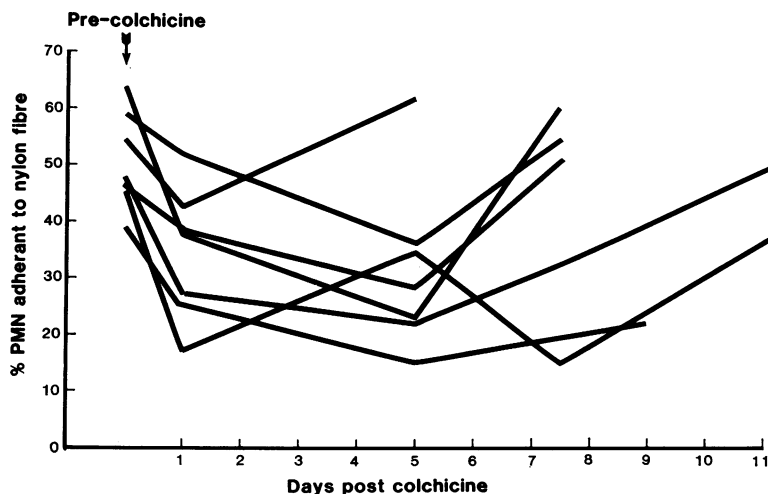‡Not significantly different from precolchicine value.

Fig. 1   *Change in PMN adherence following colchicine (percentage cells adherent to nylon fibre). Each line represents one subject.*
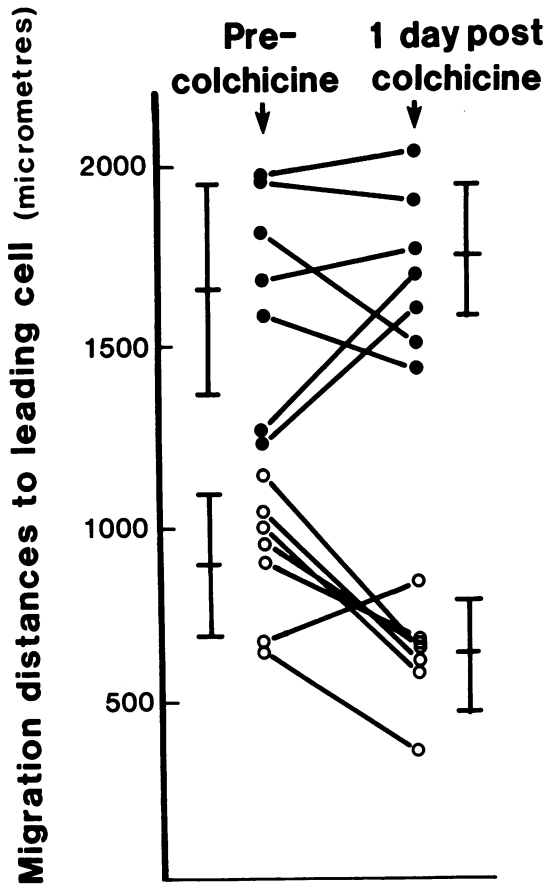
Fig. 2 *Changes in PMN motility following colchicine. Agarose plate method single cell leading front measurement. O = random migration. ● = directed migration measurements in microns ± 1 SD.*

Table 2 *Changes in PMN motility following colchicine (mean distances in µm ± SD) for single cell leading front using agarose plates*

	Precolchicine	Days postcolchicine	
		1	5
Directed	1767 (348)	1788 (192)	1853 (253)
Random	932 (190)	688 (176)*	893 (194)

*Significantly different from precolchicine value, $p < 0.01$.

Table 3 *Change in phagocytic index after colchicine (mean number of blastospores per PMN ± SD)*

Precolchicine	Days postcolchicine	
	1	5
4·96 (0·86)	4·71 (0·5)*	4·83 (0·61)*

*Not significantly different from precolchicine value.

colchicine. However, random migration fell from a mean of 932 ± 190 µm precolchicine to 688 ± 176 µm at day 1 ($p < 0.01$). By day 5 there was no significant difference from the precolchicine level.

PHAGOCYTOSIS (Table 3)
There was no change in the phagocytic index immediately following colchicine or at day 5.

Discussion

We have shown that colchicine, within the dosage used in the treatment of acute gout, causes a marked and persistent reduction in PMN adherence assessed in an in-vitro test. Since the plasma half life of colchicine is 19 minutes[2] and that of PMN only 7 hours,[7] this result can be explained only on the basis of uptake by bone marrow PMN precursors as well as circulating PMN at the time of taking the drug. This is in keeping with Ertel and Wallace's[8] finding of significant levels of colchicine present in PMN at 9 days following a single intravenous dose of 3 mg. Previous[9][10] have demonstrated a reduction in adhesion after colchicine. However, this was reported as short-lived, lasting only 6 hours in MacGregor's study.[10] Two of the 3 subjects whom Perry et al. reported on[9] showed a drop in adhesion following 5·5 mg of oral colchicine at 4 hours, which was maintained 8 hours after taking the drug. One of these subjects still had depressed adherence at 24 hours. In none of these studies was adherence measured after 24 hours. The differences between our findings and these others may relate to differences in methodology and dose of drug taken.

It seems likely that PMN adhesiveness to nylon fibre reflects the capacity of PMN to adhere to foreign surfaces generally. It is known that complement activators are released by PMN on contact with nonphagocytosable material such as nylon fibre.[11][12] Thus, through the generation of complement products (chiefly C5a) which increase PMN adhesiveness, this test is also a reflection of PMN secretory function. This being the case, our findings would be compatible with either a drug-induced reduction in secretory function of PMN (and hence less local complement activation) or with an impaired adhesive response per se. It is pertinent to note in this regard that pretreatment of leucapheresis donors with colchicine has been shown to reduce lysosomal enzyme release when PMN are harvested on nylon fibre.[13] Other workers have shown that colchicine reduces extracellular release of granular enzymes[14] and uric acid crystal-induced chemotactic factor.[15] It has been suggested that

PMN-nylon fibre interactions mirror PMN-endothelial interactions.[16] If so, it may be that the efficacy of the drug in preventing or ending acute gout may be due to interference with PMN adhesion to endothelium and thus reduction in the egress of PMN towards the joint cavity. Reduced release of crystal-induced chemotactic factor[15] or complement-activating enzymes could be other mechanisms reducing PMN recruitment.

We have been unable to show any impairment in directed PMN motility following colchicine. This in keeping with the work of Pinarello *et al.*,[17] who found no change in the chemotactic response of PMN of patients taking colchicine for familial Mediterranean fever. It also concurs with Allan and Wilkinson's suggestion[18] that colchicine affects the turning ability of the cell without altering its response to a chemoattractant. It is not clear whether or how reduced random motility in vivo might relate to the efficacy of colchicine in gout, although it is conceivable that the property of non-directed random motility is also necessary for the PMN to play its full role in urate crystal induced inflammation.

The lack of effect of the drug on phagocytosis of *Candida albicans* is in keeping with previous in-vivo findings.[9 17] Spilberg *et al.*[15] noted that although release of a urate-crystal-induced chemotactic factor was impaired by colchicine, phagocytosis per se was not impaired.

The most interesting finding in this study is the surprisingly prolonged effect of the drug on what is probably an important aspect of PMN function, namely, adhesion. It suggests that prophylaxis of acute gout may be achieved by very much less frequently administered doses of colchicine.

If PMN adherence to endothelium is the critical factor in the action of colchicine in acute gout, this does not explain the specificity of the drug in this condition. Only the urate-crystal-induced chemotactic factor hypothesis[15] invokes a mechanism unique to gout. On the other hand it is conceivable that the very acuteness of urate crystal synovitis is what makes it to some extent 'unique.' If so, endothelial adherence of PMN could be the critical factor susceptible to colchicine.

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