EFFECT OF COLCHICINE ON POLYMORPHONUCLEAR LEUCOCYTE CHEMOTAXIS IN HUMAN VOLUNTEERS

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- 1 The effect of colchicine on polymorphonuclear leucocyte chemotaxis was assessed in eight healthy volunteers. Four of them received 1.0 mg colchicine and the other four received placebo daily for 8 successive days.
- 2 Chemotaxis decreased significantly within 24 h of the ingestion of colchicine; the values remained low throughout the 8 days of treatment and returned to normal within 48 h of cessation of medication.
- 3 There were no significant changes in the placebo treated subjects.

Introduction

Directed movement of polymorphonuclear leucocytes (PMN) is one of the fundamental mechanisms of the human defence system. Abnormal PMN chemotaxis has been described in a number of disorders (Miller, 1975; Clark, 1978). PMN migration has been shown to occur in joints affected by acute gouty arthritis (McCarty, 1971). It is therefore of interest to determine whether the effect of colchicine in gout is due to inhibition of PMN chemotaxis. Several studies on this subject have produced conflicting results (Caner, 1965; Phelps, 1970; Chang, 1975). The purpose of the present investigation was to establish the extent and duration of the effect of colchicine on PMN chemotaxis in human volunteers.

Methods

Eight healthy male volunteers aged 20 to 25 years were the subjects of this study. All had normal serum electrolytes, glucose, cholesterol, renal and liver function tests, blood counts and urinalysis. Informed consent was obtained and the study was approved by the Institutional Committee for Human Experimentation. Using a double blind random method, four subjects were given one 1 mg tablet of colchicine at 08.00 h, and four others received an identical placebo tablet, daily for 8 successive days. Two additional male subjects served as controls for

standardization of the chemotactic assay. Samples of venous blood (12 ml) were drawn into heparinized tubes before and 4, 8, 12 and 24 h after the first dose. Additional samples were taken 2 to 4 h following the ingestion of the tablets on days 3, 4 and 8 and after cessation of the experiment on days 9, 10, 11, 13 and 15. Two subjects of the colchicine group took a single tablet of 0.3 or 0.5 g aspirin, respectively, during the experiment. No side effects were observed.

Chemotactic assay

The chemotactic assay was performed by a modification of the Boyden chamber technique (Boyden, 1962). PMN cells were obtained by sedimentation of the blood for one hour at room temperature, followed by ammonium chloride lysis of the erythrocytes. The PMN were washed twice with phosphate buffered saline (PBS), resuspended in RPMI-1640 medium and brought to a concentration of 2.5×10^6 cells/ml for use in the upper compartment.

The chemotactic attractant was prepared by incubating AB normal human serum with 1.5 mg/ml *E. coli* lipopolysaccharide W (Difco Laboratories, Detroit, Michigan) at 37°C for 90 min. The endotoxin serum mixture was then heated at 56°C for 30 min and centrifuged at 3,000 g for 30 min at 40°C. Aliquots of the attractant were stored at – 20°C. The chemotactic attractant was diluted four-fold in RPMI-1640 medium before being added to the lower well of Lucite chambers (0.2 ml/well). Polycarbonate

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Table 1 PMN chemotaxis in colchicine and placebo treated subjects (mean number of cells per high power field)

		Time after initial colchicine ingestion (h/days)								Time after last colchicine ingestion (h/days)				
		0	4h	8h	12h	24h	3d	4d	8d	24h	48h	72h	5d	7d
Control (subjects)*		117	115	112	121	113	92	132	98	90	89	106	92	91
Colchicine	1	108	93	113	97	40	28	—	56	—	76	—	130	—
	2	115	95	110	86	70	59	67	46	71	82	—	108	88
	3	110	105	115	126	50	63	50	65	—	102	103	110	106
	4	128	102	106	73	71	50	56	54	67	91	105	103	106
Mean ± s.d.		115 ± 9	98 ± 5.7	111 ± 3.9	95 ± 22.6	58 ± 15.3	50 ± 15.6	57 ± 8.6	55 ± 7.8	69 ± 2.8	87 ± 11.3	104 ± 1.4	112 ± 11.8	100 ± 10.4
Placebo	1	118	107	—	—	86	84	86	89	80	96	107	87	95
	2	116	108	110	101	84	115	111	—	117	—	101	87	107
	3	134	111	107	135	132	—	124	97	107	97	106	97	107
	4	121	120	123	126	116	121	—	94	114	118	105	83	91
Mean ± s.d.		122 ± 8.1	111 ± 5.7	113 ± 8.5	120 ± 17.6	104 ± 15.3	106 ± 19.9	107 ± 19.3	93 ± 4.0	104 ± 16.9	103 ± 12.4	104 ± 2.6	88 ± 5.9	100 ± 8.2
% change**		—6	—12	—2	—21	—44	—53	—47	—41	—34	—16	0	+27	0

*Mean of two untreated subjects

**%change in chemotaxis-colchicine group v placebo

Figure 1 PMN chemotaxis expressed as % of the control standard values during colchicine (○) or placebo (●) treatment and the recovery period. ↓ Initiation and cessation of medication.

membrane filters (5 μ m pore size; Nucleopore Corp., Pleasanton, California) were placed over the lower wells. An aliquot (0.2 ml) of the cell suspension in medium was added to the upper well (8 mm diameter). The chambers in duplicate, as well as a chamber with normal human serum without attractant, were incubated at 37°C for 1 h in humidified 5% CO₂ in air. The filters were then air dried, fixed with methanol and stained with Giemsa. Twenty high-power fields (HPF) were counted using a microgrid, and the mean number of PMN per HPF

which migrated through the filter was determined. The mean value of each experimental subject was expressed as percent of the control mean. In order to determine the number of cells falling off the filters, circle cover slides were placed at the bottom of the lower compartment. Cells falling from the filters were stained and counted.

Results

PMN chemotaxis decreased significantly 24 h after the ingestion of the first tablet of colchicine and remained about half of that in the control group throughout the 8 days of treatment (Table 1, Figure 1). Chemotaxis returned to normal within 48 h of cessation of therapy. Two-way analysis of variance demonstrated that the values obtained at zero time, 24 h after colchicine ingestion and 48 h after cessation of therapy, were statistically significant ($F = 34.26$, $P = 0.001$). In the placebo group, the differences at these points were not significant. Table 1 illustrates PMN chemotaxis expressed as mean number of cells per HPF migrating through the membrane in the four colchicine treated subjects compared to the four subjects who received placebo. Figure 1 shows the results in the experimental subjects expressed as percent of the control standard values. The number of cells falling off the filters was negligible. Locomotion in the absence of attractant was similar in both treated and untreated subjects and was less than 10%.

Discussion

The present study indicates that 1 mg of oral colchicine causes a prompt and significant decrease in PMN locomotion. Although our methodology did not distinguish between chemokinesis and true chemotaxis, directed movement through a filter in the Boyden chamber is usually referred to as chemotaxis (Territo & Cline, 1977). Caner (1965) was the first to show that low concentrations of colchicine 0.06–20.0 µg/ml (1.5×10^{-7} to 5.0×10^{-5} M), added *in vitro*, suppressed the chemotactic response of PMN toward *Staphylococcus albus*. Later, Phelps (1970) demonstrated that pre-treatment of dogs with intravenous colchicine (0.1–0.2 mg/kg) suppressed the generation of urate-induced chemotaxis in synovial fluid. On the other hand, Chang (1975) reported that high concentrations of colchicine (39.9 µg/ml or 10^{-4} M) applied *in vitro*, did not suppress the chemotactic response of rabbit PMN to immune-complex activated factors. Additional studies showed that very high concentrations of colchicine (399.4 µg/ml or 10^{-3} M) interfered with the random motility of PMN, while lower concentrations had no such effect (Malawista, 1965; Ramsey & Harris, 1972). Two recent studies in humans (Dinarello, Chusid, Fauci, Gallin, Dale & Wolff, 1976; Territo, Peters & Cline, 1976) have failed to demonstrate an impairment of PMN chemotaxis in colchicine treated patients with recurrent polyserositis (familial Mediterranean fever). However, Dinarello *et al.* (1976) showed that PMN and monocyte migration was depressed in colchicine treated patients, as observed at skin window sites 24 h after abrasion.

The mechanism of the effect of colchicine on cell movement is not clear. Malawista & Bensch (1967) suggested that it is related to the organization of the microtubular structure of the cell. There is ample evidence that colchicine binds to the protein tubulin, the dimer of which composes the microtubules (Wilson, Bamburg, Mizel, Grisham & Greswell, 1974). However, Wallace (1975) and Phelps & McCarty (1969) have shown that trimethyl-colchicinic acid (TMCA), a colchicine analogue effective in gout,

suppresses PMN motility, although it has no effect on microtubules. It has further been suggested that colchicine inhibits calcium release at the cytoplasmic level, thereby affecting microtubular assembly and chemotaxis (Gallin & Rosenthal, 1974).

Wallace, Omokoku & Ertel (1970) have calculated that the zero-time plasma colchicine concentration following a single intravenous injection of 2.0 mg in humans is 1.79 ± 0.74 µg% (4.5×10^{-8} M) and that this concentration decreased rapidly, the $T_{1/2}$ being only 19.3 min. However, the concentration of colchicine in white blood cells was about ten times higher than that in the plasma. Significant concentrations were measurable in the leucocytes as long as ten days after the injection (Ertel & Wallace, 1971).

The dose of colchicine used in the present study was similar to that used for long-term prophylaxis in patients with recurrent polyserositis (Levy & Eliakim, 1977). At present, no information is available on either plasma or PMN colchicine concentration during such therapy. Wallace & Ertel (1973) showed that mean peak plasma concentration after oral administration of 1 mg colchicine was 0.323 ± 173 µg% ($8.1 \pm 4.3 \times 10^{-9}$ M). Since the effect of colchicine on PMN chemotaxis occurs already after 12 to 24 h, it is of interest to investigate the time curve of colchicine PMN concentration in relation to its effect on chemotaxis. The reversal of PMN chemotaxis to normal values within 48 h of cessation of therapy is in keeping with observations that patients with recurrent polyserositis may experience an attack a few days after they have stopped taking the drug.

The finding that therapeutic doses of colchicine have a marked inhibitory effect on PMN chemotaxis in humans support Wyngaarden & Kelly's (1976) suggestion that the anti-chemotactic activity of this drug is the most significant of its effects on leucocyte functions. Indeed, this effect may be the common denominator explaining the response of various inflammatory disorders to colchicine therapy.

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