Lack of effect of colchicine on human neutrophil chemotaxis under agarose[1]

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Summary. Human polymorphonuclear neutrophil chemotaxis under agarose was assessed following in vitro treatment of cells with colchicine. No inhibition of random migration or chemotaxis was detected. The discrepancy between these results and reports utilizing micropore filter assays could be due to the need for cell deformability in the latter.

The antitubulin drug colchicine has been shown in most studies to inhibit human polymorphonuclear (PMN) chemotaxis when the cells are exposed to the drug in vitro and tested in a micropore filter assay system[2-8]. The results of these studies have been cited as evidence for a role of microtubules in the orientation of cells along a chemotactic gradient, and proposed as a possible mechanism for the anti-inflammatory effects of colchicine. Yet patients treated with colchicine have no clinical evidence of neutrophil dysfunction[9,10]. Since micropore filter assays reflect a complex expression of several aspects of PMN function including locomotion, orientation, and deformability, the in vitro effects of colchicine could be explained by any or all of these functions. In order to test the effects of the drug on locomotion and orientation we have applied the technique of chemotaxis under agarose[11], a system in which deformability would be expected to play a minor role, since cells are not required to squeeze through tortuous spaces as they do in micropore filters.

Heparinized venous blood from normal donors was sedimented with 6% Dextran T 500 for 30 min at 37 °C and the cells were washed twice in Minimum Essential Medium (MEM). Red cells were lysed by hypotonic shock. The cells were incubated at a concentration of 5×10^6 PMN per ml with colchicine (10^{-8}–10^{-6} M) in MEM with 10% fetal calf serum for 20 min, washed and resuspended at 5×10^7 PMN per ml for the chemotaxis assay.

Agarose plates were prepared as described by Nelson[11]. 3 wells, 2.4 mm in diameter and arranged radially 2.4 mm apart, were cut with a template and punch. The cells were placed in the middle well, various concentrations of the chemotactic peptide, N-formyl-methionyl-leucyl-phenylalanine (FMLP) in the outer well, and medium in the inner well. After a 3-h incubation the plates were fixed and stained. The leading fronts were measured with the aid of a microprojector, and the results expressed as μm. Random migration was determined from the distance the cells moved towards the inner well. The distance the cells moved towards the outer well was used to determine activated random migration and chemotaxis. Since the effects of colchicine could be caused by either a disturbance of locomotion itself or of the ability of cells to become oriented along the gradient, we utilized a 'checkerboard' design to distinguish activated random migration from true chemotaxis[12]. The former is defined as migration of the leading front of cells in varying concentrations of FMLP in the absence of a gradient. The results are shown in table 1. As expected, the chemotactic peptide stimulated random migration, but no effect could be demonstrated for the concentrations of colchicine tested on either spontaneous or activated random migration. These values were used to calculate the migration expected on the basis of activated migration alone for the various gradients tested according to the method of Zigmond and Hirsch[12]. The data for control and 10^{-6} M colchicine treated cells are shown in table 2. The deviation of the observed from the predicted migration represents true chemotaxis of the cells. The results demonstrate that FMLP has a true chemotactic effect, since the migration of cells in gradients with a higher concentration of FMLP in the cell well was less than that predicted on the basis of activated random alone, and exceeded the predicted for gradients with a higher concen-

Table 1.

	Control	Colchicine (M) 10^{-8}	10^{-7}	10^{-6}
Random migration (μm ± SD)	550 ± 180	580 ± 130	600 ± 130	530 ± 40
Activated random migration (μm ± SD)*	1070 ± 180	1000 ± 20	1090 ± 420	910 ± 420

* Migration in presence of 50 nM FMLP in both cell well and chemoattractant well.

Table 2. Chemotaxis of cells in varying gradients of FMLP*

	nmole FMLP in cell well	nmole FMLP in chemoattractant well 5	10	50	100
Control cells					
	5	710	800 (710)**	1000 (690)	1200 (760)
	10	760 (670)	670	1020 (730)	1160 (800)
	50	710 (1000)	760 (1000)	1070	1020 (1070)
	100	760 (1090)	760 (1090)	910 (1110)	1110
Cells treated with 10^{-6} M colchicine					
	5	640	1020 (670)	1090 (760)	1220 (800)
	10	760 (760)	780	1020 (800)	1130 (820)
	50	530 (890)	760 (890)	910	1020 (910)
	100	640 (930)	710 (930)	930 (930)	930

* Results given as leading front measurements in μm. ** Activated random migration is observed in the absence of a gradient by comparing figures on the diagonal from upper left to lower right. The gradient effect can be observed by comparing the figures on the diagonal from lower left to upper right. Numbers in parentheses represent the calculated distance the cells would be predicted to move on the basis of activated random migration alone.

tration of FMLP in the outer well. By 2-way analysis of variance FMLP had a significant effect on chemotaxis ($p < 0.01$). Comparison of the responses of 10^{-6} M colchicine treated cells with those of control cells by paired T-test failed to demonstrate any significant differences in any gradient ranging from a ratio of 2:1 to 20:1. Similar results were obtained for 10^{-7} and 10^{-8} M colchicine (data not shown).

Colchicine has been postulated to inhibit chemotaxis by disrupting the assembly and disassembly of microtubules[3]. The evidence for this hypothesis is based mostly on experiments utilizing micropore filter chambers. In those studies, cells treated with 10^{-6} M colchicine show up to 75% inhibition of chemotaxis[3,4]. Random migration, either spontaneous or stimulated under gradient free conditions, is largely unaffected. Evidence of the proposed defect in orientation has come from the ultrastructural studies of Malech[3] in which the ability of cells to respond to rapid reversal of the gradient was impaired by treatment with 10^{-6} M colchicine. The impairment was manifested by a loss of orientation of the centriole relative to the nucleus accompanied by a significant decrease in the number of centriole associated microtubules. Zigmond, however, has reported that colchicine-treated cells observed in a direct visual assay were still able to orient in a gradient[12]. Using a filter assay, Valerius[13] also found that colchicine-treated cells could orient in a gradient, but activated random migration was decreased.

Our results indicate that locomotion itself is unaffected by colchicine. Neither spontaneous nor activated random migration was significantly affected by the drug. Furthermore, the responses of the cells in varying concentrations and gradients of FMLP failed to show any impairment of chemotaxis. We conclude that colchicine, at therapeutic serum concentrations in vivo, does not affect either locomotion or chemotaxis.

The reasons for the discrepancy between our results and those observed in micropore filter systems are unclear. A major difference between the agarose and micropore filter assays is the need for cell deformability in the latter. In filter assays the cells are required to pass through a tortuous network of fibres in order to penetrate the membrane. In the agarose system the cells can move freely along the surface towards the source of the gradient. Indeed defects of cell deformability occurring either spontaneously, or following various in vitro treatments, correlate well with defective chemotaxis in micropore filter[15] assays. In conclusion, our data indicate that colchicine at tested concentrations, had no effect on cell locomotion using the agarose technique. These findings are in agreement with the conclusion of Valerius that microtubular assembly is not required for PMN orientation[13], and suggest the possibility that the effects of colchicine on PMN locomotion in filters may occur at other sites in the cell.

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IgE rheumatoid factor

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Summary. A solid phase enzyme-linked immunosorbent assay for detecting class-specific rheumatoid factors (RF) has been devised. IgE RF, which has not been reported yet, was found in sera from 3 of approximately 100 rheumatoid patients.

Rheumatoid factor (RF) activity is found in IgM, IgG, IgA and IgD[1]. However IgE RF has not been reported. We devised an enzyme-linked immunosorbent assay (ELISA) for detecting class-specific rheumatoid factors (RF). Measuring approximately 100 samples from patients with active rheumatoid arthritis (RA), we found a case of pleuritis-associated RA in which a high level of IgE-RF was observed both in the serum and in the pleural effusion. A moderate elevation of serum IgE RF was found in another 2 cases, but approximately 200 sera from normal subjects and patients with RA and SLE were negative. The ELISA was based on the solid phase sandwich method, and our previous method[2] for measuring serum IgE levels was employed with some modifications.

Polystyrene tubes were coated with rabbit IgG (RIgG) instead of anti-IgE antibody and a horseradish peroxidase (HRPO)-labelled Fab fragment of anti-IgE antibody was used in place of HRPO-conjugated anti-IgE whole antibody to avoid the interference of RF other than IgE RF[3]. RIgG which also reacts with RF was used instead of human IgG in order to avoid the reaction with antihuman IgG antibody for detecting IgG RF. When we used anti-IgE whole antibody in place of Fab fragment, a weak false positive reaction due to the binding between all classes of RF and Fc portion of the antibody was observed. Specific anti-IgE antibody (anti-ε) was prepared as described in the previous paper[2].

In order to determine the quantity of IgE RF in the patient,