EFFECT OF COLCHICINE ON VISCOELASTIC PROPERTIES OF NEUTROPHILS

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ABSTRACT The effect of colchicine (15–60 μg/ml) on the viscoelastic properties of human neutrophils was studied by the micropipette technique. The small deformation of the neutrophil in response to a step aspiration pressure was analyzed by using a three-element model in which an elastic element, K_1, is in parallel with a Maxwell element composed of another elastic element, K_2, in series with a viscous element, μ. Colchicine treatment of neutrophils caused decreases in K_2 and μ without affecting K_1. The results indicate that the integrity of the microtubules plays a significant role in providing the viscoelastic resistance (as represented by the Maxwell element in the model) of neutrophils to deforming stress.

INTRODUCTION

Leukocytes have a larger volume and less deformability than erythrocytes (1, 2). As a result, leukocytes can exert significant influences on blood flow through the microcirculation (3).

The deformation behavior that occurs when the whole leukocyte is aspirated into a micropipette or capillary tube depends on the geometric features of the cell as well as its rheological properties (1, 4, 5). When leukocytes are subjected to small deformations by using micropipettes with an internal radius smaller than ~2 μm, the response primarily reflects the intrinsic rheological properties of the cell (6). Such experiments have been performed on normal human neutrophils and the results have been successfully analyzed with a three-element model in which a Maxwell element (serial elastic and viscous element) is in parallel with another elastic element (6, 7). These viscoelastic coefficients change in response to several physicochemical treatments, such as changes in temperature, pH, and osmolality (8).

The biophysical behavior of a cell reflects its biochemical composition and organization. The cytoskeleton of a leukocyte contains microfilaments, microtubules, and intermediate filaments. The microfilaments, which are 5–8 nm thick, are composed mainly of actin; they also contain myosin, tropomyosin, and α-actinin (9, 10). The microtubules, which are 24 nm in diameter and have a hollow 15-nm core, are composed primarily of subunits of tubulin heterodimers (11). The intermediate filaments consist of fibrous polypeptides such as desmin (12). These cytoskeletal components form a cytoplasmic network that maintains cell shape and effects cell motion (13); the components can be modified by chemical treatments. Thus, cytochalasins can cause disruption of the microfilaments (14) and colchicine can cause disruption of the microtubules (11).

This investigation was performed to study the effects of colchicine on the viscoelastic properties of human neutrophils during small deformation in the micropipette. The aim is to elucidate the role of microtubules in the rheological behavior of neutrophils, thus establishing the biochemical-biophysical correlation in these cells.

METHODS

Blood samples were drawn from healthy human subjects using EDTA as an anticoagulant and allowed to stand at room temperature for 25–40 min. After the red cells sedimented, the supernatant plasma, containing white blood cells (WBCs), platelets, and a few red cells, was collected and diluted with a prefiltered, buffered saline-albumin solution to a concentration of ~50 WBCs/mm^3. The solution contained 0.9 g/dl NaCl, 0.1 g/dl EDTA, 0.25 g/dl bovine serum albumin, and 12 mM Tris, and its pH was adjusted to 7.4 by adding 1 N HCl drop by drop.

In addition to the control WBC suspensions prepared in the saline-albumin solution, WBC suspensions were prepared in the same manner in saline-albumin solutions containing colchicine (Sigma Chemical Corp., St. Louis, MO) at concentrations of 15, 30, and 60 μg/ml. For each of these colchicine concentrations, the cells were studied 15, 30, and 45 min after colchicine treatment.

The viscoelastic properties of control and colchicine-treated neutrophils were determined by using the micropipette aspiration technique described elsewhere (6, 8). About 0.5–1 ml of the cell suspension was loaded in a small round chamber located on the stage of an inverted microscope. With the use of an 100× objective (NA 1.25, oil immersion) and a 20× eyepiece, the viewing field was displayed on a video monitor through a video camera and recorded on a video recorder. Micropipettes with inner radii of 1.1–1.7 μm were filled with the saline-albumin solution and connected to a pressure regulation system and a pressure recording system. With the use of a hydraulic micromanipulator, the pipette tip was positioned near the surface of a neutrophil, which was then subjected to a step negative pressure aspiration.

The time course of deformation of the neutrophils was analyzed from sequential photographs taken from the video image during single frame

replay on the video monitor. The displacement of the cell surface into the pipette was determined by subtracting the distance that the cell reached into the pipette without deformation from the distance that the cell reached into the pipette with deformation. With the aid of an Eye Com II video-digitizer (model 109PT; Spatial Data Systems Inc., Goleta, CA) and a PDP 11/23 Minc microcomputer (Digital Equipment Corp., Marlboro, MA), the data were analyzed by using our three-element model (6). In this model, the neutrophil is treated as a standard solid consisting of an elastic element K_1 in parallel with a Maxwell element (an elastic element K_2 in series with a viscous element μ).

RESULTS

The response of human neutrophils to a step aspiration pressure consists of an initial elastic deformation followed by a slow-creeping phase (Fig. 1). The time-dependent deformation curve was analyzed by using the three-element model described in the Methods section. The values of K_1, K_2, and μ (mean ± SD) in the control measurements of this study were 308 ± 77 dyn/cm^2, 758 ± 258 dyn/cm^2, and 167 ± 67 dyn · s/cm^2, respectively. These values are not significantly different from the results obtained in our previous studies on normal human neutrophils (6).

Treatment of human neutrophils with colchicine increased the degree of deformation that occurred in response to a given step aspiration pressure. An analysis of the stress-strain relation by using the three-element model indicates that colchicine caused reductions in K_2 and μ, but it had no significant effect on K_1 (Table I). The decrease in K_2 was essentially the same over the colchicine concentration range of 15–60 μg/ml (Fig. 2). The reduction in μ was greater with 30 and 60 μg/ml colchicine than with 15 μg/ml colchicine. There was no systematic difference in the results when the durations of colchicine treatment were varied from 15 to 45 min (Table I).

The Brownian movements of the granules inside the neutrophils increased after colchicine treatment. The neutrophils developed protopodia at ~45 min after they were treated with the highest concentration of colchicine used (60 μg/ml). There were no other significant changes in cell morphology (Fig. 3).

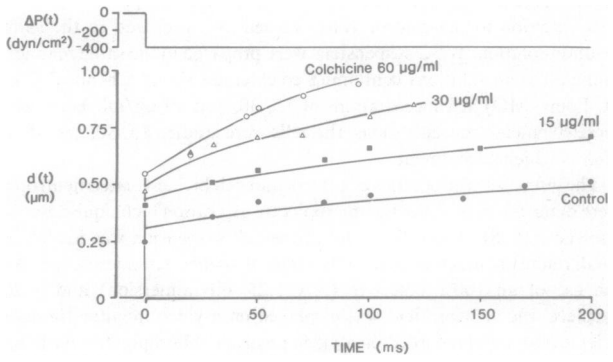

FIGURE 1 Time course of deformation of neutrophils, d(t), in response to a step aspiration pressure of 392 dyn/cm^2 applied using a micropipette with an inner radius of 1.7 μm. Closed circles represent the data from a control neutrophil. Closed squares, open triangles, and open circles represent the data from neutrophils treated for 30 min with 15, 30, and 60 μg/ml concentrations of colchicine, respectively. The solid lines are the theoretical lines obtained by using the three-element model.

TABLE I
EFFECTS OF COLCHICINE ON VISCOELASTIC CONSTANTS OF HUMAN NEUTROPHILS*

	K_1	K_2	μ
	dyn/cm^2	dyn/cm^2	dyn · s/cm^2
Control (n = 14)	308 ± 77	758 ± 258	167 ± 67
15 μg/ml Colchicine			
15 min (n = 9)	324 ± 49	595 ± 194	86 ± 53‡
30 min (n = 12)	311 ± 197	385 ± 158‡	53 ± 50‡
45 min (n = 9)	340 ± 133	585 ± 107‡	64 ± 65‡
30 μg/ml Colchicine			
15 min (n = 24)	299 ± 93	430 ± 81‡	29 ± 15‡
30 min (n = 10)	334 ± 34	418 ± 106‡	18 ± 8‡
45 min (n = 11)	315 ± 162	454 ± 200‡	64 ± 43‡
60 μg/ml Colchicine			
15 min (n = 8)	267 ± 107	585 ± 218	24 ± 12‡
30 min (n = 15)	290 ± 172	432 ± 116‡	20 ± 18‡
45 min (n = 5)	290 ± 74	508 ± 111‡	38 ± 19‡

*Values are mean ± SD, n = number of cells studies; each cell had an average of three aspiration tests under the conditions given.
‡$P < 0.01$ when compared with control.

DISCUSSION

Human neutrophils have a large excess membrane area; the determination of the stress-strain relationship of neutrophils during small deformations in micropipette tests allows the viscoelastic coefficients of cell content to be computed independent of cell geometry (6, 8). By aspirating a small portion of the cell away from the nucleus, one avoids the influence of the more rigid nucleus on the deformation behavior. The results of the small deformation micropipette tests primarily reflect the behavior of the cytoplasm, especially the cytoskeletal apparatus (6).

The biophysical behavior of biological tissues is a func-

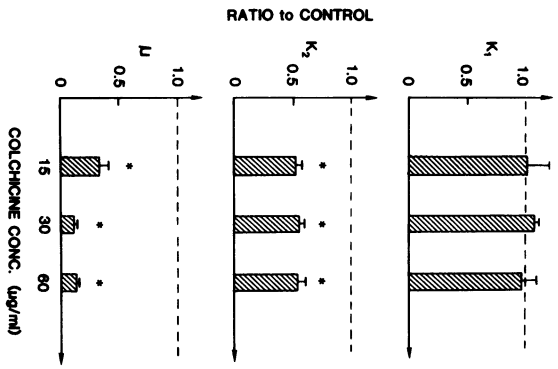

FIGURE 2 The viscoelastic parameters K_1, K_2, and μ for human neutrophils treated with three dose levels of colchicine for 30 min. The values are expressed as ratios to the constants of the control neutrophils that were run in parallel. The symbol * represents ratios that were significantly different from 1.0 ($P < 0.001$).

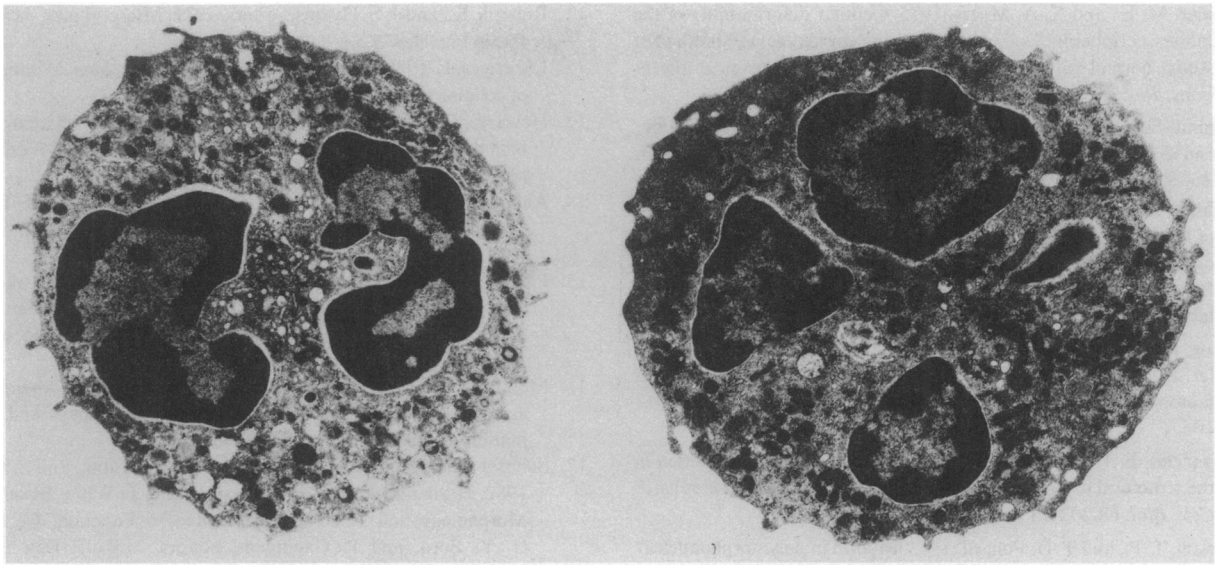

FIGURE 3 Transmission electron micrographs showing a control neutrophil (*left*) and a neutrophil treated with 30 μg/ml colchicine (*right*).

tional manifestation of their biochemical composition and molecular organization. The identification of such biochemical-biophysical correlation would help to elucidate the structure-function relationship in biological systems. This approach is exemplified by the recent investigations on the role of membrane proteins on the viscoelastic properties of the erythrocyte membrane (15, 16). In the present study, the role of microtubules in neutrophil viscoelasticity was investigated by treating the cells with colchicine, an agent that can bind tubulin and cause microtubule depolymerization (11). After colchicine treatment, selective decreases in K_2 and μ occur without a significant alteration in K_1, suggesting that the microtubules contribute to the viscoelastic behavior of the neutrophils, as reflected by the change in the Maxwell element in our rheological model. Thus, the integrity of the microtubules may play a significant role in affecting the viscoelastic resistance of neutrophils to externally applied deforming stress. The enhancement of Brownian movements of the granules of neutrophils following colchicine treatment probably is a reflection of the decrease in μ. Our previous studies (8) showed that an increase in osmolality causes increases in all three viscoelastic coefficients of human neutrophils, probably because the solid concentration of all cytoplasmic components increases. The finding that a high pH value of 8.4 causes increases in K_1 and μ, but not in K_2 (8), indicates that the three elements in the viscoelastic model can be affected differently by various types of chemical treatments. Note that the three-element model is used only to describe the stress-strain relationship for small deformations of neutrophils over a short time period (<1 s). Therefore, the major finding in this study is that the microtubule-disrupting agent, colchicine, can alter the viscoelastic properties of the neutrophil content, and further work is needed to correlate the rheological coefficients, the viscoelastic behavior, and the biochemical constituents of neutrophils.

The present investigation was performed on neutrophils suspended in EDTA. Previous studies from our laboratory have shown that in the presence of free Ca^{2+}, neutrophils undergo active deformation and the protopod regions have higher viscoelastic coefficients (17). Thus, the present results primarily reflect the action of colchicine on the neutrophil in the passive state. It would be interesting to evaluate how the rheological effects of colchicine on the neutrophil cytoskeleton are affected by Ca^{2+}. Additional investigations on the effects of different chemical agents, e.g., the microfilament-disrupting agent, cytochalasin, would help to further establish the biochemical basis of the biophysical behavior of the neutrophils.

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