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THE PHAGOCYTOSIS OF YEAST CELLS BY MONOCYTES: Effects of Colchicine, β-lumicolchicine, and γ-lumicolchicine

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ABSTRACT

The effect of microtubule antagonist (MA) colchicine and its two non-MA derivatives β-lumicolchicine and γ-lumicolchicine on the phagocytosis of yeast cells by glass-adherent monocytes were studied with a fluorescence quenching technique which allowed a distinction between the adherence and engulfment phases of phagocytosis. The engulfment of yeast cells was depressed by colchicine ($p = 0.031$) but not by the lumicolchicines. The adherence of yeast cells to the monocytes, on the other hand, was depressed by γ-lumicolchicine ($p = 0.028$).

The colchicine effect suggests that cytoplasmic microtubules play a role in the engulfment process whereas the lumicolchicine effect suggests a role of nucleoside transport over the cell membrane in the adherence process of phagocytosis.

Colchicine belongs to a group of antimitotic substances called "spindle poisons" or "microtubule antagonists" (MAs). The antimitotic action of colchicine is explained by its binding to the protein tubulin, the main building block of microtubules, organelles involved in cell shape, transport and movement. Colchicine is degraded to lumicolchicines by exposure to ultraviolet light. The lumicolchicines apparently lack activity as microtubule antagonists but inhibit nucleoside transport over the cell membrane like colchicine and podophyllotoxin (1,2).

The microtubule antagonists vincristine, vinblastine and vindesine inhibit monocyte phagocytosis of yeast cells by 10-30% (3); a finding due to an MA inhibition of microtubule function in monocyte phagocytosis. The significance of this observation is broader than merely an immunologic one, because monocyte/macrophage activity plays an important scavenger role in proteolysis associated with high-protein edemas (4,5). The aim of the present study was to elucidate the role of microtubules in monocyte phagocytosis with colchicine and two commercially available lumicolchicines as cytobiological tools.

MATERIALS AND METHODS

Definition of Terms

Monocyte migration (chemotaxis) over the glass surface towards a yeast cell was not measured in the present assay. Yeast cell *adherence* to the monocyte was defined as a visible membrane contact between yeast cell(s) and a monocyte. Yeast cell *engulfment* by a monocyte was defined as the presence of a fluorescent yeast cell within a monocyte. Engulfment and adherence together represented *total phagocytosis*, since adherence and engulfment have not been discriminated from each other in most tests of phagocytosis. The distance between yeast cells on the slide was estimated to be 20-50 μm, but

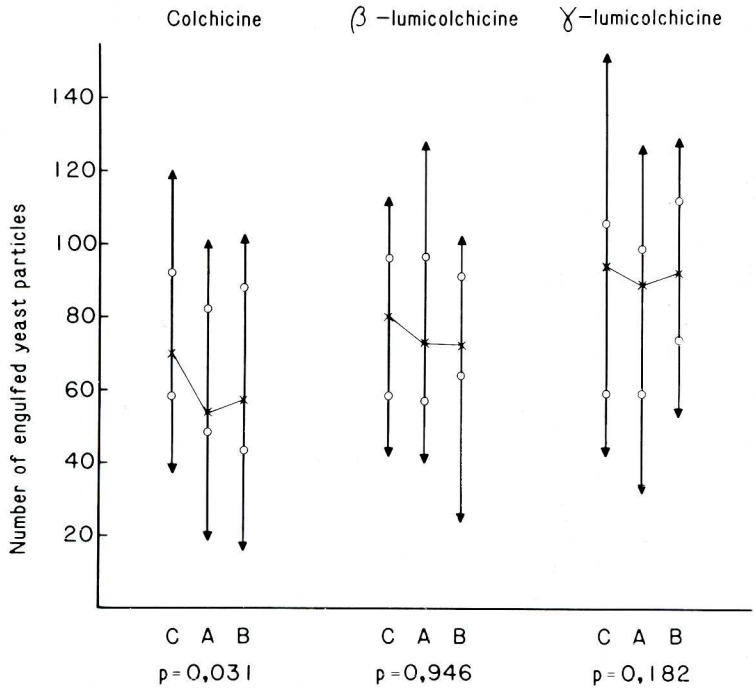

Fig. 1: The effect of colchicine and two lumicolchicines on the engulfment of yeast cells by monocytes from 18 healthy blood donors. Symbols: C = control preparations, A = 0.01 μg/ml, B = 0.10 μg/ml; Median (*), interquartile range (o) and maximum values. The data were evaluated by the Friedman two-way analysis of variance. Ordinate: number of yeast cells engulfed by 50 monocytes.

the yeast cells often lay in clusters. The distance between monocytes was estimated to be 100-500 μm. There was a surplus of non-phagocytosed yeast cells in the preparation after stopping phagocytosis.

Preparation of yeast cell suspension

Baker's yeast (Saccharomyces cerevisiae) was suspended in saline, washed twice, boiled 30 min., washed twice, and labelled with fluorescein isothiocyanate (FITC, Merck, Darmstadt, FRG) in a carbonate buffer, pH 10.2, 10^8 cells per ml of FITC buffer with a FITC concentration of 0.1 mg/ml, 60 min., +37°C. The cells were washed three times with saline, re-diluted to 10^8 cells per ml, and frozen at -17°C in 0.5 ml aliquots. Prior to the experiment, the FITC-labelled yeast cells were opsonized with human serum which had been stored at -70°C for less than two weeks. Serum, 0.5 ml, was mixed with 0.5 ml of the FITC-labelled yeast cells, and the mixture was incubated at +37°C for 30 min. Then the cell suspension was washed twice in saline and resuspended in 2 ml of saline.

Complete medium

The complete medium consisted of human serum, 165 ml, L-glutamine 20 mM, 7 ml, Ham's F10 medium 500 ml (Flow Laboratories, Irvine, Ayershire, Scotland), amphoterizine B 1.7μg/ml, streptomycin 4 μg/ml and benzylpenicillin 2.4μg/ml.

Glassware

The glassware was thoroughly washed, rinsed in demineralized water and 70% ethanol, and autoclaved at +120°C for 20 minutes.

Blood sampling

Heparinized blood, 16 IU/ml, was obtained from healthy blood donors from the Blood Transfusion Center of the hospital.

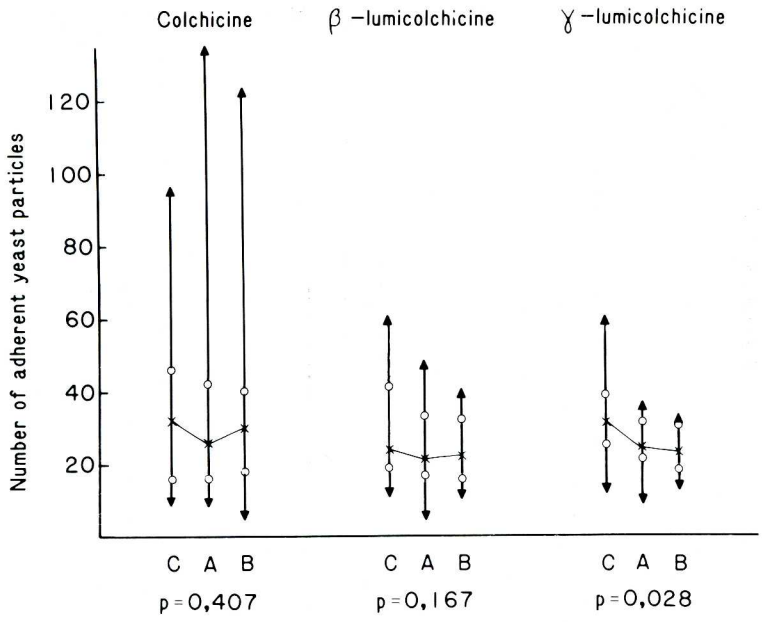

Fig. 2: The effect of colchicine and lumicolchicines on the adherence of yeast cells to the membrane of fifty monocytes (N — 18 healthy blood donors). Symbols: see legend Fig. 1.

Isolation of mononuclear leucocytes

Mononuclear leucocytes (L-MNs) were isolated from peripheral blood by the one-step metrizoate-Ficoll procedure described by Boyum (6) using Lymphoprep® (Nyegaard, Oslo, Norway). The L-MNs were then washed twice in complete medium (see above) and resuspended to a concentration of 5 x 10^6 cells per ml.

Incubation for monocyte adherence to the substrate

The L-MNs, 1 x 10^6 in 200 µl complete medium, were then seeded on sterile slides and incubated in a humidified chamber with 5% CO_2 at +37°C for 30 minutes in order to allow the monocytes to adhere to the glass surface.

Removal of lymphocytes

The slides were rinsed with pre-warmed complete medium.

Test drug suspensions

The colchicine (Fluka Ag, Buchs, Switzerland), β-colchicine and γ-colchicine (Sigma, St. Louis, MD, USA) were suspended in isotonic NaCl every day prior to experimentation.

Pre-incubation with test drugs

Control slides were again incubated in complete medium for 30 minutes at +37°C in 5% CO_2 in humidified chambers. Test slides were incubated likewise with the addition of colchicine, β-colchicine or γ-colchicine, 0.01 or 0.10 µg/ml.

Phagocytosis experiments

Yeast cells labelled with FITC and opsonized with human serum were added to control slides and test slides, 2.5 x 10^7 yeast cells in 200 µl saline. The yeast cell suspension added to the test slides contained test drug, 0.01 µg/ml or 0.10 µg/ml. Incubation was performed for 30 minutes at 37°C in 5% CO_2. Then the fluorescence of non-ingested yeast cells was quenched by dripping crystal violet (Merck, Darmstadt, FRG), 0.5 mg/ml saline, onto the slides. The staining time was ten seconds. The stain was decanted and the preparation was sealed with a coverslip. Monocyte-adherent yeast cells (brown) and

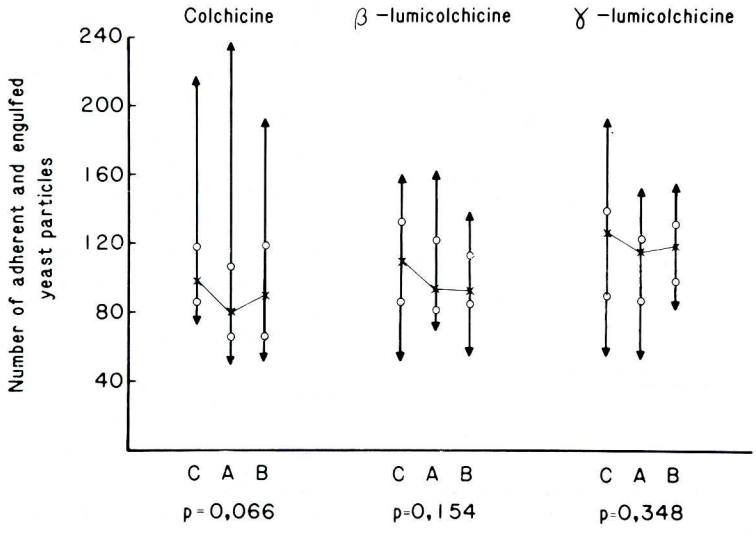

Fig. 3: The effect of colchicine and two lumicolchicines on total phagocytosis of yeast cells by fifty monocytes (N = 18 healthy blood donors). Symbols: see legend Fig. 1.

ingested yeast cells (fluorescent) were then counted in 50 monocytes at 320x magnification with a Zeiss incident-light microscope.

Statistics

The Friedman two-way analysis of variance was calculated according to Siegel (7). The basic figures were provided by the numbers of yeast cells associated with 50 monocytes. Control and test samples from a cell donor (N = 18 in each series of experiments) were run simultaneously.

RESULTS

The engulfment of yeast particles by monocytes was depressed by colchicine at a concentration of 0.01 μg/ml and 0.10 μg/ml ($p = 0.031$) but the lumicolchicines had no demonstrable effect *(Fig. 1)*.

The adherence of yeast particles to monocytes were depressed by γ-lumicolchicine ($p = 0.028$) whereas colchicine and β-lumicolchicine had no demonstrable effect *(Fig. 2)*.

A possible colchicine inhibition of total phagocytosis was not statistically significant ($p = 0.066$). The lumicolchicines also had no effect on total phagocytosis *(Fig. 3)*. The overall response on monocyte phagocytosis to these agents is shown in *Fig. 4*.

DISCUSSION

A previous study supports that monocyte phagocytosis requires prolonged membrane contact between leucocyte and test particle, in the order of magnitude of 3-5 seconds (8). A corollary to this observation is that monocyte phagocytosis occurs mainly on a surface unless monocytes and test particles are closely packed to ensure membrane contact (8). Another fundamental problem in the conventional analysis of phagocytosis is difficulty in discriminating between engulfed particles and particles only adherent to the monocyte membrane (9).

The present monolayer technique, originally introduced by Hed (10,11) for the study of neutrophil phagocytosis, overcomes some of the major objections to conventional analysis of phagocytosis. Phagocytosis occurs on a glass surface, the monocytes exert directional movement (chemotaxis) towards the yeast cell, embrace it and ingest it. The extinction of fluorescence in non-ingested yeast cells by crystal violet allows a clear distinction between membrane-adherent yeast cells and engulfed yeast cells.

Colchicine, 0.01 and 0.10 μg/ml, appears

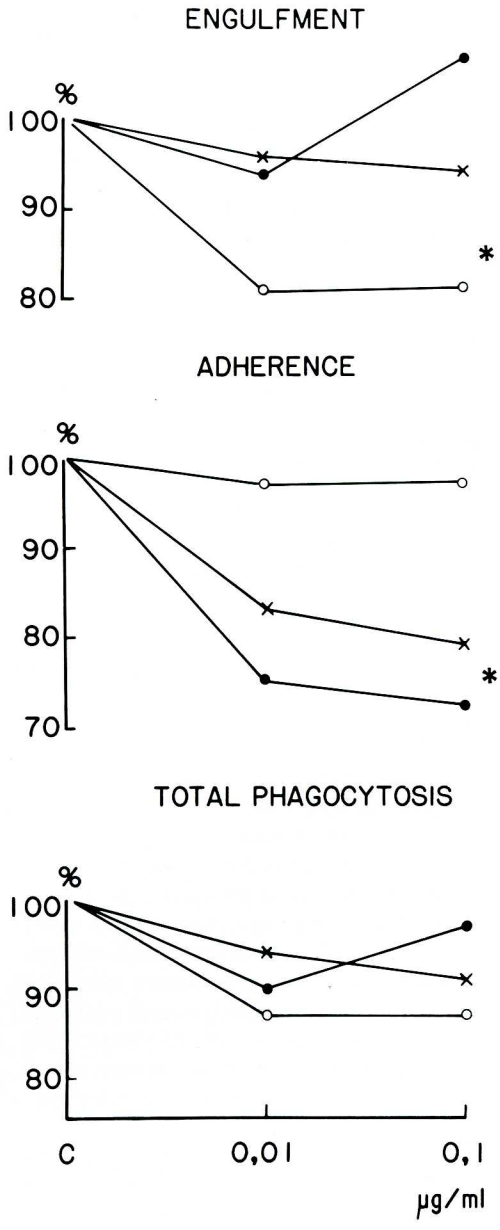

*Fig. 4: Effect of colchicine (o), β-lumicolchicine (x) and γ-lumicolchicine (•) on monocyte phagocytosis of yeast cells. The partial processes adherence and engulfment are analyzed separately. Each point represents the median value of data from 18 cell donors. *: $p < 0.05$.*

to depress monocyte engulfment and total monocyte phagocytosis of yeast cells by 10-20% (see *Fig. 4*). This observation agrees with previous findings using comparable concentrations of Vinca alkaloids (3). The possible colchicine inhibition of monocyte phagocytosis is, however, only statistically significant for engulfment.

In contrast to engulfment, colchicine has no apparent effect on yeast cell adherence to monocytes (*Fig. 4*). However, the lumicolchicines seem to depress yeast cell adherence, the γ-lumicolchicine effect being statistically significant.

It should be emphasized that the drug concentrations used in the present study were specifically low to make it unlikely that colchicine exerted an effect on nucleoside transport (2). It is therefore reasonable to assume that the colchicine inhibition of engulfment was due to interference with microtubule function.

The observed effect of colchicine and lumicolchicines on monocyte phagocytosis is compatible with the hypothesis that yeast cell adherence to monocytes is partly dependent on nucleoside transport over the cell membrane (lumicolchicine effect), and that monocyte engulfment of yeast cells is partly dependent on cytoplasmic microtubules (colchicine effect).

Although the activity of these agents on scar formation and lymphedema has not specifically been investigated, macrophage scavenger activity is important in the digestion of tissue fluid proteins in high-protein lymphedema (4,5). Considering the ability of colchicine and lumicolchicine to inhibit monocyte/macrophage reactivity, administration of these microtubule antagonists (e.g. for treatment of gout) should be used with caution in patients with high-protein lymphedema.

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