Interference of Granulocyte Function by the Vehicle of Miconazole

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The vehicle of miconazole inhibited adherence and leukotaxis, but did not affect nitroblue tetrazolium reduction and trypan blue exclusion by granulocytes at 0.33% concentration and above.

Information on the effects of miconazole, a new and effective antifungal drug, on host defense function is meager (3). Since it is agreed that intact leukocyte function is important especially in the infected host, we studied the influence of miconazole and its liquid vehicle on granulocyte functions.

Miconazole base dry powder (lot no. A 29/2) and its vehicle marked "placebo" (lot no. 618.095, containing 0.115 ml of polyethoxylated castor oil, 1.62 mg of methylparaben, 0.18 mg of propylparaben, and pyrogen-free water to make 1 ml) were kindly provided by Janssen Research and Development, Inc., New Brunswick, N.J. For our studies a stock solution was made by dissolving 41.6 mg of miconazole base powder in 10 ml of vehicle. Further dilutions of the stock solution and dilutions of the vehicle were made using medium 199.

Granulocytes were prepared from heparinized venous blood by sedimentation of erythrocytes at $1 \times g$ for 1 h and centrifugation of the leukocyte-rich plasma at $500 \times g$ for 5 to 10 min. The cell pellet obtained was then washed once in medium 199 and adjusted to a concentration of 5×10^6 granulocytes per ml. Only medium 199 was used in all experiments.

Granulocyte adherence was measured by the Hemmalini and Martin modification (Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 15th, Washington, D.C., Abstr. no. 68, 1975) of the method described by MacGregor et al. (4), using 40 mg of scrubbed nylon fibers (in LP-1 Leuko-Pac leukocyte filter; Fenwal Laboratories, Morton Grove, Ill.) packed inside each plastic tuberculin syringe, with the length of the packed column adjusted to exactly 15 mm. Washed granulocytes as prepared above were then applied over the column, and the percentage of adherence was calculated from the origi-

nal count and the filtrate count. Granulocyte migration was assessed using the "leading front technique" (8). In this method, the prepared leukotactic chambers, with the indicator cells in the upper compartment (above a micropore filter) and the chemotactic attractant in the lower compartment (below the micropore filter), were incubated in air at 37°C for only 1 h. After incubation, the filters were removed and stained. The distance (in micrometers) migrated by the leading front of cells (two or more cells in the same focal plane) was then measured by the microscope micrometer. Nitroblue tetrazolium reduction was measured by the quantitative method (1), using opsonized zymosan as the particles for engulfment.

To study the effects of the drugs on granulocyte adherence, cells were incubated at 37°C for 30 min with medium 199 containing the various concentrations of miconazole in vehicle or vehicle alone. Cells incubated with medium 199 alone were used as controls. After the incubation, the treated cells were applied over the fiber columns, and the percentage of adherence was calculated. The marked inhibition of adherence by miconazole in vehicle paralleled the effects of the corresponding concentrations of the vehicle alone (Fig. 1). These results indicate that the inhibition is solely due to the effects of the vehicle and not to the miconazole itself.

In another experiment, the effect of the drugs on leukotaxis was studied and compared. Cells treated in the same fashion but without miconazole or vehicle served as controls. After incubation, the chemotactic activity of the treated cells was tested by using 10% bacterial chemotactic factor derived from *Escherichia coli* (7) in the lower compartment. The results (Fig. 2) show a marked leukotactic inhibitory effect of miconazole dissolved in its vehicle. As with the

FIG. 1. Inhibition of granulocyte adherence by miconazole in vehicle and by vehicle alone.

studies on adherence, these results can be explained solely by the effects of the vehicle alone.

Nitroblue tetrazolium dye reduction of cells treated with the drugs was normal, indicating that neither miconazole nor its vehicle interferes with phagocytosis of opsonized zymosan or the oxidase-dependent microbicidal system of granulocytes.

Over 95% of the treated cells excluded trypan blue, suggesting no loss of viability as a result of exposure to miconazole or its vehicle.

Since cell adherence is fundamentally a surface phenomenon (5), and since polyethoxylated castor oil is a surface-active agent (a detergent or surfactant), among the three constituents of the vehicle the oil is the most likely substance

FIG. 2. Leukotactic inhibitory effect of miconazole in vehicle and of vehicle alone.

responsible for the changes in leukocyte function. As little as 0.33% of the vehicle (containing 0.00038 ml of the oil per ml of vehicle-medium 199 solution) produced notable effects on cell adherence and leukotaxis. With the allowable dose and the recommended preparation of miconazole for intravenous use, this concentration of polyethoxylated castor oil can be calculated to be achievable in serum. This agrees with reports showing the accumulation of abnormal lipids in the blood of patients receiving intravenous miconazole (2, 6).

This study stresses the importance of testing also the vehicles and adjuvants in drug studies and adds strength to the belief that regulated

cell adherence is an important factor for normal granulocyte locomotion.

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LITERATURE CITED

1. Baehner, R. L., and D. G. Nathan. 1968. Quantitative nitroblue tetrazolium test in chronic granulomatous disease. *N. Engl. J. Med.* 278:971-976.
2. Bagnarello, A. G., L. A. Lewis, M. C. McHenry, A. J. Weinstein, H. K. Naito, A. J. McCullough, R. J. Lederman, and T. L. Gavan. 1977. Unusual serum lipoprotein abnormality induced by the vehicle of miconazole. *N. Engl. J. Med.* 296:497-499.

3. Björkstén, B., C. Ray, and P. G. Quie. 1976. Inhibition of human neutrophil chemotaxis and chemiluminescence by amphotericin B. *Infect. Immun.* **14**:315-317.
4. MacGregor, R. R., P. G. Spagnuolo, and A. L. Lentnek. 1974. Inhibition of granulocyte adherence by ethanol, prednisone and aspirin measured by an assay system. *N. Engl. J. Med.* **291**:642-646.
5. Marchesi, V. T., and H. W. Florey. 1960. Electron micrographic observations on the emigration of leukocytes. *Q. J. Exp. Physiol.* **45**:343-349.
6. Niell, H. B. 1977. Miconazole carrier solution, hyperlipidemia and hematologic problems. *N. Engl. J. Med.* **296**:1479.
7. Ward, P. A., I. H. Lepow, and L. J. Newman. 1968. Bacterial factors chemotactic for polymorphonuclear leukocytes. *Am. J. Pathol.* **52**:725-736.
8. Zigmond, S. H., and J. G. Hirsch. 1973. Leucocyte locomotion and chemotaxis. New methods for evaluation, and demonstration of a cell-derived chemotactic factor. *J. Exp. Med.* **137**:387-410.