Toxicity of Amphotericin B, Miconazole, and Ketoconazole to Human Granulocyte Progenitor Cells In Vitro

TIMOTHY C. MEEKER,† MARTIN S. SIEGEL,1,2* FAITH M. SHIOTA,2 JOHN J. CROWLEY,2 AND ROBERT W. MCGUFFIN1,2

Programs in Oncology, Infectious Diseases and Epidemiology and Biostatistics, Fred Hutchinson Cancer Research Center, Seattle, Washington 98104,2 and Department of Medicine, University of Washington School of Medicine, Seattle, Washington 981951*

Received 7 September 1982/Accepted 20 October 1982

Granulocyte progenitor cells were grown in culture with amphotericin B, miconazole, and ketoconazole. Significant suppression of progenitor cell growth could be demonstrated with all three drugs at increasing concentrations. No additive suppression was seen when amphotericin B and ketoconazole were combined.

Fungal infection is a frequent and severe complication in the granulocytopenic host after marrow transplantation or conventional chemotherapy for hematological malignancy (11, 18). Antifungal therapy with either amphotericin B or miconazole is often used during the period of granulocytopenia for presumed or proven invasive fungal disease (9, 15). A new imidazole, ketoconazole, is also being investigated (15). Though leukopenia has been infrequently reported as a consequence of antifungal therapy with the above agents, one study has suggested that amphotericin B is capable of suppressing marrow granulocyte precursors (10). In addition, neutropenia has been occasionally observed during miconazole and ketoconazole therapy (4, 14). Suppression of growth of granulocyte progenitor cells in the marrow by these antifungal agents would be of diminished clinical value, though the marrow-suppressive effects of these agents would be difficult to differentiate from the known marrow toxicities (i.e., chemotherapy, radiation) of other agents being used in the granulocytopenic host. To evaluate the potential marrow-suppressive role of amphotericin B, miconazole, and ketoconazole, we studied their effects on the growth of colony-forming units (CFU) of granulocyte-monocyte cells (GM) (CFU-GM) in vitro.

Bone marrow aspirates from normal individuals were used as a source of GM. Nucleated cells from buffy coat were plated in quadruplicate at a concentration of 10² cells per ml in 0.3% agar–20% fetal calf serum–alpha medium as previously described (13, 16). Medium conditioned by phytohemagglutinin-stimulated lymphocytes was used as a source of colony-stimulating activity and was added at a dose that gave maximal stimulation of control cultures. Amphotericin B dissolved in sterile water, miconazole dissolved in dimethyl sulfoxide, and ketoconazole dissolved in 0.2 N hydrochloric acid (HCl) were added just before plating in concentrations ranging between 0.1 to 100.0 μg/ml. Control plates did not contain the agents but were otherwise identical and also included the addition of dimethyl sulfoxide, HCl, or sterile water. Plates were incubated at 37°C in an atmosphere of 5% CO₂ in air. Colonies of 40 or more cells were counted at 14 days over an inverted microscope. The mean colony count of quadruplicate plates at each drug concentration was expressed as a percentage of the colony count of control plates. The results of 10 separate runs were averaged to generate a suppression curve for each drug. In addition, 10 runs were plated in an identical fashion, with the combination of amphotericin B and ketoconazole; the concentration of amphotericin B was held constant at either 1.0 or 2.0 μg/ml, whereas the concentration of ketoconazole varied between 0.1 and 20.0 μg/ml.

The mean (± 1 standard error of the mean) growth of CFU-GM with each drug at increasing concentrations is shown in Fig. 1. All drugs suppressed CFU-GM growth with higher concentrations. Miconazole caused more significant suppression when compared with amphotericin B at 2.0, 10.0, and 20.0 μg/ml and with ketoconazole at 4.0, 10.0, and 20.0 μg/ml (P < 0.01 by the Wilcoxon rank sum test). The combination of amphotericin B and ketoconazole together did not show any additive suppression (Table 1). There was no significant difference in the percentage of suppression when the concentration

† Present address: Department of Medicine, Stanford University, Palo Alto, CA 95128.
of amphotericin B was increased from 1.0 to 2.0 μg/ml in combination with ketoconazole (P > 0.10 in all cases by the Wilcoxon rank sum test). There was also no difference in suppression when ketoconazole alone at a concentration of between 0.1 and 20.0 μg/ml was compared with the combination of ketoconazole and amphotericin (P > 0.05 in all cases by the Wilcoxon rank sum test; P < 0.10 only at 0.1 μg/ml).

When the serum levels reached after conventional doses of the three antifungal agents were used for comparison, we found a narrower toxic-to-therapeutic ratio for the imidazole compounds than for amphotericin B. Peak serum levels with amphotericin B vary between 0.5 and 2.0 μg/ml, whereas significant suppression of CFU-GM (>50%) was not observed until a concentration of 10 μg/ml was reached (2). Peak serum levels of ketoconazole vary between 2.0 to 6.0 μg/ml after doses of 200 and 400 mg, with levels in excess of 10.0 μg/ml (and up to 50.0 μg/ml) reported at higher doses (3, 6). With a half-life of approximately 3 h, ketoconazole serum levels in excess of the concentration required for 50% CFU-GM suppression (10 to 15 μg/ml) could be maintained for several hours (3). With miconazole, peak blood levels are typically less than 4.0 μg/ml, the concentration at which 50% CFU-GM suppression is observed. Levels well above this concentration are seen occasionally but fall quickly with a rapid initial-phase half-life of 30 min (17).

The existing clinical data suggest that these drugs as currently used are usually nontoxic to narrow granulocyte progenitor cells. As noted, neutropenia has been reported with miconazole but is uncommon (14). Clinical experience with ketoconazole, including a study of prophylactic ketoconazole in patients undergoing either marrow transplantation or intensive chemotherapy

### TABLE 1. Effect of ketoconazole and amphotericin B in combination on in vitro growth of CFU-GM

| Ketoconazole concn (μg/ml) | Growth of CFU with a: |  |
|---------------------------|-----------------------|--|---|
|                           | Ketoconazole          | Ketoconazole + amphotericin at 1.0 μg/ml | Ketoconazole + amphotericin at 2.0 μg/ml |
| 0.1                       | 99 ± 4.9              | 109 ± 4.8                           | 118 ± 4.4                           |
| 1.0                       | 94 ± 4.7              | 104 ± 4.0                           | 104 ± 5.2                           |
| 2.0                       | 93 ± 4.7              | 87 ± 6.5                            | 89 ± 7.3                            |
| 4.0                       | 90 ± 3.0              | 80 ± 5.7                            | 81 ± 5.8                            |
| 10.0                      | 66 ± 8.4              | 62 ± 6.2                            | 60 ± 7.7                            |
| 20.0                      | 15 ± 5.5              | 12 ± 5.0                            | 14 ± 7.0                            |

a Numbers represent the mean of 10 separate marrow results ± standard error of the mean expressed as a percentage of a control.
Amphotericin B, with a much larger clinical usage than the imidazoles, has been associated with anemia and thrombocytopenia but only rarely with leukopenia (1). A proposed mechanism for amphotericin B-associated anemia is suppression by the drug of precursors in the marrow or of erythropoietin production (10, 12). Amphotericin B-associated thrombocytopenia is thought to be mediated through marrow inhibition (5). One previous report, in which similar culture techniques were used, showed 50% CFU-GM suppression at amphotericin B concentrations between 2 and 3 μg/ml, whereas suppression was not seen in our study until levels of approximately 10.0 μg/ml were reached (10). The reasons for these different results are not clear, but clinical experience with amphotericin B is more consistent with a lack of toxicity to granulocyte precursors.

We found no evidence of augmented CFU-GM suppression when amphotericin B and ketoconazole were used together in cell culture. This drug combination may be clinically useful in the future and has been recently explored in an animal model for treatment of histoplasmosis and cryptococcosis (7).

It should be stressed that this study was carried out with normal marrow cells grown in an otherwise optimal culture environment. In the neutropenic patient, there may be impaired mechanisms for metabolizing antifungal drugs. Other agents used concomitantly may have hematological toxicity. These additional factors could amplify the marrow-suppressive effects of these antifungal agents. If higher doses of the imidazoles are used in future trials in the neutropenic host or other patient populations, attention should be paid to potential marrow toxicity.

Ketoconazole base was kindly supplied by Janssen Pharmaceuticals, New Brunswick, N.J.

This investigation was supported by Public Health Service grants CA 10829 and CA 30924 from the National Cancer Institute.

LITERATURE CITED