Loss of Melanin in Wangiella dermatitidis Does Not Result in Greater Susceptibility to Antifungal Agents

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Melanized wild-type and melanin-deficient (Mel−) strains of Wangiella dermatitidis (Kano) McGinnis were tested for in vitro susceptibility to amphotericin B, flucytosine, amorolfine, ketoconazole, flunazole, terbinafine, and itraconazole by using an agar dilution technique. Although the MICs of itraconazole obtained with seven of the eight Mel− strains were lower than those obtained with the melanized wild-type strains, there was no such trend observed with flucytosine, the other triazole tested. Furthermore, there was no apparent difference in MICs when comparing the melanized wild-type and the Mel− strains for the other drugs tested. Thus, no consistent increase in in vitro antifungal activity was found to be associated with a specific class of drug. Therefore, melanin does not appear to confer protection against some of the more important antifungal agents.

Natural infections caused by the dematiaceous (melanized) fungi are difficult to treat, and experimental systemic infections caused by these fungi in mice are among the more difficult models in which to achieve 100% survival with available chemotherapy (2). Similarly, the in vitro susceptibility values with the standard antifungal agents tested against the dematiaceous fungi are generally higher than those reported for the nonmelanized fungi (1, 5). The protective role of melanin in the dematiaceous fungi is well known and has been discussed in relation to the resistance of the melanized fungi to antifungal agents such as amphotericin B and the imidazoles (6). Thus, the presence of melanin has been one possible explanation for the relative drug resistance of the dematiaceous fungi.

Wangiella dermatitidis (Kano) McGinnis is a representative dematiaceous fungus which produces phaeohyphomycosis in humans (4); this fungus and most, if not all of the medically important dematiaceous fungi produce dihydroxynaphthalene melanin, which is synthesized via pentaketide metabolism and deposited in the cell wall (6; D. M. Dixon, P. J. Szansiszlo, and A. Polak, in G. T. Cole and H. Hoch, ed., The Fungal Spore and Disease Initiation in Plants and Animals, in press). Now that melanin-deficient (Mel−) mutants of W. dermatitidis are available, we wanted to test the hypothesis that if melanin is responsible for the drug resistance of the dematiaceous fungi, then melanin-deficient mutants should be more susceptible to antifungal agents than are the melanized wild-type strains. Our results do not support this hypothesis, thereby indicating that for the drugs studied here, there is no apparent correlation between the presence of melanin and in vitro antifungal susceptibility.

Drugs tested were amphotericin B, flucytosine, amorolfine, ketoconazole, flunazole, and terbinafine, and were obtained and prepared as described previously (2). Itraconazole was from Janssen, Beerse, Belgium, and was dissolved in polyethylene glycol 200.

The strains of W. dermatitidis used are characterized in Table 1. Inoculum was prepared from potato dextrose agar (Difco Laboratories, Detroit, Mich.) slant cultures incubated at 30°C for 1 week. Yeastlike cells were suspended in sterile saline and adjusted by hemacytometer count to give 10^9 cells per ml of molten growth medium. Yeast morphology agar (GIBCO Diagnostics, Madison, Wis.) was used for flucytosine. All other drugs were tested by using Casitone agar (containing, per liter of distilled water, 9 g of Casitone, 20 g of glucose, 10 g of yeast extract, 1 g of KH2PO4, 1 g of Na2HPO4, 10 g of sodium citrate, and 20 g of agar) and the procedure described previously (2). Slants were incubated at 30°C, and the MIC was read at 6 to 7 days as the 100% inhibition of visible fungal growth. All tests were done in duplicate, and paired samples always gave the same end-point.

The results are summarized in Table 1. Overall, the in vitro susceptibility patterns of the Mel− mutants were similar to those of the wild-type W. dermatitidis. The endpoints for the two wild-type strains, 368 and CM 26, were within two dilutions of each other for a given drug. Also, these values were consistent with those previously published for these isolates (2). All the other strains listed in Table 1 are mutants independently derived from 368. Strains 369 and 372 represent independently maintained subcultures of the same mutant (3; Dixon et al., in press) and thus could be considered internal test controls; they gave essentially the same results (within one dilution step) for all drugs except itraconazole. Both 369 and 372 appeared to be more susceptible to itraconazole than was the wild type. This was also true for five of the other six mutants examined, for which the endpoints ranged from 2 to 5 dilution steps lower than the wild type. The sole exception was UT-UV 23, which was less susceptible to this drug than was the wild type. This trend was not observed with fluconazole, the other triazole tested, for which the results obtained with the Mel− mutants were essentially the same as for the wild-type strains; this result was true for all of the other drugs tested.

The drugs tested here represent a polyene (amphotericin B), an antimetabolite of nucleic acid synthesis (flucytosine), an imidazole (ketoconazole), two triazoles (fluconazole and itraconazole), an allylamine (terbinafine), and a morpholine (amorolfine). No consistent increase in in vitro antifungal activity was found to be associated with a specific class of drug. Therefore, although melanin is known to be associated with virulence (3; Dixon et al., in press) and to confer...
increased resistance to damage from such variables as UV irradiation and desiccation, dihydroxynaphthalene melanin does not appear to confer protection against some of the more important antifungal drugs.

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LITERATURE CITED