Filamentous fungal pathogens are recognized as a major and increasing source of infection in immunocompromised hosts (3). The most common species causing disease are *Aspergillus fumigatus* (90%) followed by *Aspergillus flavus*, *Aspergillus niger*, and *Aspergillus terreus* (3). Due to the high mortality rate from mold infections and the limited efficacies of the current agents, the use of combination therapy is an interesting option in the treatment of invasive aspergillosis (11, 15). Since echinocandins and triazoles are different classes of antifungal drugs in the treatment of invasive aspergillosis (11, 15). Since echinocandins and triazoles are different classes of antifungal drugs with different modes of action, the use of this two-drug combination has recently received much attention in medical mycology (2, 8, 12, 14).

Anidulafungin is a novel cyclic lipopeptide antifungal agent of the echinocandin class, which acts via inhibition of 1,3-β-D-glucan synthesis (18). The drug end point for the echinocandins is the minimum effective concentration (MEC) (1, 5). However, determination of MEC is difficult and labor intensive and requires expertise. Warn et al. (19) demonstrated that end point reading for caspofungin and micafungin under hypoxic conditions (1% oxygen) was superior and suggests reading the MIC end points were read at 100% inhibition. Also, the metabolic activity of drug-treated hyphae was determined from their ability to reduce the tetrazolium compound 2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-2- H-tetrazolium-5-carboxanilide (XTT) as described elsewhere (4).

For selected experiments, the plates were incubated in ambient air conditions (20% oxygen). For voriconazole, MICs were read visually with no growth end point, and for anidulafungin, the MEC instead of the MIC was defined microscopically (6).

The synergy tests were evaluated by using MIC end points of each drug. The fractional inhibitory concentration (FIC) of each drug for an individual isolate was calculated as the ratio of the concentration of the drug in combination that achieves the MIC end point to the MIC of the drug alone using that end point. FIC index (FICI) values were interpreted as follows: FICI of ≤0.5, synergistic; FICI of >0.5 to ≤4, indifferent; and FICI of >4, antagonistic (7). All tests were performed twice and in duplicate.

In this study we determined the in vitro antifungal activity of anidulafungin either alone or in combination with voriconazole against conidia and hyphae of *Aspergillus* spp. under hypoxic conditions. We found that the growth of *Aspergillus* spp. under these conditions in the presence of anidulafungin was almost totally inhibited, and the end point was clear and plain to define. The isolates tested were susceptible to anidulafungin and voriconazole, displaying lower MICs (≤0.06 μg/ml) for anidulafungin than those found for voriconazole (≤2 μg/ml) under hypoxic conditions, as shown in Tables 1 and 2.

A subset of *Aspergillus* isolates (*n* = 10) tested under ambi-
ent air conditions demonstrated that MECs and MICs for conidia (n = 10) and hyphae (n = 10) of anidulafungin and voriconazole correlated to 93% and 90% (data not shown) with the MICs tested under hypoxic conditions. Hence, the hypoxic condition had no influence on the MICs of voriconazole. MECs and MICs of anidulafungin were also similar

As shown in Table 1, the in vitro interaction of anidulafungin with voriconazole in combination for a conidial suspension of *Aspergillus* spp. exerted partly synergy and a lack of antagonism. Similar results were obtained by others (14). Yet this report is the first to demonstrate the interaction of anidulafungin and voriconazole against hyphae of *Aspergillus*. Results for the majority of isolates were indifferent, whereas results for 4 of the 27 tested isolates showed antagonism (Table 2). Presently we lack in vivo data for this drug combination. The use of animal models to evaluate the usability of anidulafungin combined with voriconazole for combination therapy is warranted.

The fact that oxygen levels in wounds and deep-tissue foci are much lower (<1% oxygen) than in healthy tissues (16) may support testing under hypoxic conditions. Under hypoxic conditions, similar to those that might occur in tissue infected with *Aspergillus* (17), the end point for anidulafungin was clear and easy to define. The MEC and MIC were found to be similar, yet the MIC was easier to read.

### Table 1. MIC and FICI results for conidia of *Aspergillus* spp. tested at 48 h

| Species     | MIC (μg/ml) of drug | FICI | Result |
|-------------|---------------------|------|--------|
|             | VRC<sup>a</sup>  | ANID<sup>b</sup> |        |        |
| A. fumigatus| 1                   | 0.03 | 2.1    | I      |
| A. fumigatus| 1                   | 0.06 | 0.7    | I      |
| A. fumigatus| 0.5                 | 0.06 | 0.8    | I      |
| A. fumigatus| 1                   | 0.06 | 0.7    | I      |
| A. fumigatus| 0.5                 | 0.06 | 0.8    | I      |
| A. fumigatus| 1                   | 0.01 | 0.9    | I      |
| A. fumigatus| 1                   | 0.03 | 1.6    | I      |
| A. fumigatus| 0.5                 | 0.06 | 0.8    | I      |
| A. fumigatus| 1                   | 0.03 | 1.6    | I      |
| A. fumigatus| 1                   | 0.03 | 1      | I      |
| A. terreus  | 1                   | 0.03 | 1.4    | I      |
| A. terreus  | 2                   | 0.06 | 1.2    | I      |
| A. terreus  | 1                   | 0.06 | 2.1    | I      |
| A. terreus  | 2                   | 0.06 | 2.1    | I      |
| A. terreus  | 1                   | 0.01 | 2      | I      |
| A. terreus  | 1                   | 0.01 | 0.9    | I      |
| A. terreus  | 1                   | 0.06 | 2.1    | I      |
| A. terreus  | 1                   | 0.06 | 2.1    | I      |
| A. terreus  | 1                   | 0.06 | 2.1    | I      |
| A. terreus  | 1                   | 0.06 | 2.1    | I      |
| A. flavus   | 1                   | 0.06 | 0.7    | I      |
| A. flavus   | 1                   | 0.06 | 1.5    | I      |
| A. flavus   | 1                   | 0.06 | 1.6    | I      |
| A. flavus   | 1                   | 0.06 | 0.8    | I      |
| A. flavus   | 1                   | 0.06 | 1.5    | I      |
| A. flavus   | 1                   | 0.06 | 0.8    | I      |
| A. niger    | 2                   | 0.03 | 2      | I      |
| A. niger    | 1                   | 0.03 | 1.8    | I      |
| A. niger    | 1                   | 0.03 | 0.5    | I      |
| A. niger    | 1                   | 0.03 | 2      | I      |
| A. niger    | 1                   | 0.03 | 1.8    | I      |
| A. niger    | 2                   | 0.008| 0.8    | I      |

<sup>a</sup> VRC, voriconazole.

<sup>b</sup> ANID, anidulafungin.

<sup>c</sup> I, indifferent; S, synergistic.

### Table 2. MIC and FICI results for hyphae of *Aspergillus* spp. tested at 48 h

| Species     | MIC (μg/ml) of drug | FICI | Result |
|-------------|---------------------|------|--------|
|             | VRC<sup>a</sup>  | ANID<sup>b</sup> |        |        |
| A. fumigatus| 1                   | 0.06 | 4.6    | A      |
| A. fumigatus| 1                   | 0.06 | 7.2    | A      |
| A. fumigatus| 1                   | 0.03 | 2.6    | I      |
| A. fumigatus| 1                   | 0.06 | 6.7    | A      |
| A. fumigatus| 2                   | 0.06 | 1.9    | I      |
| A. fumigatus| 1                   | 0.03 | 4.6    | A      |
| A. fumigatus| 1                   | 0.03 | 1      | I      |
| A. fumigatus| 1                   | 0.03 | 1      | I      |
| A. fumigatus| 1                   | 0.03 | 1      | I      |
| A. fumigatus| 1                   | 0.03 | 1.5    | I      |
| A. terreus  | 1                   | 0.03 | 2      | I      |
| A. terreus  | 1                   | 0.06 | 2.6    | I      |
| A. terreus  | 1                   | 0.06 | 1      | I      |
| A. terreus  | 1                   | 0.06 | 1.3    | I      |
| A. terreus  | 2                   | 0.06 | 1.3    | I      |
| A. terreus  | 1                   | 0.06 | 1.1    | I      |
| A. terreus  | 1                   | 0.03 | 1      | I      |
| A. terreus  | 2                   | 0.01 | 0.8    | I      |
| A. terreus  | 1                   | 0.03 | 0.8    | I      |
| A. flavus   | 2                   | 0.06 | 1.2    | I      |
| A. flavus   | 2                   | 0.03 | 1      | I      |
| A. flavus   | 2                   | 0.06 | 1      | I      |
| A. flavus   | 2                   | 0.03 | 1      | I      |
| A. flavus   | 2                   | 0.06 | 1      | I      |
| A. flavus   | 2                   | 0.06 | 1      | I      |
| A. flavus   | 2                   | 0.03 | 1      | I      |
| A. niger    | 2                   | 0.01 | 0.7    | I      |
| A. niger    | 1                   | 0.03 | 1.8    | I      |
| A. niger    | 1                   | 0.01 | 1      | I      |
| A. niger    | 1                   | 0.01 | 0.9    | I      |
| A. niger    | 1                   | 0.03 | 1.8    | I      |
| A. niger    | 1                   | 0.01 | 0.9    | I      |

<sup>a</sup> VRC, voriconazole.

<sup>b</sup> ANID, anidulafungin.

<sup>c</sup> I, indifferent; A, antagonistic.
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