Effect of Granulocyte Colony-Stimulating Factor Combination Therapy on Efficacy of Posaconazole (SCH56592) in an Inhalation Model of Murine Pulmonary Aspergillosis

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Using an inhalation model of pulmonary aspergillosis, we observed modest differences in the survival rates of mice treated with granulocyte colony-stimulating factor (G-CSF) and posaconazole (POS) and those treated with POS alone. This finding is in contrast to a previous report that suggested that G-CSF had a significant antagonistic effect on the antifungal activity of POS.

Invasive aspergillosis is most prevalent, and often fatal, in patients with defective macrophage and neutrophil function, such as leukopenic cancer patients and bone marrow transplant recipients (1, 15). Consequently, these patients receive therapy with immunomodulators such as granulocyte colony-stimulating factor (G-CSF) to boost host defense against inhaled Aspergillus spp. (10, 19). It has been suggested that G-CSF not only augments neutrophil activity (14, 21, 22) but may also improve the effects of antifungal drugs (6, 24, 28). However, a recent report demonstrated that posaconazole (POS)–G-CSF cotreatment of corticosteroid-immunocompromised mice infected intranasally with Aspergillus fumigatus resulted in significantly increased mortality compared with that resulting from POS monotherapy (9).

By use of an inhalation model of murine aspergillosis, male CF-1 mice (17 to 18 g; Charles River Laboratories) were infected with A. fumigatus clinical isolate ND158 or ND208 (isolate 94-2766 used by Graybill et al. [9]) or Aspergillus flavus isolate ND83 by inhalation as previously described (17, 20). Mice were immunocompromised with cortisone acetate (100 mg/kg of body weight/day administered subcutaneously on days –1, 0, 1, and 6). Recombinant human G-CSF (Amgen Inc., Thousand Oaks, Calif.) was administered intraperitoneally from day –3 to day 5 at 125 or 600 μg/kg/day. Sterile water for injection (control) or POS (Schering-Plough Research Institute, Kenilworth, N.J.) was given orally on days 1 to 9 at 5, 25, or 100 mg/kg/day for A. fumigatus infection or at 0.2, 1, or 10 mg/kg/day for A. flavus infection.

The infective dose was determined 2 h postinfection by plating serial dilutions of lung homogenates from individual mice on Sabouraud dextrose agar plates. Up to three individual experiments were performed per strain. A 30-s exposure of the mice in the inhalation chamber resulted in a mean infectious dose of 4.68 × 104 ± 2.09 × 106 CFU (A. fumigatus ND158), 9.33 × 107 ± 0.8 × 107 CFU (A. fumigatus ND208), or 1.26 × 106 CFU (A. flavus ND83) for the lungs of each mouse. Intranasal infection with A. fumigatus ND208 yielded 1.6 × 107 CFU for the lungs of each mouse.

The survival of the mice was monitored throughout the treatment. Mean survival data for all treatment groups are summarized in Table 1. Representative survival curves from individual experiments are shown in Fig. 1. Similar trends were observed with the intranasal and inhalation routes of infection and with different strains and species of Aspergillus. G-CSF monotherapy at 125 or 600 μg/kg/day was not protective against Aspergillus infection since all mice died by day 10. However, POS monotherapy was protective, enhancing survival from 0 to 41.7% (inhaled A. fumigatus), 83.3% (inhaled A. flavus), or 30% (intranasal A. fumigatus). Similarly, POS increased the survival of mice treated with G-CSF from 0% without POS to 22.2 to 66.6% (125 μg of G-CSF/kg/day) and to 19.4 to 66.7% (600 μg of G-CSF/kg/day) with POS (Table 1). Thus, in contrast to a previous study by Graybill et al. (9), we observed a dose-dependent increase in survival irrespective of whether POS was used alone or with G-CSF (Table 1).

A comparison of survival of Aspergillus-infected mice treated with POS alone with survival of those treated with POS and G-CSF suggested a trend towards a decrease in survival of as much as 10 to 15%. However, an equivalent increase in survival was observed in some cases for individual experiments (e.g., for groups to which POS was given at 5 mg/kg/day with or without G-CSF [data not shown]). Moreover, at higher doses of POS, an increase in G-CSF from 125 to 600 μg/kg/day appeared to have either no effect or a modestly beneficial effect (Table 1). Overall, the differences between groups treated with POS alone and those treated with POS in combination with G-CSF were modest (−10%; P > 0.05). These findings are in contrast to an earlier report that suggested that POS monotherapy was more efficacious than the combination of POS and G-CSF, with a 20 to 50% difference in mortality between the monotherapy and combination therapy groups (9).

To determine the effect of POS–G-CSF combination therapy on the fungal burden in the lungs, the surviving mice from the high-dose POS groups were sacrificed on day 10 postinfection. The results for individual and combined drug experiments are shown in Table 2 and Fig. 2. It is interesting that in contrast

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burden data. Therefore, no clear conclusions can be drawn from the lung mean lung burden (Table 2). However, it should be noted that some cases there was either no change or a decrease in the data collected varied per individual study (n).

Interestingly, A. flavus ND83 infection, which resulted in the lowest mortality (>70% survival) (Fig. 1c), also resulted in the least difference in mean numbers of CFU per lung between treatment groups (P > 0.05) (Fig. 2c), suggesting that G-CSF has no significant effect on the antifungal activity of POS under these conditions. While lower mortality appears to present a practical constraint of the inhalation route, this possibility could not be further substantiated by using a lower infecting dose of A. fumigatus, due to practical constraints of the inhalation model. Furthermore, in our hands, intranasal administration of A. fumigatus also resulted in >70% mortality at a dose for which >70% survival was observed by Graybill et al. (9), highlighting some limitations of in vivo analyses.

Several factors may contribute to the differences in the survival rates and pathogen burdens between the present study and the previous study by Graybill et al. (9). The mouse strain, age, and weight and the route of infection have all been shown to affect pathogen clearance and survival in pulmonary disease models of cryptococcosis (16) and coccidioidomycosis (4).

Both the mouse weight (18 versus 30 g) and the strain used (CF-1 versus CD-1 [ICR]; both with the H-2d haplotype) differed between this and the previous study by Graybill et al. (9). However, no difference in susceptibility to Aspergillus infection or response to G-CSF or triazoles has been documented for these strains. Differences due to the Aspergillus strain used can be ruled out, since we observed similar trends with one strain of A. flavus and two strains of A. fumigatus, including the same clinical isolate, ND208 (94-2766), used by Graybill et al. (9).

To address the possibility that the route of infection may play a role in susceptibility and response to therapy, we repeated the study using an intranasal route of infection with A. fumigatus ND208. An interesting difference was found in the clearance of A. fumigatus ND208 infection after intranasal or inhalation administration (Fig. 2d; Table 2). This finding may suggest possible differences in infectivity or mechanisms and efficiency of pathogen clearance following different routes of infection. It is conceivable that the infectivity and dissemination of Aspergillus may differ in the lungs depending on
FIG. 1. Kaplan-Meier survival curves showing percentages of CF-1 mice infected with *Aspergillus* either by inhalation in a chamber (*A. fumigatus* ND158 [a], *A. fumigatus* ND208 [b], and *A. flavus* ND83 [c]) or by intranasal administration (*A. fumigatus* ND208 [d]). Each graph represents a single study (with 10 to 12 mice). Treatment consisted of sterile water for injection as a control (○), 600 μg of G-CSF/kg/day (×), or POS monotherapy at 100 mg/kg/day (*A. fumigatus*) or 10 mg/kg/day (*A. flavus*) (●). Combination therapy consisted of treatment with POS at 100 mg/kg/day for *A. fumigatus* or 10 mg/kg/day for *A. flavus* ND83 and G-CSF at either 125 μg/kg/day (■) or 600 μg/kg/day (▲). P values for all combinations were >0.05 by Wilcoxon and log rank test analyses.

### TABLE 2. Mean lung burden by group of surviving *Aspergillus*-infected mice following combination therapy with G-CSF and POS

<table>
<thead>
<tr>
<th>Infection isolate</th>
<th>Expt</th>
<th>Mean lung burden (log CFU ± SD) after POS therapy plus indicated dose of G-CSF&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0 μg/kg/day</td>
</tr>
<tr>
<td><em>A. fumigatus</em> ND158</td>
<td>1</td>
<td>2.97 ± 0.39 (5)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1.24 ± 1.75 (2)</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>2.65 ± 0.59 (3)</td>
</tr>
<tr>
<td><em>A. fumigatus</em> ND208</td>
<td>1</td>
<td>1.30 ± 1.84 (2)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1.56 ± 1.53 (5)</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>ND</td>
</tr>
<tr>
<td><em>A. flavus</em> ND83</td>
<td>1</td>
<td>2.04 ± 1.12 (10)</td>
</tr>
<tr>
<td><em>A. fumigatus</em> ND208 IN</td>
<td>1</td>
<td>0 (3)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Values in parentheses are numbers of surviving mice. POS was given at 100 mg/kg/day to mice infected with *A. fumigatus* and at 10 mg/kg/day to mice infected with *A. flavus*, with or without G-CSF. IN, intranasal infection; NS, no survivors; ND, not determined.

<sup>b</sup> P = 0.0495. Unless otherwise indicated, no significant difference was observed between the lung burdens of surviving mice in combination therapy and those in monotherapy groups on day 10 postinfection (P > 0.05).
whether the infective dose is administered in solution or by aerosol, and as a result, the host response may also be affected. Generally, aerosolized infections result in diffuse bronchopneumonia with even distribution and replication primarily in lung tissue, whereas intranasal administration often results in upper respiratory tract infection (2). Thus, diffuse Aspergillus infection resulting from aerosol administration may contribute to the increased mortality and lung burden observed in the present study.

Triazoles, including POS, inhibit lanosterol 14α-demethylase. The net result is a depletion of ergosterol and the accumulation of methylated precursors in the fungal membrane (25). These changes in membrane composition may result in increased sensitivity to oxygen-dependent immune mechanisms such as neutrophil attack (5, 24). For example, ex vivo studies have shown that POS had a synergistic effect on the fungicidal activity of neutrophils against Scedosporium species (6) similar to properties of another triazole, voriconazole, against A. fumigatus (26, 27). Similarly, fluconazole in combination with granulocyte macrophage-CSF increased monocyte killing of Candida albicans (3). Thus, the combination of POS and G-CSF offers the possibility of direct inhibition of fungal growth, triazole-induced susceptibility to neutrophil-mediated killing, and enhancement of innate immunity by G-CSF (11, 18, 19, 23).

In vivo studies using G-CSF–triazole combination therapy have reported either a beneficial response (7, 8, 13, 28) or no significant difference (12, 13) in the survival of infected mice. For example, fluconazole–G-CSF combination therapy had no effect on survival (~10% difference) (12). In another study, the same combination prolonged the survival of neutropenic mice at a low infecting dose of Candida (7). However, at high infecting doses, the differences in survival were marginal to none (10 to 30% decrease). In the present study, we report that combining G-CSF with POS does not substantially affect the antifungal efficacy of POS in a murine model of invasive aspergillosis, with differences observed in ranges similar to those of previous studies that reported a lack of effect of G-CSF on triazole efficacy.

**REFERENCES**