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Inhaled Voriconazole for Prevention of Invasive Pulmonary Aspergillosis[▽]

Justin A. Tolman,[1] Nathan P. Wiederhold,[1,2*] Jason T. McConville,[1] Laura K. Najvar,[3,4]
Rosie Bocanegra,[3,4] Jay I. Peters,[4,5] Jacqueline J. Coalson,[6] John R. Graybill,[3]
Thomas F. Patterson,[3,4] and Robert O. Williams III[1*]

*The University of Texas at Austin College of Pharmacy, Austin, Texas[1]; The University of Texas Health Science Center at
San Antonio, Pharmacotherapy Education and Research Center, San Antonio, Texas[2]; The University of
Texas Health Science Center at San Antonio, Department of Medicine, Division of Infectious Diseases,
San Antonio, Texas[3]; South Texas Veterans Health Care System, San Antonio, Texas[4]; The University of
Texas Health Science Center at San Antonio, Department of Medicine, Division of
Pulmonary Diseases/Critical Care Medicine, San Antonio, Texas[5]; and
The University of Texas Health Science Center at San Antonio,
Graduate School of Biomedical Sciences, Department of
Pathology, San Antonio, Texas[6]*

**Targeted airway delivery of antifungals as prophylaxis against invasive aspergillosis may lead to high lung
drug concentrations while avoiding toxicities associated with systemically administered agents. We evaluated
the effectiveness of aerosolizing the intravenous formulation of voriconazole as prophylaxis against invasive
pulmonary aspergillosis caused by *Aspergillus fumigatus* in an established murine model. Inhaled voriconazole
significantly improved survival and limited the extent of invasive disease, as assessed by histopathology,
compared to control and amphotericin B treatments.**

Invasive aspergillosis is a significant cause of morbidity and mortality in heavily immunocompromised patients and is associated with significant hospital costs and therapy complications in those with multiple comorbidities (3, 5, 9). Targeted pulmonary delivery by aerosolization of antifungals has recently gained attention, as this strategy may lead to high local concentrations at the primary site of infection (1, 4, 6). Our objective was to assess an inhaled aqueous solution of voriconazole as prophylaxis against invasive pulmonary aspergillosis. We hypothesized that this strategy would prevent invasive disease and improve survival in an established murine model.

This study was approved by the Institutional Animal Care and Use Committee at the University of Texas Health Science Center at San Antonio, and all animals were handled in accordance with the American Association for Accreditation of Laboratory Animal Care. Outbred ICR mice (Harlan) were immunosuppressed by cyclophosphamide and cortisone acetate and inoculated via an aerosol chamber with *Aspergillus fumigatus* clinical isolate Af293 as previously described (7, 8). Mice were assigned to the following groups: (i) inhaled voriconazole (5 ml of a 6.25-mg/ml formulation of commercially available voriconazole IV containing 100 mg/ml sulfobutyl ether-β-cyclodextrin sodium via 20 min of aerosolization twice daily; Pfizer, Inc.) or aerosolized sulfobutyl ether-β-cyclodextrin sodium as a control (5 ml of a 100-mg/ml solution via 20 min of aerosolization twice daily; Captisol, CyDex Pharmaceuticals,

Inc.) as prophylaxis begun 2 days prior to pulmonary inoculation or (ii) amphotericin B deoxycholate treatment (1 mg/kg intraperitoneally daily) begun 1 day after inoculation. Voriconazole and control mice received aerosolized solutions in a nose-only dosing chamber by an Aeroneb Pro micropump nebulizer system. All agents were continued until day 7 postinoculation. Mice were then monitored off therapy until day 12. Animals that appeared moribund were euthanized, and death was recorded as occurring the next day.

For fungal burden analysis, 12 mice from each group were euthanized on day 8 and the lung tissue was harvested. Lungs were homogenized in sterile saline, and serial dilutions were plated onto potato dextrose agar. Following 24 h of incubation at 37°C, colonies were enumerated and numbers of CFU per gram of lung tissue were calculated. Pulmonary fungal burdens were also quantified by real-time quantitative PCR (qPCR) as
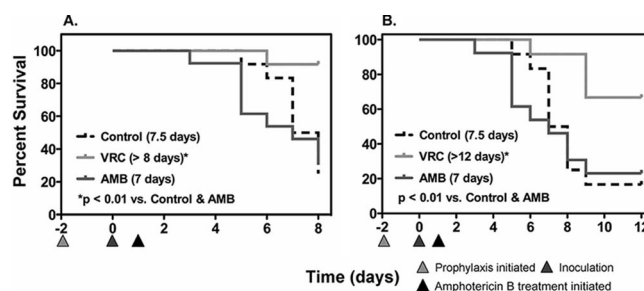

FIG. 1. Survival curves of immunosuppressed mice that received aerosolized voriconazole (VRC; 6.25 mg/ml twice daily), amphotericin B deoxycholate (AMB), or a control (aerosolized sulfobutyl ether-β-cyclodextrin sodium, 100 mg/ml twice daily) and were challenged by pulmonary inoculation with *A. fumigatus*. (A) Survival on therapy (day 7; *n* = 24 per study group). (B) Survival after therapy was discontinued (*n* = 12 per study group).

* Corresponding author. Mailing address for Nathan P. Wiederhold:
UTHSCSA, PERC MSC 6220, 7703 Floyd Curl Drive, San Antonio,
TX 78229. Phone: (210) 567-8340. Fax: (210) 567-8328. E-mail:
wiederholdn@uthscsa.edu. Mailing address for Robert O. "Bill"
Williams III: The University of Texas at Austin, 1 University Station,
A1920, Austin, TX 78712. Phone: (512) 471-4681. Fax: (512) 471-7474.
E-mail: williro@mail.utexas.edu.
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TABLE 1. Pulmonary fungal burdens of the mice used in this study

Group[a]	Median $\log_{10}$ CFU/g (range)	Median $\log_{10}$ CE/g (range)
1 h SAC[b]	3.99 (3.55–4.45)	
Control	4.41 (3.56–4.91)	5.66 (4.47–5.95)
Voriconazole	4.21 (3.62–4.68)	5.24 (4.45–5.98)
Amphotericin	4.33 (3.59–5.07)	5.56 (5.08–5.89)

[a] Mice received aerosolized voriconazole (6.25 mg/ml twice daily), amphotericin B deoxycholate, or a control (aerosolized sulfobutyl ether-β-cyclodextrin sodium, 100 mg/ml twice daily) and were challenged by pulmonary inoculation with *A. fumigatus*.
[b] 1 h SAC, animals sacrificed 1 h after inoculation.

previously described (2). DNA was extracted from 90 μl of lung homogenate with a commercially available kit (DNeasy Tissue Kit; Qiagen), and fungal DNA was measured by qPCR with a probe and primers specific for the *A. fumigatus FKS* gene (GenBank accession no. U79728) and reported as the number of conidial equivalents (CE) per gram of tissue (11). Two additional mice per group were selected and euthanized on days 8 and 12 for histopathology. Lungs were placed into 10% (vol/vol) formaldehyde, processed, and embedded in paraffin wax, and coronal sections were obtained. Sections were stained with hematoxylin and eosin and viewed by light microscopy. Survival was plotted by Kaplan-Meier analysis, with differences in median and percent survival analyzed by the log rank and chi-square tests, respectively. Differences in fungal burden (numbers of CFU and CE per gram) were assessed by analysis of variance with Tukey's posttest for multiple comparisons. A *P* value of ≤0.05 was considered statistically significant.

Mice that received aerosolized voriconazole had a survival advantage over controls and those treated with amphotericin B, with survival on therapy significantly improved in the aerosolized voriconazole prophylaxis group (92%) compared to that of controls (25%; *P* < 0.05) and those treated with amphotericin B (31%; *P* < 0.05) (Fig. 1). This survival benefit was maintained once therapy was discontinued, with 67% of the animals that received voriconazole surviving until day 12, compared to 17% of the controls (*P* < 0.05) and 23% of those treated with amphotericin B (*P* < 0.05). No survival difference was observed between the control and amphotericin B groups. The median survival time of mice that received aerosolized voriconazole (>12 days) was also significantly longer than that of mice that received the control or amphotericin B, 7.5 and 7 days, respectively (*P* < 0.01). Although survival in the amphotericin B treatment group was poor, the survival at day 4 is consistent with previously reported data after 4 days of treatment with the same dose (12). Furthermore, survival rates have only reached 50% when the dose of amphotericin B deoxycholate has been increased to 3 mg/kg/day or when high-dose liposomal amphotericin B (10 mg/kg/day) has been used in this animal model (11). These results demonstrate the difficulty in achieving favorable treatment outcomes in heavily immunocompromised hosts once disease is established.

Although survival was improved in animals that received aerosolized voriconazole, this benefit was not explained by reductions in tissue burden. As shown in Table 1, no significant differences in median tissue burden, as measured by CFU count or qPCR, were observed between any of the groups (Table 1). However, marked differences in lung histopathology were found (Fig. 2). Animals that received the control or amphotericin B had more severe invasive disease and marked abnormalities within the lungs compared to those administered aerosolized voriconazole. Specifically, lungs from control and amphotericin B-treated animals had increased epithelial dis-
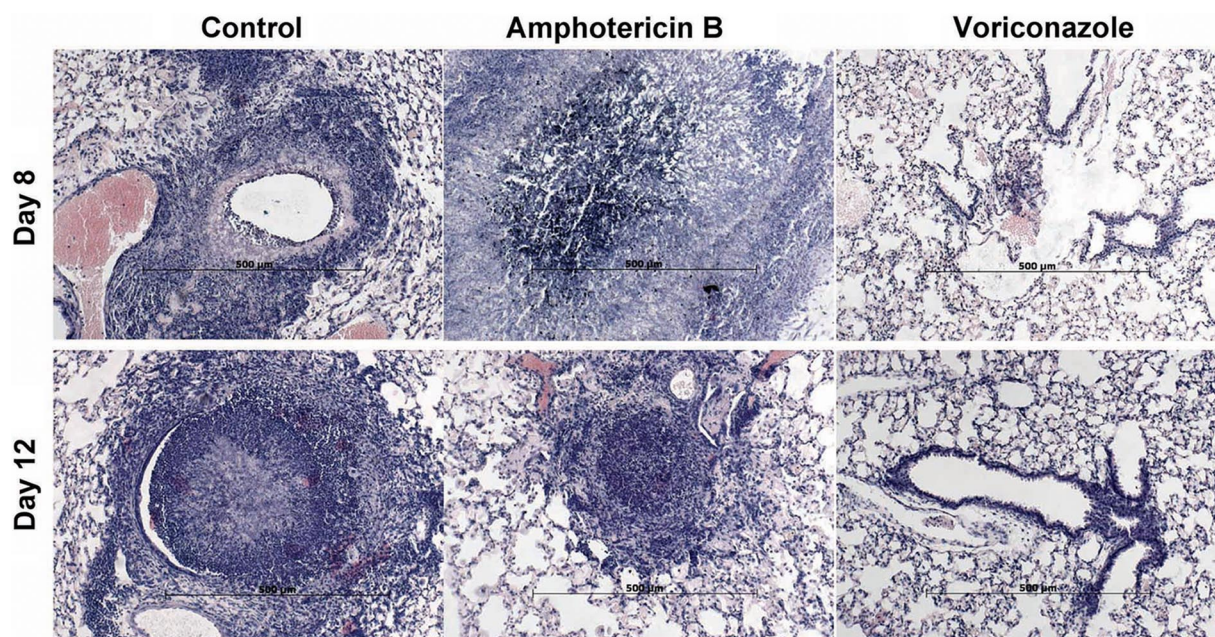

FIG. 2. Representative histopathology, on days 8 and 12 postinoculation, of lungs from mice that received a control (aerosolized sulfobutyl ether-β-cyclodextrin sodium), intraperitoneal amphotericin B deoxycholate, or aerosolized voriconazole. Lung sections were stained with hematoxylin and eosin and viewed by light microscopy at ×20 magnification.
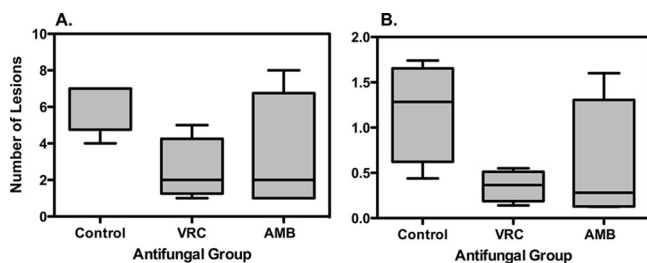
FIG. 3. Box plots of the numbers of necrotic foci observed by histopathology in lungs per animal (A) and per lung field (B) in mice that received a control (aerosolized sulfobutyl ether-β-cyclodextrin sodium), prophylaxis with aerosolized voriconazole (VRC), or treatment with intraperitoneal amphotericin B deoxycholate (AMB). Boxes represent the 25th and 75th percentiles, and horizontal lines within the boxes represent the median values.

ruption, congestion, necrosis, angioinvasion, and vascular lesions within the small airways on day 8. The extent of pulmonary lesions was variable in mice that received amphotericin B, indicating inconsistent in vivo activity (Fig. 3). In contrast, mice that received aerosolized voriconazole had fewer signs of invasive disease and markedly improved histological findings. Similar findings were also noted on day 12 postinoculation, supporting the finding that the protective effects of aerosolized voriconazole are maintained once prophylaxis is stopped. Thus, the improved survival may be attributed to reductions in the extent of invasive disease following aerosolized voriconazole. Furthermore, both aerosolized voriconazole and sulfobutyl ether-β-cyclodextrin sodium are well tolerated, with no lung injury or inflammatory changes on histology in uninfected mice (data not shown).

Our results suggest that aerosolized voriconazole may be effective for targeted delivery to the lungs. This is encouraging, as we adapted the commercially available intravenous formulation with adjustments to ensure that the osmolality (293.2 mosM/kg) and pH (6.4 to 6.8) were within physiologically acceptable ranges for pulmonary delivery (10). Furthermore, peak lung voriconazole concentrations of 6.73 μg/g have been achieved in uninfected mice following multiple inhaled doses, with lower peak values observed in serum (2.32 μg/ml) (10). Although these results are promising, additional studies are warranted to evaluate the efficacy of inhaled voriconazole as prophylaxis and treatment to further support its therapeutic use.

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