Successful Therapy for Cerebral Phaeohyphomycosis Due to Dactyalaria gallopava in a Liver Transplant Recipient

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A 68-year-old liver transplant recipient who was being treated with FK 506 and immunosuppressive steroid therapy was admitted to our medical center because of a tonic-clonic seizure. Computed tomography of the head revealed multiple discrete cerebral abscesses, and culture of fluid drained intraparatively yielded a dematiaceous fungus. The isolate was susceptible to amphotericin B and itraconazole but was resistant to fluconazole and fluconazole. The patient was successfully treated with a prolonged course of amphotericin B colloidal dispersion and itraconazole, as evidenced by both clinical and radiographic resolution of disease over a 2-year follow-up.

Dactyalaria constricta variety gallopava sensu Georg et al. is a thermophilic dematiaceous hyphomycete (this organism is also known as D. gallopava) [1]. Recently, this fungus has undergone taxonomic evaluation by several investigators and has been reclassified as D. constricta variety gallopava sensu Dixon and Salkin [2] and Ochroconis gallopava sensu Cannon [3]. It has been isolated from heated environments (30°C-70°C) and acidic environments (pH 2-6) [4] including thermal-spring and nuclear-reactor effluents, self-heated waste piles, thermal soils, and chicken litter [5, 6]. The fungus has caused neurological infections, such as encephalitis and brain abscesses, in young captive or domesticated birds, including turkey poults, chickens, owls, and gray trumpeter swans [1, 7, 8]. To our knowledge, only three cases of D. gallopava infection in humans have been reported previously [9-11]. We describe a liver transplant recipient with cerebral abscesses caused by D. gallopava who was successfully treated with amphotericin B colloidal dispersion and itraconazole. This is the fourth report of human infection (and the third report of neurological infection) caused by this organism.

Case Report

A 68-year-old man for whom cryogenic cirrhosis was diagnosed underwent orthotopic liver transplantation in July 1990. His postoperative course was uneventful, and allograft function while he received a maintenance immunosuppressive regimen of FK 506 (8 mg/d) and prednisone (5 mg/d) was excellent. He was discharged on the 45th day after surgery and then was examined monthly in the outpatient clinic.

He was well until February 1992, when he experienced a tonic-clonic seizure after bruising the left side of his forehead. On admission to our medical center, the patient was alert and coherent. His vital signs were normal: blood pressure, 158/90 mm Hg; pulse rate, 84; respiratory rate, 24; and temperature, 36.1°C. Physical examination revealed an apical grade 2/6 systolic ejection murmur, a palpable spleen tip, and contusions of the left forehead and left elbow. The chest was clear to auscultation. A funduscopic examination revealed no retinal pathology. No focal sensory or motor deficit was observed.

Laboratory studies indicated the following: mild leukopenia (total white blood count, 3.6 × 10^9/L [3,600/mm^3]); normal differential blood cell count (absolute neutrophil count, 2,448/mm^3); platelet count, 140 × 10^9/L; creatinine level, 200 mmol/L; and glucose level, 10.3 mmol/L. Serum electrolyte levels and results of liver function tests and coagulation studies were all within normal limits. Chest radiography demonstrated a new noncavitory nodule in the left midlung, subsegmental atelectasis in the left lower lobe, and a slight pleural effusion in the right lobe.

On the day of admission, contrast-enhanced computed tomography (CT) of the head revealed three hypodense, ring-enhancing lesions (1-1.7 cm in diameter) in the left frontal, left parietal, and right periventricular white matter adjacent to the corona radiata (figure 1). All three lesions were located <1 cm from the lateral ventricles. Mild surrounding edema was evident, but no mass effect was present. A lumbar puncture yielded clear fluid; analysis of the fluid revealed 4 leukocytes, a glucose concentration of 7.0 mmol/L (126 mg/dL), and a mildly increased protein concentration of 1.26 g/L. Gram and acid-fast staining of the fluid did not show...
any microorganisms. A test for CSF cryptococcal antigen was negative. Serum titers of antibody to toxoplasma and human immunodeficiency virus type 1 were negative. Trans-thoracic two-dimensional echocardiography revealed no cardiac vegetations.

On the second day of hospitalization, 0.5 mL of thick brown pus was aspirated through a CT-guided stereotactic needle from the left parieto-occipital lesion. Gram staining of the material showed numerous white blood cells and septate hyphal elements with acute-angle branching; these hyphal elements were initially thought to be an *Aspergillus* species. Grocott-Gomori methenamine–silver nitrate staining revealed similar findings (figure 2). Therapy with intravenous amphotericin B (50 mg/d) was begun immediately. However, progressive azotemia developed by the fourth day of therapy, and the patient’s treatment was changed to an investigational preparation of amphotericin B (amphotericin B colloidal dispersion [Liposome Technology, Menlo Park, CA]) under a compassionate protocol approved by the University of Pittsburgh’s Institutional Review Board. The maintenance dosage of amphotericin B colloidal dispersion was 2 mg/(kg·d).

In addition, flucytosine (1.5 g every 8 hours) was added to the therapeutic regimen during the first week of treatment. The dose of FK 506 was tapered to 2 mg/d, and prednisone therapy was continued at a dose of 5 mg/d. On the eighth day of hospitalization, the fungal isolate was identified as *D. gallopava* by definitive analysis of morphological criteria and thermal or cycloheximide tolerance criteria. During discussions with the patient’s family, we learned that he kept a pet parakeet at home and used mail-order potting soil to graft plants. Samples of potting soil and litter from the parakeet’s cage were cultured [12].

Subsequently, the patient’s condition in the hospital stabilized, and he did not have any further seizures or other neurological sequelae. On hospital day 28 CT demonstrated slight shrinkage of the abscesses and partial resolution of the edema. The patient received a total dose of amphotericin B colloidal dispersion of 8.5 g and a 4-week course of flucytosine therapy. His medication was switched to itraconazole (200 mg twice per day) during the last week of hospitalization, and he was discharged 57 days after admission. He received maintenance doses of itraconazole over the subsequent year and remained asymptomatic. Follow-up CT performed 18 months after hospital discharge revealed progressive resolution of the cerebral abscesses.

### Methods

The abscess aspirate was cultured using standard laboratory methods on 5% sheep blood agar, Columbia colistin–nalidixic acid (CNA) agar, and eosin–methylene blue agar (BBL Microbiology Systems, Cockeysville, MD) for facultative bacteria and on CDC ( Centers for Disease Control and Prevention) anaerobic and Columbia CNA agars incubated in Gas Pack jars (BBL Microbiology Systems) for anaerobic bacteria. Fungal cultures were performed on Sabouraud dextrose (SAB) agar and mycosphil agar with penicillin (100

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**Figure 1.** Computed tomogram of the brain of a liver transplant recipient with cerebral phaeohyphomycosis due to *Dactyliaria gallopava*; this scan demonstrates the extent of disease at the time of admission.

**Figure 2.** Stereotactic brain biopsy specimen of the parieto-occipital lesion in a liver transplant recipient with cerebral phaeohyphomycosis due to *Dactyliaria gallopava*; the specimen demonstrates collections of darkly stained fungal elements within necrotic tissue (Grocott-Gomori methenamine–silver nitrate stain; original magnification, × 500).
U/mL) and gentamicin (100 μg/mL) and were incubated at 28°C.

The isolate was identified by morphological examination of lactophenol cotton blue-stained scotch-tape preparations of the growth from the original SAB agar slants. Thermotolerance was measured by inoculating the isolate onto SAB agar and incubating it at 45°C. Cycloheximide tolerance was demonstrated using Mycosel agar (BBL Microbiology Systems).

The isolate was then examined at the Fungus Testing Laboratory (University of Texas Health Science Center at San Antonio) with use of a standard procedure for molds. Susceptibility testing was accomplished before the proposed standard for susceptibility testing of yeasts was proposed by the National Committee for Clinical Laboratory Standards in 1992 [13]. Maceroboth dilution tests with antibiotic medium 3 (DIFCO, Detroit) were used to determine the susceptibility of the isolate to amphotericin B and amphotericin B colloidal dispersion; maceroboth dilution tests with Synthetic Amino Acid Medium-Fungal (American Biorganics, North Tonawanda, NY) were used to determine the isolate’s susceptibility to itraconazole, ketoconazole, fluconazole, and fluocytosine. A 0.1-mL volume of each agent was placed in labeled polystyrene snap-cap tubes (Falcon, Cockeysville, MD) and stored at –70°C.

The mold isolate was subcultured to potato flakes agar and allowed to mature for 5 days. The slant was covered with sterile distilled water. The surface was scraped with a sterile wooden applicator to produce a conidial suspension. The slant tube was centrifuged and allowed to sit until large hyphal clumps had settled. A 1:100 dilution of the suspension was made. Conidia were counted with use of a hemocytometer; the count was then adjusted to 1 × 10⁵ conidia/mL. The desired medium was then made from a 1:10 dilution of conidia, and 0.9 mL of this suspension was placed into each of the drug tubes in sequence from the lowest to the highest concentration. The tubes were incubated at 25°C.

The MIC was determined at the first 24-hour interval, when growth could be detected in the drug-free control tube. The MIC was defined as the lowest concentration at which inhibition occurred in the first drug-treated tube. Minimum lethal concentrations (MLCs) were determined by placing a 0.1-mL volume from each tube, beginning with the MIC tube and proceeding to the highest concentration tube, onto a quadrant of a SAB agar plate, which was incubated at 25°C. The MLC was defined as the lowest concentration at which five or fewer colonies grew. MICs and MLCs were determined again in the next consecutive 24-hour period. Twenty-four hours later this isolate was tested against a standard antifungal agent panel (amphotericin B, ketoconazole, fluconazole, fluocytosine) and then against an alternate antifungal agent panel (amphotericin B colloidal dispersion, itraconazole) (A. W. F., personal communication).

Figure 3. Slide of Daetnyaria gallopava featuring conidia attached to hyphae by denticles (lactophenol cotton blue-stained isolate from culture on potato flakes agar for 5 days; original magnification, ×1,250).

Results

After 48 hours of incubation in 5% CO₂, pale-yellow-to-tan colonies were present on the sheep blood agar and CNA agar plates. Gram staining of these colonies demonstrated only yeast forms, and the isolate was subcultured to mycologic media for further study. Our laboratory received the original specimen on a Thursday, and because fungal cultures are not routinely examined on weekends, growth was not detected on the mycologic media until the first examination on the following Monday. Numerous tan colonies with a “suede” texture were present on both fungal media. Over the next several days, the color of the colonies darkened to gray. A diffusing bordeaux burgundy pigmentation was visible in the agar after 4 days of incubation. The cultures yielded no other microorganisms.

Examination of the lactophenol cotton blue-stained mounts revealed branching, separate dematiaceous hyphae with conidia characteristic of Daetnyaria species. These conidia had two cells, with a marked constriction at the apical cell, and were attached to the hyphae by a dentele. The isolate grew at 45°C and was inhibited by cycloheximide. A slide of the isolate cultured on potato flakes agar is shown in figure 3.

Cultures of litter from the parakeet’s cage and the potting soil failed to yield Daetnyaria or other fungal species. The results of susceptibility testing of the isolate are shown in table 1. After 24 hours of incubation, there was sufficient growth for determining the MICs of amphotericin B, ketoconazole, fluconazole, and fluocytosine. Incubation for >48 hours was required for determining the MICs of amphotericin B colloidal dispersion and itraconazole. This isolate was susceptible to amphotericin B and itraconazole and was resistant to fluconazole and fluocytosine on the basis of achievable serum levels in humans.
Table 1. Susceptibility pattern of Dactyliaria gallopava isolated from a liver transplant recipient with cerebral phaeohyphomycosis.

<table>
<thead>
<tr>
<th>Antifungal drug</th>
<th>SB (µg/mL)</th>
<th>MIC (µg/mL) 24 h</th>
<th>MIC (µg/mL) 48 h</th>
<th>MIC (µg/mL) 72 h</th>
<th>MLC (µg/mL) 24 h</th>
<th>MLC (µg/mL) 48 h</th>
<th>MLC (µg/mL) 72 h</th>
</tr>
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<tbody>
<tr>
<td>Amphotericin B</td>
<td>2.31</td>
<td>0.14</td>
<td>0.14</td>
<td>...</td>
<td>0.14</td>
<td>0.14</td>
<td>...</td>
</tr>
<tr>
<td>Amphotericin B colloidal dispersion</td>
<td>2.31</td>
<td>...</td>
<td>0.14</td>
<td>0.14</td>
<td>...</td>
<td>0.14</td>
<td>0.14</td>
</tr>
<tr>
<td>Itraconazole</td>
<td>5</td>
<td>0.018</td>
<td>3.2</td>
<td>3.2</td>
<td>80.88</td>
<td>80.88</td>
<td>32.75</td>
</tr>
<tr>
<td>Ketoconazole</td>
<td>5</td>
<td>0.8</td>
<td>20.0</td>
<td>80.0</td>
<td>80.0</td>
<td>32.75</td>
<td>80.0</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>10</td>
<td>20.0</td>
<td>20.0</td>
<td>20.0</td>
<td>80.0</td>
<td>80.0</td>
<td>32.75</td>
</tr>
<tr>
<td>Fluconosine</td>
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<td>80.68</td>
<td>80.68</td>
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NOTE: SB = susceptibility breakpoint; MLC = minimum lethal concentration; ... = not performed.

Discussion

D. gallopava belongs to a group of fungi collectively termed dematiaceous because of a dark pigment (dihydroxy-naphthalene melanin) present in the cell wall [14]. In humans the dematiaceous fungi cause either skin lesions, with epidermal sclerotic bodies seen histologically (chromoblastomycosis or chromomycosis), or systemic disease, often with CNS involvement (phaeohyphomycosis) [15]. Dixon and colleagues [16] reviewed the existing literature regarding infections with one dematiaceous fungus, Xylohypha bantiana, and found that CNS involvement occurred in 26 of 30 cases in healthy and immunocompromised patients. Although infections due to the dematiaceous fungi occur infrequently, these organisms are being increasingly recognized as opportunistic pathogens that can cause serious disease in immunocompromised patients [17].

The genus Dactyliaria contains several species with common morphological features. Initially, Georg and co-workers [1] named an organism recovered from diseased turkeys as Diplorhinotrichum gallopavum in 1964. In 1968 Bhatt and Kendrick [18] examined this fungus and reclassified it as D. gallopava. The fungus was reclassified again some 15 years later in 1983 and was placed into the genus Ochroconis as O. gallopavum [19]. Three years later Dixon and Salkin [2] presented data based on morphology, physiology, and thermostolerance that resulted in naming the organism D. consticta variety gallopavum. Most recently, Cannon [3] argued that the fungus originally named D. gallopavum is most appropriately named O. gallopavum because of the secretion of conidia via a rhexolytic process (rupture of the cell wall below the basal septum of the conidium), rather than a shizolytic one (fission through a double septum) which is believed by some authors to be characteristic of the genus Dactyliaria.

Such mycologic nuances continue to the present, and authorities do not agree on the proper name or taxonomy of the organism. We believe that the fungus should remain classified in the genus Dactyliaria as D. gallopava. Given the emotion evoked by matters of mycologic nomenclature and the advent of contemporary molecular biological techniques that will assist in resolving such matters, the debate as to the most appropriate name for this mold will undoubtedly continue [20].

D. gallopava has caused fatal encephalitis or brain abscess in owls, turkey poults, and other avian species [1, 6-8]. However, Dactyliaria species have rarely been pathogenic in humans. Of the four previously reported cases of human infection with Dactyliaria, three were due to D. gallopava (O. gallopavum) and one was due to Dactyliaria consticta variety consticta. The first reported case of infection caused by O. gallopavum occurred in a 58-year-old woman who developed multiple subcutaneous abscesses during therapy for acute myeloblastic leukemia [10]. She was treated with local drainage and fluconosine, and her condition improved clinically. She died 6 months later of leukemia, and there was no evidence of residual infection at autopsy. The second case involved a 62-year-old diabetic man with T cell chronic lymphocytic leukemia; his disseminated disease, which was due to Dactyliaria in the lungs, liver, kidney, spleen, and brain, was diagnosed at autopsy [11]. The third case involved a fatal mycotic brain abscess caused by D. gallopavum; it occurred in a patient with large-cell malignant lymphoma [9]. The only report of human infection due to D. consticta variety consticta was a case of pulmonary abscess in a heart transplant recipient cured with amphotericin B therapy alone [21].

We report the fourth case of human infection due to D. gallopava, which is also the third case of neurological disease. This case shares several common epidemiologic and clinical features with the previous cases of infection due to Dactyliaria or Ochroconis. Long-standing native or iatrogenic T cell-mediated immunosuppression was clearly a predisposing condition for the development of invasive disease. Although the incidence of severe infectious complications seems to be less among patients treated with FK 506 than among those treated with cyclosporine, the pattern and spectrum of infectious pathogens are similar [22]. It is of interest
that neutropenia was not a preexisting condition in any of the patients. The patients’ occupations appear to be related; all patients were exposed to unspecified environmental sources potentially harboring Dactylyaria [11, 21].

Our patient was exposed to potting soil and a pet parakeet, but D. gallopava was not isolated from either source. Nevertheless, avian zoonosis remains a possibility because only the litter from the parakeet’s cage was cultured. The rarity of documented dactylyaria infection among immunocompromised patients may thus represent a relatively decreased prevalence of this fungus in the environment, a relatively narrow environmental distribution, or an inherently low pathogenic potential. Limited clinical experience suggests that the lungs are a portal of entry for D. gallopava, with secondary hematogenous dissemination to visceral organs. Direct cutaneous inoculation also may be a feasible mechanism of infection by this soilborne fungus.

Although the pulmonary lesion in our patient was not confirmed histologically, the appearance of a lung nodule at presentation that resolved over the course of therapy indirectly supports a primary pulmonary focus of infection [22]. Finally, a propensity for involvement of the CNS or neurotropism, is suggested by the encephalitic properties of dactylyaria infection in birds and by the formation of a cerebral abscess in our patient and in two of the other patients. Such neurotropism may, in fact, merely be a consequence of the relative degree of organ perfusion coupled with the ability of Dactylyaria to disseminate through the blood [24, 25].

The optimal therapy for D. gallopava infection cannot be deduced from our limited clinical experience. The location of the lesions near the cerebral ventricles in our patient precluded surgical drainage and necessitated medical therapy alone. Although the patient responded to antifungal treatment, his response was slow, and he required long-term therapy. A more rapid response might have occurred had adjunctive surgical drainage been possible.

This case offers little information concerning the most effective antifungal agent for the treatment of phaeohyphomycosis caused by Dactylyaria. Antifungal susceptibility tests are not routinely used prospectively, because there is an inconsistent correlation between in vitro results and clinical outcome as well as differences in strain susceptibility. However, in this case it appears that amphotericin B and itraconazole inhibited growth at levels much below the susceptibility breakpoint. In vivo testing of mice infected with D. gallopava demonstrated that this fungus was more susceptible to amphotericin B than to flucytosine and fluconazole; however, in vivo efficacy was greatest for flucytosine [23].

The role of in vitro drug susceptibility tests in predicting therapeutic efficacy remains less clear for antifungal agents than for antibacterial agents. Itraconazole was selected on the basis of the susceptibility tests reported here and the observation that dematiaceous fungi as a group are highly susceptible to this drug (M. G. R., personal communication).

On the other hand, there is both in vitro and in vivo evidence that D. constricta variety constricta infection appears to have been treated successfully with amphotericin B [15, 21]. Judicious tapering of our patient’s immunosuppressive drugs to minimally tolerated doses may have contributed to his clinical improvement.

The diagnosis of phaeohyphomycosis in predisposed patient populations is facilitated by a high level of clinical suspicion, but since this infection is caused by a heterogeneous grouping of dematiaceous molds, yeasts, and yeastslike cells, its diagnosis must be confirmed by the presence of pseudohyphal or hyphal elements. These filamentous elements may cause cutaneous, corneal, subcutaneous, or systemic disease [15]. This case highlights the importance of establishing the etiology of cerebral and pulmonary lesions in immunocompromised patients on the basis of culture results. Too often, clinicians may rely on histologic staining alone, which is inadequate for discriminating between dematiaceous fungi and other more common fungal pathogens such as Aspergillus. As the identity of the pathogen now has important therapeutic implications, fungi should be isolated and identified by culture in all such cases.

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