FAILURE OF AMPHOTERICIN B COLLOIDAL DISPERSION IN THE TREATMENT OF PARACOCCIDIOIDOMYCOSIS

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Abstract. Although amphotericin B desoxycholate is considered the most effective treatment for disseminated Paracoccidioides brasiliensis infections, little is known about the efficacy of lipid-based formulations of amphotericin B in this infection. In this study, we treated four adults with the juvenile form of paracoccidioidomycosis with 3 mg/kg/day of amphotericin B colloidal dispersion for at least 28 days. Although all of the patients initially responded by clinical observation, all four patients relapsed within six months. The use of amphotericin B colloidal dispersion for the initial induction of paracoccidioidomycosis failed to cure this infection. Possible reasons for failure include dose, duration, or reduced efficacy of this lipid preparation. For many fungal infections, lipid-based preparations have been shown to have a therapeutic-toxic advantage, but our experience with Paracoccidioides infections suggests that more careful studies will need to be performed before they can be recommended for use in this mycosis.

Paracoccidioidomycosis is a systemic infection caused by the dimorphic fungus Paracoccidioides brasiliensis. Found only in Central and South America, the disease has an estimated annual incidence of 1–3 per 100,000 in endemic regions. However, subclinical exposure, manifested by skin test positivity, is much more common, being as high as 82% among young adults in parts of Brazil.

After initial invasion, P. brasiliensis may remain quiescent for the life of the host or emerge in one of two dominant forms. The first is the juvenile form, an acute or subacute disease with rapid hematogenous and/or lymphatic spread through the reticuloendothelial system. More common is the adult form, a chronic disease with gradually progressive pulmonary lesions and frequent involvement of skin, mucous membranes, and gastrointestinal tract.

Prior to the advent of antimicrobial therapy in the 1940s, paracoccidioidomycosis was a fatal disease. Fortunately, a number of effective drugs currently exist. These include sulfonamides,azole derivatives, and amphotericin B. Sulfonamides are inexpensive and well tolerated. However, because several years of daily therapy are required, compliance is problematic and as a result, cure rates are only 70%. The azoles, especially itraconazole, have proven to be highly effective with 94–98% cure rates. The expense of these medications, however, limits their use in a disease requiring six months of treatment and targeting rural workers in developing areas.

Amphotericin B desoxycholate (Fungizone; Apothecon, Princeton, NJ) is often considered the most effective treatment, with rapid and predictable responses. However, significant side effects limit its clinical utility. Because of this, lipid-based formulations of amphotericin B have generated considerable interest. These drugs contain the amphotericin B moiety incorporated into a lipid preparation, which improves their therapeutic index and tissue distribution.1,2

Lipid-based formulations of amphotericin B are very appealing for the treatment of paracoccidioidomycosis because they can be administered at high doses with minimal toxicity. This would theoretically allow for treatment of this serious infection with a brief hospitalization followed by a short course of maintenance treatment, resulting in lower rates of noncompliance and relapse. Unfortunately, the only data available fail to demonstrate these advantages in chronic paracoccidioidomycosis (Macedo V and others, unpublished data). In that study, five patients with chronic paracoccidioidomycosis were treated with 4 mg/kg/day of AmBisome® (Nexstar, San Dimas, CA), a preparation of small unilamellar lipid vesicles containing lipophilic amphotericin B. All five patients initially responded to therapy but relapsed after the treatment was stopped.

In an effort to confirm and extend these observations, we undertook a study of four patients with the juvenile or subacute form of paracoccidioidomycosis to determine the efficacy of lipid-based amphotericin B in the treatment of this aggressive disease. Because the more acute juvenile form of paracoccidioidomycosis is felt to warrant intravenous therapy, amphotericin B colloidal dispersion was used. We report here the results of this experience.

PATIENTS, MATERIALS, AND METHODS

Patients. Over a one-month period, four adult patients with diffuse lymphoid paracoccidioidomycosis were enrolled in an open label study using amphotericin B colloidal dispersion (Amphocil®; Sequus Pharmaceuticals, Menlo Park, CA) after providing informed consent. The diagnosis of paracoccidioidomycosis was based upon culture of P. brasiliensis from clinical samples. Complete blood counts, serum electrolytes, and renal and hepatic function were monitored weekly. The study was reviewed and approved by the Ethical Review Board of the Biomedical Center of the Universidade Federal do Espirito Santo prior to its initiation.

Complete clinical response was defined as a resolution of systemic signs and symptoms of infection as indicated by resolution of fever, reduction in the size of mucosal lesions and/or lymphadenopathy. Partial clinical response was defined as improvement in systemic signs and symptoms of infection without complete resolution. Relapsed infection was defined as recurrence of paracoccidioidomycosis (as confirmed by culture) during the follow-up period of a patient that had previously experienced complete clinical response.

Medications. All patients received intravenous amphotericin B colloidal dispersion, 3 mg/kg/day for 28 days. Amphotericin B colloidal dispersion was supplied as a yellow powder in sterile vials containing 100 mg of amphotericin

837
B. The contents of each vial were suspended in 20 ml of distilled water and subsequently diluted with 5% dextrose in water, according to the manufacturer’s instructions. Reconstituted drug was diluted with 5% dextrose in water to achieve a final concentration of 0.14 mg/ml and administered over a 1.5–2.0 hr period.

Sulfonamide therapy was defined as trimethoprim/sulfamethoxazole (80 mg/400 mg) given orally twice a day.

RESULTS

The patient characteristics and results of therapy are shown in Table 1. All patients were male agricultural laborers in the third decade of life who presented with weight loss and fever. The median duration of symptoms was four months (range = 3–6 months). All patients had diffuse lymphadenopathy. Three patients had oral mucosal lesions, and three patients had hepatomegaly by physical examination. One patient was positive for human immunodeficiency virus (HIV). He was also the only one of the four patients to have received prior therapy for paracoccidioidomycosis.

The diagnosis of paracoccidioidomycosis was confirmed in all four patients by culture, direct smear, and serology. Paracoccidioides brasiliensis was cultured from biopsies of oral mucosal lesions in three patients and by lymph node aspiration from one patient (patient 4).

Complete clinical response. Three patients, including the one with HIV infection, had a complete clinical response at the completion of 28-day treatment with amphotericin B colloidal dispersion. All three of these patients defervesced and had a reduction in their mucosal lesions within 10 days of treatment. Mucosal lesions and lymphadenopathy had completely resolved by the completion of therapy in all three patients. Unfortunately, all three patients relapsed with recurrent lymphadenopathy and mucosal ulcerations within six months. In all three cases, culture of the recurrent mucosal ulcerations yielded P. brasiliensis. All three patients received 6–12 months of sulfonamide therapy. Twelve months after beginning sulfonamide therapy, all three patients remain asymptomatic.

Partial clinical response. One patient (patient 4) had persistent fever, lymphadenopathy, and hepatomegaly despite 28 days of therapy with amphotericin B colloidal dispersion. Because of persistent infection, a six-month course of sulfonamide therapy was initiated. Over the next four weeks, the patient also exhibited a complete clinical response, with resolution of fever, hepatomegaly, and lymphadenopathy. One year after beginning sulfonamide therapy, the patient remains symptom-free.

DISCUSSION

Lipid-based formulations of amphotericin B derivatives show great promise as alternative agents for the treatment of serious mycoses. Preliminary studies found a greater therapeutic index for several preparations compared with amphotericin B desoxycholate (Fungizone®). Early clinical trials demonstrated effectiveness of lipid-based amphotericin B in coccidioidomycosis (Hostetler JS and others, unpublished data), febrile neutropenia unresponsive to antibacterial drugs,3 cryptococcosis in acquired immunodeficiency syndrome (AIDS),4 and visceral leishmaniasis.5,6 Of note, many of these patients had failed prior therapy with either azoles or Fungizone.

Because of the difficulty in treating paracoccidioidomycosis and the favorable results obtained in other mycoses, we believed that a trial of lipid-based amphotericin B for this disease was indicated. Since treatment failures using lipid-based amphotericin B to treat chronic paracoccidioidomycosis have been demonstrated (Macedo V and others, unpublished data), we chose the more subacute, juvenile form of the disease with the expectation that the more rapid fungal growth characteristic of juvenile paracoccidioidomycosis would result in effective short course therapy. Our results were disappointing. Although three of the four patients initially had a complete response, all relapsed within six months.

There are several possible reasons for the treatment failures with this regimen. First, the dose administered (3 mg/kg) may have been inadequate to eradicate paracoccidioidomycosis. However, this dose has been previously demonstrated to be effective in the treatment of other yeastlike fungi such as Candida.7

Second, it is possible that other lipid-based formulations of amphotericin B may have been more effective than amphotericin B colloidal dispersion. There are three commercially available lipid preparations (amphotericin B colloidal dispersion, amphotericin B lipid complex, and liposomal amphotericin), and they may have different efficacies.1 For example, the liposomal form of amphotericin B (AmBisome; Nexstar) was found to be as much as 4–8 times less active than the conventional form against several isolates of C. albicans in vitro and in a murine model.8

Third, inadequate delivery to sites of infection is another possible explanation for the failure of lipid-based formulations of amphotericin B. The drug concentrates primarily in cells of the reticuloendothelial system (e.g., liver and spleen). Much lower levels of lipid-based amphotericin B have been detected in lymph nodes,2 which are commonly

### Table 1

Demographics of patients treated for juvenile paracoccidioidomycosis with amphotericin B colloidal dispersion (ABCD)*

<table>
<thead>
<tr>
<th>Patient</th>
<th>Underlying disease</th>
<th>Previous therapy</th>
<th>Mucosal disease</th>
<th>Cutaneous disease</th>
<th>Hepatomegaly</th>
<th>Lymphadenopathy</th>
<th>Total amount of ABCD (gm)</th>
<th>Initial outcome</th>
<th>Time to relapse (months)</th>
<th>Effective treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>HIV+</td>
<td>Amphotericin B</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>5.0301</td>
<td>CR</td>
<td>6</td>
<td>Sulfonamide</td>
</tr>
<tr>
<td>2</td>
<td>HIV−</td>
<td>None</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>6.032</td>
<td>CR</td>
<td>3</td>
<td>Sulfonamide</td>
</tr>
<tr>
<td>3</td>
<td>HIV−</td>
<td>None</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>4.175</td>
<td>CR</td>
<td>2</td>
<td>Sulfonamide</td>
</tr>
<tr>
<td>4</td>
<td>HIV−</td>
<td>None</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>5.4</td>
<td>PR</td>
<td>0</td>
<td>Sulfonamide</td>
</tr>
</tbody>
</table>

* HIV = human immunodeficiency virus; CR = complete clinical response; PR = partial clinical response.
involved in juvenile paracoccidioidomycosis. A similar situation occurs with when attempting to treat leishmaniasis with lipid preparations of amphotericin B. Although lipid preparations of amphotericin B are highly effective against visceral leishmaniasis\textsuperscript{8,9} (which primarily infects the reticuloendothelial system), they are ineffective against cutaneous leishmaniasis\textsuperscript{10} (which primarily infects the dermis).

A fourth possible reason for treatment failure is the long doubling time of this dimorphic fungus, which may necessitate a longer treatment course. It is possible that our duration of therapy was inadequate no matter how acute the disease or effective the drug. Indeed, several other dimorphic fungi (histoplasmosis, blastomycosis, and coccidioidomycosis) require induction therapy followed by prolonged courses of antifungal agents.

Finally, host immunity may have played an important role in the failure of amphotericin B colloidal dispersion. Because paracoccidioidomycosis is an important opportunistic infection among patients with AIDS,\textsuperscript{11} it is possible that the treatment failure of the HIV-positive patient was due in part to his immunocompromised condition. In addition, patients with acute paracoccidioidomycosis have reduced cell-mediated immunity, including a low CD4:CD8 ratio, a reduced absolute number of T lymphocytes, and decreased lymphocyte responsiveness to \textit{P. brasiliensis} cell wall antigen.\textsuperscript{12–15} Patients with acute paracoccidioidomycosis also demonstrate an impaired Th1 lymphocyte response, the major mechanism of host defense against \textit{P. brasiliensis}.\textsuperscript{16} A gradual correction of these abnormalities usually accompanies treatment, but may require months.\textsuperscript{12,13} Thus, impaired host immunity may have contributed to the observed failure of amphotericin B colloidal dispersion. It is also possible that amphotericin B in the deoxycholate preparation has more interaction with these immune cells than when it is encased in a lipid preparation. Therefore, prolonged or high doses of lipid formulations may be needed to allow for the return of cell-mediated immunity to eradicate residual slowly dividing fungi and prevent relapse.

While lipid-based formulations of amphotericin B may have an important place in the future of antifungal therapy, these results should raise concern about their use in short-course treatment of paracoccidioidomycosis. On the basis of available data, we suggest that lipid preparations of amphotericin B are as yet insufficiently studied for the treatment of paracoccidioidomycosis. Further studies with higher doses, different preparations, longer duration of therapy, or courses of lipid-based amphotericin B preparations followed by extended courses of itraconazole will be needed before these agents can be recommended for use in paracoccidioidomycosis.

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P. \textit{BRASILIENSIS} AND LIPID-ASSOCIATED AMPHOTERICIN B 839