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Successful Treatment of Cryptococcal Meningitis with Amphotericin B Colloidal Dispersion: Report of Four Cases

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Four patients with cryptococcal meningitis were treated with amphotericin B colloidal dispersion because of nephrotoxicity from prior treatment with conventional amphotericin B. The limited experience presented here suggests that amphotericin B colloidal dispersion is efficacious for the treatment of cryptococcal meningitis, despite being undetectable in cerebrospinal fluid, and offers a potential therapeutic alternative for patients who cannot tolerate conventional amphotericin B.

Conventional amphotericin B alone or in combination with flucytosine has a well-defined role in the treatment of most systemic fungal infections, including cryptococcal meningitis (1, 2). However, nephrotoxicity remains one of the main adverse effects of prolonged therapy. In an attempt to overcome this problem, newer amphotericin B preparations with different lipid vehicles have been created, such as amphotericin B lipid complex, AmBisome, and amphotericin B colloidal dispersion (ABCD). Although these lipid-associated amphotericin B preparations can be given at higher doses than conventional preparations (1 to 6 mg/kg of body weight/day) (1, 4, 5, 7), their therapeutic ratios, unlike that for conventional amphotericin B, have not been established. In addition, the central nervous system pharmacokinetics of these lipid-associated amphotericin B preparations have not been systematically studied. Although human immunodeficiency virus (HIV)-infected patients with cryptococcal meningitis have been reported to respond favorably to treatment with both amphotericin B lipid complex (10 of 13 evaluable patients had cerebrospinal fluid [CSF] culture conversion) (3) and AmBisome (12 of 18 patients had CSF culture conversion in a median of 11 days) (4), two treatment failures with ABCD were described, raising concern about the use of the latter preparation for HIV infection (5). We describe the outcomes for four patients with cryptococcal meningitis who were treated with ABCD or Amphocil (Liposome Technology, Inc., Menlo Park, Calif.). In addition, we also present the results of measurements of the levels of amphotericin B in the sera and CSF of three of these patients (Table 1).

Patient 1. A 52-year-old man with diabetes mellitus type II and heart transplantation (day 90) was admitted because of pneumonia. He was receiving cyclosporine, azathioprine, and prednisone. A chest radiograph demonstrated bilateral interstitial infiltrates. Bronchoalveolar lavage specimens grew out both *Cryptococcus neoformans* and *Aspergillus fumigatus*. The serum cryptococcal antigen (CA) titer was 1:4,190 by the enzyme-linked immunosorbent assay (ELISA) microtiter method (Meridian Diagnostics, Cincinnati, Ohio). Amphotericin B therapy was started at a dosage of 0.46 mg/kg/day. Eight days later (total amphotericin B dose, 200 mg), he developed somnolence. Examination of the CSF revealed a leukocyte count (WBC) of $<5 \times 10^6$ cells per liter, a glucose level of 48 mg/dl, a protein level of 0.51 g/liter, and a CA titer of 1:120. India ink preparations and fungal culture were negative. The patient was then switched to ABCD at a dosage of 4 mg/kg/day because of renal toxicity during amphotericin B therapy. Two weeks later, he had a normal sensorium and his CSF CA titer had decreased to 1:70. Fungal culture remained negative. After 5 weeks of therapy, examination of his CSF revealed a WBC of $<5 \times 10^6$ cells per liter, a glucose level of 107 mg/dl, a protein level of 0.34 g/liter, and a CA titer of 1:20. Fungal culture yielded no growth. He was discharged after the completion of 6 weeks of ABCD therapy and received no further antifungal therapy. Five weeks later, he was readmitted because of severe dyspnea and somnolence. A chest radiograph demonstrated bilateral diffuse interstitial infiltrates. He refused aggressive diagnostic and therapeutic measures and died 2 days later. No autopsy was performed. Blood cultures yielded *C. neoformans*. Sputum cultures resulted in heavy growth of *A. fumigatus.*

Patient 2. A 50-year-old man with chronic liver disease with portal hypertension (positive hepatitis C serology) was admitted because of headache. His sensorium was normal, and there was no papilledema. Examination of the CSF revealed a WBC of 59×10^6 cells per liter, a glucose level of 4 mg/dl, a protein level of 0.61 g/liter, a CA titer of 1:8,940, and a positive India ink result. Fungal culture grew *C. neoformans*. Amphotericin B at a dosage of 0.3 mg/kg/day in combination with flucytosine at 150 mg/kg/day was started. Six days later (total amphotericin B dose, 198 mg), he was switched to ABCD at a dosage of 4 mg/kg/day (400 mg/day) because of amphotericin B nephrotoxicity. Flucytosine was discontinued after 14 days of therapy because of bloody diarrhea. After 1 week of ABCD therapy, his CSF examination revealed a WBC of 17×10^6 cells per liter, a glucose level of 65 mg/dl, a protein level of 0.80 g/liter, a CA titer of 1:4,260, and a positive India ink result; however, fungal culture was negative. The level of amphotericin B in the CSF, as measured 6 h after a dose (tested by bioassay with *Chrysosporium pruinosum* to avoid flucytosine interference), was 1.2 μg/ml. Five weeks later, examination of the CSF revealed a WBC of 16×10^6 cells per liter, a glucose level of 63 mg/dl, a protein level of 0.24 g/liter, a CA titer of 1:1,830, and a positive India ink result. Fungal culture was negative. After the completion of 8 weeks of therapy, ABCD was discontinued. Examination of the CSF revealed a WBC of $<5 \times 10^6$ cells per liter, a glucose level of 48 mg/dl, a protein level of 0.27 g/liter, a CA titer of 1:1,580, and a positive India ink result. Fungal culture remained negative. Three months later, he was

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TABLE 1. Summary of patient information

Patient	Initial complaint	Underlying condition	Initial CSF finding			Time of treatment			Serum creatinine level (μmol/liter)			Amphotericin level (μg/ml)[a]		Time to culture conversion[b]	CSF CA titer at end of therapy[c]	Outcome
			WBC (cells per liter)	India ink	CA titer	Culture	Amphotericin B (total amt of drug)	Flucytosine	ABCD (dosage)	Baseline	After amphotericin B	After ABCD	Serum	CSF			
1	Somnolence	Transplantation	$<5 \times 10^6$	Negative	1:120	Negative	8 days (200 mg)	None	6 wk (4 mg/kg/day)	141.4	247.5	159.1	ND	ND	NA	1:20	Relapse after 5 wk and death
2	Headache	Liver disease	59×10^6	Positive	1:8,940	Positive	6 days (198 mg)	14 days	8 wk (4 mg/kg/day)	79.5	176.8	203.3	ND and 0.54	1.2 and <0.125	13 days	1:1,580	No relapse for >12 mo
3	None	Leukemia	$<5 \times 10^6$	Negative	1:10	Positive	7 days (442 mg)	None	6 wk (4 mg/kg/day)	53	176.8	194.4	0.54	<0.125	18 days	Negative	Died of unrelated causes
4	Headache	HIV infection	14×10^6	Negative	1:70	Positive	6 days (320 mg)	None	6 wk (6 mg/kg/day)	70.7	185.6	88.4	1.1	<0.125	15 days	1:5	No relapse for >7 mo

[a] Amphotericin levels were measured by bioassay at the Fungus Testing Laboratory, University of Texas Health Science Center at San Antonio, San Antonio, Tex. Patient 2 had two measurements (after weeks 1 and 4 of ABCD therapy, respectively). ND, not done.
[b] The time to culture conversion includes the time for the conventional amphotericin B therapy. For patient 3, the first follow-up lumbar puncture was performed 2 weeks after ABCD therapy. NA, not available.
[c] The CA titer for patient 1 was measured 1 week prior to the end of therapy. All CA titers were measured by the ELISA microtiter method.

asymptomatic. Examination of the CSF revealed a WBC of 10×10^6 cells per liter, a glucose level of 64 mg/dl, a protein level of 0.20 g/liter, a CA titer of 1:120, and a negative India ink result and fungal culture. After 12 months of therapy were completed, he was asymptomatic. His serum CA titer had decreased to 1:4. He died 6 months later from group A streptococcal bacteremia and septic shock (no autopsy or CA titer was done). Amphotericin B levels in serum and CSF obtained during the fourth week of ABCD therapy 23 h after a dose were 0.54 μg/ml and <0.125 μg/ml, respectively (the lower limit of detection for the assay is 0.125 μg/ml).

Patient 3. A 38-year-old man with diabetes mellitus type II and acute lymphocytic leukemia was admitted for consolidation chemotherapy. He received L-asparaginase, vincristine, and prednisone. He developed fever and a painful ulceration of the right naris while neutropenic. Biopsy of his nasal septum revealed hyphae invading blood vessels. Fungal culture yielded both *Exserohilum* and *Fusarium* species. In addition, blood cultures grew *C. neoformans*. The serum CA titer was 1:54,940. He denied any headache, and his sensorium was normal. Examination of the CSF revealed a WBC of $<5 \times 10^6$ cells per liter, a glucose level of 186 mg/dl, a protein level of 0.24 g/liter, a CA titer of 1:10, and a negative India ink result. Fungal culture yielded *C. neoformans*. After having received amphotericin B for 7 days (total dose, 442 mg), he was switched to ABCD at a dosage of 4 mg/kg/day (340 mg/day) because of nephrotoxicity. Two weeks later, his neutrophil count had recovered. His nasal lesion had healed. Examination of the CSF revealed a WBC of $<5 \times 10^6$ cells per liter, a glucose level of 93 mg/dl, a protein level of 0.18 g/liter, and a CA titer of 1:3. Fungal culture was negative. Levels of amphotericin B in serum and CSF obtained 7 h after a dose were 0.54 μg/ml and <0.125 μg/ml, respectively. After 6 weeks of ABCD therapy, his CSF CA titer became negative. Two weeks later, the patient died of complications related to chemotherapy. No autopsy was performed.

Patient 4. A 43-year-old man seropositive for HIV for 7 years with a CD4 count of 171 cells per mm^3 was admitted because a thin-walled, left upper lobe lung cavitary lesion and headache. His sensorium was normal. Examination of the CSF revealed a WBC of 14×10^6 cells per liter, a glucose level of 53 mg/dl, a protein level of 0.44 g/liter, a CA titer of 1:70, and a negative India ink preparation. Culture yielded *C. neoformans*. Bronchoalveolar lavage culture also yielded *C. neoformans*. The serum CA titer was 1:16,370. Amphotericin B at a dosage of 0.7 mg/kg/day was initiated. After six doses (total, 320 mg), he was switched to ABCD at a dosage of 6 mg/kg/day (500 mg/day) because of amphotericin B nephrotoxicity. After 10 days of ABCD therapy, an examination of his CSF revealed a WBC of $<5 \times 10^6$ cells per deciliter, a glucose level of 79 mg/dl, a protein level of 0.33 g/liter, and a CA titer of 1:20. Fungal culture was negative. The patient received ABCD six times a week during the last 3 weeks of treatment as an outpatient. After 6 weeks of ABCD therapy, an examination of his CSF revealed a WBC of $<5 \times 10^6$ cells per liter, a glucose level of 77 mg/dl, a protein level of 0.13 g/liter, and a CA titer of 1:5. Fungal culture remained negative. The left lung cavitary lesion had resolved by chest X ray. The patient was then started on fluconazole at 200 mg/day as maintenance therapy. Follow-up serum CA titers at 1 month and 7 months after ABCD therapy termination had decreased to 1:1,250 and 1:150, respectively. Four months later, he died from pneumonia (work-up and autopsy were refused). Levels of amphotericin B in serum and CSF obtained during the second week of ABCD therapy 2 h after a 500-mg dose were 1.1 μg/ml and <0.125 μg/ml, respectively.

ABCD, a novel formulation that combines conventional amphotericin B and sodium cholesteryl sulfate, is designed to decrease toxicity while maintaining efficacy. A murine model of disseminated cryptococcal infection demonstrated efficacy comparable to that of conventional amphotericin B (6); however, early reports of treatment failure in patients with HIV-associated cryptococcal meningitis discouraged the use of ABCD for this entity (4). The limited experience presented here suggests that ABCD is efficacious for the treatment of cryptococcal meningitis, despite having undetectable levels in the CSF. Amphotericin B was measured in the CSF of only one patient. A possible explanation is that this particular patient had the highest degree of meningeal inflammatory response, which allowed for greater penetration of the drug. Inflammation diminished with the continuation of therapy, and 3 weeks later, levels of the drug in the CSF were undetectable.

Although all four patients may have had an initial therapeutic benefit from conventional amphotericin B, their treatment course (6 to 8 days) was not long enough to explain the sustained response seen. ABCD was well tolerated (Table 1). Only patient 2 missed three doses (two of them because of the lack of intravenous access). The encouraging results obtained in this experiment may be explained by the use of a higher dosage (4 to 6 mg/kg/day) than that (3 mg/kg/day) used in previous experiments, the milder degree of immunosuppression, or the lower organism load of our patients.

In summary, this report illustrates an alternative form of therapy for cryptococcal meningitis for patients who cannot tolerate conventional amphotericin B. The ability to deliver higher doses continuously may translate into increased efficacy. Another interesting question raised by this type of amphotericin B preparation is whether combination therapy with flucytosine may be better tolerated by such populations as HIV-infected patients, which could potentially improve on current outcomes.

The patients reported on this paper were part of a multicenter study involving open-label treatment with Amphocil. This study involves patients with renal impairment and systemic fungal infection and is supported by Liposome Technology, Inc., Menlo Park, Calif.

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REFERENCES

1. **Coker, R. J., M. Viviani, B. G. Gazzard, B. Du Pont, H. D. Pohle, S. M. Murphy, et al.** 1993. Treatment of cryptococcosis with liposomal amphotericin B (AmBisome) in 23 patients with AIDS. AIDS **7:**829–835.
2. **Dismukes, W. E., G. Cloud, H. A. Gallis, T. M. Kerkering, G. Medoff, P. C. Craven, et al.** 1983. Treatment of cryptococcal meningitis with combination amphotericin B and flucytosine for four as compared with six weeks. N. Engl. J. Med. **317:**334–341.
3. **Gallis, H. A., R. H. Drew, and W. W. Pickard.** 1990. Amphotericin B: 30 years of clinical experience. Rev. Infect. Dis. **12:**308–329.
4. **Graybill, J. R., P. K. Sharkey, D. Vincent, E. Johnson, P. F. Havard, R. Eng, A. Kolokathis, D. Mildvan, R. Pollard, L. Kerr, B. Zalewska, and R. Gupta.** 1991. Amphotericin B lipid complex (ABLC) in treatment (Rx) of cryptococcal meningitis (CM) in patients with AIDS, abstr. 289, p. 147. *In* Program and abstracts of the 31st Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Washington, D.C.
5. **Grünewald, T., A. Dormann, and B. Ruf.** 1992. Treatment failure of amphotericin B colloidal dispersion (ABCD) in AIDS-associated cryptococcal meningitis—report of two cases, abstr. 1209, p. 312. *In* Program and abstracts of the 32nd Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Washington, D.C.
6. **Hostetler, J. S., K. V. Clemons, L. H. Hanson, and D. A. Stevens.** 1992. Efficacy and safety of amphotericin B colloidal dispersion compared with those of amphotericin B deoxycholate suspension for treatment of disseminated murine cryptococcosis. Antimicrob. Agents Chemother. **36:**2656–2660.
7. **Liposome Technology, Inc.** Data on file. Liposome Technology, Inc., Menlo Park, Calif.