Clinical Roundtable Monograph

Clinical Advances in Hematology & Oncology August 2010

Lipid-based Amphotericin B Formulations in Opportunistic Infections

Discussants

Cornelia Lass-Flörl, MD
Professor of Medicine
Division of Hygiene and Medical Microbiology
Department of Hygiene, Microbiology and Social Medicine
Medical University of Innsbruck
Innsbruck, Austria

Nina Singh, MD
Professor of Medicine
Division of Infectious Diseases
VA Pittsburgh Healthcare System and
University of Pittsburgh
Pittsburgh, Pennsylvania

Ivica Knezovic, MD
Head, Unit for Infectious Diseases of Infancy
Department of Pediatric Infectious Diseases
University Hospital for Infectious Diseases
Zagreb, Croatia

Abstract

Opportunistic fungal and parasitic infections are an important cause of morbidity and mortality. Although these infections can affect both immunocompromised and immunocompetent individuals, HIV-infected patients and stem cell transplant recipients are at special risk. Although several therapeutic options exist to treat these infections, drug resistance is becoming an increasing issue which should be considered when selecting a treatment. Among the treatment options available, amphotericin B is characterized by broad-spectrum activity. While dose-related adverse events often occur with administration of the conventional formulation of amphotericin B, newer lipid-based formulations of amphotericin B, eg, amphotericin B colloidal dispersion, are associated with less toxicity. Further, these lipid-based formulations have better solubility, an important characteristic when treating infections that have invaded the central nervous system. This monograph discusses the application of the lipid-based amphotericin B formulations amidst the changing and emerging epidemiology of opportunistic fungal and parasitic infections, focusing on those caused by zygomycetes, cryptococcus, and leishmania.

Supported through funding from Three Rivers Pharmaceuticals and Torrex Chiesi Pharma

The Changing Epidemiology of Opportunistic Fungal and Parasite Infections

Cornelia Lass-Flörl, MD

Emerging Pathogens and Resistance Patterns

Presently, there is a shift in the epidemiology of invasive fungal diseases throughout Europe and the Americas.[1,2] This change has become apparent through the use of surveillance programs such as SENTRY and ARTEMIS DISK. The SENTRY Antimicrobial Surveillance Program evaluated the activity of contemporary antifungal agents against *Candida* species, *Cryptococcus* species, and *Aspergillus* species.[3] The ARTEMIS DISK Global Antifungal Surveillance Study (1997–2007) performed a 10.5-year analysis of the susceptibility of *Candida* species and non-*Candida* species to the antifungal agents fluconazole and voriconazole.[4,5] The first wave of this changing epidemiology appeared during the late 1980s, for multifactorial reasons. A primary cause is the increased reliance on fluconazole as a prophylactic and therapeutic agent. For example, the increased usage of fluconazole likely explains the shift in appearance from *Candida albicans* to non-*Candida albicans*, namely *Candida glabrata*.[6]

Currently, the predominant species are non-*Candida albicans Candida* strains, as well as several species of molds, mainly those that cause Pithomyces and Fusarium infections.[4,5,7] Various populations are at risk for the development of invasive fungal diseases, particularly patients who are undergoing allogeneic stem cell transplantation and patients receiving other treatments for hematologic malignancies.

Similarly, an increase in azole-resistant molds, such as *Aspergillus fumigatus*, has also become apparent.[8] A particular increase in the incidence of *Aspergillus fumigatus* has been reported in the United Kingdom, as well as the Netherlands, where it has increased remarkably from 2% to 8%.[9] This increased incidence is attributable to many factors, the foremost of which is likely the heightened and extensive usage of azoles and/or azole-containing fungicides.[10]

Rates of Zygomycosis, Cryptococcal, and Leishmaniasis Infections

Zygomycosis, an uncommon and frequently fatal mycoses that is caused by the *Zygomycetes* fungi, now accounts for an ever-increasing number of hospital infections.[11,12] Zygomycosis currently represents 2% of solid organ transplant patients and up to 8% of hematopoietic stem cell transplant patients.[13] Epidemiologic reports have documented an increased incidence of zygomycosis in hospitals and other clinical centers.[14,15] For example, more than 30% of fungal infections at the Innsbruck Medical Center in Austria are due to zygomycosis. Many of these infections occur in patients already being treated with systemic antifungal therapies, such as azoles including voriconazole.

The primary risk groups for cryptococcal diseases are immunocompromised patients, such as those with HIV infection or solid organ transplant recipients; however, many individuals who do not fall into these categories are also susceptible.[16] Several epidemiologic studies estimate that the global burden of HIV-associated cryptococcosis is approximately 1 million cases annually. Additionally, solid organ transplant patients are another significant risk group for cryptococcal disease. Interestingly, cryptococcosis seems to affect children to a lesser degree.[17] Among HIV-infected children in the United States, the incidence of cryptococcosis is limited to 0.5–1%,[18,19] although the incidence of cryptococcosis among HIV-infected children in other countries, including Thailand and South Africa, is somewhat higher.[20,21]

Two cryptococcal species are especially important in the clinical setting. *Cryptococcus neoformans* has a worldwide distribution and is responsible for the vast majority of cryptococcal infections in immunosuppressed hosts, such as HIV-infected patients. Conversely, *Cryptococcus gattii* causes

Disclaimer
Funding for this Clinical Roundtable Monograph has been provided by Three Rivers Pharmaceuticals and Torrex Chiesi Pharma. Support of this monograph does not imply the supporter's agreement with the views expressed herein. Every effort has been made to ensure that drug usage and other information are presented accurately; however, the ultimate responsibility rests with the prescribing physician. Millennium Medical Publishing, Inc, the supporter, and the participants shall not be held responsible for errors or for any consequences arising from the use of information contained herein. Readers are strongly urged to consult any relevant primary literature. No claims or endorsements are made for any drug or compound at present under clinical investigation.

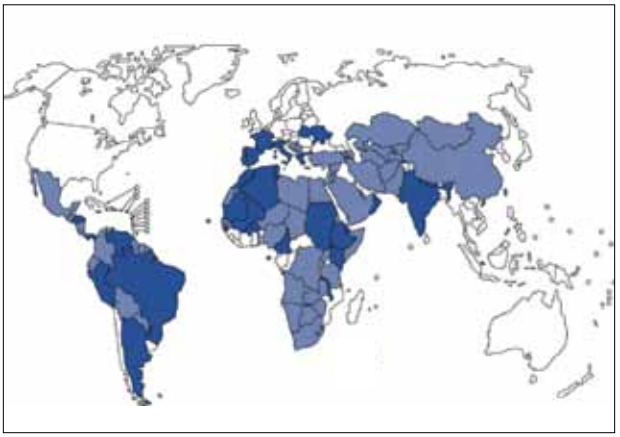

Figure 1. Geographical distribution of leishmaniasis. Light blue=leishmaniasis; dark blue=co-infection.

Data from WHO.

70% of infections among immunocompetent hosts. Unlike *Cryptococcus neoformans*, *Cryptococcus gattii* occurs only rarely in HIV-infected patients.

Leishmaniasis disease is caused by the *Leishmania* species of protozoa. The overall global prevalence of leishmaniasis is estimated to be approximately 12 million cases; nearly 2 million of these patients will develop a clinical syndrome related to leishmaniasis.[22] Leishmaniasis is extremely common in many American regions, as well as in endemic zones such as Latin America, Africa, the Indian subcontinent, the Middle East, and Mediterranean regions (Figure 1). In Europe, reemergence of leishmaniasis is a common issue, due to 3 primary scenarios. The first of these is the introduction of exotic *Leishmania* species or strains into Europe due to an increase in worldwide travel. Secondly, a natural spread of visceral and cutaneous leishmaniasis is caused by *Leishmania infantum* and *Leishmania tropica* from the Mediterranean region of Europe, where these species are endemic. Third, a reemergence of diseases in the Mediterranean region is caused by an increase in the number of immunosuppressed patients.

Lipid Formulations of Amphotericin B—Broad-spectrum Coverage and Rare Mycologic Resistance

Invasive infections, especially those caused by the emerging pathogens discussed here, are an important cause of morbidity and mortality worldwide. The conventional amphotericin B formulation has long been a gold standard for the treatment of many infections. However, poor solubility and dose-related toxicities have prompted the development of lipid formulations of amphotericin B. These lipid formulations of amphotericin B offer a broad range of coverage of fungal and protozoa strains, including the emerging pathogens discussed. Lipid formulations of amphotericin B are now often suggested as first-line therapy due to their broad-spectrum activity against these and other emerging pathogens.[23,24]

References

1. Lai CC, Tan CK, Huang YT, Shao PL, Hsueh PR. Current challenges in the management of invasive fungal infections. *J Infect Chemother.* 2008;14:77-85.
2. Lass-Flörl C. The changing face of epidemiology of invasive fungal disease in Europe. *Mycoses.* 2009;52:197-205.
3. Messer SA, Moet GJ, Kirby JT, Jones RN. Activity of contemporary antifungal agents, including the novel echinocandin anidulafungin, tested against Candida spp., Cryptococcus spp., and Aspergillus spp.: report from the SENTRY Antimicrobial Surveillance Program (2006 to 2007). *J Clin Microbiol.* 2009;47:1942-1946.
4. Pfaller MA, Diekema DJ, Gibbs DL, et al. Results from the ARTEMIS DISK Global Antifungal Surveillance Study, 1997 to 2007: a 10.5-year analysis of susceptibilities of Candida Species to fluconazole and voriconazole as determined by CLSI standardized disk diffusion. *J Clin Microbiol.* 2010;48:1366-1377.
5. Pfaller MA, Diekema DJ, Gibbs DL, et al. Results from the ARTEMIS DISK Global Antifungal Surveillance Study, 1997 to 2007: 10.5-year analysis of susceptibilities of noncandidal yeast species to fluconazole and voriconazole determined by CLSI standardized disk diffusion testing. *J Clin Microbiol.* 2009;47:117-123.
6. Neu N, Malik M, Lunding A, et al. Epidemiology of candidemia at a Children's hospital, 2002 to 2006. *Pediatr Infect Dis J.* 2009;28:806-809.
7. Horn DL, Neofytos D, Anaissie EJ, et al. Epidemiology and outcomes of candidemia in 2019 patients: data from the prospective antifungal therapy alliance registry. *Clin Infect Dis.* 2009;48:1695-1703.
8. Brakhage AA. Systemic fungal infections caused by Aspergillus species: epidemiology, infection process and virulence determinants. *Curr Drug Targets*. 2005;6:875-886.
9. Snelders E, van der Lee HA, Kuijpers J, et al. Emergence of azole resistance in Aspergillus fumigatus and spread of a single resistance mechanism. *PLoS Med.* 2008;5:e219.
10. Verweij PE, Snelders E, Kema GH, Mellado E, Melchers WJ. Azole resistance in Aspergillus fumigatus: a side-effect of environmental fungicide use? *Lancet Infect Dis*. 2009;9:789-795.
11. Antoniadou A. Outbreaks of zygomycosis in hospitals. *Clin Microbiol Infect.* 2009;15(suppl 5):55-59.
12. Meis JF, Chakrabarti A. Changing epidemiology of an emerging infection: zygomycosis. *Clin Microbiol Infect.* 2009;15(suppl 5):10-14.
13. Abstract 12 presented at: 2nd International Forum on Zygomycosis; May 28-30, 2010; Porto Heli, Greece.
14. Ambrosioni J, Bouchuiguir-Wafa K, Garbino J. Emerging invasive zygomycosis in a tertiary care center: epidemiology and associated risk factors. *Int J Infect Dis.* 2010 Mar 22. [Epub ahead of print]
15. Bitar D, Van Cauteren D, Lanternier F, et al. Increasing incidence of zygomycosis (mucormycosis), France, 1997-2006. *Emerg Infect Dis.* 2009;15:1395-1401.
16. Li SS, Mody CH. Cryptococcus. *Proc Am Thorac Soc*. 2010;7:186-196.
17. Severo CB, Xavier MO, Gazzoni AF, Severo LC. Cryptococcosis in children. *Paediatr Respir Rev.* 2009;10:166-171.
18. Abadi J, Nachman S, Kressel AB, Pirofski L. Cryptococcosis in children with AIDS. *Clin Infect Dis*. 1999;28:309-313.
19. Gonzalez CE, Shetty D, Lewis LL, Mueller BU, Pizzo PA, Walsh TJ. Cryptococcosis in human immunodeficiency virus-infected children. *Pediatr Infect Dis J*. 1996;15:796-800.
20. McCarthy KM, Morgan J, Wannemuehler KA, et al. Population-based surveillance for cryptococcosis in an antiretroviral-naive South African province with a high HIV seroprevalence. *AIDS*. 2006;20:2199-2206.
21. Likasitwattanakul S, Poneprasert B, Sirisanthana V. Cryptococcosis in HIV-infected children. *Southeast Asian J Trop Med Public Health*. 2004;35:935-939.
22. Desjeux P. Leishmaniasis: current situation and new perspectives. *Comp Immunol Microbiol Infect Dis.* 2004;27:305-318.
23. Chu P, Sadullah S. The current role of amphotericin B lipid complex in managing systemic fungal infections. *Curr Med Res Opin.* 2009;25:3011-3020.
24. Gullo A. Invasive fungal infections: the challenge continues. *Drugs.* 2009;69(suppl 1):65-73.

Lipid-based Polyenes for Selected Mycoses, Including Cryptococcosis and Zygomycosis

Nina Singh, MD

Therapy for Cryptococcosis

Cryptococcosis is currently one of the most important opportunistic infections worldwide and is especially common in HIV-infected individuals.[1] Importantly, the mortality rate attributed to cryptococcal meningitis in Sub-Saharan Africa is approximately 1.5 times higher than deaths due to tuberculosis (530,000 vs 350,000).[1] However, the impact of cryptococcal infection is not limited to HIV-infected individuals. Cryptococcal disease is an important cause of central nervous system (CNS) mycoses in solid organ transplant (SOT) recipients; a recent study by Van de Beek and colleagues found that cryptococcal meningitis was the most common CNS infection among heart transplant recipients.[2] The overall incidence in SOT patients is approximately 2.8%, and cryptococcosis accounts for 8% of all invasive fungal infections in SOT patients.[3,4] Conversely, cryptococcal disease is not a significant threat in hematopoietic stem cell transplant recipients.[5]

Polyenes are regarded as the preferred primary agents for the treatment of CNS cryptococcosis. Guidelines from the Infectious Diseases Society of America recommend amphotericin B deoxycholate with flucytosine for the treatment of cryptococcal meningoencephalitis.[6] In selected hosts—for example, SOT recipients—lipid formulations of amphotericin B are recommended as induction therapy for CNS and severe non-CNS disease, largely because these agents incur a lower risk of drug-associated nephrotoxicity in these patients.[7] Superiority of lipid polyenes over amphotericin B deoxycholate has not been incontrovertibly documented. A randomized double-blind trial compared 2 doses of liposomal amphotericin B (3 mg/kg/day [n=86] or 6 mg/kg/day [n=94]) with the amphotericin B deoxycholate formulation (0.7 mg/kg/day [n=87]).[8] The rate of efficacy was similar among all 3 treatment groups. However, the liposomal formulations were associated with fewer adverse events, including infusion-related reactions (P<.001 for both liposomal formulations compared with amphotericin B deoxycholate) and nephrotoxicity (P=.004 for the 3 mg/kg/day dosage of liposomal amphotericin B compared with amphotericin B deoxycholate). In a study of SOT recipients, after controlling for factors that portend poor outcome, such as renal failure at baseline and fungemia, treatment with lipid formulations of amphotericin B was independently associated with improved survival compared with amphotericin B deoxycholate (hazard ratio [HR], 0.11; 95% confidence interval [CI], 0.02–0.57; P=.008; Figure 1).[7]

A meta-analysis of the efficacy of lipid-based amphotericin B compared with amphotericin B deoxycholate showed that the all-cause mortality rate was reduced by 28% with the lipid-based agent (odds ratio [OR], 0.72; 95% CI, 0.54–0.97).[9] However, this reduction in death was not found to be attributed to differences in efficacy. In a separate study, the clinical response rate (resolution of signs and symptoms) in 55 HIV-infected patients with cryptococcal meningitis was demonstrated to be improved with amphotericin B lipid complex compared with amphotericin B deoxycholate (86% vs 65%).[10] Although this increase in clinical response was not replicated in a prior study of 28 HIV-positive patients with cryptococcal meningitis, it was shown that lipid amphotericin B formulations sterilized cerebral spinal fluid cultures significantly more rapidly than traditional amphotericin B.[11]

Therapy for Zygomycosis

Emerging data suggest that zygomycosis is increasingly becoming a significant opportunistic mycosis in immunocompromised hosts. For example, a report and review of English-language publications by Almyroudis and colleagues (Table 2) reported an incidence as high as 16 cases per 1,000 organ transplant recipients, although this incidence rate ranged largely across different transplant types.[12] The disease most frequently manifests clinically as pulmonary zygomycosis; in one study, 53% of SOT patients presented with this condition.[13] Although not yet conclusively shown, it appears that iron overload or iron supplementation may be a risk factor for greater disease severity and higher incidence of disseminated zygomycosis in the liver as compared to other SOT patients.[14]

Polyenes are regarded as the standard of care for the treatment of zygomycosis. Based on improved outcomes, several studies support the use of lipid formulations of amphotericin B to treat zygomycosis in both SOT patients and patients with other conditions.[14] A retrospective study of patients with a hematologic malignancy with zygomycosis demonstrated significantly improved survival among those patients who received lipid formulations of amphotericin B compared with amphotericin B deoxycholate (67% vs 39%; P=.02).[15] A recent study comprising 41 cases of zygomycosis reported that liposomal amphotericin B was associated with significantly improved response rates (P=.012) and survival rates (P=.004).[16] In a prospective, matched, case-controlled study, SOT recipients receiving liposomal amphotericin B

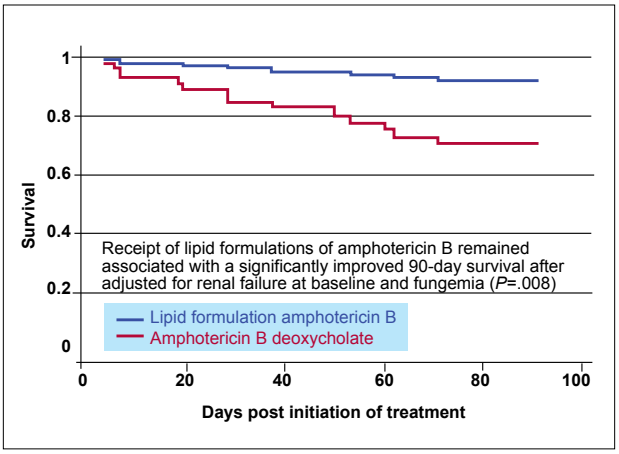

Figure 1. Cox proportional hazards regression survival curves with the receipt of lipid formulations of amphotericin B and amphotericin B deoxycholate.

Data from Sun HY et al. *Clin Infect Dis.* 2009;49:1721-1728.

had higher success rates (OR 4.0, 95% CI, 0.63–26).[8] Additionally, in a review of SOT recipients with rhino-orbital-cerebral zygomycosis, lipid formulations of amphotericin B (OR 0.09, 95% CI, 0.02–0.50) were associated with improved survival even when controlled for CNS involvement and the era in which the disease was diagnosed.[17] Interestingly, the addition of echinocandins (which are generally ineffective as single agents) to the lipid polyenes against zygomycosis led to superior outcomes compared to the lipid polyene alone. For example, a combination of amphotericin B lipid complex and caspofungin significantly improved the success rate (*P*=.009) and survival time (*P*=.01) compared with amphotericin B lipid complex for the treatment of rhino-orbital-cerebral zygomycosis.[18]

The advantages of lipid formulations of amphotericin B as the therapeutic agents for these mycoses may result from better brain penetration and, therefore, more efficient reduction of fungal burden.[19-21]

The Balance Between Pro- and Anti-inflammatory Responses

It has become evident that while a robust inflammatory response plays a critical role in eradicating invading microbes, dysregulated immunity can be detrimental to the host if an optimal balance between the inflammatory and anti-inflammatory reactions is disrupted. In addition to improved CNS penetration, distinct immunomodulatory characteristics of specific antifungal agents may also be responsible for conferring some of the differences observed in outcomes. Cell-based studies show that while amphotericin B deoxycholate stimulates increased transcription and expression of inflammatory cytokines (including TNF-alpha and IL-6), chemokines, and ICAM-1, the lipid polyenes either downregulate or have no effect on inflammatory cytokine gene expression.[22,23] These results may be explained by the fact that amphotericin B deoxycholate causes upregulation of signal transduction via TLR-2, and lipid polyenes divert TLR receptor signaling from TLR-2 to TLR-4. The resulting lower induction of overzealous inflammation and tissue damage during fungal infection may be advantageous to patient outcome.

Empty liposomes per se, through lesser molecular interaction with TLR-2, have been found to attenuate the antifungal immunopathology. In this vein, Lewis and colleagues reported that in an animal model of aspergillosis, pretreatment with empty liposomes (with no direct antifungal properties) was nearly as effective as lipid formulations of amphotericin B and superior to amphotericin B deoxycholate.[24] These improvements included enhanced fungal clearance, reduced lung injury, and improved survival.

Table 2. Clinical Manifestations and Time of Onset of Zygomycosis According to Type of Organ Transplantation

Clinical Manifestation	Heart* (n=18)	Lung (n=4)	Liver (n=19)	Kidney† (n=75)	Total (n=116)
Rhino-sinusitis ± orbits	1 (5.5%)	1 (25%)	3 (15.8%)	15 (20%)	20 (17.2%)
Rhinocerebral	2 (11.1%)	0	1 (5.3%)	13 (17.3%)	16 (13.8%)
Pulmonary	7 (38.8%)	2 (50%)	0	19 (25.3%)	28 (24.1%)
Gastrointestinal	4 (22.2%)	0	2 (10.5%)	7 (9.3%)	13 (11.2%)
Cutaneous	2 (11.1%)	0	8 (42.1%)	8 (10.6%)	18 (15.5%)
Renal	0	0	0	6 (8%)	6 (5.2%)
Disseminated	2 (11.1%)	1 (25%)	5 (26.3%)	7 (9.3%)	15 (12.9%)
Time of onset, median	60 days	180 days	18.5 days	60.5 days	60 days
Time of onset, range	6–912 days	30–730 days	1–1,095 days	7–2,920 days	1–2,920 days

*Includes 2 heart-lung transplantations.
†Includes 2 pancreas-kidney transplantations.
Data from Almyroudis NG et al.[12]

References

1. Park BJ, Wannemuehler KA, Marston BJ, Govender N, Pappas PG, Chiller TM. Estimation of the current global burden of cryptococcal meningitis among persons living with HIV/AIDS. *AIDS*. 2009;23:525-530.
2. van de Beek D, Patel R, Daly RC, McGregor CG, Wijdicks EF. Central nervous system infections in heart transplant recipients. *Arch Neurol*. 2007;64:1715-1720.
3. Singh N, Dromer F, Perfect JR, Lortholary O. Cryptococcosis in solid organ transplant recipients: current state of the science. *Clin Infect Dis*. 2008;47:1321-1327.
4. Husain S, Wagener MM, Singh N. Cryptococcus neoformans infection in organ transplant recipients: variables influencing clinical characteristics and outcome. *Emerg Infect Dis*. 2001;7:375-381.
5. Sun HY, Wagener MM, Singh N. Cryptococcosis in solid-organ, hematopoietic stem cell, and tissue transplant recipients: evidence-based evolving trends. *Clin Infect Dis*. 2009;48:1566-1576.
6. Perfect JR, Dismukes WE, Dromer F, et al. Clinical practice guidelines for the management of cryptococcal disease: 2010 update by the Infectious Diseases Society of America. *Clin Infect Dis*. 2010;50:291-322.
7. Sun HY, Alexander BD, Lortholary O, et al. Lipid formulations of amphotericin B significantly improve outcome in solid organ transplant recipients with central nervous system cryptococcosis. *Clin Infect Dis*. 2009;49:1721-1728.
8. Hamill RJ, Sobel JD, El-Sadr W, et al. Comparison of 2 doses of liposomal amphotericin B and conventional amphotericin B deoxycholate for treatment of AIDS-associated acute cryptococcal meningitis: a randomized, double-blind clinical trial of efficacy and safety. *Clin Infect Dis*. 2010;51:225-232.
9. Barrett JP, Vardulaki KA, Conlon C, et al. A systematic review of the antifungal effectiveness and tolerability of amphotericin B formulations. *Clin Ther*. 2003;25:1295-1320.
10. Sharkey PK, Graybill JR, Johnson ES, et al. Amphotericin B lipid complex compared with amphotericin B in the treatment of cryptococcal meningitis in patients with AIDS. *Clin Infect Dis*. 1996;22:315-321.
11. Leenders AC, Reiss P, Portegies P, et al. Liposomal amphotericin B (AmBisome) compared with amphotericin B both followed by oral fluconazole in the treatment of AIDS-associated cryptococcal meningitis. *AIDS*. 1997;11:1463-1471.
12. Almyroudis NG, Sutton DA, Linden P, Rinaldi MG, Fung J, Kusne S. Zygomycosis in solid organ transplant recipients in a tertiary transplant center and review of the literature. *Am J Transplant*. 2006;6:2365-2374.
13. Sun HY, Aguado JM, Bonatti H, et al. Pulmonary zygomycosis in solid organ transplant recipients in the current era. *Am J Transplant*. 2009;9:2166-2171.
14. Sun HY, Singh N. Emerging importance of infections due to zygomycetes in organ transplant recipients. *Int J Antimicrob Agents*. 2008;32(suppl 2):S115-S118.
15. Gleissner B, Schilling A, Anagnostopolous I, Siehl I, Thiel E. Improved outcome of zygomycosis in patients with hematological diseases? *Leuk Lymphoma*. 2004;45:1351-1360.
16. Ruping MJ, Heinz WJ, Kindo AJ, et al. Forty-one recent cases of invasive zygomycosis from a global clinical registry. *J Antimicrob Chemother*. 2010;65:296-302.
17. Sun HY, Forrest G, Gupta KL, et al. Rhino-orbital-cerebral zygomycosis in solid organ transplant recipients. *Transplantation*. 2010;90:85-92.
18. Reed C, Bryant R, Ibrahim AS, et al. Combination polyene-caspofungin treatment of rhino-orbital-cerebral mucormycosis. *Clin Infect Dis*. 2008;47:364-371.
19. Clemons KV, Sobel RA, Williams PL, Pappagianis D, Stevens DA. Efficacy of intravenous liposomal amphotericin B (AmBisome) against coccidioidal meningitis in rabbits. *Antimicrob Agents Chemother*. 2002;46:2420-2426.
20. Takemoto K, Yamamoto Y, Ueda Y. Influence of the progression of cryptococcal meningitis on brain penetration and efficacy of AmBisome in a murine model. *Chemotherapy*. 2006;52:271-278.
21. Albert MM, Stahl-Carroll L, Luther MF, Graybill JR. Comparison of liposomal amphotericin B to amphotericin B for treatment of murine cryptococcal meningitis. *J Mycol Med*. 1995;5:1-6.
22. Sau K, Mambula SS, Latz E, Henneke P, Golenbock DT, Levitz SM. The antifungal drug amphotericin B promotes inflammatory cytokine release by a Toll-like receptor- and CD14-dependent mechanism. *J Biol Chem*. 2003;278:37561-37568.
23. Bellocchio S, Gaziano R, Bozza S, et al. Liposomal amphotericin B activates antifungal resistance with reduced toxicity by diverting Toll-like receptor signalling from TLR-2 to TLR-4. *J Antimicrob Chemother*. 2005;55:214-222.
24. Lewis RE, Chamilos G, Prince RA, Kontoyiannis DP. Pretreatment with empty liposomes attenuates the immunopathology of invasive pulmonary aspergillosis in corticosteroid-immunosuppressed mice. *Antimicrob Agents Chemother*. 2007;51:1078-1081.

Treatment of Visceral Leishmaniasis With Lipid-based Amphotericin B

Ivica Knezovic, MD

Epidemiology, Diagnosis, Clinical Signs, and Symptoms of Leishmaniasis

Leishmaniasis is a parasitic disease caused by a protozoan from the genus *Leishmania*. The epidemiology and presentation of leishmaniasis differs worldwide based upon the type of protozoa present in particular geographic regions. Although the genus *Leishmania* can be classified taxonomically into subgenus categories, tropism of the specific *Leishmania* species results in a specific clinical syndrome, of which a few have been identified. These clinical syndromes include a visceral, mucocutaneous, and cutaneous form. Considerable overlap of these syndromes can occur.

There is a worldwide distribution of leishmaniasis, all forms of which have been found to be present in 88 countries.[1] Specifically, the visceral form is present in 62 countries, including 16 developed countries. However, it is a major disease in developing countries, with more than 90% of cases reported in India, Bangladesh, Nepal, Ethiopia, Sudan, and Brazil. Approximately 350 million people worldwide are at risk for leishmaniasis infection. Each year, there are an estimated 500,000 new cases of visceral leishmaniasis, and 1–1.5 million new cases of cutaneous leishmaniasis. If left untreated, the visceral form of leishmaniasis can be fatal in up to 70–90% of cases. Notably, up to 100% of patients in developing countries may die from infection, depending on the particular geographic region and available healthcare facilities and treatment options.

The incubation period of visceral leishmaniasis is a minimum of 2 weeks and a maximum of 8 months. In most cases, it ranges between 2–6 months. Mammals, primarily dogs and other canids, serve as the main reservoir for the *Leishmania* parasite. The sand fly serves as the vector for *Leishmania*, although the genus changes depending on location (the genus *Phlebotomus* is found in the Eastern Hemisphere [Old World], and the genus *Lutzomyia* is found in the Western Hemisphere [New World]).[2] One exception

Table 1. Signs and Symptoms of Visceral Leishmaniasis

Sign/Symptom	%
Fever	100
Splenomegaly	96
Hepatomegaly	72
Anemia	44
Thrombocytopenia	24
Pancytopenia	16
Leukopenia	8
Neutropenia	8
Anorexia	8
Diarrhea	4
Abdominal pain	4
Paleness	4
Hypoproteinemia	4
Meningeal syndrome	4

Data from Cruz I et al. *J Clin Microbiol.* 2006; 44:2343-2347.

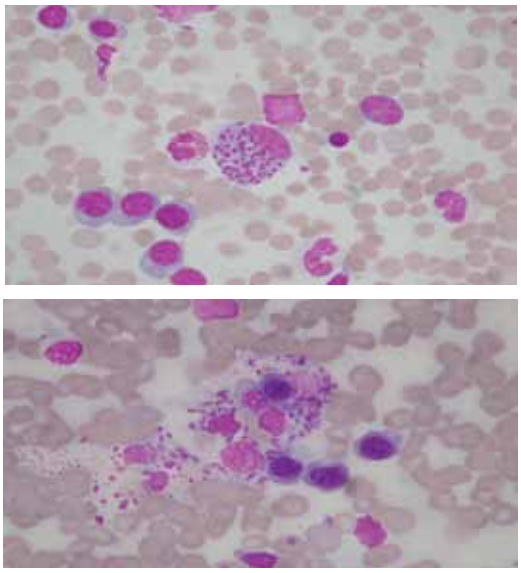

Figure 1. A) Intracellular amastigotes. Giemsa-stained bone marrow aspirate. Magnification x1000. B) Extracellular amastigotes. Giemsa-stained bone marrow aspirate. Magnification x1000.

occurs in India, where a human reservoir exists that allows human-to-human transmission via the sand fly vector. Transmission begins with a sand fly bite; rarely, transmission may occur through the use of shared needles in intravenous drug use or with sexual activity.[3,4]

Special consideration must be made for those leishmaniasis-infected patients with HIV infection. Coinfection is apparent in 34 countries, mainly restricted to those in Africa, Asia, Europe, and South America.[1] In southern Europe alone, an estimated 70% of adult visceral leishmaniasis infections occur in HIV-infected patients. Coinfection occurs more frequently with low (<200 cells/mm^3) CD4-positive lymphocyte counts.

The clinical presentation of the visceral form of leishmaniasis in nearly all patients is based primarily on the presence of fever, hepatomegaly and/or splenomegaly, pancytopenia, hypergammaglobulinemia, and cachexia (Table 1).[5] Other symptoms that may be present include enlarged lymph nodes and secondary infections, mostly bacterial. Atypical presentations, including respiratory disorders or diarrhea, sometimes occur in HIV-infected patients. In persons who become immunocompromised, latent visceral leishmaniasis can reactivate and become apparent.

The primary abnormal laboratory findings that accompany visceral leishmaniasis are predominantly restricted to cytopenias (including anemia, leukopenia, neutropenia, thrombocytopenia) and hypergammaglobulinemia.

Several methods may be used to diagnose visceral leishmaniasis.[6] Identification of the clinical presentation is the first step in diagnosis. However, definitive diagnosis is made with microscopic visualization.[5] It is generally performed in specimens of bone marrow aspirates, or biopsy samples from the spleen, liver, or, in some cases, the lymph node. From these samples, microscopic identification of amastigotes can be made in May-Grünwald-Giemsa–stained smears (Figure 1). This diagnosis can also be confirmed with a positive culture demonstrating promastigotes in Novy-MacNeal-Nicolle (NNN) medium. Other diagnostic tools include serologic tests, such as the indirect immunofluorescence antibody test (IFAT), enzyme-linked immunosorbent assay (ELISA), direct agglutination test (DAT) and rK39 antigen-based immunochromatographic test (ICT). Molecular techniques, such as polymerase chain reaction (PCR)—including linear-nested PCR and semi-nested PCR—are associated with 90–99% sensitivity and up to 100% specificity and can be implemented using typical specimens or blood.

Successful Case Studies of Visceral Leishmaniasis Treated With Lipid-Based Amphotericin B, Including Amphotericin B Colloidal Dispersion

All amphotericin B formulations, including both conventional and lipid-based formulations that are administered intravenously, have a proven efficacy in the treatment of visceral leishmaniasis.[7] However, in regions where resistance is not an issue, the standard therapy for leishmaniasis revolves around the use of pentavalent antimony compounds, derived from the heavy metal antimony. These include sodium stibogluconate (SbV) and meglumine antimoniate, both of which have been a standard of therapy for more than 60 years. These agents, administered either intramuscularly or intravenously, are long-lasting; however, they are associated with significant and possibly fatal toxicities.[8]

Intravenously-administered pentamidine is an effective alternative to pentavalent antimony compounds, but it can be toxic, with reversible and irreversible diabetes mellitus occurring in 10% of patients.[9] Today, the use of pentamidine is limited to the prevention of relapses in immunocompromised patients previously successfully treated with another agent.[10]

Resistance to pentavalent antimony compounds is frequent, occurring in up to 60% of patients who receive the agent. Resistance is regularly observed in India, and therefore clinicians in this region have particular experience with using alternative compounds in the treatment of leishmaniasis, such as lipid-based and traditional formulations of amphotericin B.[11] Oral miltefosine and parenteral paromomycin are effective alternative and/or additional drugs in the treatment of visceral leishmaniasis.[12]

During the last 2 decades, numerous clinical studies have been performed worldwide, proving the effectiveness of lipid amphotericin B formulations.[13] However, in immunocompromised patients (eg, HIV-infected), frequent relapses of visceral leishmaniasis can occur after initial healing.

Lipid-based formulations of amphotericin B are an alternative first-line treatment option for visceral leishmaniasis. Furthermore, these compounds are considered to be the first-line treatment for Mediterranean and Indian visceral leishmaniasis. Although conventional amphotericin B is less expensive, it is associated with far greater toxicity (eg, renal toxicity) and more infusion-related reactions than lipid-based amphotericin B formulations.[14,15] Overall, liposomal amphotericin B and amphotericin B colloidal dispersion (amphotericin B cholesteryl sulfate complex for injection) are more effective than amphotericin B lipid complex, which is more effective than conventional amphotericin B.[16]

In the Department of Pediatric Infectious Diseases of the University Hospital for Infectious Diseases in Zagreb, Croatia, we have treated 7 children for visceral leishmaniasis from 2000–2009. These children, aged 9 months to 4.5 years, each had confirmed visceral leishmaniasis and were all immunocompetent and previously healthy. Of these 7 children, 4 were successfully treated with pentavalent antimony compounds, and 3 were treated with amphotericin B colloidal dispersion. Treatment with amphotericin B colloidal dispersion was associated with a very good efficacy and no side effects.

Lipid formulations of amphotericin B are designed to deliver the active drug to target sites (the reticulo-endothelial system), and are highly effective against *Leishmania*. There are some concerns about infusion-related reactions, which have been reported in 63–68% of patients.[16] Similarly, the incidence of fevers associated with these amphotericin B formulations is 56–60%. Other studies have also reported respiratory distress in children younger than 5 years.[17] However, our experience in 3 children did not include either infusion-related reactions or respiratory distress. It is possible that these adverse events could be diminished by slowing the infusion rate and/or administering premedication.

Although lipid-based amphotericin B formulations are noted to be expensive, they are highly cost effective compared with the hospitalization expenses required for 28–30 days of antimony compound treatment. Recommended dosage of lipid-based amphotericin B formulations varies in different countries/regions, according to the susceptibility of the particular *Leishmania* strain. Several dosage schemes have been proposed. Recommended dosage of amphotericin B colloidal dispersion is currently 2 mg/kg/day IV over 7 days.[13]

Our 3 cases of young children treated with amphotericin B colloidal dispersion can be seen as an example of successful treatment. In this small number of patients, we did not observe any of the adverse reactions previously mentioned. If patients are closely monitored in a hospital setting, lipid-based formulations of amphotericin B should be the first-line treatment for leishmaniasis. According to our clinical experience, amphotericin B colloidal dispersion is a good alternative to liposomal amphotericin B in the treatment of visceral leishmaniasis in immunocompetent children.

References

1. Desjeux P. Leishmaniasis: current situation and new perspectives. *Comp Immunol Microbiol Infect Dis.* 2004;27:305-318.
2. Sharma U, Singh S. Insect vectors of Leishmania: distribution, physiology and their control. *J Vector Borne Dis.* 2008;45:255-272.
3. Quinnell RJ, Courtenay O. Transmission, reservoir hosts and control of zoonotic visceral leishmaniasis. *Parasitology.* 2009;136:1915-1934.
4. Bañuls AL, Hide M, Prugnolle F. Leishmania and the leishmaniases: a parasite genetic update and advances in taxonomy, epidemiology and pathogenicity in humans. *Adv Parasitol.* 2007;64:1-109.
5. Cruz I, Chicharro C, Nieto J, et al. Comparison of new diagnostic tools for management of pediatric Mediterranean visceral leishmaniasis. *J Clin Microbiol.* 2006;44:2343-2347.
6. Peres LC, Saggioro FP, Dias LB Jr, et al. Infectious diseases in paediatric pathology: experience from a developing country. *Pathology.* 2008;40:161-175.
7. Kafetzis DA. An overview of paediatric leishmaniasis. *J Postgrad Med.* 2003;49: 31-38.
8. Thakur CP, Narayan S. A comparative evaluation of amphotericin B and sodium antimony gluconate, as first-line drugs in the treatment of Indian visceral leishmaniasis. *Ann Trop Med Parasitol.* 2004;98:129-138.
9. Thakur CP, Kumar M, Pandey AK. Comparison of regimes of treatment of antimony-resistant kala-azar patients: a randomized study. *Am J Trop Med Hyg.* 1991;45:435-441.
10. Patel TA, Lockwood DN. Pentamidine as secondary prophylaxis for visceral leishmaniasis in the immunocompromised host: Report of four cases. *Trop Med Int Health.* 2009;14:1064-1070.
11. Singh N. Drug resistance mechanisms in clinical isolates of Leishmania donovani. *Indian J Med Res.* 2006;123:411-422.
12. Moore EM, Lockwood DN. Treatment of visceral leishmaniasis. *J Global Infect Dis.* 2010;2:151-158.
13. Murray HM. Treatment of visceral leishmaniasis in 2004. *Am J Trop Med Hyg.* 2004; 71:787-794.
14. Sundar S, Mehta H, Suresh AV, et al. Amphotericin B treatment for Indian visceral leishmaniasis: conventional versus lipid formulations. *Clin Infect Dis.* 2004; 38: 377-383.
15. Barratt G, Legrand P. Comparison of the efficacy and pharmacology of formulations of amphotericin B used in treatment of leishmaniasis. *Curr Opin Infect Dis.* 2005;18:527-530.
16. Sundar S, Mehta H, Chhabra A, et al. Amphotericin B colloidal dispersion for the treatment of Indian visceral leishmaniasis. *Clin Infect Dis.* 2006;42:608-613.
17. Berman J, Dietze R. Treatment of visceral leishmaniasis with amphotericin B colloidal dispersion. *Chemotherapy* 1999;45(suppl 1):54-66.