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AMPHOTERICIN B: A QUICK REFERENCE FOR PRACTICING CLINICIANS

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Amphotericin B is a polyene antifungal agent derived from *Streptomyces nodosus*.[1] Although it was discovered more than 60 years ago, it remains a first-line agent for the treatment of many life-threatening invasive fungal infections.

Amphotericin B is amphipathic (i.e. it possesses both hydrophilic and hydrophobic moieties), and hence is insoluble in water.[1] Aqueous solubility is therefore achieved by formulation with deoxycholate (D AmB) or a lipid carrier. The former has been available since the late 1950s, but its clinical utility is limited by a high frequency of adverse effects, particularly nephrotoxicity.[1,2] There are various lipid formulations (Table 1) which reduce the parent drug's nephrotoxicity while retaining the drug's activity and also providing a better therapeutic index for the drug. [1,2] All are administered intravenously (IV). Aerosolized formulations should be a valuable alternative, because administration by inhalation ensures a high drug concentration in the respiratory tract, while potentially minimizing systemic toxicities. [3–5]

Message from the Editor

We start herewith a series on antifungal drugs previously published by Dr Atul Patel in the Asia Fungal Working Group Newsletter and reproduced after appropriate permissions. This newsletter discusses Amphotericin B with important practical tips for practising clinicians. We then discuss two interesting cases which highlight the need for accurate identification of fungi as well as anti fungal susceptibility testing. Readers are urged to use newer methods such as MALDI TOF and send isolates to reference laboratories for the same.

We announce three important upcoming events. Please save the dates. We would also like to draw your attention to the FISF online course in fungal infections and the two day teaching course in fungal infections. Please visit the FISF website for further details.

Finally recommending you to browse the newsletter from the Clinical Infectious Diseases of India (CIDS) at the website www.cids.org which will keep you updated with infectious diseases apart from mycology.

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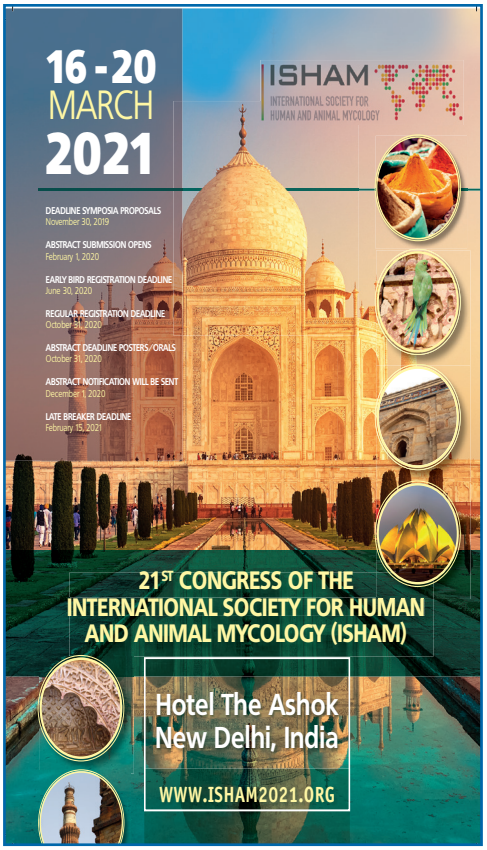

Table 1: Lipid formulations of amphotericin B

Formulation	Complex
Amphotericin B lipid complex (ABLC)	• Complexed with dimyristoyl phosphatidylcholine and dimyristoyl phosphatidylglycerol
Amphotericin B colloidal dispersion (ABCD)	• Complexed with cholesteryl sulphate
Liposomal amphotericin B (LAmB)	• Complexed with hydrogenated soy phosphatidylcholine, distearoyl, phosphatidylglycerol and cholesterol

Spectrum of activity

It acts by disruption of the cell membrane and is toxic to mammalian cells. Amphotericin B has a broad spectrum of activity, against most fungi including yeast and filamentous fungi. Fungi with intrinsic resistance include some candida spp.., *Trichosporon* spp., *Aspergillus terreus, Scedosporium* spp., and *Malassezia furfur*.[2]

Pharmacokinetic parameters

Pharmacokinetics properties differ between the various formulations. For DAmB, peak plasma concentrations typically range from 0.5 to 2 mg/L, with slow excretion by the kidneys over weeks or months.[6] Among the formulations with a lipid carrier, ABLC and ABCD are rapidly cleared from the bloodstream and taken up primarily by the reticuloendothelial system, with peak plasma levels ≤ 5 mg/L when given at therapeutic doses.[7] By contrast, LAmB given at similar doses is associated with peak plasma levels up to 200 times higher.[7] However, for all three lipid carrier formulations, the pharmacokinetics are non-linear, such that there are greater than (LAmB) or less than (ABLC, ABCD) proportional increases in serum concentrations with increasing dose.[8–10] **LAmB achieves 4 -7 fold higher brain parenchymal concentration compared to other preparations, makes this molecule useful for treatment of fungal infections involving CNS.**

Indications

Amphotericin B is the drug of choice for Mucormycosis, Cryptococcosis, Empirical therapy of Febrile neutropenia in adults and children, Severe endemic mycosis (histoplasmosis, coccidioidomycosis, paracoccidioidomycosis), Candida infections of the CNS/ Endocarditis/Neonates/ fluconazole resistant UTI as well as Kala azar and Amebic meningoencephalitis. It is an alternative agent for treatment of invasive aspergillosis when voriconazole cannot be used and is also used both aerosolized and intravenously as prophylaxis in cancer patients and patients undergoing hematopoetic stem cell transplant (HSCT).

Dosage recommendations

The dosing of IV amphotericin B is dependent both on the formulation used and the specific indication. **The drug should be diluted in 5% dextrose and not normal saline.**

DAmB should be given over a period of around 4–6 hours. Because patient tolerance can vary substantially, the dosage should be individualized and adjusted according to clinical status.[3] A test dose may be desirable to assess tolerance. In patients with good cardio-renal function and a well-tolerated test dose, therapy is usually initiated at 0.25 mg/kg/day, gradually increasing to 0.5–1.3 mg/kg/day (higher doses for mucormycosis).[6]

Clinicians can initiate DAmB with required dose of 0.7 -1.0mg/kg/day from day one rather gradually escalating dosage. Two simple clinical tips to reduce DAmB associated toxicities are 1. Prehydration with 500 ml of intravenous normal saline before starting DAmB infusion 2. Prolong infusion of DAmB for 7-10 hours. Prehydration with saline increases circulating volume and maintains glomerular perfusion while prolonged infusion reduces allergic infusion associated reactions. Clinicians should avoid concomitant saline infusion into the same line as DAmB as saline may precipitate DAmB.

With ABLC, the recommended daily dosage for adults and pediatric patients is 5 mg/kg/day, administered at a rate of 2.5 mg/kg/hour.8 Meanwhile, with ABCD, the recommended dose for adults and pediatric patients is 3–4 mg/kg/day, initially at a rate of 1 mg/kg/hour, although this can be shortened to a minimum of 2 hours if there is no evidence of intolerance or infusion-related reactions.[9] Finally, LAmB may be given to adults and children at doses of 3–10 mg/kg/day (3 mg/kg/day in aspergillosis and 5-10 mg/kg/day for mucormycosis) initially administered over 2 hours, although this can be reduced to around 1 hour if the drug is well-tolerated.[10] The drug may need filtration prior to administration. Prehydration with NS can also be used for the lipid formulations.

There is another indigenously developed preparation of lipid formulation amphotericin B that needs sonication prior to administration and dose recommended is 1-3 mg/kg/day. Clinical data with this preparation is sparse.

Drug–drug interactions

Drug interactions associated with amphotericin B are often based on their potential to cause nephrotoxicity.[3] For example, compounds such as aminoglycosides, polymyxins, cyclosporine, pentamidine and antineoplastic agents can enhance the potential for drug-induced renal toxicity, and should be used concomitantly with great caution.[6,8–10] Intensive monitoring of renal function and electrolytes is recommended in patients requiring any combination of nephrotoxic medications.

Furthermore, concurrent use of flucytosine with amphotericin B may increase the toxicity of flucytosine, possibly by increasing its cellular uptake and/or impairing its renal excretion.[6,8–10]

Therapeutic drug monitoring

At present, there is no evidence to support the routine use of therapeutic drug monitoring with amphotericin B.[11] However, this could change in future as our understanding of the exposure–response relationships improve.

Adverse drug reactions

The most common adverse events associated with amphotericin B are transient chills and/or fever during infusion of the drug.[6,8–10] Infusion-related reactions are due to toll-like receptor (TLR)-2 activation, resulting in a proinflammatory cytokine response. Pre-treatment with nonsteroidal anti-inflammatory agents, antihistamines, corticosteroids, meperidine may reduce such reactions.

However, the clinical use of DAmB is limited primarily by nephrotoxicity (40%). Toxicity is dose dependent. In some cases, this can be associated with permanent renal impairment, particularly when given alongside other nephrotoxic drugs.[2] Lipid carrier formulations have lower rates of nephrotoxicity (15%), and these effects are only weakly correlated with dose.[2] Hence, higher effective dosages can be given with lipid-formulated amphotericin B compared with DAmB, with a lower risk of treatment-limiting renal dysfunction.

Hypokalemia, hypomagnesemia are common with use of amphotericin B and need constant monitoring and correction.

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THE NEEDLE AND BLACK MAGIC

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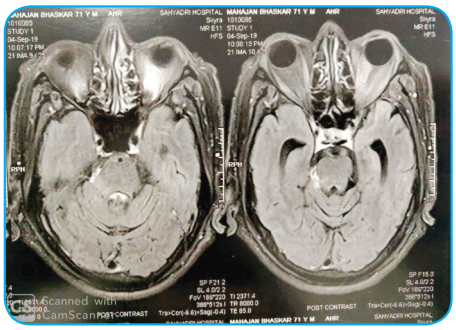
Mr. M, a 71 year old gentleman with no previous comorbidities, received an epidural steroid injection to relieve lower back pain due to lumbar spondylosis. A month later, he developed severe back pain and bilateral lower limb weakness, which worsened over the next month.MRI brain showed meningeal enhancement in the perimesencephalic cistern and a lesion in the pons and cerebellar peduncle (Figure 1).

Figure 1: MRI showing the lesions in pons and cerebellar peduncle



His serial CSF analyses and treatment received before presenting to us are as depicted in Table 1

Table 1: CSF analysis and Treatment

Date	Cells	Protein	Sugar	Culture	CSF BioFire	Treatment
5/9	940, 68% N	90	16	Candida ciferii	CMV	LAmB + FC for 10 days
11/9	1080, 92%N	159	28	-	-	
20/9	1200, 85%N	195	18	-	-	
30/9	30, 95%L	50	37	-	-	Ceftriaxone + Vancomycin for 3 weeks
18/10	430, 80%N	186	19	-	Quantitative CMV negative	
19/10	Started on empiric ATT (HRZE) and was referred to a neurosurgeon for placement of VP shunt, who referred him for ID opinion					

We discuss the differential diagnosis in a tabular format (Table 2)

Table 2: Differential diagnosis for the chronic meningitis

D/D	Points in favor	Points against
CMV	Myeloradiculitis CSF PMN pleocytosis	Patient not heavily immuno-compromised Can be detected as a false positiveby Biofire film array in CMV infected individuals (latent infection)
Pyogenic meningitis due to pneumococcus	-	Negative Biofire Subacute/ chronic meningitis Cerebritis Not cured with 3 weeks of ceftriaxone + Vancomycin
TB	Chronic meningitis	Chronic meningitis but not progressed to unconsciousness, infarcts etc despite steroids without ATT
Candida ciferii	Chronic meningitis possible- Temporally associated with receipt of steroid injection Some benefit had occurred with AmB + FC	-

The 1st 3 entities have no relation to the receipt of steroid injection in the spine. On further enquiry, we found that the isolate, which was found as Candida ciferii by VITEK2, was identified by MALDI-TOF as Exophiala spp, a dematiaceousyeast. This fungus has been previously reported as a cause of infections following contaminated injections.[1,2] Considering all the points, *Exophiala dermatitidis* infection seemed to be the most likely cause of chronic meningitis and brain stem involvement. It is a relatively resistant organism for which voriconazole would be helpful, but had not been received so far.[3]

The isolate was again identified as *Exophiala dermatitidis* by phenotypic methods at PGI Chandigarh, with AFST (antifungal susceptibility testing) showing susceptibility to voriconazole (Figure 2).

Figure 2: Identification of the fungus at a reference lab

DEPARTMENT OF MEDICAL MICROBIOLOGY (MYCOLOGY DIVISION)
POST GRADUATE INSTITUTE OF MEDICAL EDUCATION RESEARCH, CHANDIGARH 160012 (INDIA)

Antifungal Susceptibility Report

Sample ID	:	IL-4066
Date of Report	:	31-10-2019
Identification	:	Exophiala dermatitidis
Method of Identification	:	Phenotypic identification
Antifungal susceptibility method	:	CLSI method (M38, A2)

Sr. No.	Antifungal	MIC(μg/ml)
1	Amphotericin B	2
2	Fluconazole	0
3	Voriconazole	0.12
4	Itraconazole	0.5
5	Posaconazole	0.25
6	Caspofungin	4
7	Anidulafungin	4
8	Micafungin	16

Note :- S : Susceptible. I : Intermediate, R : Resistant, SDD : Susceptible Dose Dependent

Since he was on Rifampicin for 10 days, starting voriconazole immediately would have led to lower voriconazole levels (due to interactions with Rifampicin). He was asked to stop ATT and start voriconazole after 7-10 days, by which time the enzyme induction effect of Rifampicin would have worn off.

The case underscores the need to carefully consider the differential diagnosis, use appropriate methods for identification of the organism & its susceptibility, avoid ineffective empiric therapy, remember drug interactions&pay strict attention to infection control.

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RELAPSING CRYPTOCOCCAL MENINGITIS COMPLICATED BY DRUG INDUCED QUADRIPLEGIA

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A 28-year old HIV-negative male, bird-handler by occupation developed three episodes of cryptococcal meningitis in the past 18 months. Miliary pulmonary TB was diagnosed in him 24 months ago for which he received anti-tuberculosis therapy (ATT) for 9 months. There was complete radiologic resolution of disease In the later part of his ATT treatment, there was onset of his first episode of cryptococcal meningitis. The illness could have been associated with his steroid intake at the time of diagnosis of miliary TB disease. He was treated elsewhere with only fluconazole for a short duration and relapsed some time later. He was retreated again with fluconazole monotherapy again for a short time.

At his third relapse he presented to our hospital. CSF India Ink was positive. The stain isolated during third relapse was *Cryptococcus neoformans grubii* identified by MALDI-TOF (Matrix assisted laser desorption-ionization Time of Flight) at a reference mycology laboratory with fluconazole MIC=4 (Figure 1). He had a normal CD4 count. We initiated treatment with IV amphotericin B deoxycholate (D AmB) to which there was a peculiar intolerance; although he was young and having a normal baseline creatinine. Repeated attempts to challenge him with amphotericin B deoxycholate failed due to quick rise in serum creatinine. He could not afford IV liposomal amphotericin B therapy. Therefore, we had to treat him with an alternative oral regimen of high-dose fluconazole at 800 mg per day and 5-flucytosine (5-FC) at 25 mg/kg q6h for 6 weeks. Towards the end of fluconazole/5-FC therapy, he developed rapid onset ascending hyporeflexic flaccid quadruparesis with hypokalemia. This was most probably due to oral 5-FC and recovered with its discontinuation and potassium replacement. At his discharge, we plan to continue his oral fluconazole treatment at 400 mg per day for 2 months (consolidation) and then at 200 mg per day for another 12 months.

Sample ID	:	IL-4139
Date of Report	:	26-11-2019
Identification	:	*Cryptococcus meformans grubii*
Method of Identification	:	MALDI-TOF MS
Antifungal Susceptibility method	:	CLSI method (M27, A3)

Sr. No.	Antifungal	MIC(μg/ml)
1	Amphotericin B	0.5
2	Fluconazole	4
3	Voriconazole	0.25
4	Itraconazole	0.12
5	Posaconazole	0.12
6	Caspofungin	16
7	Anidulafungin	16

Figure 1: CSF isolate identification by MALDI TOF and antifungal susceptibility

This case brings out several important points discussed below.

Treatment duration: Duration of induction treatment of cryptococcal meningitis in non-HIV non-transplant patients is typically 4-6 weeks of amphotericin B and flucytosine as compared to two weeks in HIV-infected patients. The maintenance phase of treatment with fluconazole lasts one year or longer and there is no CD4 count threshold based upon which treatment can be stopped in HIV-negative individuals[1]. This patient was treated inappropriately with fluconazole monotherapy for short duration which led to his relapses.

Fluconazole resistance in cryptococci: There are no accepted clinical breakpoints (CBP) to define fluconazole resistance in cryptococcal isolates. To facilitate detection of emerging resistance Pfaller et al established an MIC of 8 mg/L as an ECV (epidemiological cut-off value for fluconazole in Cryptococcus neoformans). In a study done by Bongomin F et al, using this cut off value it was estimated that current fluconazole resistance in cryptococci is about 18.7%.[2] In cases of relapsed meningitis, the relapse isolates should be checked for changes in the fluconazole and flucytosine minimum inhibitory concentrations (MICs) from the original isolate; a ≥3-dilution difference suggests development of direct drug resistance. Otherwise, an MIC of the persistent or relapse isolate ≥16 mg/mL for fluconazole or ≥32 mg/mL for flucytosine may be considered resistant, and alternative agents should be considered[1].

Polyene nephrotoxicity: The underlying mechanism for amphotericin B-induced acute kidney injury (AKI) remains poorly understood and may be immunologically mediated. This was studied in 58 patients who received AmB D, circulating serum interleukin IL-6, IL-8 and IL-10 were measured at baseline, week 1 and week 2 of antifungal treatment and correlated to the development of renal impairment, this showed persistence of an elevated pro-inflammatory cytokine milieu is associated with a predisposition to drug-related kidney injury.[3] This patient may have had an immune mediated nephrotoxicity as he was young and had normal renal function and nephrotoxicity developed very quickly each time he was challenged with AmB.

Short-course high-dose polyene treatment as a treatment option: This was evaluated in AMBITION trial in HIV infected patients in Botswana[4]. Comparison was done between (1) a single dose (10 mg/kg) of L-AmB given with 14 days of fluconazole (1200 mg/day) and flucytosine (100 mg/kg/day) and (2) 7 days amphotericin B deoxycholate (1 mg/kg/day) given alongside 7 days of flucytosine (100 mg/kg/day) followed by 7 days of fluconazole (1200 mg/day). Arm 1 was found to be non-inferior to arm 2. Since our patient had tested negative for HIV infection multiple times, he was not a suitable candidate for short-course high-dose polyene treatment.

Hypokalemia and 5-flucytosine: Hypokalemia occurs with an unmeasured frequency in patients receiving 5-FC. The mechanism is not fully understood. Our patient had loss of all superficial & deep tendon reflexes except one sided ankle jerk which was regularly elicitable. Neurological adverse reactions associated with 5-FC include ataxia, hearing loss, headache, paresthesias, Parkinsonism, vertigo, sedation, convulsions and peripheral neuropathy[5].

Conclusions

Treatment of cryptococcosis in non immunocompromised hosts is more challenging than the HIV infected with higher mortality rates. This is possibly attributed to delayed diagnosis and possibly host immune response. New trial data is also lacking unlike the HIV infected host. Paradoxical reactions needing steroids especially with *C. gatti* have also been described. Lastly, drug adverse effects are not uncommon and often require deviation from the standard regimes.

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About FISF

The purpose of the Fungal Infections Study Forum is to conduct educational activities, undertake epidemiological and clinical studies and to promote research activities on invasive fungal infections. The results of such research would benefit the clinicians, mycologists and the general public. The trust was formed in view of emergence of Invasive fungal infections (IFIs) in India which is posing a serious challenge to the haematologists, critical care providers, ID specialists, pulmonologists, neurologists, medical mycologists and many other clinicians handling serious and immunocompromised patients.The trust is the independent working consisting of clinicians and mycologists and instituted on 3rd March 2012 at Mumbai, India. To know more about us visit www.fisftrust.org.