CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

50-740/SE1-002

MICROBIOLOGY REVIEW

OCT 4 1999

MICROBIOLOGY REVIEW

DIVISION OF SPECIAL PATHOGENS AND IMMUNOLOGIC DRUG PRODUCTS (HFD-590)

NDA #: 50-740

REVIEWER	: Shukal Bala
CORRESPONDENCE DATE	: 07-06-99
CDER RECEIPT DATE	: 07-07-99
REVIEW ASSIGN DATE	: 07-13-99
REVIEW COMPLETE DATE	: 10-05-99

SPONSOR: Fujisawa Healthcare, Inc.
Three Parkway North
Deerfield, IL 60015

SUBMISSION REVIEWED: SE1-SE2

DRUG CATEGORY: Anti-fungal

INDICATION: Treatment of acute cryptococcal meningitis in immunocompromised patients

DOSAGE FORM: Liposomal formulation for systemic injection

PRODUCT NAMES:

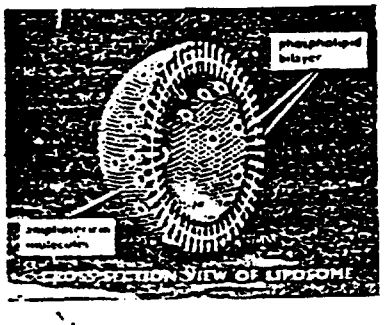
a. **PROPRIETARY:** AmBisome^{R}

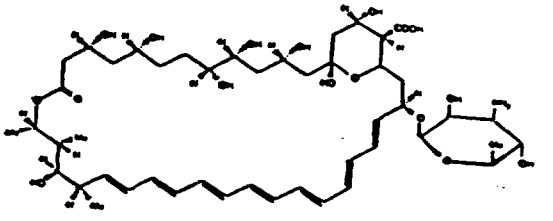
b. **NONPROPRIETARY:** VS104

c. **CHEMICAL:** [1R(1R*,3S*,5R*,6R*,9R*,11R*,15S*,16R*,17R*,18S*,19E,21E,23E,25E,27E,29E,31E,33R*,35S*,36R*,37S*)]-33-[(3-amino-3,6-dideoxy-ß-D-manno-pyranosyl)oxy]-1,3,5,6,9,11,17,37-octahydroxy-15,16,18-trimethyl-13-oxo-14,39-dioxa-bicyclo[33.3.1]nonatriaconta-19,21,23,25,27,29,31-heptaene-36-carboxylic acid

STRUCTURAL FORMULA:

Molecular weight: 924.09
Empirical Formula: $C_{47}H_{73}NO_{17}$





(amphotericin B)

SUPPORTING DOCUMENTS: NDA # 50-740, [redacted]

BACKGROUND:

The subject of this NDA supplement is AmBisome, a liposomal preparation of Amphotericin B, for the treatment of cryptococcal meningitis in immunocompromised patients. AmBisome is approved in the United States for treatment of systemic fungal infections, empiric therapy for presumed fungal infections and treatment of visceral leishmaniasis.

Amphotericin B is a well known anti-fungal agent. An intravenous formulation of the drug is approved for the treatment of potentially life threatening fungal infections, which include but are not limited to Aspergillosis, Cryptococcosis, Blastomycosis, systemic Candidiasis, Coccidioidomycosis, and Zygomycosis. Amphotericin B has an affinity for the sterol component of the cell membrane. Binding of the drug to this site alters the membrane permeability leading to cell death.

SUMMARY:

The non clinical studies demonstrating the antifungal activity of AmBisome were reviewed earlier (for details see Microbiology review dated 6/30/97). In this submission the sponsor has included 2 additional published reports demonstrating the activity of AmBisome against *Cryptococcus neoformans in vitro* and *in vivo*. A brief summary of representative studies follows:

Studies *in vitro*

In a study by Hossain *et al.*, 1998 (Antimicrob. Agents Chemotherapy 42: 1722) the *in vitro* activity of AmBisome was tested by the micro-dilution method using [redacted] medium according to NCCLS guidelines using 18 isolates of *C. neoformans*. The minimum inhibitory concentrations (MIC) were determined after 72 hours of incubation. The results in Table 1 show the mean MIC_{50} and MIC_{90} values to be 0.5 and 1.0 ug/ml respectively.

TABLE 1 MICs of antifungal agents for 18 strains of *C. neoformans*

Drug	MIC (μg/ml)*		
	Range	50%	90%
AmPH-B	[redacted]	1	2
Fungizone		0.5	0.5
AmBisome		0.5	1
MS-718		0.063	0.125

* The MICs of antifungal agents were determined by the microdilution method modified from the macrodilution method of the National Committee for Clinical Laboratory Standards (19). The 50% and 90% MICs are the MICs at which 50 and 90% of the isolates were inhibited, respectively.

Overall, the *in vitro* studies show that AmBisome exhibits activity against a wide range of fungal species including *C. neoformans* comparable to that of amphotericin B. Whether repeated exposure of the organisms to AmBisome *in vitro* will alter drug susceptibility is not known.

Studies *in vivo*

BALB/c mice were infected with 10^5 cfu of strain YC-11, serotype A of *C. neoformans* by the intratracheal route. Mice were administered AmBisome, amphotericin B or the lipid nanosphere formulation of amphotericin B (NS-718) by the intravenous route for 5 days beginning 2 hours after infection. The results showed that AmBisome was effective in improving survival of infected mice compared to the vehicle treated group. However, the lipid nanosphere formulation of amphotericin B was the most effective. A group of 10 mice were sacrificed on day 7 of infection and the lungs processed for the measurement mycological burden. The results in Table 2 show that AmBisome was not effective in reducing the mycological burden under the experimental conditions tested. NS718 was, however, effective in reducing mycological burden at the high dose.

TABLE 2. *C. neoformans* cell count in murine lung with pulmonary cryptococcosis after drug treatment[a]

Treatment	No. of cells (log10 CFU/g) after dose of	
	0.8 mg/kg	2.6 mg/kg
Control (5% dextrose)	7.4 ± 0.04	7.4 ± 0.04
NS-718	6.7 ± 0.38[b,c]	<3.0[b]
AmBisome	7.3 ± 0.58	7.6 ± 0.09
Fungizone	5.9 ± 0.51[b,c]	ND[d]

[a] Drug or diluent was injected intravenously into 10 mice in each group, and the number of *C. neoformans* cells was counted 7 days after infection. The values shown are the means ± standard deviations.
[b] $P < 0.05$, compared to the respective control value (Scheffe multiple-comparison test).
[c] $P < 0.05$, compared to the respective AmBisome value (Scheffe multiple-comparison test).
[d] $P < 0.05$, compared to the respective NS-718 value (Scheffe multiple-comparison test).
[e] ND, not done.

In another study (Clemons and Stevens, 1998, Antimicrob Agents Chemotherapy, 42: 899), the activity of AmBisome was compared with other lipid formulations of amphotericin B [AMPHOTEC (ABCD) and ABELCET (ABLC)] in a systemic murine infection model for *C. neoformans*. For this, CD-1 mice were infected intravenously with 6.25 x 10^5 cfu of *C. neoformans* (strain 9759). Intravenous treatment with AmBisome or another antifungal agent was initiated after 4 days of infection and continued for 14 days. The infected untreated mice died between day 15 – 34 post-infection. AmBisome was effective in improving survival (measured up to 49 days post-infection) compared to vehicle or amphotericin B treated animals. ABCD and AmBisome were more effective than ABLC. On day 49 post-infection, various organs from the surviving animals were processed for measurement of mycological burden. The results in Table 3 show that although AmBisome was effective in reducing the mycological burden, these organs were not free of cryptococcal infection. Overall, the activity of AmBisome and ABCD was comparable.

No studies were conducted in immunocompromised animals.

Table 3: Recovery of C. neoformans from the organs of surviving mice treated with Fungizone, ABCD, AmBisome (AmBi), or ABLC

Formulation and dose (mg/kg)	No. of survivors/no. cured	Geometric mean log10 CFU/organ (no. free of infection) [95% confidence interval]				
		Brain	Spleen	Liver	Kidney	Lung
None	8/0	1.74 (0)	0 (1)	0.96 (0)	0 (1)	[illegible]
Fungizone, 1	1/0	1.52 (0)	0.71 (0)	0 (1)	0 (1)	0 (1)
ABCD						
1	5/0	5.92 (0) [4.4–7.9]	2.60 (0) [0.9–4.3]	3.74 (0) [3.0–4.5]	3.07 (0) [2.3–3.8]	4.03 (0) [3.0–5.0]
5	10/0	5.55 (0) [4.7–6.4]	1.30 (3) [0.5–2.3]	1.62 (3) [0.5–2.7]	2.06 (3) [0.6–3.3]	2.60 (1) [1.0–4.0]
10*	9/0	4.05 (0) [2.6–5.3]	0 (9)	0.16 (8) [0–0.5]	0.3 (7) [0–1.0]	0.70 (0) [0–1.5]
AmBi						
1	3/2	3.72 (1) [0–11.7]	1.5 (3) [0–4.0]	1.81 (1) [0–3.7]	1.30 (1) [0–3.7]	2.67 (1) [0–5.3]
5	10/0	5.52 (0) [4.3–6.5]	1.53 (3) [0.6–2.4]	0.27 (9) [0–0.9]	1.53 (3) [0.6–2.5]	2.37 (2) [1.1–3.7]
10	10/0	4.12 (0) [3.0–5.2]	0.87 (3) [0.2–1.5]	0 (10)	0.44 (7) [0–1.0]	1.54 (2) [0.7–2.4]
ABLC						
1	2/1	2.45 (1) [0–33]	0 (2)	0 (2)	0 (2)	0 (2)
5	5/0	6.59 (0) [5.8–7.3]	1.81 (1) [0.0–3.6]	2.13 (0) [1.1–3.1]	1.99 (1) [0.2–3.8]	2.54 (0) [2.2–4.1]
10	9/0	6.48 (0) [5.8–7.1]	1.10 (4) [0–2.4]	0.51 (7) [0–1.5]	2.68 (0) [1.8–3.6]	3.19 (0) [2.2–4.1]

* There were only nine mice in this group

Mechanism of Action

Studies showing the mechanism of action of the drug were reviewed earlier (for details see Microbiology review dated 6/30/97). No new studies have been conducted since then.

THE LABEL PROPOSED BY THE SPONSOR

No changes have been made in the microbiology section of the label.

CONCLUSIONS:

AmBisome, a liposomal preparation of amphotericin B, is an approved antifungal agent in the United Kingdom, some parts of Europe, Mexico and the United States. Amphotericin B is a well known antifungal agent. In this submission the sponsor seeks approval of AmBisome for treatment acute cryptococcal meningitis in immunocompromised patients.

The antifungal activity of free amphotericin B is well known. The activity of the new liposomal formulation was investigated *in vitro* and *in vivo* and compared with that of the free drug. The *in vitro* studies indicate that the minimal inhibitory concentrations for AmBisome against different fungal species including *Cryptococcus neoformans* are comparable to those observed using the free form of the drug.

The *in vivo* activity of AmBisome was tested against a wide range of fungal species as a prophylactic and/or therapeutic agent including *C. neoformans*. AmBisome improves the percent survival and reduces fungal burden in immunocompetent mice infected with *C. neoformans*. In the pulmonary model of murine cryptococcal infection, AmBisome was not effective in reducing the mycological burden in the lung under the experimental conditions tested. In the systemic model of *C. neoformans*, AmBisome was effective in prolonging survival, however, its impact on mycological burden varied by organ system. For example, the fungal burden was reduced in liver and spleen but not in the brain. It should also be noted that immunosuppressed animals were not used to establish fungal infection in the studies. Immunosuppression would mimic more closely the situation observed in immunocompromised patients. A role for host defense mechanisms in conferring protection or enhancing drug efficacy cannot be ruled out. Also, it is known that the fungal infections would be more severe in immunosuppressed animals and that the activity of the drug decreases with the severity of infection.

RECOMMENDATIONS:

This NDA is approved with respect to Microbiology.

/S/
Shukal Bala
Microbiologist, HFD-590

CONCURRENCES:

HFD-590/ Deputy Dir /S/ Signature 10/26/99 Date
HFD-590/Micro TL /S/ Signature 10/26/99 Date
CC:
HFD-590/Original NDA 50-740
HFD-590/Division File
HFD-590/MO
HFD-590/Pharm
HFD-590/Chem
HFD-590/Review Micro
HFD-590/CSO/Bacho