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Liposomal amphotericin B (AmBisome) in the treatment of fungal infections in neutropenic patients

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The use of high-dose chemotherapy and the subsequent prolonged neutropenia in patients with haematological diseases has resulted in an increased incidence of fungal infections. The diagnosis and treatment of these infections in neutropenic patients pose major therapeutic problems. The only drug with proven efficacy in the treatment of deep-seated fungal infections, including invasive aspergillosis, is amphotericin B. Unfortunately, this drug has adverse side effects, most importantly dose-dependent nephrotoxicity; furthermore, some patients fail to show a response to amphotericin B. We have treated 20 patients undergoing myeloablative chemotherapy and/or bone marrow transplantation for haematological diseases with liposomal amphotericin (AmBisome) for proven or suspected aspergillosis. Eighteen patients had diffuse interstitial pneumonitis and two patients had suspected fungal liver abscesses. Five patients had mycologically proven fungal infection and of these, three patients (60%) showed a complete response to liposomal amphotericin. Eleven patients received liposomal amphotericin because of the failure of conventional amphotericin B to eradicate proven or suspected fungal infection. Five of these 11 patients (45%) showed a complete clinical response to liposomal amphotericin. Eight patients received liposomal amphotericin because of pre-existing renal impairment or nephrotoxicity caused by conventional amphotericin B. Four of these patients (50%) showed a response to liposomal amphotericin. Recovery from probable fungal infection in this group of patients occurred when there was complete remission of underlying disease and recovery of neutrophil counts, when they were concurrently treated with liposomal amphotericin.

Introduction

Fungi are being increasingly recognized as significant pathogens with a high mortality rate in patients with neutropenia and haematological malignancy; this may be a result of the increasing use of more intensive chemotherapy regimens and bone marrow transplantation in these patients. The fungi usually isolated in such cases are *Candida* spp. and *Aspergillus* spp. In our experience, and that reported by other workers, a major cause of morbidity and mortality is aspergillus infection in the neutropenic patient, with invasive aspergillosis being the main problem (Gerson *et al.*, 1984). The problems posed by invasive aspergillosis are encountered in both the diagnosis and the treatment of the condition. Invasive pulmonary aspergillosis usually presents as a diffuse interstitial pulmonary infiltrate in a patient with neutropenia. Other causes of

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such a pulmonary infiltrate include cytomegalovirus pneumonitis, *Pneumocystis carinii* pneumonia, bacterial infection (including tuberculosis), haemorrhage, pneumonitis induced by drugs or radiation, graft versus host disease and the primary disease itself (Rubin & Greene, 1988). The clinical presentation of all these conditions may be similar and there are as yet few sensitive tests that can differentiate these various causes (Bustamante & Wade, 1990). Although open lung biopsy, when carried out, may allow a definitive diagnosis in 65% of cases (Greenman, Goodall & King, 1975; Singer *et al.*, 1979) and thus be regarded as the 'gold standard', it nevertheless carries a high morbidity rate, especially in patients who may be pancytopenic with low platelet counts (Stover *et al.*, 1984). The main agent for treatment of aspergillosis is amphotericin B but, even so, in neutropenic patients with proven aspergillosis the mortality rate has been greater than 90% (Denning & Stevens, 1990). The main limitation to the use of amphotericin B is its toxicity. Amphotericin B causes severe acute and chronic side effects including fever, chills and rigors, and electrolyte disturbances. Long term administration invariably leads to some degree of renal impairment (Hamilton-Miller, 1973). Furthermore, it has been shown that even in the presence of high tissue levels of amphotericin B, fungal infections often progress (Christiansen *et al.*, 1985). Liposomal amphotericin has few of the side effects reported with amphotericin B and the enhanced therapeutic index may allow a greater efficacy against aspergillus infection (Lopez-Berestein, 1986).

In order to investigate the safety profile and to obtain preliminary data on the efficacy of AmBisome (unilamellar liposomal amphotericin B), we have treated 20 neutropenic patients, who were undergoing treatment for haematological malignancy, with this preparation.

Patients and methods

Patient selection and details

Between January 1990 and February 1991 we admitted 149 patients with haematological disorders to our unit, the majority with acute leukaemia or lymphoma. Of these, 111 patients underwent bone marrow transplantation (10 allogeneic and 101 autologous transplants), 37 others received treatment for acute leukaemia and one patient had aplastic anaemia. Patients received amphotericin B for presumed fungal infection if they had persistent fever for 72 h when neutropenic and showed no response to broad spectrum antibacterial chemotherapy, or if they had a clinically suspected or proven fungal infection. Fifty of the 111 patients treated on the Bloomsbury Bone Marrow Transplant unit developed an interstitial pneumonitis (i.e. diffuse interstitial pulmonary infiltrate with associated hypoxia) during their treatment; of these 50 patients, 37 (33% of the total) had suspected fungal pneumonitis. Eighteen of these 37 patients received liposomal amphotericin (AmBisome) and a further two patients received liposomal amphotericin for suspected fungal liver abcesses on abdominal ultrasound or computerized tomographic (CT) scan. The characteristics of the patients receiving liposomal amphotericin are summarized in Table I. There were 14 males and six females. The median age of these patients was 33 years (range 7–72). The underlying haematological condition was acute myeloid leukaemia in 11 patients, acute lymphoblastic leukaemia in one patient, Hodgkin's disease in three patients, non-Hodgkin's lymphoma in three patients, chronic granulocytic leukaemia in one patient and aplastic anaemia in one

patient. Eight patients underwent bone marrow transplantation (seven autologous transplants and one bone marrow transplant from a matched unrelated donor), 11 patients received myeloablative chemotherapy and the patient with aplastic anaemia received horse antilymphocyte globulin (ALG).

Indications for liposomal amphotericin B

The reason for commencing AmBisome was failure of conventional amphotericin B (i.e. no clinical response or progression of suspected/proven fungal infection after 350 mg of amphotericin B iv) in 11 patients and renal toxicity due to conventional amphotericin B or previous renal failure in eight patients. One patient received AmBisome to facilitate outpatient care since he was otherwise fit for discharge (Table I).

Diagnosis of fungal infection

The diagnosis of fungal infection was confirmed by isolation of *Aspergillus* spp. from bronchoalveolar lavage in two patients (10%), sputum positive for *Aspergillus* spp. in two patients (10%), pleural aspirate positive in one patient (5%) and radiological appearances on chest radiograph and CT scanning (cavitating lesions highly suggestive of fungal infection) in four patients (20%). Eleven of the 20 patients (55%) had no mycologically or radiologically proven fungal infection at the time of treatment with amphotericin but were treated on the basis of clinical suspicion alone. However, two of these patients had disseminated aspergillosis at post-mortem. Thus only five of 20 (25%) of the patients had a mycologically proven fungal infection at the time of commencement of the liposomal amphotericin therapy.

Administration of liposomal amphotericin B

Liposomal amphotericin B (AmBisome) was administered according to a standard protocol (see Ringdén *et al.*, 1991, this supplement).

Results

Overall, of the 20 patients receiving liposomal amphotericin (AmBisome), 10 (50%) showed resolution of their fungal infection and 10 (50%) of patients showed no response (see Tables II and III). The median length of treatment with conventional amphotericin B before this was replaced with liposomal amphotericin was 10 days (range 0–23 days) in the patients showing a response to liposomal amphotericin, in comparison with 14 days (range 6–30 days) in those showing no response. The median length of treatment with liposomal amphotericin in the responding group was 10 days (range 4–96 days) and the median dose of liposomal amphotericin was 760 mg (range 300–7900). The median length of treatment with liposomal amphotericin in the group showing no response was 6 days (range 1–9) and the median dose was 500 mg (range 30–1180). The median neutrophil count (at the time of clinical resolution of the fungal infection) in the group showing a response to liposomal amphotericin was $1{\cdot}4 \times 10^9$/L (range 0·25–10·0) and $0{\cdot}3 \times 10^9$/L (range 0·1–10·8) in the non-responding group. Of the ten patients showing a response to liposomal amphotericin, only one patient (no. 3,

Table I. Details of patients, including diagnoses

Patient no	Age (years)	Sex	Haematological diagnosis and treatment	Reasons for suspecting fungal infection	Neutrophil count at time of suspected infection ($\times 10^9$/L)	Mode of diagnosis confirmation
Patients with proven fungal infection						
1	30	M	Burkitt's lymphoma (ABMT)	BIS-basal	< 0·5	Aspergillus isolated from sputum
2	45	F	AML (ABMT)	BIS; R pleural effusion	< 0·5	Aspergillus isolated from pleural aspirate
3	19	M	Aplastic anaemia (ALG)	BIS-diffuse	< 0·5	Aspergillus isolated from BAL
4	67	M	AML (induction chemotherapy)	BIS-diffuse	< 0·5	Aspergillus isolated from BAL
5	46	M	AML (induction chemotherapy)	BIS; cavitating lesion on CT scan; sinusitis	< 0·5	Aspergillus isolated from sputum
Patients with suspected fungal infection						
6	72	M	AML (induction chemotherapy)	BIS; cavitating lesions	< 0·5	BALND
7	52	M	AML (induction chemotherapy)	Cavitating lesion on CT scan	< 0·5	BAL negative; no biopsy done

8	26	F	AML (induction chemotherapy)	Cavitating lesion on X-ray	< 0·5	BALND
9	27	M	Hodgkin's disease (ABMT)	RIS-basal	> 1·0	BALND
10	27	M	Hodgkin's disease (ABMT)	BIS. R pleural effusion	< 0·5	BALND
11	28	F	Hodgkin's disease (ABMT)	BIS-diffuse	< 0·5	BALND
12	60	F	AML/MDS (induction chemotherapy)	BIS	< 0·5	BALND
13	52	F	AML (ABMT)	BIS	< 0·5	BAL negative
14[a]	45	M	NHL (chemotherapy)	BIS	0·5–1·0	BALND
15	27	F	NHL (ABMT)	BIS	< 0·5	BALND
16	16	M	AML (consolidation chemotherapy)	BIS	< 0·5	BALND
17	16	M	ALL (induction chemotherapy)	BIS	< 0·5	BALND; BAL negative
18	31	M	CGL (MUD)	BIS	1·0–2·0	BALND
19	35	F	AML (induction chemotherapy)	PUO; abnormal LFTs	0·5–2·0	liver biopsy negative; small abcess on CT scan
20	7	M	AML (consolidation chemotherapy)	PUO; abnormal LFTs	< 0·5	liver abscesses on ultra-sound

[a]Received anti-tuberculous treatment in addition; ABMT, autologous bone marrow transplant; ALG, given anti-lymphocyte globulin; ALL, acute lymphoblastic leukaemia; AML, acute myeloid leukaemia; BAL, broncho-alveolar lavage; BALND, broncho-alveolar lavage not carried out because of clinical condition and low platelet count; BIS, bilateral interstitial shadowing on chest X-ray; CGL, chronic granulocytic leukaemia; CT, computerized tomography; LFTs, liver function tests; MDS, myelodysplasia; NHL, non-Hodgkin's lymphoma; PUO, pyrexia of undetermined origin; RIS, right-side interstitial shadowing on chest X-ray.

Table II. Results of treatment

Patient no	Total dose of and duration of treatment with conventional amphotericin	Reasons for commencing liposomal amphotericin	Total dose and duration of treatment with liposomal amphotericin	Neutrophil count ($\times 10^9$/L) at the time of resolution of fungal infection or death	Status of underlying diseases	Outcome and summary of the effect of liposomal amphotericin
Patients with proven fungal infection						
1	430 mg, 9 days	progression of chest signs	730 mg, 10 days	1·0	remission	resolution of pneumonitis–success
2	260 mg, 5 days	progression of chest signs	760 mg, 9 days	0·4	remission	resolution of pneumonitis–success
3	750 mg, 15 days	progression of chest signs	800 mg, 7 days	0·6	aplastic anaemia	resolution of pneumonitis–success
4	1000 mg, 20 days	progression of chest signs	500 mg, 5 days	0·2	not evaluable	died from respiratory failure–failure
5	1170 mg, 30 days	progression of chest signs; polyuria	660 mg, 5 days	0·4	active disease	died from sepsis and AML–failure
Patients with suspected fungal infection						
6		pre-existing renal impairment	4290 mg, 56 days	4·5	remission	resolution of one cavitating lesion–success
7	670 mg, 12 days	renal impairment	1000 mg, 11 days	0·7	remission	resolution of pneumonitis and cavitating lesion–success
8	405 mg, 14 days	renal impairment	700 mg, 7 days	2·7	remission	resolution of pneumonitis–success
9	995 mg, 23 days	out-patient treatment	400 mg, 4 days	1·9	not evaluable	resolution of pneumonitis–success

10	610 mg, 25 days	renal impairment	220 mg, 7 days	0·4	not evaluable	died from renal failure, respiratory failure and sepsis–failure
11	900 mg, 15 days	progression of chest signs	500 mg, 8 days	7·0	active disease	died from respiratory failure–failure
12	360 mg, 6 days	renal impairment	540 mg, 9 days	0·3	active disease	died from respiratory failure–failure
13	330 mg, 5 days	progression of chest signs	2750 mg, 41 days	0·2	remission	complete resolution–success
14	400 mg, 6 days	progression of chest signs	300 mg, 4 days	0·25	active disease	died from respiratory failure and progressive disease–failure
15	600 mg, 6 days	renal impairment	500 mg, 5 days	0·2	remission	died from respiratory failure–failure
16	600 mg, 12 days	progression of chest signs	60 mg, 1 day	0·2	not evaluable	died from *Pseudomonas* sepsis–failure
17[a]	530 mg, 8 days	progression of chest signs	1180 mg, 8 days	0·1	not evaluable	died from respiratory failure–failure
18[b]	610 mg, 17 days	renal impairment	500 mg, 8 days	10·8	remission	died from liver failure–failure
19	750 mg, 5 days	no resolution of fever with conventional amphotericin	4290 mg, 96 days	10·0	remission	resolution of fever and liver abscesses, disease in remission–success
20	350 mg, 4 days	renal impairment	300 mg, 8 days	3·4	remission	resolution of fever and liver abscesses in remission–success

[a]Disease state not evaluable.
[b]Aspergillus diagnosed post-mortem; AML, acute myeloid leukaemia.

Table III. Response to liposomal amphotericin in all patients

	Resolution	No resolution
Number	10 (50%)	10 (50%)
Median time on conventional amphotericin (days)	10 (0–23)	14 (6–30)
Total dose of conventional amphotericin (mg)	418 (0–995)	605 (360–1170)
Median neutrophil count ($\times 10^9$/L)	1·4 (0·2–10·0)	0·3 (0·1–10·8)
Median time on liposomal amphotericin (days)	10 (4–96)	6 (1–9)
Total dose of liposomal amphotericin (mg)	760 (300–7900)	500 (30–1180)

with aplastic anaemia) had non-responsive disease when response was assessed and all the responsive patients survived the neutropenic phase. In contrast, ten patients showed no response to liposomal amphotericin and all died. The cause of death was respiratory failure due to interstitial pneumonitis in eight patients, pseudomonas septicaemia in one patient and hepatic failure due to graft versus host disease in one patient. In the non-responsive group, three of ten patients had active haematological disease at the time of death (acute myeloid leukaemia, two patients; non-Hodgkin's lymphoma, one patient). Two patients were in remission (chronic granulocytic leukaemia, one patient; non-Hodgkin's lymphoma, one patient). Five patients were not evaluable for their disease status at death (acute myeloid leukaemia, two patients; Hodgkin's disease, two patients; acute lymphoblastic leukaemia, one patient). Disease status was not evaluable in some patients owing to lack of marrow recovery in the patients with leukaemia and refusal of post-mortem in the patients with Hodgkin's disease.

Patients with mycologically proven infection (Table IV)

Of the five patients with fungal infection mycologically proven during life, three (60%) showed complete resolution of their fungal infection (assessed clinically). The median neutrophil count (at the time of clinical resolution of the fungal infection) in this group was $0{\cdot}6 \times 10^9$/L (range 0·4–1·0) and the median total dose of liposomal amphotericin received was 760 mg (range 730–800). Two of the three patients were in remission from their underlying disease but one patient still had aplastic anaemia. In the two patients (40%) showing no resolution, the neutrophil counts (at the time of death) were 0·2 and $0{\cdot}4 \times 10^9$/L respectively, and the total doses of liposomal amphotericin received were 600 and 660 mg, respectively. A further two patients were shown to have disseminated aspergillosis at post mortem. These patients (nos 17 and 18) died from respiratory failure due to interstitial pneumonitis and liver failure due to acute graft-versus-host

Table IV. Response to liposomal amphotericin in patients with mycologically proven fungal infection

	Resolution	No resolution
Number	3 (60%)	2 (40%)
Median neutrophil count ($\times 10^9$/L)	0·6 (0·4–1·1)	0·3 (0.2–0.4)
Total dose of liposomal amphotericin	750 mg (730–760)	630 mg (600–660)

Table V. Response to liposomal amphotericin in patients showing no response to previous conventional amphotericin therapy

	Resolution	No resolution
Number	5 (45%)	6 (55%)
Median neutrophil count ($\times 10^9$/L)	0·6 (0·2–10·0)	0·2 (0·1–7·0)
Total dose of liposomal amphotericin	800 mg (730–7900)	550 mg (30–1180)

disease. Thus, seven patients had documented aspergillosis and three of seven (43%) showed resolution of their fungal infection.

Patients showing no response to previous conventional amphotericin B therapy (Table V)

Of the 11 patients who received liposomal amphotericin as a result of failure of conventional amphotericin B therapy, five (45%) showed a complete response after changing to liposomal amphotericin. The median neutrophil count in this group of patients was $0{\cdot}6 \times 10^9$/L (range 0·2–10·0) at the time of resolution of the fungal infection; indeed, the neutrophil count had recovered (i.e. was $>0{\cdot}5 \times 10^9$/L) in three patients. The median total dose of liposomal amphotericin received was 800 mg (range 730–7900). Of the five patients showing a response to liposomal amphotericin after failing on conventional amphotericin B, four were in disease remission and one patient (no. 3) had aplastic anaemia with a neutrophil count of $0{\cdot}6 \times 10^9$/L. Six patients (55%) showed no response to salvage treatment with liposomal amphotericin. The median neutrophil count in this group was $0{\cdot}2 \times 10^9$/L (range 0·1–7·0). Only one patient showed neutrophil recovery. Two patients had active underlying haematological disease at the time of death but four patients were not evaluable for disease status for the reasons explained in the previous section. The total dose of liposomal amphotericin received in the non-responsive group was 550 mg (range 30–1180).

Toxicity (Table VI)

There were no acute fevers, chills or rigors in the patients receiving liposomal amphotericin. Furthermore, there was no impairment in liver function attributable to

Table VI. Response to liposomal amphotericin in patients with renal toxicity during previous conventional amphotericin therapy

	Resolution	No resolution
Number	4 (50%)	4 (50%)
Median creatinine before liposomal amphotericin	165 μmol/L (177–224)	175 μmol/L (112–224)
Median creatinine during liposomal amphotericin	141 μmol/L (121–169)	200 μmol/L (195–265)
Total dose of liposomal amphotericin	850 mg (300–4290)	520 mg (226–540)

liposomal amphotericin. One patient received liposomal amphotericin via a peripheral cannula without thrombophlebitis.

Eight patients received liposomal amphotericin because of either pre-existing renal impairment before amphotericin B treatment (one patient) or renal and electrolyte disturbances which developed during treatment with conventional amphotericin B (seven patients). Four of the eight patients receiving liposomal amphotericin showed a complete resolution of their fungal infection, whereas the other four patients all died: three from progressive interstitial pneumonitis leading to respiratory failure, and one from liver failure due to graft-versus-host disease. Two patients had active haematological disease at the time of death, one patient was in remission and one patient was not evaluable. In the patients who did show resolution, the median creatinine before administration of liposomal amphotericin was 165 μmol/L (range 117–224) and during the treatment the median was 141 μmol/L (range 121–169). The median dose of liposomal amphotericin received was 850 mg (range 300–4290). In the patients showing no response, the median creatinine was 175 μmol/L (range 112–224) before treatment and 200 μmol/L (range 195–265) during treatment. The median dose of liposomal amphotericin received was 520 mg (range 226–540). No patient suffered clinically significant renal failure whilst receiving liposomal amphotericin.

Discussion

The difficulty in arriving at a definitive diagnosis of aspergillosis is highlighted by this study, in which only five of 20 patients (25%) had a mycologically proven fungal infection, but all required treatment with amphotericin. The use of invasive procedures like transbronchial biopsy (Stover *et al.*, 1984) or open lung biopsy in invasive pulmonary aspergillosis may not be justified in our group of patients since these carry a high morbidity, especially in patients who have received extensive treatment so that there is a potential for life-threatening septicaemia and haemorrhage. The recently described ELISA technique for detecting aspergillus antigen in urine or blood reported by Rogers, Haynes & Barnes (1990) may be a more promising and less invasive approach.

The fact that 18 of 37 patients (who originally had a suspected fungal pneumonitis) proceeded to receive liposomal amphotericin highlights the difficulties associated with the use of conventional amphotericin B. The response of five of 11 patients to salvage treatment with liposomal amphotericin, after the failure of conventional amphotericin B, is an encouraging result. Furthermore the response to liposomal amphotericin in three of five patients (60%) with proven aspergillosis compares favourably with a greater than 90% mortality reported in neutropenic patients (Denning & Stevens, 1990). However, in the whole group of 20 patients, half the patients still did not respond to liposomal amphotericin. This group of patients had a longer period of treatment with conventional amphotericin B and had a lower median neutrophil count. It may be that these patients received salvage liposomal amphotericin too late or that they had a more serious underlying illness with slower neutrophil regeneration. Indeed the neutrophil count in the group responsive to liposomal amphotericin was higher (1·7 *vs* $0{\cdot}3 \times 10^9$/L) and the majority of the patients were in disease remission (eight patients as against two patients). The relative roles of the drug, neutrophil regeneration and disease status are difficult to define in such a retrospective analysis but it is clear that the latter two variables make an important contribution in the eradication of fungal

infection from neutropenic patients. Overall, of the ten patients showing a response to liposomal amphotericin B, eight had recovered their neutrophil count ($> 0{\cdot}5 \times 10^9$/L) and eight had achieved remission from their underlying disease. Similar findings have been reported by Fisher *et al.* (1981) who observed that recovery, in patients with leukaemia and invasive aspergillosis, after treatment with conventional amphotericin B, was associated with complete remission of the leukaemia and recovery of the neutrophil counts. Response to liposomal amphotericin in our selected group of patients who had 'failed' conventional amphotericin B treatment was optimal in those patients who had shown neutrophil recovery or recovery from their underlying condition. Thus it appears that liposomal amphotericin will further salvage a group of neutropenic patients who fail conventional amphotericin B providing the neutrophil count is recovering and the underlying disease is in remission.

The successful use of liposomal amphotericin in the patients with renal impairment highlights the experience of other studies with this or similar preparations (Lopez-Berestein *et al.*, 1985; Tollemar, Ringdén & Tydén, 1990). Indeed in our experience the drug was well tolerated with few side effects.

Although our experience of the use of liposomal amphotericin (AmBisome) is encouraging, prospective randomized trials comparing this preparation with conventional amphotericin B are required to assess both the efficacy and the cost-benefit effect of this new drug. However such trials may be difficult to implement, especially if definitive evidence of fungal infection is required before treatment.

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