British Journal of Haematology, 1997, **98**, 711–718

A randomized comparison of liposomal versus conventional amphotericin B for the treatment of pyrexia of unknown origin in neutropenic patients

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Received 27 January 1997; accepted for publication 15 May 1997

Summary. One hundred and thirty-four adults and 204 children were randomized in two prospective, parallel comparative multicentre trials to receive either conventional amphotericin B 1 mg/kg/d (c-AMB), liposomal amphotericin B 1 mg/kg/d (L-AMB1) or liposomal amphotericin B 3 mg/kg/d (L-AMB3). Patients were entered if they had a pyrexia of unknown origin (PUO) defined as temperature of 38°C or more, not responding to 96 h of systemic broad-spectrum antibiotic treatment, and neutropenia ($< 0{\cdot}5 \times 10^9$/l). The safety and toxicity of liposomal amphotericin B was compared with that of conventional amphotericin B. Efficacy of treatment was assessed, with success defined as resolution of fever for 3 consecutive days (< 38°C) without the development of any new fungal infection. Clinical and laboratory parameters were collected for safety analysis. In both the paediatric and adult populations, L-AMB treated patients had a 2–6-fold decrease in the incidence ($P \leqslant 0{\cdot}01$) of test-drug-related side-effects, compared to c-AMB. Severe trial-drug-related side-effects were seen in 1% of L-AMB treated patients, in contrast to 12% of patients on c-AMB ($P < 0{\cdot}01$). Nephrotoxicity, in the patient subset not receiving concomitant nephrotoxic agents, defined as a doubling from the patients baseline serum creatinine level, was not observed in the L-AMB1 arm whereas the incidence was 3% in patients on L-AMB3 and 23% in those on c-AMB ($P < 0{\cdot}01$). Moreover, time to develop nephrotoxicity was longer in both L-AMB arms than c-AMB ($P < 0{\cdot}01$). Severe hypokalaemia was observed less frequently in both L-AMB arms ($P < 0{\cdot}01$).

Analysis was by intention-to-treat and included all patients randomized. Success was defined by a minimum of 3 consecutive days with fever (< 38°C) continuing to study end indicated by recovery of neutrophils to $0{\cdot}5 \times 10^9$/l. Addition of systemic antifungal therapy or development of systemic fungal infection were failures as was persistent fever to study end. Efficacy assessments indicated success in 49% of the total group treated with c-AMB. 58% of patients responded to L-AMB1 and 64% to L-AMB3. A statistically significant difference was found between c-AMB and L-AMB3 ($P = 0{\cdot}03$) but a Kaplan-Meier analysis of time to defervescence of fever showed there was no significant difference between the arms.

It was concluded that liposomal amphotericin at either 1 or 3 mg/kg/d was significantly safer than conventional amphotericin B in children and adults. The main aim of this open-label study was to compare safety between the three trial arms. However, we provide evidence for an equivalent or possibly superior efficacy of liposomal amphotericin with regard to resolution of fever of unknown origin. Subsequent trials should compare amphotericin preparations in defined fungal infections.

Keywords: neutropenia, PUO, fungal infection, amphotericin B, AmBisome.

Invasive fungal disease remains one of the major causes of morbidity and mortality in the immunocompromised host (Pizzo *et al*, 1982). Diagnosis is notoriously difficult and often based upon the exclusion of bacterial and viral infection. Only a small proportion of patients treated with antimycotic agents have documented fungal infection which can be mycologically demonstrated by either positive histology or

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microbiological culture. The definitive diagnosis of fungal disease is usually made at autopsy. Therefore antifungal treatment, especially in the immunocompromised host, is most often started at the first clinically suggestive sign of invasive mycosis (EORTC, 1989). This might include some combination of antibiotic unresponsive fever, unexplained or suggestive pulmonary infiltrates, fungal colonization of the gastrointestinal tract or central venous catheter line, or a positive fungal antigen test. Since under such circumstances the treatment is empirical, it is essential that a broad-spectrum antimycotic agent is applied. Ideally this should be done using an algorithm such as that proposed by Fraser & Denning (1993). Until now, intravenous amphotericin B has been the only available broad-spectrum antimycotic agent. It has proven activity against most *Candida* and *Aspergillus* species, the most prevalent fungal pathogens in the neutropenic population in Europe. However, the use of amphotericin B is hampered by its safety profile, with infusion-related side-effects and nephrotoxicity, limiting its use (Goodwin *et al*, 1995).

Several new formulations of amphotericin B have become available to address some of these drug-related toxicities. One such is AmBisome® (Nexstar Pharmaceuticals, Boulder, Colorado, U.S.A.), which is a liposomal formulation of amphotericin B in which the drug is incorporated in the membrane of small (< 100 nm) unilamellar vesicles (SUVs). These vesicles are composed of hydrogenated soy phosphatidyl choline, cholesterol and distearoyl phosphatidyl glycerol (Adler-Moore & Profitt, 1993). The pharmacokinetic behaviour of this product has been well described (Janknegt *et al*, 1992). It is characterized by its small volume of distribution of about 10 litres, high peak serum levels, and its increased area under the time versus serum concentration curve (AUC) in contrast to the native drug.

In vitro experiments show that the liposomal structure adheres to the fungal cell wall, after which it decomposes and releases amphotericin B (Adler-Moore, 1994). If such a mechanism were reproduced *in vivo* it would lead to a higher concentration of the drug at the site of infection with limited release in those uninfected organs where it might otherwise cause toxicity.

Another explanation for the limited toxicity of the bound drug can be found in the stability of the liposome in the circulation. The fungicidal capacity of amphotericin B is based on its affinity for ergosterol in the fungal cell membrane, but it will also bind cholesterol which is the major lipid in the normal mammalian cell membrane. The low plasma levels of free amphotericin B when administered as AmBisome® would be expected to significantly reduce the normally observed side-effect pattern of the conventional formulation. This has been extensively documented in animal experiments (Profitt *et al*, 1991). The LD50 of conventional amphotericin B in rodents is approximately 2 mg/kg. The LD50 for AmBisome®, in these models, is > 100 mg/kg. These results have been confirmed in several smaller phase II and III clinical trials (de Marie *et al*, 1994; Meunier *et al*, 1991).

We now report the results of two prospective, open label, randomized, multicentre trials, comparing conventional

Table I. Baseline demographics.

	c-AMB	L-AMB1	L-AMB3
No. of patients			
Adults	39	48	47
Children	63	70	71
Age (year) (median)			
Adults	45	43	42
Children	7	7	7
Gender male			
Adults	26	27	31
Children	39	36	40
Caucasian			
Adults	33	38	38
Children	53	56	58
Weight (kg) (median)			
Adults	69	73	72
Children	26	26	27
Height (cm) (median)			
Adults	172	169	171
Children	121	120	118
ANC $< 0{\cdot}5 \times 10^9$/l			
Adults	38	44	46
Children	61	70	71
ANC $0{\cdot}5–1{\cdot}0 \times 10^9$/l			
Adults	1	2	1
Children	2	0	0
ANC $> 1 \times 10^9$/l			
Adults	0	2	0
Children	0	0	0
T $\geqslant$ 38°C			
Adults	39	46	45
Children	60	67	71
Acute lymphoblastic leukaemia			
Adults	7	9	14
Children	31	33	34
Acute myeloblastic leukaemia			
Adults	13	12	9
Children	13	14	9
Lymphoma			
Adults	5	4	8
Children	3	6	6
Others			
Adults	14	23	16
Children	16	17	22
At entry fungal colonization			
Adults	8	11	5
Children	4	5	6
At entry confirmed mycosis			
Adults	0	3	2
Children	0	0	0

ANC, absolute neutrophil count; c-AMB, conventional amphotericin B; L-AMB, liposomal amphotericin B.

Table II. Prior and concomitant medication (number of patients).

	Pre study			During study*		
Class of drug	c-AMB	L-AMB1	L-AMB3	c-AMB	L-AMB1	L-AMB3
Teicoplanin/vancomycin	78	80	82	87	86	75
Aminoglycosides	66	76	67	60	68	69
Quinolones	44	47	45	47	48	46
Cephalosporins, third generation	60	53	61	60	68	66
Penicillins (other)	31	31	27	26	21	25
Piperacillin	32	47	36	26	36	30
Colistin	10	10	13	9	10	13
Beta lactam combinations	12	10	11	15	22	24
Metronidazole	17	22	25	29	29	35
Sulphonamides	28	29	20	27	24	24
Other macrolides	10	5	2	16	13	8
Cephalosporins, second generation	4	5	6	4	2	7
Monobactam	4	2	2	6	4	2
Cephalosporins, first generation	1	2	0	0	0	1
Antivirals	37	41	47	36	39	47
Systemic antifungals†	32	29	36	2	4	1
Non-systemic antifungals	66	75	52	48	51	40

*Drugs started either before and continued or started during trial therapy.
†Other than the study drugs.

amphotericin B, 1 mg/kg/d (c-AMB) with liposomal amphotericin B, 1 or 3 mg/kg/d (L-AMB1, L-AMB3), for the empirical treatment of pyrexia of unknown origin (PUO) in neutropenic adults and children. This is the first large randomized study reported in this clinical setting.

PATIENTS AND METHODS

Patients

Neutropenic patients ($< 0{\cdot}5 \times 10^9$/l) were eligible for randomization in the study following 96 h of fever, defined as a temperature $\geqslant 38°C$, not responding to broad-spectrum antibacterial therapy. The study was performed according to the principles of 'Good Clinical Practice', under a UK Clinical Trials Exemption Certificate (CTX) (038130002) and ethics committee approvals were obtained at each participating centre. Informed consent was obtained from every subject or legal representative prior to inclusion. 19 European centres participated in these trials and randomly assigned 204 paediatric and 134 adult patients. There were 102 c-AMB1 (1 mg/kg/d), 118 L-AMB1 (1 mg/kg/d) and 118 L-AMB3 (3 mg/kg/d) patients (total 338). 10 adult patients entered the study with missing or non-verifiable enrolment criteria. 10 adults and six children presented with either no fever or no neutropenia at study entry. Confirmed mycosis was present in four adults at study entry. In the L-AMB1 group one patient had an *Aspergillus* infection defined by X-ray and positive culture on bronchoscopy. Two patients on L-AMB1 had multiple positive blood cultures for *Candida* species. One patient on L-AMB3 had fever and *Candida* species isolated from stool, pharynx, mouth and midstream urine samples. Four adults and 11 children continued concomitant systemic antifungal treatment after enrolment. Most patients had profound neutropenia and presented with underlying haematological malignancies.

Baseline demographic features did not reveal statistically significant differences, and are listed in Table I. Antifungal prophylaxis with itraconazole, fluconazole and/or an oral polyene was allowed until the day of enrolment. Antibacterial treatment before and during study was at the discretion of the physician. Other non-antimicrobial therapy was added as indicated. Table II summarizes the relevant anti-bacterial, anti-viral and anti-fungal therapies administered before and during the study. There were no differences observed in the number of patients receiving antibiotic or antiviral therapy before or during study amongst the treatment arms.

Methods

The combined results of two open-label randomized multicentre trials are reported. Study 104-10 was conducted in adult patients and study 104-14 was done in children. Inclusion and exclusion criteria were identical. The only difference in the conduct of the trials was that adult patients who developed severe and irreversible renal dysfunction on c-AMB were allowed to switch to L-AMB1. This treatment switch was not permitted in the paediatric protocol. For clarity, all tables and figures differentiate between the results in adults and those obtained in children. In addition, where appropriate, the results for the combined groups are presented. The primary end point of this trial was safety, and sample size calculations were based on an assumed difference in serious toxicity of 15% in

favour of both L-AMB arms (one-sided, alpha = 0·05, power = 80%). Fisher exact and chi-square tests were used to compare discrete variables. One-way ANOVA analysis was applied to compare quantitative data between the three study arms. In the case of differences, the Bonferroni correction was used to compare the groups two-by-two. Time-to-event data were analysed by the Kaplan-Meier analysis and differences were tested with the log-rank test. All statistical data analysis was performed using SAS Stat® statistical software.

Efficacy, the secondary endpoint, was assessed according to the following definitions. Response was defined by a minimum of 3 consecutive days without fever (< 38°C) which continued until study end, indicated by recovery of neutrophils to $0{\cdot}5\times10^9$/l. The addition of another systemically active antifungal medication during study was considered a failure. Patients who developed a systemic fungal infection on study were equally considered as failures. Patients who remained febrile at study end were also classified as treatment failures. Efficacy assessments were made on the originally assigned treatment arms. In the case of a treatment switch, for reason of toxicity, response could be assigned only if the response criteria were fulfilled before the treatment switch. The study end was defined as resolution of fever (< 38°C), recovery of neutrophils to above or equal to $0{\cdot}5\times10^9$/l for 3 consecutive days, patient death, unresolved toxicity, patient or physician request to withdraw.

L-AMB (AmBisome®, NeXstar Pharmaceuticals Inc., San Dimas, Calif., U.S.A.), was provided free of charge. C-AMB (Fungizone®, Bristol-Myers Squib), was commercially obtained. The reconstitution and administration of both study drugs was performed according to the instructions of the respective manufacturer's recommendations. A gradual dose increase of c-AMB was allowed over 2–3 d until a total planned daily dose of 1 mg/kg was reached. Test doses of c-AMB were allowed. L-AMB treatment was started immediately at the randomized study dose, without prior test doses. There were no restrictions on the use of premedications such as antihistamines, steroids, pethidine or paracetamol. Dose reduction for nephrotoxicity was allowed to 50% of the prescribed daily dose. After recovery of renal function, the dose could be restored stepwise to the originally assigned level.

Randomization was done centrally and each participating centre was provided with a set of blinded, numbered envelopes which required sequential opening. The arms were balanced per centre and per block of six patients.

Safety assessment focused on the number and severity of reported adverse reactions, and on the required blood chemistry and haematology laboratory assessments. All patients receiving study medication were evaluated for safety ($n = 338$). Patients were followed until resolution of fever for at least 3 consecutive days and recovery of neutrophil count to $> 0{\cdot}5\times10^9$/l.

Analysis for efficacy was carried out on an intention-to-treat (ITT) basis. This analysis included 133 adults and 202 children in whom efficacy parameters were collected ($n = 335$).

RESULTS

Safety results

Sixty-four percent of patients treated with c-AMB experienced side-effects (Table III). In contrast, 36% and 43% of subjects experienced side-effects on L-AMB1 and L-AMB3 respectively ($P < 0{\cdot}01$). Severe and test-drug-related side-effects occurred in 11 of the c-AMB-treated patients; nephrotoxicity in seven, rigors, fever and skin rash in two, and one each of hypokalaemia and dyspnoea. Only three patients in the L-AMB arms developed severe and related adverse reactions ($P < 0{\cdot}01$). These were on L-AMB1 one encephalopathy and on L-AMB3 convulsions in one and hypokalaemia in one. Analysis of all subsets in both the adult and paediatric patient populations showed a statistically significant difference in favour of L-AMB. The most frequently reported side-effects were: first-dose reactions of fever and rigors, hypokalaemia and renal toxicity. 27 allergic reactions were seen in 1146 administered doses of c-AMB; 12 of these reactions were considered by the reporting physician to be severe or life-threatening. In the L-AMB1 arm, 11 reactions were seen over 1669 doses, one of which was classified as severe. 14 instances were observed out of 1762 doses of L-AMB3; two were severe ($P < 0{\cdot}01$). The most prevalent symptoms during these allergic reactions were: rash, flushing, bronchospasm, facial oedema, rigors and back pain.

Table III. Incidence of clinical adverse events (percentage of patients).

	c-AMB	L-AMB1	L-AMB3	*P*
Adverse events (% of patients)				
Adults	79	46	48	< 0·01
Children	54	29	39	0·01
Total	64	36	43	< 0·01
Related adverse events (% of patients)				
Adults	44	6	11	< 0·01
Children	36	6	17	< 0·01
Total	39	6	14	< 0·01
Severe and related adverse events (% of patients)				
Adults	16	0	0	< 0·01
Children	8	1	1	0·06
Total	12	1	1	< 0·01
Allergic reactions (% of total no. of doses)				
Adults	2	0·3	0·4	< 0·01
Children	2	1	1	< 0·01
Total	2	0·6	0·8	< 0·01

Dose reduction or study drug discontinuation occurred on 35 occasions in the c-AMB arm, nine and six of such events were observed in the L-AMB1 and L-AMB3 arms respectively ($P < 0{\cdot}01$). The mean daily received doses were c-AMB $0{\cdot}77 \pm 0{\cdot}22$ in the children, and $0{\cdot}78 \pm 0{\cdot}16$ in adults mg/kg/d. For L-AMB1 these were $1{\cdot}05 \pm 0{\cdot}31$ and $1{\cdot}03 \pm 0{\cdot}15$ and for L-AMB3 $2{\cdot}95 \pm 0{\cdot}17$ and $2{\cdot}95 \pm 0{\cdot}35$ mg/kg/d respectively. Biochemical safety analysis, based on the

Table IV. Incidence of blood chemistry abnormalities (percentage of patients).

	c-AMB	L-AMB1	L-AMB3	*P*
Alkaline phosphatase (⩾ 1000 IU/l)				
% of patients				
Adults	5	0	0	0·09
Children	0	0	2	0·44
Total	3	0	1	0·29
Bilirubin (⩾ 35 μmol/l)				
% of patients				
Adults	29	12	15	0·12
Children	10	11	12	0·95
Total	20	11	14	0·31
Transaminases (⩾ 110 IU/l)				
% of patients				
Adults	18	7	21	0·18
Children	17	17	23	0·80
Total	18	11	22	0·23
Potassium (< 2·5 mmol/l)				
% of patients				
Adults	38	18	21	0·07
Children	26	10	11	0·02
Total	31	13	15	< 0·01
Sodium (⩾ 150 mmol/l)				
% of patients				
Adults	18	7	15	0·27
Children	3	2	2	0·72
Total	9	4	7	0·27
Sodium (< 130 mmol/l)				
% of patients				
Adults	18	20	17	0·93
Children	3	6	9	0·42
Total	9	12	13	0·72

objective laboratory measurements (Table IV), revealed no statistically significant differences in the prevalence of hyper- (⩾ 150 mmol/l) or hypo-natraemia (< 135 mmol/l). There was a significant reduction in hypokalaemia in the children ($P = 0·02$) and the total group ($P < 0·01$), despite clinician freedom to prescribe potassium supplements and to give potassium-sparing diuretics. Potassium supplements were used in 44 patients receiving c-AMB, 31 on L-AMB1, and 37 on L-AMB3. Potassium-sparing diuretics were used in 32 patients on c-AMB, nine on L-AMB1, and 20 on L-AMB3.

Significantly more potassium supplements were used with c-AMB than with L-AMB1 ($P = 0·0009$) but the reduction in use with L-AMB3 compared with c-AMB is marginal ($P = 0·07$). Overall, more potassium supplements were used with c-AMB than with the liposomal product ($P = 0·01$). Similarly, potassium-sparing diuretics were more often used with c-AMB than with L-AMB1 ($P = 0·001$) and compared with L-AMB3, but the latter was not significant ($P = 0·08$). Overall there was significantly less usage of potassium-sparing diuretics with the liposomal compounds ($P = 0·001$). Liver function assessment, documented by elevated bilirubin, alkaline phosphatase and transaminase serum levels, did not show any differences amongst the treatment arms.

Table V. Incidence of nephrotoxicity with and without concurrent other nephrotoxic drugs (percentage of patients).

	c-AMB	L-AMB1	L-AMB3	*P*
All patients (%) ($n = 305$)				
Adults	31	12	13	0·05
Children	21	8	11	0·10
Total	24	10	12	< 0·01
With concomitant nephrotoxic drugs (%) ($n = 224$)				
Adults	32	18	16	0·32
Children	22	10	15	0·27
Total	26	13	15	0·11
Without concomitant nephrotoxic drugs (%) ($n = 81$)				
Adults	29	0	7	0·05
Children	17	0	0	0·10
Total	23	0	3	< 0·01

Nephrotoxicity, defined as a 100% or more increase in baseline serum creatinine, was significantly more prevalent in the c-AMB arm and seen in 24% of patients versus 10% and 12% of patients in the L-AMB1 and L-AMB3 arms respectively ($P < 0·01$) (Table V). Fig 1 shows the Kaplan-Meier lifetest analyses of time until nephrotoxicity in the combined adult and paediatric population. A significant difference in the rate of development of renal dysfunction was seen in favour of both L-AMB arms ($P < 0·01$). A subset analysis on the prevalence of renal dysfunction was performed correcting for the concomitant use of other nephrotoxic drugs, that is platinum derivatives, aminoglycosides, vancomycin and cyclosporin A. The results indicate that the incidence of nephrotoxicity in the c-AMB arm was not influenced by the use or absence of concomitant nephrotoxic agents. In contrast, nephrotoxicity was almost absent in the L-AMB arms in patients not receiving other nephrotoxic medication (23% *v* 3%, Table V). Treatment cross-over was allowed for uncontrolled nephrotoxicity in the adult population. We observed eight adult patients who switched from c-AMB to L-AMB1. Seven of these patients had a subsequent decrease or stabilization of their serum

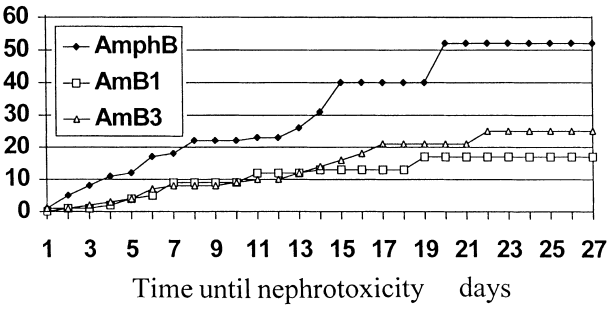

Fig 1. The Kaplan-Meier lifetest analysis of the time until nephrotoxicity in the combined adult and childhood population shows a significantly reduced rate of development of renal dysfunction in favour of both L-AMB arms ($P < 0·01$).

creatinine. One patient had a further mild increase in serum creatinine level. There were no treatment switches observed from L-AMB to c-AMB.

Efficacy

The intention-to-treat analysis (ITT) was performed on all patients in whom follow-up parameters were available. 133 adults and 202 children, a total of 335, could be assessed for this efficacy analysis (temperature charts were unavailable in one adult on L-AMB1 and two children on c-AMB). Confirmed mycoses was present in four adults at entry. One patient on L-AMB1 had an *Aspergillus* species isolated on bronchoscopy and failed treatment. Two on L-AMB1 had positive blood cultures for *Candida*, of whom one responded. *Candida* was grown from several superficial sites plus a midstream urine sample from one patient on L-AMB3 who did respond to treatment. Treatment success was observed in 49 (49%) of patients in the c-AMB arm, 68 (58%) and 75 (64%) of the L-AMB1 and L-AMB3 arms respectively ($P = 0{\cdot}09$). L-AMB3, however, was significantly more efficacious than c-AMB ($P = 0{\cdot}03$). The observed difference between L-AMB1 and c-AMB did not reach statistical significance (Table VI). However, a Kaplan-Meier analysis of time-to-event failed to show a statistically significant difference between the trial arms for time to resolution of fever. The median time until response was 7 d in the c-AMB group, 8 d and 10 d in the L-AMB1 and L-AMB3 arms ($P = 0{\cdot}16$). The reasons for failure were as follows. A systemically active alternative antifungal agent was added to the regimen in two patients on c-AMB, four on L-AMB1 and one on L-AMB3. Fungal infection was the reason for failure in two patients on c-AMB, three on L-AMB1 and one on L-AMB3, the details being given below. All other failures were due to failure to remain afebrile for 3 d until the study end. Two patients on c-AMB (one adult and one child) developed a deep fungal infection with positive blood cultures for *C. albicans* after the start of the study. Two children on L-AMB1 also had positive *Candida* blood cultures. One patient on L-AMB1 and one on L-AMB3 (both children) developed pulmonary aspergillosis (on X-ray and bronchoscopy) 2 and 3 weeks respectively after the last dose of study drug. One child on L-AMB3 had positive blood cultures for *C. albicans.* This child had positive cultures 1 d after the study drug started, and died within a week. Autopsy revealed extensive pulmonary tissue damage due to *Pneumocystis carinii* infection.

Table VI. Efficacy results (number and percentage of patients responding to trial drugs).

Response	c-AMB	L-AMB1	L-AMB3	*P*
Number (%)				
Adults	18/39 (46%)	23/47 (49%)	30/47 (64%)	0·20
Children	31/61 (51%)	45/70 (64%)	45/71 (63%)	0·22
Total	49/100 (49%)	68/117 (58%)	75/118 (64%)	0·09
		$P = 0{\cdot}03$		

Eighteen patients died while on the trial; 15 of these were adults. Disease progression (nine) and disease-related complications (six) were the reported cause in 15 and there were three other events, which were cardiac arrhythmia, ARDS and cardiac failure.

The median time to neutrophil recovery was 6 d for c-AMB, 8 d for L-AMB1 and 7 d for L-AMB3 (n.s.). Success despite continued neutropenia ($< 0{\cdot}5 \times 10^9$/l) was observed in 32 (32%) of patients on c-AMB, 42 (36%) in L-AMB1, and 72 (61%) in the L-AMB3 arm. This difference reached statistical significance between c-AMB and L-AMB3 ($P = 0{\cdot}03$).

DISCUSSION

These are the first prospective randomized trials comparing liposomal to conventional amphotericin B in patients with PUO. They provide confirmation of the superior safety profile of liposomal amphotericin B over its conventional formulation in both adult and paediatric patients. Moreover, this is the first prospective randomized clinical trial in neutropenic children with antibiotic unresponsive PUO. Since the study design was almost identical it was decided to present the results of the two patient populations both separately and combined.

The overall drug-related toxicity at equivalent doses is 2–6-fold decreased, for the AmBisome arms compared with the conventional formulation, as demonstrated by the number of reported drug-related adverse reactions. The side-effects seen with L-AMB are significantly less severe compared to those observed in the amphotericin B arm. Severe drug-related adverse reactions were almost absent in patients treated with L-AMB. The observed incidence of L-AMB-related toxicity seems dose dependant. However, the incidence and severity of adverse reactions noted at the 3 mg/kg/d dose still remains well below that observed for the conventional formulation. Of the objective parameters measured, nephrotoxicity, defined as a doubling of the serum creatinine level from the patient baseline, is clearly more prevalent in the c-AMB arm. Moreover, the nephrotoxicity observed in the L-AMB arm is likely to be related to the use of concomitant nephrotoxic agents as opposed to the test drug. This was demonstrated by the almost complete absence of renal toxicity, for both L-AMB arms, in the subgroup of patients not receiving concomitant nephrotoxic agents (0% for L-AMB1 and one (3%) for L-AMB3). The incidence of renal toxicity observed in the c-AMB arm was not influenced by the presence or absence of concomitant nephrotoxic drugs. This indicates the dominant toxic potency of c-AMB. The clinical impact of this toxicity profile goes beyond direct patient discomfort and may influence the efficacy of this drug. 29 treatment interruptions for 1 or more days were observed. Dose reductions were seen on nine separate occasions. The mean daily dose of c-AMB, in both the adult and paediatric populations, was 0·76 mg/kg or 24% below the required dose. Patients on L-AMB received the full required dose throughout the study. c-AMB seemed to be less toxic in children. Predisposing factors, such as previous exposure to nephrotoxic risk factors (hypertension, diabetes, environmental), are more likely to have occurred in the adult patient population.

Renal toxicity caused by c-AMB could be reversed in the majority of cases when patients were switched to L-AMB. This additional finding in the adult patients confirms the renal safety of the liposomal drug. Hypokalaemia occurred significantly more frequently in the c-AMB arm than the L-AMB arms. The use of potassium supplements in the three study arms was difficult to assess quantitatively, but significantly more patients on c-AMB received replacement. Similarly, fewer patients received potassium-sparing diuretics with AmBisome and thus the effect on avoidance of severe hypokalaemia with the liposomal product is, if anything, underestimated. The efficacy of L-AMB1 is equal to that of c-AMB in PUO patients. There is, however, a statistically significant difference in favour of L-AMB3 in the intention-to-treat analysis. This difference was confirmed in the second per-protocol analysis which excluded protocol violators. The effectively administered mean dosage of c-AMB (0·76 mg/kg/d) may play a role in this finding. The influence of c-AMB toxicity on the treatment for the primary disease was not addressed in this trial. However, interactions may be present and it may influence the administration of drugs such as cyclosporin A or the platinum derivatives in addition to important antimicrobials such as the aminoglycosides and vancomycin. The survival analysis in this study is limited to the mean study duration of 30 d and thus no differences could be detected. 88 (87·5%) patients survived on c-AMB, 106 (89·8%) on L-AMB1, and 106 (89·8%) in the L-AMB3 arm ($P = 0{\cdot}90$).

Neutrophil recovery is a major predictor for response in the neutropenic patient. Of major interest are those patients responding to treatment without neutrophil recovery. A Kaplan-Meier analysis for time until neutrophil recovery did not reveal any differences between the study arms (data not shown). Treatment success occurred in 32% of the c-AMB group where no neutrophil recovery to $>0{\cdot}5\times10^9$/l was observed. In this subset success occurred in 36% and 61%, respectively, of L-AMB1 and L-AMB3 treated patients. The difference was statistically significant between c-AMB and L-AMB3 ($P = 0{\cdot}03$).

During recent years a significant amount of effort has been made to develop a safe alternative to amphotericin B (Janknegt *et al*, 1992). The main objective was to provide a drug with a maintained broad spectrum of antifungal activity and reduced toxicity. This safety advantage could then perhaps be used to allow increased dosage with the ultimate goal of improved efficacy. This trial has provided, for the first time, evidence that this aim is within reach. The preliminary evidence for equivalent or possibly superior efficacy of AmBisome, especially in those patients not recovering from neutropenia, combined with the significant differences in several safety parameters, such as the near absence of nephrotoxicity, will allow the exploration of higher doses and use in other indications. From that perspective, a liposomal formulation of amphotericin B is a promising candidate for decreasing fungal-disease-related mortality. Further studies, including comparison with the other lipid-based formulations of amphotericin B are warranted in patients with confirmed mycoses and these studies should include a cost–benefit analysis of these expensive agents. AmBisome, at the lower dose (1 mg/kg/d), is recommended for the treatment of patients with antibiotic unresponsive fever in whom the conventional drug is contraindicated.

ACKNOWLEDGMENTS

Investigators and institutions of the 104-10 study group. Principal investigator: H. G. Prentice, The Royal Free Hospital and School of Medicine, London. Participating investigators and institutions: M. Aoun, Institut Jules Bordet, Brussels; A. J. Bell, Royal Bournemouth Hospital, Bournemouth; J. Ellegaard, Aarhus Amt Hospital, Aarhus; A. A. Fauser, Medizinische Universitatsklinik, Freiburg; R. Waldner, Hanusche Krankenhaus, Vienna; R. Herbrecht, Hospices Civils de Strasbourg, Strasbourg; S. Kvaloy, The Norwegian Institute for Cancer Research, Oslo; D. Catovsky, The Royal Marsden Hospital, London; S. A. Schey, Guy's Hospital, London; J. P. Thys, Hopital Erasme, Brussels; J-M. Tangen, Ulleval Hospital, Oslo; J. Bergheim, Haukeland Hospital, Bergen.

Investigators and institutions of the 104-14 study group. Principal investigator: I. M. Hann, Great Ormond Street Hospital for Children, London. Participating investigators and institutions: A. Oakhill, A. Foot, Bristol Royal Hospital for Sick Children, Bristol; R. F. Stevens, Royal Manchester Children's Hospital, Pendlebury; P. J. Darbyshire, The Children's Hospital, Birmingham; B. E. S. Gibson, Royal Hospital for Sick Children, Glasgow; C. R. Pinkerton, The Royal Marsden Hospital, Sutton Surrey.

These trials were made possible through an educational grant and free AmBisome supplies, by Nexstar Inc., Boulder, Col., U.S.A.

The authors express their sincere appreciation to the physicians and the nursing staff of all participating centres. Our special thanks to David Denning and Chris Kibbler for their inspiring support in the study analysis.

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