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Pharmacokinetics of liposomal amphotericin B in neutropenic cancer patients

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Abstract

This study was conducted to characterise the pharmacokinetics of a liposomal pharmaceutical product of amphotericin B (LAB) in three neutropenic cancer patients complicated by suspected fungal infections. LAB was administered at a constant dose of 50 mg/day over 1–6 h by intravenous infusion, and blood samples were obtained between two infusion intervals without complicating the systemic therapy of the patients. Quantitative analysis of amphotericin B (AB) in plasma was established by a validated reversed-phase high-performance liquid chromatographic (HPLC) assay. Model independent pharmacokinetic parameters were estimated using area and moment analysis. Administration of LAB to the first patient (day 1) diagnosed as malignant melanoma resulted in a mean maximum concentration (C_{max}) of 679 ± 6 ng/ml and a mean minimum concentration (C_{min}) of 139 ± 9 ng/ml of AB. Pre-dose, C_{max} and C_{min} values of AB, after multiple LAB dosing to the other two patients both having acute myeloblastic leukemia were found to be 440 ± 6, 1140 ± 10, 409 ± 11 ng/ml (day 19) and 408 ± 3, 1180 ± 10, and 283 ± 1 ng/ml (day 9), respectively. The area under the plasma concentration–time curve (AUC) and the mean residence time (MRT) calculated between the two infusion intervals were 6180 ng h/ml, 7.79 h (day 1) for the first patient; 13 700 ng·h/ml, 10.5 h (day 19) and 14 000 ng·h/ml, 9.93 h (day 9) for the other two patients, respectively. The pharmacokinetic profiles and non-compartmental parameters calculated were comparable for both patients diagnosed with acute myeloblastic leukemia after multiple dosing at steady state, which also demonstrated a twofold increase in their AUC values compared with the AUC of the first patient. Although C_{min} values supported the assumption that there was AB accumulation in plasma and this accumulation could be increased at high doses, LAB was administered safely to these patients and well tolerated at the doses given.

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1. Introduction

The polyene antibiotic amphotericin B (AB) formulated as a mixed-micellar dispersion with sodium deoxycholate in its conventional dosage form (Fungizone®, Bristol-Myers Squibb, USA) remains the agent with the broadest spectrum of antifungal action and it has been used for the treatment of systemic fungal infections for more than 30 years (de Marie et al., 1994; Brajtburg and Bolard, 1996). However, it is well known that therapeutic doses of Fungizone® often cause serious acute side effects including headache, chills, fever, nausea, vomiting, and hypokalemia. In addition, symptoms of nephrotoxicity, including elevated serum creatinine and urea levels, become apparent upon prolonged administration of this formulation. Consequently, these acute and chronic toxic effects of AB have limited its potential use for systemic therapy (Sculier et al., 1988; Proffitt et al., 1991; Mills et al., 1994; Boswell et al., 1998). During the last ten years several strategies, including chemical modification of AB molecule and changes in delivery systems, have been used with the aim to reduce AB toxicity and to improve its therapeutic effectiveness (Brajtburg and Bolard, 1996). In this respect, novel lipid-based delivery systems of AB, such a liposomal formulation (AmBisome®, NeXtar Pharmaceuticals, USA), a lipid-complex of AB with phospholipids (ABELCET®, Liposome Company Inc, USA) and AB–cholesteryl sulfate complex (Amphocil®, Sequus Pharmaceuticals Inc, USA), have been developed and they are currently in therapeutic use (Collette et al., 1991; Meunier et al., 1991; Wang et al., 1995; Brajtburg and Bolard, 1996; Adedoyin et al., 1997; Bhamra et al., 1997). Preclinical and clinical studies have demonstrated that these lipid-based delivery systems of AB have different pharmacokinetic and pharmacodynamic properties and they are reported to be less toxic than Fungizone® (Kan et al., 1991; Meunier et al., 1991; Proffitt et al., 1991; Wang et al., 1995; Wong-Beringer et al., 1998). All of these formulations have different physicochemical and pharmaceutical properties in terms of their size, structure, shape, lipid composition, and molar AB content (Cellat, 1995; Brajtburg and Bolard, 1996) and these differences have important roles in determining their biological fate (Brajtburg and Bolard, 1996; Cohen, 1998). Although the exact mechanisms in the reduction of AB toxicity are not perfectly defined, the complex-forming behaviour of AB with lipids, the distribution of AB between the lipid in the formulation and lipoproteins, and their tissue distribution, levels in blood, uptake by macrophages and penetration to the site of infection are thought to be responsible in altering disposition characteristics of AB and its toxicity (Lopez-Berestein et al., 1989; Joly et al., 1990; Collette et al., 1991; Ringden et al., 1991; Khutorsky, 1992; Legrand et al., 1992; Brajtburg and Bolard, 1996; Slain, 1999). In a recent study (Kennedy and Wasan, 1999), dealing with the plasma lipoprotein distribution of AB and a lipid-complexed product of AB, it was suggested that the association of AB with dipalmitoyl phosphatidylcholine and dipalmitoyl phosphatidylglycerol to form drug–lipid complexes, and the distribution of drug–lipid complex among plasma lipoproteins of different human subjects should be taken into consideration in evaluating the pharmacokinetics, toxicity and activity of these products. In this respect, there has been an increased research interest in these lipid-based formulations of AB in order to describe the parameters which influence the pharmacokinetics and mechanisms of therapeutic or toxic effects of AB (Brajtburg and Bolard, 1996; Cohen, 1998; Wong-Beringer et al., 1998). On the other hand, comparative clinical studies correlating pharmacokinetics and efficacy of these formulations are also needed, especially for making a decision, in administering the most effective formulation in a sufficient dose with a cost-effective manner, because all of the novel formulations of AB are expensive and their relative efficacy is unknown. Recently, the liposomal AB (LAB) (AmBisome®) has been reported as an appropriate alternative to conventional AB for empirical antifungal therapy in patients with per-

sistent fever and neutropenia, while the therapy with LAB associates less infusion related toxicity and less nephrotoxicity (Walsh et al., 1999). In several other clinical studies, LAB is demonstrated to be safe and well tolerated, and the most frequently reported side effects are hypokalemia, nephrotoxicity and infusion-related reactions, but these occur significantly less often after LAB than after conventional formulation of the drug (Wong-Beringer et al., 1998; Walsh et al., 1999). Due to the limited efficacy data, not only LAB but the other lipid based formulations of AB are suggested to be used as second-line therapy for patients (Wong-Beringer et al., 1998; Slain, 1999). LAB is also in current use in the clinics of our country. It has been widely administered to neutropenic patients having suspected or proven fungal infections at different infusion rates and intervals, but it is mostly used as a second-line therapy for those patients who cannot tolerate or benefit from other antifungal agents. The dose recommended by the manufacturer of AmBisome® has been between 3 and 5 mg/kg/day, whereas this dose was reported to be 3–6 mg/kg/day by intravenous (i.v.) infusion for a mean duration of empirical therapy of 11 days in neutropenic patients (Walsh et al., 1999). In this respect, for a single day therapy at least three vials of the liposomal product should be required and most of the previously published pharmacokinetic studies or clinical evaluations were also conducted at the recommended doses. Although the administration of LAB to patients at high doses for longer periods of time is among recommendations, depending on the disease conditions, doses lower than 3–5 mg/kg/day can also be administered and there has been no pharmacokinetic data available after i.v. infusion of LAB at these doses. In addition to that, the possible accumulation risk of AB after LAB administration at different doses has never been mentioned. In this respect, during clinical applications evaluation of AB pharmacokinetics and determination of its concentrations in biological fluids either for this purpose or for therapeutic monitoring may be essential for optimising the dose and dosing intervals between i.v. infusions. This point is thought to be further relevant in providing a more cost-effective therapy and in preventing the drug accumulation during LAB application at high doses (Cellat 1995; Eldem et al., 1996). Consequently, the aim of the present study was to report the preliminary clinical pharmacokinetic properties of LAB in neutropenic cancer patients who were under treatment with LAB (AmBisome®) due to suspected or proven invasive fungal infections at doses of 50 mg/day by i.v. infusion over 1–6 h.

2. Materials and methods

2.1. Patient characteristics and sample collection

The present study was established according to a protocol approved by the Institutional Review Board of Hacettepe University and informed consent was obtained from each patient prior to initiation of the study. The three neutropenic cancer patients having suspected fungal infections and previously treated with Fungizone® were selected according to the patient selection criteria among patients admitted to the Department of Internal Medicine at Hacettepe University Hospital in Ankara. LAB provided as a lyophilised powder was initially reconstituted with sterile water for injection and further diluted with 5% glucose in water according to the manufacturer's instructions and used immediately. On the day in which blood sampling was performed for pharmacokinetic analysis, the first patient (female, age 53 years) diagnosed as malignant melanoma (MM) was administered the first daily dose of AmBisome® (50 mg) by i.v. infusion over 1 h. The second patient (female, age 30 years) who had acute myeloblastic leukemia (AML), was under antifungal treatment for 18 days and on the day of analysis the next daily dose of AmBisome® (50 mg) was infused over 4 h. The third patient (male, age 40 years) also diagnosed as AML, was initially given Fungizone®, but after 5 days the treatment was changed to Ambisome® due to side effects. Consequently, during the sample collection day, the forth infusion dose over 6 h was administered to this patient, after receiving three previous infusion doses of LAB. Blood samples were obtained between the two infusion intervals

without complicating the systemic therapy of the patients. In this respect, venous blood samples (5 ml) were taken from each of the patients at the time zero immediately before starting the infusion, at the midpoint, at the end of the infusion, and at 0.25, 0.5, 1, 2, 3, 6, 9, 12 h post infusion. The last samples from the first, second and third patient were obtained at 18, 19 and 19.75 h post infusion, respectively, which also corresponded to the beginning of the next infusion doses of the patients. All of the samples were collected into Vacutainer® tubes containing tripotassium EDTA, thoroughly mixed and plasma was separated by centrifugation at $1500 \times g$ for 10 min. The plasma obtained were divided into two fractions and kept frozen at -20°C under light protection till analysis by high-performance liquid chromatography (HPLC).

2.2. Quantitative analysis of plasma amphotericin B by HPLC

Samples were analysed for AB concentrations by a sensitive and specific reversed-phase HPLC assay after sample pre-treatment by a solid phase extraction procedure and detecting UV absorbance at 407 nm. In this respect, a modified HPLC method was developed depending on the previously published procedures (Granich et al., 1986; Gondal et al., 1989; Wang et al., 1992). The modifications and the validation protocol of the assay were described in detail elsewhere (Cellat, 1995; Eldem and Arican-Cellat, in press). Briefly, during the solid phase extraction of plasma samples Sep-Pak columns (C-18, 100 mg, Waters, Millipore, Milford, MA) of 1 ml capacity were used. The columns were first conditioned and 1 ml of the plasma sample was loaded to the column. After washing three times with 3 ml of methanol-phosphate buffer (pH 7.4; 11 mM) (40:60, v/v) and applying vacuum, 1 ml of acetonitrile-disodium EDTA (2.5 mM) (pH 4.2; 60:40, v/v) containing 1-amino-4-nitronaphtalene as an internal standard was added to the extraction column. The elution of AB was established together with the internal standard at this final step and 100 μl of the extract was used in the HPLC analysis. The chromatographic separation was performed on a 5 μm Bondapak C-18 reversed-phase column (300×4.6 mm i.d.) (Waters) in less than 10 min by using acetonitrile-disodium EDTA (20 mM) (45:55, v/v) at pH 5.0 as mobile phase. A Shimadzu (Kyoto, Japan) HPLC system consisting of a Model LC-6A pump, a Model SPD-6AV U.V.-visible spectrophotometric detector and a Chromatopac CR3A integrator were used during the chromatography that was operated at ambient temperature at a flow-rate of 1.0 ml/min. The validation of the assay showed that the calibration curve was linear over the concentration range 10–2000 ng/ml of AB with the detection and quantitation limits of 5 and 10 ng/ml of AB, respectively. The within-day and day-to-day relative standard deviations (RSD) were less than 2% ($n = 15$) and 6.54% ($n = 45$), whereas the mean extraction recovery of AB from the plasma at three different concentration covering the calibration range was $98.1\% \pm 1.1\%$ ($n = 15$). During the analysis, nine quality control samples at three different concentrations were used. The individual and overall percent recovery of AB from the quality control samples with RSD values not more than 15% provided the acceptance criteria for the measurements of the samples. In this respect, the RSD values for all measurements on within-day and day-to-day basis were less than 15% with an overall RSD of 4.88% ($n = 27$) at 3 different days. Duplicate analysis were performed for each plasma fraction of the patients and AB concentration at the time points defined were expressed as a mean of four measurements (mean ± SEM; RSD). The HPLC assay was demonstrated to be specific for AB without having any interactions due to the endogenous plasma components and co-administered drugs during the therapy of the patients.

2.3. Pharmacokinetic analysis

The plasma AB concentration versus time data of the patients were analysed using a non-compartmental method (Gibaldi, 1984; Wagner, 1993). The mean maximum concentration (C_{max}) and the mean minimum concentration (C_{min}) prior to the next i.v. infusion dose were those values observed from the data and the C_{min} was

also the last measurable concentration (C_t) at time t (last measured time point). The area under the plasma concentration versus time curve from time zero to the time t (AUC_{0-t}) was determined by trapezoidal method and extrapolated to infinity ($AUC_{t-\infty}$) by calculating the remaining area from the relationship of C_t/k_{el}. The terminal half-life ($t_{1/2}$) was calculated from the slope of the regression line that best fit the terminal part of the log-linear concentration–time curve. AUMC was the area under the first moment curve (plasma concentration × time vs. time) and it was either calculated between the time zero and time t ($AUMC_{0-t}$) or between the time zero to infinity ($AUMC_{0-\infty}$). Mean residence time (MRT) was also determined between the time zero and time t (MRT_{0-t}) and between the time zero and to infinity ($MRT_{0-\infty}$) by using the relationship of AUMC/AUC. The total body clearance (CL) and the volume of distribution at steady state (V_{ss}) were determined from the equations $Dose/AUC_{0-\infty}$ and $Dose_{i.v.inf.}(AUMC)/(AUC)^2 - Dose_{i.v.inf.}(T)/2(AUC)$, respectively and T was the duration of infusion.

3. Results and discussion

In the present study, administration of LAB appeared to be clinically safe and well tolerated in all the three patients after either single or multiple dosing. The mean plasma AB concentration versus time data after LAB administration to the patients were illustrated in Fig. 1. Immediately after the completion of infusion, the plasma AB concentration of the first patient was 649 ± 14 ng/ml (RSD 4.24%), whereas the concentrations at 0.25 and 18 h post infusion were corresponded

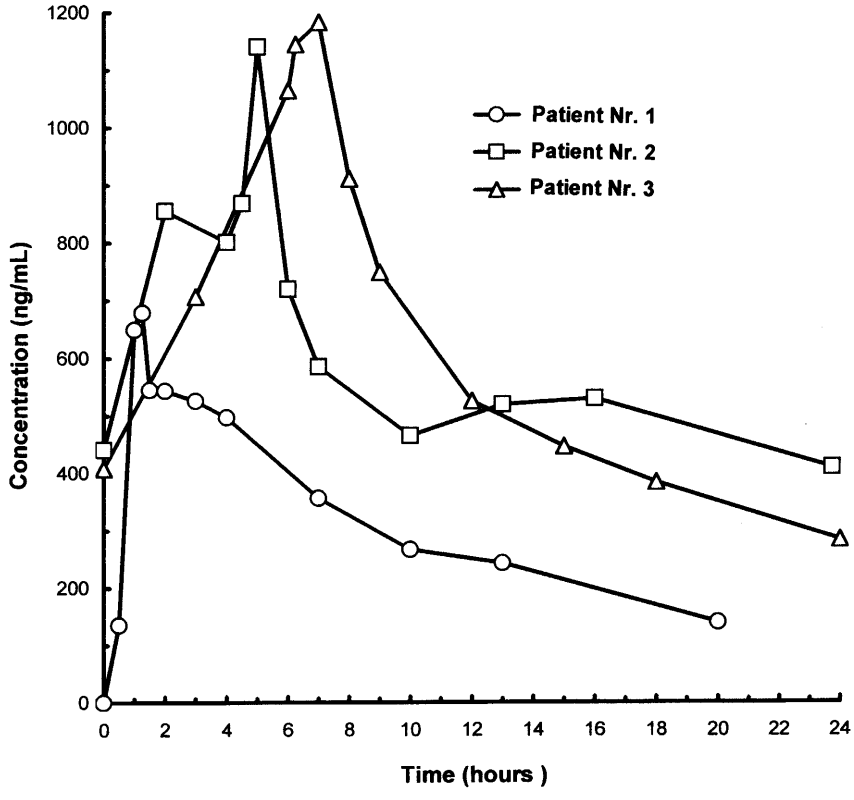

Fig. 1. Plasma concentration time profiles of amphotericin B in neutropenic cancer patients after single (first patient) and multiple (second and the third patients) application of AmBisome® by i.v. infusion over different infusion times.

to C_{max} (679 ± 6 ng/ml, RSD 1.79%) and C_{min} (139 ± 9 ng/ml, RSD 13.3%), respectively. The infusion times for the second and third patients were 4 and 6 h, respectively, and the concentrations of AB at the end of these infusion times were determined as 801 ± 18 ng/ml (RSD 4.53%) and 1060 ± 10 ng/ml (RSD 2.65%). Since the duration of infusions were different, the time at 1 h post infusion was corresponded to at the 5 and 7 h from the time zero, and C_{max} was observed at these corresponding times for these patients. In this respect, C_{max} was 1140 ± 10 ng/ml (RSD 1.12%) for the second patient, whereas it was 1180 ± 10 ng/ml (RSD 2.26%) for the third patient. The plasma AB concentration at time zero (pre-dose) (440 ± 6 ng/ml, RSD 2.55%) and C_{min} at 19.75 h (409 ± 11 ng/ml, RSD 5.46%) were similar for the second patient indicating that AB plasma concentrations were in steady state, while LAB was infused for 19 days. Contrary to this finding, pre-dose and C_{min} values (at 18 h post infusion) of the third patient (day 9) were 408 ± 3 ng/ml (RSD 1.25%) and 283 ± 1 ng/ml (RSD 0.870%), respectively. Although infusion over 4 and 6 h produced similar C_{max} following multiple dosing, C_{min} of the third patient was found lower than the other. The third patient was previously treated with Fungizone® for 5 days and then with AmBisome® for three days. The low C_{min} observed was attributed to the different pharmacokinetics of the two formulations. However, AUC_{0-t}, $AUMC_{0-t}$ and MRT_{0-t} calculated from the plasma concentration versus time data were almost same for these two patients (Table 1). AUC_{0-t}, $AUMC_{0-t}$ and MRT_{0-t} values for the second and third patients were 13 700 ng·h/ml, 143 000 ng·h^2/ml, 10.5 h, and 14 000 ng·h/ml, 139 000 ng·h^2/ml and 9.93 h, respectively. There were more than a two fold increase in the AUC_{0-t} of the second and the third patients when compared to the AUC_{0-t} of the first patient (6180 ng·h/ml) who was on the first day of the treatment. The plasma concentrations measured between two infusion intervals were used for finding the terminal elimination phase for the first patient by extrapolating to infinity and AUC, AUMC and MRT between the time zero and to infinity and CL, k_{el}, V_{ss} and $t_{1/2}$ values were calculated (Table 1). Consequently, administration of the first dose in this patient resulted in a wide tissue distribution by demonstrating a high V_{ss} (81 L) which explained the two fold lower C_{max} than the other patients. The calculation of AUC, AUMC and MRT for the second and the third patients were conducted between the time zero and time t (prior to the next infusion doses) (Table 1). As observed from the profiles, AB plasma concentrations were still high. Consequently, the terminal phases for these patients did not reflect the elimination phases, and the extrapolation was not performed. In order to estimate the terminal elimination rate constants correctly, sufficiently long sample-collection periods without applying the next infusion doses were necessary and this was not clinically applicable. Apart from C_{min} values, most of the pharmacokinetic parameters calculated were comparable for the two patients having AML after multiple dosing at the doses administered. However, the pharmacokinetic data obtained in this study could not be compared with the previously published studies. While in these studies, relatively high doses of LAB, different lipid-based products other than LAB, and formulations prepared specially for research purposes with different liposomal characteristics were administered either to patients or healthy volunteers (Sanders et al., 1991; Gokhale et al., 1993; Villani et al., 1996; Adedoyin et al., 1997). Only in a recent study (Walsh et al., 1998) safety, tolerance and multidose (1.0, 2.5, 5.0 and 7.5 mg/kg/day) plasma pharmacokinetics of AmBisome® after i.v. infusion over 1 h were investigated in neutropenic adult patients receiving cancer chemotherapy and bone marrow transplantation. According to the results of this study, administration of AmBisome® was well tolerated with minimal infusion related toxicity (Walsh et al., 1998). All of the non-compartmental pharmacokinetic parameters calculated in this study were higher than our results, even at the lowest dose (1 mg/kg/day) that was applied in the first day of the treatment. The plasma pharmacokinetic profile of AmBisome® in this study was characterised with non-linear saturation kinetics that was consistent with reticuloendothelial uptake and redistribution. Although the pharmacokinetic profiles and some of the phar-

Table 1
Non-compartmental pharmacokinetic parameters of amphotericin B in neutropenic cancer patients after AmBisome® administration by single and multiple doses of 50 mg/day

Patient	C_{max} (ng/ml)[a]	C_{min} (ng/ml)[a]	AUC_{0-t} (ng·h/ml)	$AUMC_{0-t}$ (ng·h^2/ml)	$AUC_{0-\infty}$ (ng·h/ml)	$AUMC_{0-\infty}$ (ng·h^2/ml)	MRT_{0-t} (h)	$MRT_{0-\infty}$ (h)	V_{ss} (L)	CL (ml/h)	k_{el} (h^{-1})	$t_{1/2}$ (h)
1	679 ± 6 (1.79)	139 ± 9 (13.3)	6180	48 100	7900	106 000	7.79	13.3	81.0	6310	0.0775	8.94
2	1140 ± 10 (1.12)	409 ± 11 (5.46)	13 700	143 000	–	–	10.5	–	–	–	–	–
3	1180 ± 10 (2.26)	283 ± 1 (0.870)	14 000	139 000	–	–	9.93	–	–	–	–	–

[a] Mean ± SEM (RSD %), $n = 4$.

macokinetic parameters of each dosage group on the first and last days of the therapy were illustrated, C_{max} and through levels were lacking. It was mentioned that through concentrations were relatively constant for a given patient and dosage, suggesting negligible plasma accumulation, but the data dealing with this accumulation was not presented. However, this data and comparison of the pharmacokinetic profiles for each dose group separately, for example by comparing the profiles corresponding to the first and the last infusions of one dose group in the same figure, could be not only a useful information, but a clear demonstration for the extent of drug accumulation at different doses. In our preliminary study, the pharmacokinetic profiles of the same patient at the beginning and at the end of therapy could not be examined, but due to the through levels observed, the assumption of AB accumulation was among our findings, which indicated an important need of therapeutic AB monitoring during LAB administrations.

4. Conclusion

Although the number of patients in this study was limited, the data indicated some important results for future studies. The dose administered to the patients in this study were lower than the recommended range, but it was one of the most practically applied doses in our country, not only for safety aspects, but also due to high cost of the LAB as well. Consequently, the pre-dose, C_{max}, C_{min}, the plasma AB concentrations immediately at the end of infusions and the non-compartmental pharmacokinetic parameters calculated after LAB administration at a daily dose of 50 mg showed that clinically relevant plasma concentrations was achievable and this was observed especially within the second patient's pharmacokinetic profile. Determination of AB plasma levels during the treatment, preferentially AB concentration at the end of infusion, C_{max}, C_{min} (trough levels) values either at the beginning of the therapy or at steady-state conditions could be suggested as a versatile approach for the optimisation of AB i.v. infusion doses. This would probably reduce the total dose required and hence the risk for drug accumulation at high doses and the cost of therapy with a great extent with LAB. In this respect, reliable, validated and harmonised assays should be established for AB routine analysis in clinical laboratories and monitored therapeutically for dosage adjustments.

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