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A comparative study of plasma concentrations of liposomal amphotericin B (L-AMP-LRC-1) in adults, children and neonates

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Abstract

Amphotericin B (AmpB) incorporated into small unilamellar liposomes prepared from soya phosphatidylcholine and cholesterol (L-AMP-LRC-1) has been shown to be safe and effective in patients with systemic fungal infections. In this report, we compared the plasma levels of AmpB in adults, children and neonates following administration of L-AMP-LRC-1. A 1.0 mg/kg dose of L-AMP-LRC-1 in adult patients resulted in peak concentrations of 1.02 ± 0.14 mg/l (mean $\pm$ S.D.) on day 1, which increased to 1.66 ± 0.19 mg/l on day 28 after continued therapy. The area under the plasma concentration–time curve also increased from 13.05 ± 1.52 on day 1 to 19.85 ± 5.41 mg h/l on day 28. In children, the peak plasma concentration following 1.0 mg/kg per day dose of L-AMP-LRC-1 increased from 0.63 ± 0.20 on day 1 to 1.10 ± 0.53 mg/l on day 28. While in neonates, the levels increased from 0.54 ± 0.17 on day 1 to 0.73 ± 0.29 mg/l on day 28. These levels of AmpB in children and neonates were found to be significantly lower than in adults. This may be due to higher volume of distribution, since 1.0 mg/kg per day dose of L-AMP-LRC-1 was found to be effective in neonates.

Keywords: Liposomes; Amphotericin B; Pharmacokinetics

Systemic fungal infections affecting 3–4% of premature neonates (< 1500 g birth weight), is one of the serious infections associated with high morbidity and mortality (Baley et al., 1981; Johnson et al., 1984). Since its introduction in 1958, amphotericin B (AmpB) remains the drug of choice for serious fungal infections, despite the frequent occurrence of acute and chronic toxicity that often necessitate changes in, or premature discontinuation of therapy. The most effective

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dosage of AmpB is often quite variable and to a certain extent depends on the type and severity of the infection. The dose usually ranges from 0.6 per day to 1.5 mg/kg on alternate days. Paediatric dose recommendations for AmpB have been extrapolated from adult experience and has ranged from 0.5 to 1.0 mg/kg per day (Sande and Mandell, 1985). Repeated daily intravenous infusion of 1.0 mg/kg AmpB in adults have produced peak serum concentrations of 1.5–2.1 mg/l and trough concentration of 0.1–0.15 mg/l (Atkinson and Bennett, 1978). In children, peak and trough serum concentrations after a dose of 0.75 mg/kg were 0.52 and 0.09 mg/l, respectively, suggesting a more rapid clearance of AmpB and that equal dose would produce lower peak levels in children than in adults (Starke et al., 1987). In neonates, with similar dosage regimen, an extreme individual variability for the half life, volume of distribution and clearance for AmpB has been observed.

AmpB encapsulated in liposomes prepared from a variety of lipids has been reported to be safer and more effective than conventionally administered AmpB in animals (New et al., 1981; Lopez-Berestein et al., 1983; Trembley et al., 1984; Adler-Moore et al., 1991) and in patients (Lopez-Berestein et al., 1985; Sculier et al., 1988; Ringden et al., 1991). We have previously shown that liposomal AmpB (L-AMP-LRC-1) prepared using soya phosphatidylcholine and cholesterol was safe (Gokhale et al., 1993a) and effective in patients with systemic fungal infections and leishmaniasis (Bodhe et al., 1999). In particular, it was found to be safe in adult patients with renal disease (Gokhale et al., unpublished data) and effective in a patient with chronic disseminated candidiasis resistant to conventional AmpB (Gokhale et al., 1993b). The pharmacokinetic study of L-AMP-LRC-1 in adult patients showed that the peak plasma concentration following 1.0 mg/kg dose was 1.027 mg/l with a volume of distribution of 2.285 l/kg (Gokhale et al., 1993a). In this study, we report the effect of multiple doses of L-AMP-LRC-1 on the pharmacokinetics of AmpB in adults and compare the plasma levels of AmpB in adults, children and in neonates following administration of liposomal AmpB.

Patients with histopathologically/microbiologically proven invasive fungal infection in whom AmpB administration was indicated were enrolled in the study after informed consent. Liposomal AmpB was administered in escalating doses of 0.1 mg, 0.4 mg and 1.0 mg/kg per day on consecutive days and the dose of 1.0 mg/kg per day was continued till the end of therapy. The drug was infused over 60 min. In neonates, the drug was administered using a syringe infusion pump (Terumo®, Model No 538 Japan).

AmpB concentrations in plasma were determined by high performance liquid chromatography (HPLC; Nilsson-Ehle et al., 1977) and by microbiological assay using *Paeciliomyces variotti* ATCC 22319 (MTCC 1368; Bindschadler and Bennett, 1969). The HPLC method estimated total AmpB concentrations in plasma including the liposome bound and free. We compared the concentrations of AmpB obtained by HPLC method and those obtained by the bioassay method and found a good linear relationship ($r = 0.963$, $P < 0.001$) suggesting that the total AmpB concentration in plasma was bioactive.

The peak plasma AmpB concentrations on day 1 in adult patients receiving 1.0 mg/kg of liposomal AmpB, ranged from 0.83 to 1.22 mg/l (1.02 ± 0.14 mg/l, Mean $\pm$ S.D., $n = 12$) and the 24 h trough concentrations ranged from 0.20 to 0.32 mg/l (0.26 ± 0.03 mg/l, $n = 12$). In these patients the peak and trough concentrations after 28 days of therapy were 1.66 ± 0.19 and 0.48 ± 0.07 mg/l, respectively. The decrease in plasma concentration followed a biexponential pattern. The $t_{1/2\alpha}$ and $t_{1/2\beta}$ was 0.95 ± 0.79 h (Mean $\pm$ S.D.) and 15.57 ± 1.80 h, respectively, which increased to 1.02 ± 0.71 and 30.18 ± 10.80 h, respectively, after continued therapy. The pharmacokinetic parameters in adult patients on day 1 and day 28 are summarized in Table 1.

The plasma concentrations with L-AMP-LRC-1 were found to be comparable to conventional AmpB (Atkinson and Bennett, 1978). In contrast to these observations, AmBisome, a commercially available liposomal preparation of AmpB demonstrated significantly higher plasma levels at equivalent doses with a corresponding decrease in the volume of distribution (Coukell and Brogden,

1998). These differences could be attributed to the negatively charged lipid present in the liposome composition of AmBisome since both L-AMP-LRC-1 (100–130 nm mean diameter, unpublished data) and AmBisome (45–80 nm mean diameter) are small unilamellar formulations (Boswell et al., 1998). Interestingly, both AmBisome and L-AMP-LRC-1 had comparable efficacy in *Aspergillus fumigatus* murine model despite differences in their pharmacokinetics (Kotwani et al., unpublished data). Lopez-Berestein and Rosenblum (1992) used multilamellar vesicles with negative charge and observed rapid clearance of the liposome drug complex from circulation with a half-life of less than 15 min.

Multiple dose studies with AmBisome in patients have demonstrated that the C_{max} after the second dose was approximately twice that measured after the first dose and remained constant thereafter (Janknegt et al., 1992). AmpB levels after multiple doses of L-AMP-LRC-1 showed about 1.6 fold increase in C_{max} and area under the plasma concentration–time curve. Perhaps, like AmBisome, the steady state was attained within a few days of therapy.

The peak plasma concentration of AmpB in children (age range 1–12 years) receiving 1.0 mg/kg dose of L-AMP-LRC-1 ranged from 0.36 to 0.95 mg/l (0.63 ± 0.20 mg/l, Mean $\pm$ S.D., $n = 11$) and 24 h trough concentrations ranged from 0.16 to 0.6 mg/l (0.24 ± 0.12 mg/l, $n = 11$) on day 1. Of the eleven children, only three continued to receive liposomal AmpB until day 28 due to their systemic fungal infections (1-aspergilloma, 2-candidemia). The peak and trough plasma concentrations on day 28 in three children ranged from 0.58 to 1.64 mg/l (1.10 ± 0.53 mg/l, Mean $\pm$ S.D.) and 0.27 to 0.37 mg/l (0.32 ± 0.05 mg/l), respectively. In the remaining eight children with candiduria who responded to L-AMP-LRC-1 in 21 days, the peak and trough plasma concentrations on day 21 ranged from 0.50 to 1.15 mg/l (0.87 ± 0.22 mg/l, Mean $\pm$ S.D.) and 0.054 to 0.80 mg/l (0.34 ± 0.22 mg/l), respectively.

In seventeen neonates (age range 14–30 days), weighing 1.1–2.25 kg, the peak plasma concentrations on day 1 ranged from 0.34 to 1.0 mg/l (0.54 ± 0.17 mg/l, Mean $\pm$ S.D.) and the 24 h trough concentrations ranged from 0.18 to 0.32 mg/l (0.22 ± 0.04 mg/l) after a 1.0 mg/kg dose of L-AMP-LRC-1. After 28 days of therapy, the peak plasma concentrations ranged from 0.43 to 1.29 mg/l (0.73 ± 0.29 mg/l, Mean $\pm$ S.D.) and the 24 h trough concentrations ranged from 0.2 to 0.66 mg/l (0.32 ± 0.13 mg/l).

Table 2 gives the comparative mean plasma levels in adults, children and neonates receiving 1.0 mg/kg of L-AMP-LRC-1 on day 1 and day 28. The peak plasma AmpB concentrations increased significantly from day 1 to 28 in adults ($P < 0.001$), children ($P < 0.03$) and neonates ($P < 0.001$) demonstrating cumulative dose effects until steady-state was reached. The peak plasma concentration was also significantly greater in adults on day 1 compared with children ($P < 0.001$) or neonates ($P < 0.001$). Similar results were observed on day 28 of therapy. However, plasma concentrations in children and neonates were comparable on day 1 and 28.

Studies have shown that, in general, neonates have larger volume of distribution compared with adults resulting in higher tissue levels of the drug (Bauchanan, 1987). The pharmacokinetics of liposomal AmpB in children or neonates has not been

Table 1
Pharmacokinetic parameters of AmpB in adult patients following 1.0 mg/kg per day i.v. dose of L-AMP-LRC-1 on days 1 and 28

	C_{max} (mg/l)	$AUC_{0\rightarrow\infty}$ (mg h/l)	$t_{1/2\alpha}$ (h)	$t_{1/2\beta}$ (h)	Cl (ml/h per kg)	V_d (l/kg)
Day 1	1.02 ± 0.14	13.05 ± 1.52	0.95 ± 0.79	15.57 ± 1.80	77.71 ± 9.49	1.74 ± 0.29
Day 28	$1.66^* \pm 0.19$	$19.85^* \pm 5.41$	1.02 ± 0.71	$30.18^* \pm 10.80$	$53.62^* \pm 13.53$	$2.21^{**} \pm 0.68$

Values are mean $\pm$ S.D. ($n = 12$). The AUC was corrected for residual concentration from the previous dose. C_{max}, peak plasma concentration; AUC, area under the plasma concentration-time curve; $t_{1/2\alpha}$, distribution half-life; $t_{1/2\beta}$, elimination half-life; Cl, total body clearance; V_d, volume of distribution. Days 1 vs. 28; *, $P < 0.001$; **, $P < 0.04$.

Table 2
Plasma AmpB concentrations in adults, children and neonates following 1.0 mg/kg per day dose of L-AMP-LRC-1 on days 1 and 28

	Amphotericin B concentrations (mg/l)			
	Adults	Children	Neonates	
Day1				
C_{max}	1.02 ± 0.14	0.63 ± 0.20	0.54 ± 0.17	Adults vs. children, $P<0.001$ Adult vs. neonates, $P<0.001$ Children vs. neonates, NS
C_{min}	0.26 ± 0.03	0.24 ± 0.12	0.22 ± 0.04	Adults vs. children, NS Adult vs. neonates, $P<0.01$ Children vs. neonates, NS
Day 28				
C_{max}	1.66 ± 0.19*	1.10 ± 0.53**	0.73 ± 0.29†	Adults vs. children, $P<0.008$ Adult vs. neonates, $P<0.001$ Children vs. neonates, NS
C_{min}	0.48 ± 0.07*	0.32 ± 0.05	0.32 ± 0.13†	Adults vs. Children, $P<0.004$ Adult vs. Neonates, $P<0.001$ Children vs. neonates, NS
	Days 1 vs. 28; *$P<0.001$	Days 1 vs. 28; **$P<0.03$	Days 1 vs. 28; †$P<0.001$	

Values are mean $\pm$ S.D. C_{max}, peak plasma concentration; C_{min}, trough plasma concentration; NS, not significant.

described. However, the pharmacokinetic studies of an amphotericin B lipid complex (ABLC) made up of large ribbon-like structures (size range, 1.6–11 μm) showed lower C_{max} and higher clearance in children (Walsh et al., 1997) as compared with adult patients (Adedoyin et al., 1997). In studies using conventional AmpB, Benson and Nahata (1989) found an inverse correlation between age and clearance of AmpB. Similarly, Starke et al. (1987) found that the serum levels of AmpB were approximately half in neonates and children as compared with an equivalent dose in adults. They also compared the pharmacokinetics between the neonates and children and found that neonates had serum half-lives longer than older children while the children had rapid clearance of the drug. The increase in tissue-to-plasma ratio observed in neonates has been attributed to diminished plasma protein binding and increased extracellular fluid volume. It is, however, not known, if increased AmpB clearance from plasma is due to more rapid urinary or biliary elimination, drug decomposition, or increased drug metabolism.

In our study, due to ethical reasons, it was not possible to calculate the pharmacokinetic parameters in neonates and children because of limited blood samples. However, our results clearly show that plasma AmpB levels in children and neonates were lower than in adults. This may be because of greater volume of distribution in the neonates and greater clearance in the older children. Additional studies showed that the minimum fungicidal concentration of AmpB was in the range of 0.3–1.0 mg/l in the Candida strains isolated from these neonates. Interestingly, despite the lower levels of AmpB in neonates, 1.0 mg/kg per day dose of l-AMP-LRC-1 was found to be effective (Kotwani et al., unpublished data).

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