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Rapid Communication

Comparison of the in vivo antifungal activity of amphotericin B-Solulan C24, amphotericin B-Myrj 59 and amphotericin B-Synperonic A50 with fungizone

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Summary

Three associations of amphotericin B with the surfactants, Solulan C24, Myrj 59 and Synperonic A50, were compared with the commercial preparation, Fungizone (amphotericin B-sodium deoxycholate), in treating systemic candidiasis in NMRI male mice. Following an intravenous injection, there was no difference between the four treatment groups in the survival of infected animals. When amphotericin B was injected intraperitoneally, the associations with Myrj 59 and Synperonic A50 were as active as Fungizone but that with Solulan C24 was shown to be more active.

Amphotericin B (Am B) remains the drug of choice in the treatment of systemic mycoses despite the more recent development of azole derivatives. Because of the poor water solubility of the drug, the commercial preparation, Fungizone, consists of amphotericin B solubilized with sodium deoxycholate (Bartner et al., 1957). However, the clinical use of this product is impaired by its toxicity and especially its nephrotoxicity (Maddux and Barriere, 1980).

Our previous studies have shown that three nonionic surfactants (Solulan C24, polyoxyethyleneglycol (24) derivative of cholesterol (PC), Myrj 59, polyoxyethyleneglycol (100) stearate (M 59) and Synperonic A50, polyoxyethyleneglycol (50) alkyl C13-C15 ether, (S A50)) are able to solubilize amphotericin B in water (Tasset et al., 1990, 1991a,b). In vitro, the three surfactants decrease the haemolytic activity of Am B without increasing its minimal inhibitory concentration against *Candida albicans* (Tasset et al., 1990, 1991a, and unpublished results). They also modify the nephrotoxicity of Am B in rats. After 6 days of daily i.p. injections, the formulations containing PC or S A50 were more nephrotoxic than Fungizone. In contrast, the association of Am B with M 59 was less nephrotoxic than the commercial preparation in this model (Tasset et al., 1991b).

The aim of the present study was to verify whether the associations of Am B with PC, M 59 or S A50 remain as active as the commercial preparation. For this purpose, the antifungal activity of the three formulations was compared

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with that of Fungizone against systemic candidiasis in mice. The intravenous and intraperitoneal routes of administration were selected.

NMRI male mice (23–26 g) were purchased from Iffa Credo (Les Oncins, France). They were maintained under conditions of 12 h day light cycle and had free access to food and water.

Fungizone and Am B were obtained from Squibb (Brussels, Belgium). Solulan C24, Myrj 59 and Synperonic A50 were gifts from Amerchol (Edison, NJ), Codibel (Brussels, Belgium) and ICI (Middelsbrough, U.K.), respectively.

The animal model for infection was elaborated as followed: a wild strain of *C. albicans* was suspended in saline. Groups of 8–10 animals were infected by injecting via the tail vein 0.2 ml of this suspension containing various numbers of *C. albicans* (5×10^7, 10^7, 5×10^6, 10^6 or 5×10^5). Animals were then observed for lethality.

The Fungizone solution was prepared by simple addition of 5% glucose to the freeze-dried powder. The Am B-Myrj 59, Am B-PC and Am B-S A50 formulations were prepared as described (Tasset et al., 1991a). Briefly, Am B and Myrj 59, PC or S A50 were dissolved in dimethylformamide and the solvent was evaporated under vacuum at 60–65°C. Clear solutions of Am B were obtained by addition of water to the dried products and these preparations were freeze-dried. Just before use, the clear solutions of Am B (0.25 mg/ml) were reconstituted by addition of a 5% glucose solution buffered with phosphate (pH 7.5) (0.39 mM) to the freeze-dried product.

Groups of 8–10 mice were injected via the tail vein with 5×10^7 *C. albicans*. After 30 h, the infected mice were randomly assigned to the different groups. They received a single intravenous or intraperitoneal injection of the different Am B formulations (0.9 mg/kg intravenously, determined as the maximum tolerated dose in these mice, or 5 mg/kg intraperitoneally). Controls were treated with the glucose-buffered solution.

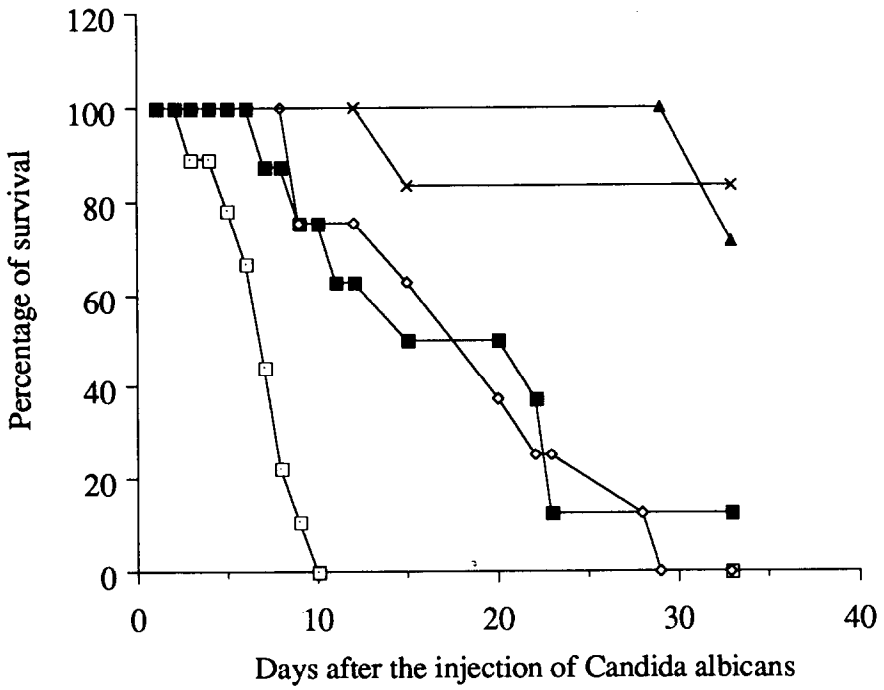

Fig. 1. Evolution of the survival of mice infected by an intravenous injection of *Candida albicans* (⊡) 5×10^7; (■) 10^7; (◇) 5×10^6; (▲) 10^6, (×) 5×10^5.

The efficacy of the Am B formulations was monitored by observing the survival of the animals during a period of 75 days.

The log-rank test of Mantel and a χ^2 test were used to determine statistical difference of survival curves and of long-term survivals between the different groups, respectively ($p < 0.05$).

The effect of the five sized inocula of *C. albicans* on the survival of the mice was firstly assessed. As shown in Fig. 1, the lethality of the animals increased on increasing the number of *C. albicans* injected. Following injection of the highest inoculum (5×10^7 *C. albicans*), all the mice died after 10 days. This inoculum was selected to compare the activity of the different Am B formulations.

Infected mice receiving Fungizone intravenously had an increased mean survival time in comparison with controls (Table 1). By day 21, all the control mice were dead but 60% of the animals receiving Fungizone were still alive. Mice treated with a single intravenous injection of Am B-M 59, Am B-S A50 or Am B-PC had similar survival curves to those of Fungizone-treated animals (Fig. 2 and Table 1).

TABLE 1

Mean survival time (M.S.T.) and increase in life span (I.L.S.) of mice infected with 5×10^7 Candida albicans and treated intravenously with different Am B formulations (0.9 mg / kg)

Treatments	M.S.T. (days)	I.L.S.(%)
Controls	9	
Fungizone	34	378
Am B-M 59	27	300
Am B-PC	35	389
Am B-S A50	30	333

Intraperitoneal treatment of infected mice with Fungizone results in a survival curve superior to that of controls. After 75 days, two of the eight mice in the Fungizone-treated group were still alive in comparison with 100% mortality after 21 days in the control group. As shown in Fig. 3 and Table 2, the mice receiving Am B-PC, Am B-S A50 or Am B-M 59 intraperitoneally had greater long-term survival than mice treated with Fungi-

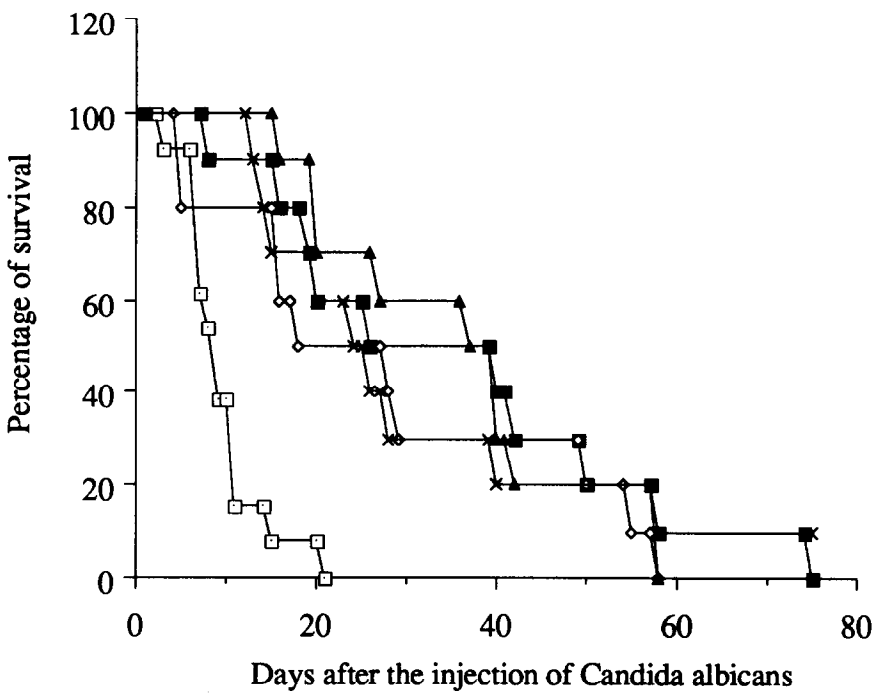

Fig. 2. Evolution of the survival of the mice infected with 5×10^7 *Candida albicans* after a single intravenous injection of Am B (0.9 mg/kg) (⊡) Controls; (■) Fungizone; (◇) Am B-M 59; (▲) Am B-PC; (×) Am B-S A50

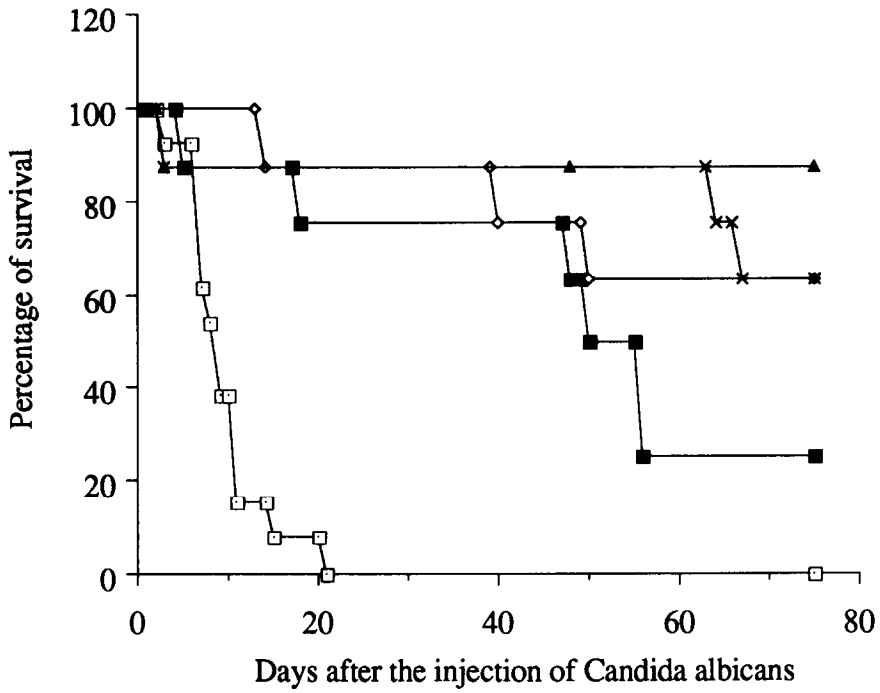

Fig. 3. Evolution of the survival of the mice infected with 5×10^7 *Candida albicans* after a single intraperitoneal injection of Am B (5 mg/kg): (⊡) Controls; (■) Fungizone; (◇) Am B-M 59; (▲) Am B-PC; (×) Am B-S A50

zone but only the Am B-PC-treated group showed a significant increase.

The results of this study demonstrate that the associations of Am B with M 59 and S A50 are as active as Fungizone when injected intravenously or intraperitoneally in this model of systemic candidiasis. The association with PC was more active than the commercial preparation by the intraperitoneal but not the intravenous route of administration. It remains to be determined whether this increased activity can be explained by increased absorption of Am B from the intraperitoneal cavity.

In conclusion, Am B-PC, Am B-M 59 and Am B-S A50 are at least as active as Fungizone against systemic candidiasis in mice. Studies are now in progress to evaluate the place of these three new formulations in the therapy with Am B.

TABLE 2

Long-term survivals (L.T.S.) of mice infected with 5×10^7 Candida albicans and treated intraperitoneally with different Am B formulations (5 mg / kg)

Treatments	L.T.S.
Controls	0
Fungizone	25
Am B-M 59	62.5
Am B-PC	87.5 [a]
Am B-S A50	62.5

[a] Different from Fungizone-treated group ($p < 0.05$).

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