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Comparison of Nephrotoxicities of Different Polyoxyethyleneglycol Formulations of Amphotericin B in Rats

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The aim of the present study was to assess whether amphotericin B (AmB)-Myrj 59, AmB-polyoxyethyleneglycol 24 cholesterol (PC), and AmB-Synperonic A50 (SA50) were less nephrotoxic than AmB-deoxycholate (DC). Rats were treated with the different AmB formulations (10 mg/kg of body weight) intraperitoneally or with the surfactants alone. A group of control rats receiving the vehicle was also examined. After 6 days of daily intraperitoneal injections of AmB-DC, decreased body weight and glomerular filtration rate as well as increased degree of diuresis, uremia, microalbuminuria, and *N*-acetyl-β-D-glucosaminidase excretion in urine were noted. Urinary excretion of potassium and sodium was also decreased in AmB-DC-treated rats. Most of these effects were more pronounced with AmB-PC and AmB-SA50. In contrast, AmB-Myrj 59 was less nephrotoxic than AmB-DC. Indeed, after 6 days of treatment with AmB-Myrj 59, the natriuria, kaliuria, albuminuria, and glomerular filtration rates were unchanged compared with those of controls. Moreover, the body weight loss and uremia increase of the rats treated by AmB-Myrj 59 were less than those of the rats treated with the commercial preparation. Among the surfactants, only PC was toxic for the rats. The intrinsic toxicity of PC and the higher systemic exposure to AmB could contribute to increased toxicities of AmB-PC and AmB-SA50, respectively. AmB-Myrj 59 was less nephrotoxic than AmB-DC at equivalent areas under the plasma concentration-time curves. These preliminary results suggest that this formulation could be a good alternative to the commercial product.

Amphotericin B (AmB) is a polyene antibiotic which is widely used in the treatment of systemic mycoses despite two major disadvantages. First, AmB is poorly water soluble, and the commercial preparation (Fungizone) consists of a colloidal solubilization of the drug in a micellar solution of deoxycholate (AmB-DC). Second, toxicity of the polyene limits its clinical use. Particularly, the chronic nephrotoxicity which affects up to 80% of the patients treated with AmB-DC (13) can sometimes lead the clinician to lower the dose or to prematurely discontinue therapy (8, 46). Usually, renal function returns to normal upon withdrawal of therapy (5), although sometimes permanent damage is sustained (49).

The mechanism of this nephrotoxicity is not yet well understood. It is characterized by an increase in the tubular permeability, leading to the inability of the kidneys to concentrate the urine (5, 13, 16, 38); to distal tubular acidosis (11, 16, 20, 38); and to potassium, sodium, and magnesium wasting (4, 11, 16, 37, 38). This effect is probably due to the direct interaction of AmB with the cell membrane (12, 15) and could be related to the ability of the antibiotic to alter the membrane permeability (1). AmB also induces a decrease in the glomerular filtration rate and in the renal blood flow (11, 13, 14, 16, 39). These hemodynamic effects are probably the results of the activation of intrarenal mechanisms (tubuloglomerular feedback) (18) and/or of the release of mediators such as thromboxane A_2 (46).

Our previous works have shown that three surfactants, polyoxyethyleneglycol 24 cholesterol (PC), Myrj 59, and Synperonic A50 (SA50), were able to solubilize AmB in water. Cholesterol is known to decrease the in vitro hemolytic acitivity of AmB by decreasing its affinity for the cellular membranes (29a). However, we previously showed that cholesterol is not necessary in the structure of the surfactant to diminish or even suppress the hemolytic activity of AmB. These surfactants were all able to decrease AmB and AmB-DC hemolytic activities without decreasing their in vitro and in vivo antifungal activities against *Candida albicans* (50–52). The aim of the present study was to assess whether AmB-Myrj 59, AmB-PC, and AmB-SA50 are less nephrotoxic than AmB-DC. Therefore, these new formulations and AmB-DC were injected daily for 6 days to different groups of five to six rats (10 mg/kg of body weight) intraperitoneally. The survival of the animals; body weight; diuresis; glomerular filtration rate; and levels of uremia, albuminuria, NAG urinary excretion, kaliuria, and natriuria were monitored during the 6 days of the treatment.

MATERIALS AND METHODS

Chemicals. Fungizone (a colloidal solubilization of AmB in a micellar solution of deoxycholate [AmB-DC]) and AmB were obtained from Squibb (Brussels, Belgium). PC (polyoxyethylenegylcol 24 cholesterol), Myrj 59 (polyoxyethyleneglycol 100 stearate), and SA50 [polyoxyethyleneglycol 50 alkyl (C_{13}-C_{15}) ether] were gifts from Amerchol (Edison, N.J.), Codibel (Brussels, Belgium), and ICI (Middlesbrough, England), respectively. Methylumbelliferyl-*N*-acetyl-glucosaminide and bovine serum albumin were purchased from Sigma Chemical Co. (St. Louis, Mo.), and glycine was purchased from UCB (Braine l'Alleud, Belgium). The solvents, EDTA, the phosphate, and citrate salts were of analytical grade and were obtained from UCB. ^{51}Cr-EDTA

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was obtained from Amersham Corp. (Arlington Heights, Ill.).

Drug preparation. The solution of AmB-DC was prepared daily by the addition of a 5% glucose solution to the commercial freeze-dried product to obtain a final concentration of 2 mg of AmB per ml.

The AmB-PC, AmB-Myrj 59, and AmB-SA50 complexes were prepared as previously described (51, 52): 150 mg of AmB was dissolved in 150 ml of dimethylformamide. Seven hundred and fifty milligrams of PC, 450 mg of Myrj 59, or 1.125 g of SA50 was then added to this solution, which was rotoevaporated under vacuum at 60 to 65°C to dryness. Afterwards, 150 ml of water was added to the film, and a clear solution was obtained after manual shaking and, if necessary, ultrasonication in a water bath sonicator. These preparations were then freeze-dried (Lyovac GT 2) and analyzed for their AmB content by a high-performance liquid chromatography (HPLC) method (21, 41). The linear regression curve was established by the unweighted method of least squares for AmB concentrations varying from 5 to 20 μg/ml. The interday and intraday coefficients of variation are less than 3.3% for $n = 4$. Just before use, a 5% glucose solution buffered with phosphate (3.12 mM) at pH 7.5 was added to the freeze-dried powder, and after manual shaking and bath sonication, if necessary, a clear solution containing 2 mg of AmB per ml was reconstituted.

DC, PC, Myrj 59, and SA50 were solubilized in the 5% glucose buffered solution at the concentrations of 1.62, 10, 6, and 15 mg/ml, respectively, equivalent to those concentrations in the AmB formulations.

Animals. Sprague-Dawley male rats (2 months old, 316 ± 14 g) were purchased from Iffa Credo (Les Oncins, France). They were housed in individual cages and randomly assigned to treatment and control groups. The animals were maintained in conditions of 12-h daylight cycles and had free access to food and water.

Toxicological studies. Repeated intravenous injections of AmB in rats are impaired by the appearance of phlebitis, and many authors have previously used the intraperitoneal route of administration to study the nephrotoxicity of AmB in rats. Moreover, AmB is not metabolized and its intraperitoneal injection is not limited by a first hepatic pass. Therefore, we chose this route of administration to compare the nephrotoxicities of the different AmB preparations. Groups of 5 to 10 rats were intraperitoneally injected daily for 6 days with the different AmB formulations (10 mg of AmB per kg) or with the surfactants alone (5 ml of surfactant solution per kg). Controls were treated with the 5% glucose buffered solution (5 ml/kg). The 6-day period of administration was selected on the basis of preliminary results, indicating that nephrotoxicity does not increase further after a second week of treatment (50).

Before the beginning of the treatment and every other day during the administration of the different drug preparations, the animals were housed in metabolic cages (permitting urine collection without contamination by the feces) and urine samples were collected over a 24-h period. A blood sample was also collected from the tail vein before the first injection and 24 h after the last one. The following parameters were monitored: survival; body weight; 24-h urinary volume; glomerular filtration rate; blood urea nitrogen concentration; degree of microalbuminuria; and urinary excretion of sodium, potassium, and NAG.

The clearance of ^{51}Cr-EDTA was used to estimate the glomerular filtration rate (44). Rats were injected intravenously with a solution of 20 μCi of ^{51}Cr-EDTA. One hour after the injection, a blood sample was collected and the serum radioactivity was measured.

The clearance of ^{51}Cr-EDTA was calculated as follows: $CL = V/t[\ln (S_0/S_t)]$, where $S_0 = I/V$, $V = 0.264\ BW - 1.92 \times 10^{-4}\ BW^2 + 1.03$, CL = clearance of ^{51}Cr-EDTA, V = distribution volume of ^{51}Cr-EDTA, S_t = amount of radioactivity in the serum at 60 min, I = injected amount of radioactivity, and BW = body weight of the rat.

The blood urea nitrogen concentration was determined by the Berthelot's reaction kit (Boehringer, Mannheim, Germany), and the amount of microalbuminuria was determined by a latex agglutination assay (6).

The activity of NAG in urine samples was determined with methylumbelliferyl-*N*-acetylglucosaminide as a substrate (1.6 mg/ml). This assay was performed in a citrate buffer at pH 5 (0.05 M) (31) containing 0.1 mg of bovine serum albumin per ml (33). The urine samples were diluted 100 times in this buffer, and 100, 250, or 500 μl was incubated in a final volume of 1 ml for 15 min at 37°C. The reaction was stopped by the addition of 3 ml of glycine buffer, pH 10.65 (0.02 M) (31). The methylumbelliferone produced was quantified by fluorimetry. Dialysis of urine samples was not necessary since dilution is able to overcome the inhibition problems (7, 55, 58). The sodium and potassium contents in urine and serum samples were determined by flame photometry on a Greiner 300 instrument (Greiner, Langenthal, Switzerland).

Pharmacokinetic studies. Different groups of rats received a single intraperitoneal injection of the different AmB formulations (AmB-DC, AmB-Myrj 59, or AmB-SA50) (10 mg/kg). Blood samples were collected from the tail veins at different times after the drug administration (1, 2, 4, 6, 8, 10, 13, 24, 48, 109, and 168 h), and the sera were frozen until analysis for their total AmB content.

The AmB serum concentration was evaluated by an HPLC method. The elution solvent was a methanol-EDTA (0.005 M) mixture (80:20, vol/vol), and the column was a reverse-phase μBondapak C_{18} (41). Solvent was delivered at a rate of 1.5 ml/min, and AmB was detected by a UV detector at 405 nm. Serum was treated as follows: to 100 μl of serum, 300 μl of methanol was added. This mixture was vortexed for 30 s and allowed to stand for 30 min at room temperature (10). After centrifugation at 700 × *g* for 15 min, the supernatant was filtered and 100 μl was injected in the chromatograph. The linear regression curve was established by the unweighted method of least squares for AmB concentrations varying from 25 to 100 ng/ml. The interday coefficient of variation is less than 7% for $n = 4$. Hemolysis does not interfere with the AmB determination. Since the polyene is stable in frozen serum for several months, the AmB concentrations were estimated from frozen samples (10). The serum AmB concentration after a single injection was estimated by calculating with the trapezoidal rule the area under the curve of the evolution of the serum drug concentration.

Statistics. Data are presented as means ± standard errors of the means. A one-way analysis of variance and a Scheffé *F* test were used to assess statistical significance. Differences were considered significant if P was <0.05.

RESULTS

The aim of the present study was to assess whether AmB-Myrj 59, AmB-SA50, or AmB-PC are less nephrotoxic than AmB-DC.

TABLE 1. Evolution of the weight of the rats during 6 days of daily intraperitoneal injections of different AmB formulations or surfactants

Treatment[a]	Body wt (g, mean ± SEM) (n) on the following day:			
	0	2	4	6
Control	320 ± 3 (10)	333 ± 3 (10)	343 ± 3 (10)	346 ± 4 (10)
AmB-DC	311 ± 5 (6)	278 ± 7[b] (6)	265 ± 10[b] (6)	264 ± 9[b] (6)
AmB-Myrj 59	308 ± 7 (5)	280 ± 9[b] (5)	291 ± 4[b,c] (5)	312 ± 4[b,c] (5)
AmB-PC	318 ± 4 (5)	284 ± 5[b] (4)	266 (2)	252 (2)
AmB-SA50	325 ± 6 (5)	293 ± 7[b] (4)	276 ± 11[b] (4)	264 ± 10[b] (4)
Deoxycholate	312 ± 4 (5)	321 ± 3[b] (5)	330 ± 7 (5)	337 ± 8 (5)
Myrj 59	310 ± 7 (5)	325 ± 6 (5)	335 ± 5 (5)	343 ± 9 (5)
PC	315 ± 6 (5)	295 ± 4[b] (5)	264 ± 11[b] (5)	247 ± 9[b] (4)
SA50	323 ± 3 (5)	334 ± 2 (5)	343 ± 5 (5)	355 ± 2 (5)

[a] Doses of AmB preparations were 10 mg/kg of body weight. Surfactant doses were as follows (milligrams per kilogram): deoxycholate, 8.2; Myrj 59, 30; PC, 50; SA50, 75.
[b] $P < 0.05$, compared with the control group.
[c] $P < 0.05$, AmB-treated group compared with AmB-DC-treated group.

Toxicological studies. The effects of different AmB formulations and of surfactants on the growth and viability of the rats are summarized in Table 1. In the control group, the body weight of the rats increased with time (an 8% increase after 6 days). In contrast, rats treated with AmB-DC lost weight; this was also true for those treated by AmB-Myrj 59, AmB-SA50, and AmB-PC. On day 2, the body weight of the rats treated with each AmB formulation was more than 10% lower than that of the controls. On day 6, the body weight of the rats treated with AmB-DC, AmB-SA50, or AmB-PC was more than 20% smaller than that of the controls, whereas with the AmB-Myrj 59 treatment, the body weight of the animals was only 9% lower. Myrj 59, DC, and SA50 had no effect on the growth of the rats, but PC induced after 6 days a 29% decrease in their body weight compared with that of the controls. After 6 days of daily injections, one rat of 5 in the AmB-SA50 group and three rats of five in the AmB-PC group died, but the other treatments with AmB (AmB-DC and AmB-Myrj 59) were not lethal. Among the surfactants tested, only PC induced the death of one rat of five. All the rats receiving PC alone had ascites at the end of the 6 days of treatment, but none of the other treatments induced such a symptom.

As shown in Table 2, the four formulations containing AmB produced a significant diuretic effect from the fourth day of treatment with AmB-DC, AmB-SA50, and AmB-PC or even from the second day with AmB-Myrj 59. However, none of the surfactants alone increased the 24-h urinary volume of the rats.

After 6 days of treatment, the animals treated with AmB-DC showed a decrease in their glomerular filtration rate compared with that of the controls. The AmB-PC and AmB-SA50 treatments led to a more marked decrease in the glomerular filtration rate, while the AmB-Myrj 59 treatment induced no change. The treatment of the rats with the surfactants alone did not modify their glomerular filtration rate. However, a determination of the glomerular filtration rate of the rats treated with PC was not possible because of ascites that had developed in these animals (Fig. 1).

After 6 days of AmB-DC administration, the rats had a fourfold increase in their serum urea concentration. AmB-Myrj 59 induced only a twofold increase in the serum urea concentration, whereas AmB-SA50 and the AmB-PC induced 4- and 10-fold increases, respectively. Among the surfactants, only PC modified the uremia of the rats (Fig. 1).

The treatment of the rats with each of the AmB preparations increased microalbuminuria, but the magnitude of this increase varied with the AmB formulation. Albuminuria increased less with AmB-Myrj 59 (a 1.9-fold increase after 6 days [not significant]) than with AmB-DC (a 6.6-fold increase), while the amount of albuminuria was much higher with the AmB-SA50 and AmB-PC treatments (Fig. 2).

TABLE 2. Evolution of the 24-h urinary volume of the rats during 6 days of daily intraperitoneal injections of different AmB formulations or surfactants

Treatment[a]	Vol (ml/24 h, mean ± SEM) (n) on the following day:			
	0	2	4	6
Control	10.8 ± 1.3 (10)	11.6 ± 1.5 (10)	11.6 ± 0.9 (10)	13.1 ± 1.3 (10)
AmB-DC	14.3 ± 3.1 (6)	14.4 ± 2.0 (6)	38.8 ± 4.6[b] (5)	38.5 ± 1.6[b] (6)
AmB-Myrj 59	14.8 ± 4.3 (5)	25.6 ± 3.3[b,c] (5)	28.8 ± 2.9[b] (5)	33.1 ± 2.3[b] (5)
AmB-PC	10.6 ± 2.1 (5)	14.0 ± 6.4 (4)	21.9 (2)	27.5 (2)
AmB-SA50	10.5 ± 0.6 (5)	11.8 ± 2.9 (5)	26.5 ± 7.2[b] (4)	32.2 ± 3.4[b] (4)
Deoxycholate	8.7 ± 0.7 (5)	9.5 ± 1.6 (5)	9.4 ± 1.0 (5)	10.6 ± 1.6 (3)
Myrj 59	10.1 ± 1.5 (5)	10.4 ± 1.2 (5)	8.4 ± 0.8 (4)	10.7 ± 2.1 (5)
PC	9.9 ± 1.7 (5)	13.1 ± 3.2 (5)	5.9 ± 1.4[b] (5)	7.1 ± 1.3[b] (4)
SA50	11.3 ± 1.6 (5)	12.6 ± 1.7 (5)	12.5 ± 1.7 (5)	14.4 ± 2.8 (5)

[a] Doses are the same as those for Table 1.
[b] $P < 0.05$, compared with the control group.
[c] $P < 0.05$, AmB-treated group compared with AmB-DC-treated group.

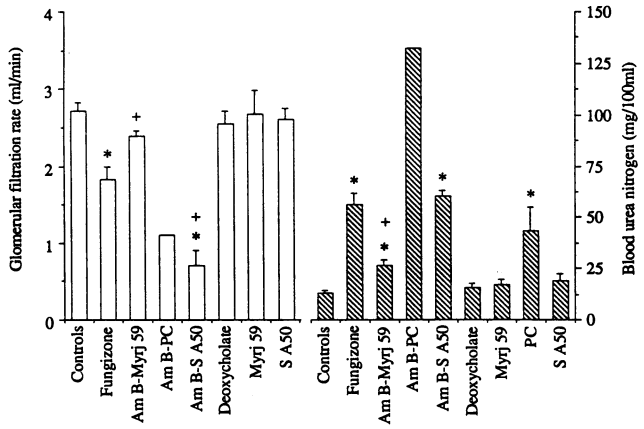

FIG. 1. Glomerular filtration (open bars) and blood urea nitrogen (striped bars) of the rats after 6 days of daily intraperitoneal injections of different AmB formulations (10 mg/kg) (AmB-DC, AmB-Myrj 59, AmB-PC, and AmB-SA50) or surfactants (milligrams per kilogram) as follows: deoxycholate, 8.2; Myrj 59, 30; and SA50, 75). *, $P < 0.05$, compared with controls; $^{+}$, $P < 0.05$, AmB-treated group compared with AmB-DC-treated group. *n* values are shown in Table 1. Bars indicate standard errors of the mean.

The urinary excretion of NAG increased in a similar way after injection of each of the AmB formulations. At the end of the treatment, rats excreted about fivefold more NAG than did the controls (Fig. 2).

After 2 days of treatment, the animals receiving AmB had decreased the urinary excretion of sodium and potassium. However, the kaliuria was less decreased for the rats treated with AmB-Myrj 59. After 6 days, the levels of kaliuria and natriuria of the rats receiving AmB-Myrj 59 were not different from those of the controls. In contrast, rats receiving AmB-DC, AmB-PC, or AmB-SA50 had a significant decrease in the urinary excretion of sodium and potassium (Table 3).

Pharmacokinetic studies. The AmB serum concentration-time curves obtained after a single intraperitoneal injection of AmB-DC, AmB-Myrj 59, or AmB-SA50 are represented in Fig. 3, and the areas under the concentration-time curves from 0 h to infinity ($AUC_{0-\infty}$) are represented in Table 4. AmB-DC, AmB-Myrj 59, and AmB-SA50 intraperitoneal injections gave different AmB serum concentration profiles. The injection of AmB-Myrj 59 led to a higher maximum concentration in serum (C_{max}) and a smaller time to maximum concentration of the drug in serum (T_{max}) than those for AmB-DC. However, the $AUC_{0-\infty}$ and the serum concentration 24 h after the injection were not statistically different. After the AmB-SA50 injection, two peaks in concentration were noted, and the AUC, the C_{max}, and the serum concentration 24 h after the injection were higher than those obtained with AmB-DC.

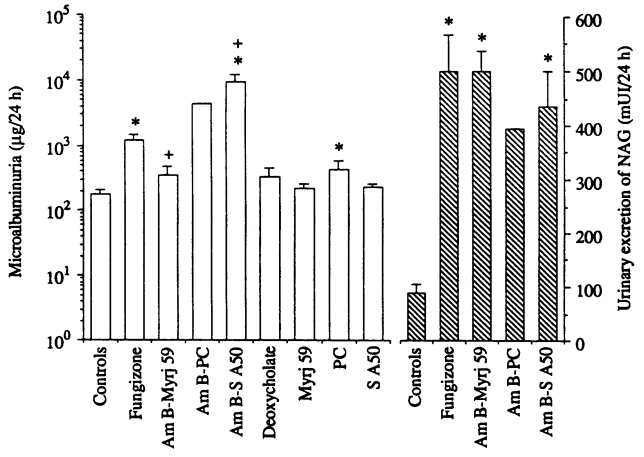

FIG. 2. Microalbuminuria (open bars) and NAG (striped bars) excretion in 24-h urine samples of rats after 6 days of daily intraperitoneal injections of different AmB formulations as described in the legend to Fig. 1. Symbols and *n* values are the same as for Fig. 1.

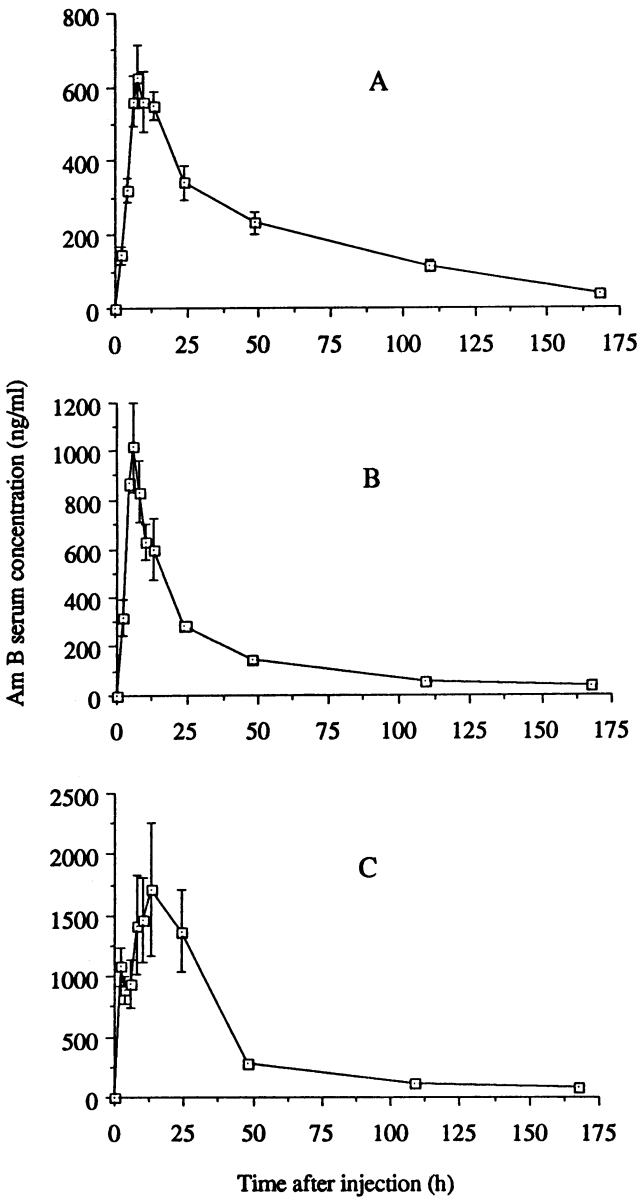

FIG. 3. Evolution of AmB serum concentrations in rats after a single intraperitoneal injection of AmB-DC (A), AmB-Myrj 59 (B), or AmB-SA50 (C) in doses of 10 mg/kg of body weight. *n* values are the same as those shown in Table 4.

TABLE 3. Evolution of the 24-h natriuria and kaliuria of the rats during 6 days of daily intraperitoneal injections of different AmB formulations or surfactants

Excreted material and treatment[a]	Urinary excretion (meq/24 h, mean ± SEM) on the following day[b]:			
	0	2	4	6
Sodium				
Control	1.47 ± 0.08	1.40 ± 0.15	1.44 ± 0.16	1.48 ± 0.16
AmB-DC	1.49 ± 0.06	0.49 ± 0.11[c]	0.79 ± 0.08[c]	0.73 ± 0.13[c]
AmB-Myrj 59	1.20 ± 0.10[c,d]	0.39 ± 0.04[c]	0.88 ± 0.12[c]	1.40 ± 0.23[d]
AmB-PC	1.42 ± 0.10	0.22 ± 0.12[c]	0.37	0.65
AmB-SA50	1.40 ± 0.11	0.71 ± 0.17[c]	ND	0.57 ± 0.12[c]
Deoxycholate	1.39 ± 0.13	1.19 ± 0.22	1.35 ± 0.08	1.23 ± 0.29
Myrj 59	1.32 ± 0.14	1.35 ± 0.25	1.21 ± 0.17	1.46 ± 0.16
PC	1.50 ± 0.26	0.08 ± 0.02[c]	0.02 ± 0.00[c]	0.03 ± 0.00[c]
SA50	1.35 ± 0.18	1.43 ± 0.16	1.31 ± 0.13	1.53 ± 0.15
Potassium				
Control	2.89 ± 0.18	2.79 ± 0.22	3.10 ± 0.13	3.50 ± 0.19
AmB-DC	2.80 ± 0.14	0.52 ± 0.08[c]	1.37 ± 0.19[c]	1.92 ± 0.26[c]
AmB-Myrj 59	2.51 ± 0.30	0.89 ± 0.11[c,d]	2.03 ± 0.23[c]	3.23 ± 0.320[d]
AmB-PC	2.83 ± 0.15	0.61 ± 0.16[c]	0.76 ± 0.27[c]	1.40
AmB-SA50	2.73 ± 0.13	0.49 ± 0.08[c]	ND	1.17 ± 0.31[c]
Deoxycholate	2.77 ± 0.29	2.34 ± 0.28	2.81 ± 0.17	3.55 ± 0.31
Myrj 59	2.48 ± 0.08	2.55 ± 0.25	2.57 ± 0.14[c]	3.27 ± 0.39
PC	2.88 ± 0.37	1.62 ± 0.14[c]	1.11 ± 0.06[c]	0.95 ± 0.08[c]
SA50	2.55 ± 0.34	2.63 ± 0.20	2.94 ± 0.18	3.77 ± 0.14

[a] Doses are the same as those for Table 1.
[b] *n* values are the same as those shown in Table 1. ND, not determined.
[c] $P < 0.05$, compared with the control group.
[d] $P < 0.05$, AmB-treated group compared with AmB-DC-treated group.

DISCUSSION

Renal toxicity limits the clinical use of AmB, and the aim of the present work was to assess, in rats, whether AmB-Myrj 59, AmB-SA50, and AmB-PC were less nephrotoxic than the commercial preparation of the antibiotic.

We showed that AmB-DC decreased the weight of the animals. This effect has been previously reported by other authors (28) and could be a consequence of an increase in diuresis (not completely compensated by the drink), an increase in the catabolism of the proteins, or a decrease in food intake. AmB-DC increased the serum urea concentration, and such a symptom, common with AmB treatment (23, 28, 43, 57), can be due not only to a decrease in the glomerular filtration rate but also to an increase in the catabolism of the proteins. In the present study, rats treated by AmB-DC also had increased diuresis, which has been reported in rats (15, 19, 23, 53) and which is probably the result of the toxicity of the drug on the collecting tubule (3). AmB is also known to decrease the glomerular filtration rate, and, in our study, AmB-DC decreased this filtration rate in the rats. However, under circumstances of increased tubular permeability, the true glomerular filtration rate cannot be estimated. AmB-DC is also known to increase the urinary excretion of sodium and potassium, but in the present work, sodium and potassium excretions were decreased. This contradictory effect has also been reported by other investigators for the urinary excretion of these two ions (15, 23, 36a). For potassium, it was explained partially by the slight decrease in the glomerular filtration rate. NAG is a lysosomal enzyme, and its activity in urine was increased in rats treated with AmB-DC. This increase was also reported when a liposomal formulation of the drug was used in rabbits (26) and reflects injury to proximal tubule cells. AmB-DC induced a rise in the albuminuria, and this increase can be due to a defective reabsorption of filtered plasma proteins in the proximal tubule or to the destruction of the integrity of the glomerular capillary wall. Moreover, the decrease in the glomerular filtration rate probably leads to the underestimation of this effect.

TABLE 4. Serum AmB concentrations 24 h after a single intraperitoneal injection of different AmB formulations in rats

Treatment[a] (*n*)	Serum AmB concn (ng/ml, mean ± SEM)	$AUC_{0-\infty}$ (ng · h/ml, mean ± SEM)
AmB-DC (4)	340.8 ± 46	31,943 ± 3,590
AmB-Myrj 59 (3)	277.6 ± 24.2	27,273 ± 2,955
AmB-SA50 (4)	1,368.3 ± 335.4[b]	70,727 ± 12,760[b]

[a] Doses were 10 mg/kg of body weight.
[b] $P < 0.05$, compared with AmB-DC-treated group.

The results of the present study show that AmB-Myrj 59 is partially less nephrotoxic than AmB-DC. After 2 days of treatment, AmB-Myrj 59 is as nephrotoxic as AmB-DC, as demonstrated by increased diuresis, loss of body weight, and decreased urinary excretion of sodium and potassium. However, after 6 days of treatment, the renal function was partially recovered in the AmB-Myrj 59 group but not in the AmB-DC group. Indeed, the degrees of kaliuria, natriuria, and microalbuminuria and the glomerular filtration rate in rats treated with AmB-Myrj 59 were not different from those of the controls; the degree of uremia and body weight of the rats were less modified than those of the animals receiving AmB-DC. However, diuresis and NAG urinary excretion were not different from the AmB-DC-treated group. These results suggest that, during AmB-Myrj 59 treatment, the glomerular filtration rate but not the membrane tubular permeability can be restored.

AmB-PC and AmB-SA50 were more toxic than AmB-DC, as evidenced by greater modifications in albuminuria, ure-

mia, glomerular filtration rate, and lethality. The intrinsic toxicity of PC probably explains the higher modification of the renal parameters of the rats treated with AmB-PC in comparison with those treated by AmB-DC.

Since AmB-DC is not completely absorbed from the intraperitoneal cavity, we checked whether the differences between the toxicities of the different AmB preparations were correlated with differences in the absorption of the polyene. The intraperitoneal injection of AmB-SA50 gave a higher serum concentration of AmB than did the injection of AmB-DC: the AUC of the evolution of serum concentrations of AmB and the serum concentration 24 h after the administration of the drug were not different from those of Fungizone. However, the shapes of the curves are notably different with a significant higher C_{max}, and this may underscore the protective effect of Myrj 59 on AmB toxicity. Moreover, a previous study had already shown that the intraperitoneal administration of AmB-SA50 in mice infected with *C. albicans* was more active than AmB-DC (50). Both of these observations suggest higher systemic exposure, which could explain the higher nephrotoxicity of AmB-SA50. However, the intraperitoneal injection of AmB-Myrj 59 did not give a lower area under the concentration-time curve than AmB-DC. Moreover, we previously showed that the intraperitoneal injection of this AmB formulation was not less active than AmB-DC in infected mice (50). Therefore, AmB-Myrj 59 seems to be a promising alternative to AmB-DC therapy. However, the reason for this improvement remains to be determined.

Several attempts to decrease the nephrotoxicity of AmB have already been proposed. Both the slow infusion of the antibiotic (45, 54) and the administration of the drug on alternate days lower the incidence of nephrotoxicity (32). More recently, sodium loading of the organism which can interfere with the tubuloglomerular feedback response has been shown to reduce the hemodynamic problems associated with antibiotic therapy (2, 8, 9, 22–25, 42). Another approach to decrease AmB nephrotoxicity consists of acting either on the drug itself to obtain less toxic AmB derivatives or on the galenical preparation of the drug. Earlier studies have shown that the methylester derivative of AmB was less nephrotoxic in different animal models (27, 28, 30), but, unfortunately, its clinical use was impaired by neurotoxicity (17). It was also reported that a liposomal formulation of the antibiotic gave some protection against renal toxicity. A recent work evaluating the acute nephrotoxicity of the drug in rabbits shows protection by liposome encapsulation of AmB against the glomerular filtration rate decrease and ion tubular permeability increase induced by the antibiotic, but the excretion of *N*-acetyl-β-D-glucosaminidase (NAG) was increased in the rabbits treated by the liposomal formulation compared with AmB-DC (26). This suggests that the liposomal encapsulation of AmB does not completely prevent the nephrotoxicity of the polyene. Only results of clinical trials give us information about the influence of liposome encapsulation on the chronic nephrotoxicity of the polyene, and they suggest partial improvement of renal tolerance (29, 34–36, 40, 48, 56). Kirsh et al. (29) showed some protection of an emulsion formulation on the AmB concentration ability after a single injection of the drug in mice, but we have no data on nephrotoxicity after several injections. Among the formulations of AmB tested, AmB-Myrj 59 is less nephrotoxic than Fungizone in this model, but AmB-SA50 and AmB-PC are both more nephrotoxic. When compared with the liposomal and the emulsion formulations of the antibiotic, AmB-Myrj 59 could be a good alternative.

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