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RESEARCH PAPER

Effect of Various Lipid–Bile Salt Mixed Micelles on the Intestinal Absorption of Amphotericin-B in Rat

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ABSTRACT

Amphotericin-B (AmB), a lipophilic polyene antibiotic, is the drug of choice in the treatment of many serious mycotic diseases. The solubility and gastrointestinal membrane permeability (P_{app}) of AmB in mixed micellar systems were examined. Membrane permeability was determined using a rat gut perfusion method. The mixed micellar systems studied contained the bile salt in association with fatty acid. All mixed micellar systems enhanced the absorption of AmB relative to the simple micelle. These results have shown that mixed micelles can enhance the absorption of AmB to a greater extent relative to nonmicellar and simple micellar systems. Maximum enhancement (>20-fold) in the rate of AmB absorption was obtained with the sodium desoxycholate/soya lecithin 40:40 mM system. These results offer a possible explanation for the reported enhancement in gastrointestinal absorption of AmB when coadministered with lipid–bile salt mixed micelles, and these systems can be used as a vehicle for designing novel drug delivery systems for poorly absorbable drug(s).

INTRODUCTION

Amphotericin-B (AmB), a lipophilic polyene antibiotic (1), is the drug of choice in the treatment of disseminated fungal infections occurring as a result of reduced immunocompetence seen in chemotherapy and AIDS patients. These infections are frequently fatal. Because of AmB's poor therapeutic index, it is currently used only to treat severe infections. Chemical modifications of AmB have so far proven unsuccessful (2).

A new lipid-based formulation of AmB is available for severe systemic fungal infection when conventional AmB or other antifungal agents are not successful. Systemic fungal infections are most common in immunocompromised patients, those undergoing cancer chemotherapy, or patients with AIDS (3,4).

The bioavailability of poorly absorbable drugs can be improved by increasing the dissolution rate of drugs in the presence of bile salts which are prerequisites for absorption (5,6). Bile salts are biological detergents that have amphiphilic properties and form membrane-toxic simple micelles in aqueous media (7). Bile salts are unstable upon dilution, but in the presence of phospholipids, bile salts form thermodynamically stable mixed micelles which are less toxic (8).

In the gastrointestinal tract (GIT) during lipid digestion, bile salts are found associated with phospholipids, fatty acids, and monoglycerides. In combination with bile salts, these lipoidal compounds form mixed micelles (9). Many studies have reported enhanced bioavailability of drugs when administered in combination with mixed micellar solutions (10–20). Because AmB is poorly absorbable from the GIT, it requires suitable formulation for oral administration.

Therefore, recent attempts have focused on the development of mixed micellar systems as vehicles to improve the absorption of AmB from the GIT in rat. In addition, the effects of lipid–bile salt mixed micelles on absorption with reference to apparent permeability coefficient (21) and rate of absorption (22) were investigated.

MATERIALS AND METHODS

Materials

Sodium taurocholate, sodium cholate, and sodium desoxycholate were obtained from Loba Chemie Pvt. Ltd., India. Oleic acid, monoolein, and soya lecithin were from Fluka India Ltd. All other chemicals were of analytical grade.

Methods

The mixed micellar solutions were prepared by addition of the 40 mM fatty acid to 40 mM solution of bile salt in phosphate buffer at pH 7.4. All systems were prepared by adding the AmB in excess to the system and sonicating the system for 3 min at 37°C. The solutions were then filtered through a 0.45-μm filter (Millipore).

Absorption studies were conducted according to the method of Komiya et al. (23) by perfusing AmB with various systems through the cannulated upper intestine of the anesthetized rat. Male albino rats weighing 200–250 g were used throughout the study.

Absorption studies were performed using a rat intestinal perfusion technique (24). The experimental apparent permeability coefficient was calculated for each system using the following expression:

$$P_{app} = -(Q/2\ \pi rl) \cdot \ln [c(l)/c(o)] \quad (1)$$

where Q is the flow rate (ml sec^{-1}) and $c(l)/c(o)$ represents the fraction of drug remaining in the intestinal lumen of length l and effective lumenal radius r.

The rate of absorption (R) mg sec^{-1} was also calculated for each system according to the following equation:

$$R = c(o) - c(l) \cdot Q \quad (2)$$

where $c(o) - c(l)$ is the difference in concentration of drug entering and leaving the lumen.

Perfusion samples were assayed for AmB with the Beckman DB-G grating spectrophotometer at 382 nm.

RESULTS AND DISCUSSION

The solubility and gastrointestinal absorption of AmB were determined in phosphate buffer solution at pH 7.4; subsequently, similar studies were performed using a range of simple micellar and mixed micellar systems. The solubility of AmB increased quickly and linearly in different bile salt solutions at 30–40 mM concentration of bile salt, as noted in Table 1. Hence, 40 mM concentration of bile salts was chosen for preparing micellar and mixed micellar systems in the present study. The maximum AmB concentration was observed in the system composed of sodium desoxycholate and monoolein. A more than fourfold (280.64 ± 2.64 mg ml^{-1}) increase in solubility as compared to a nonmicellar system (62.84 ± 1.42 mg ml^{-1}) was recorded.

The concentration of AmB was also determined in mixed micellar systems and recorded in Table 2. A more than fourfold increase in solubility of AmB was observed in the system composed of sodium desoxycholate with oleic acid (246.62 ± 3.11 mg ml^{-1}) and soya lecithin (214.78 ± 43.14 mg ml^{-1}). The solubility of AmB in mixed micellar systems after 1 hr at 37°C is also shown in Fig. 1.

It can be inferred that in general, the solubility of AmB improved in the mixed micellar system prepared with sodium desoxycholate with oleic acid in comparison with other systems.

The apparent permeability coefficient (P_{app}) and absorption rate (R) of AmB were determined using a rat intestinal perfusion technique. These parameters were calculated by using Eqs. (1) and (2), respectively, and the data are shown in Table 3. The P_{app} in various mi-

Table 1

Solubility of Amphotericin-B in Bile Salt Solutions at 37°C

Bile Salts	Concentration of Bile Salts (mM)	Concentration of AmB μg/ml (±SD)
Sodium taurocholate	10	60.42 ± 1.22
	20	64.58 ± 2.04
	30	78.46 ± 1.24
	40	107.92 ± 2.62
	50	112.64 ± 3.24
Sodium cholate	10	54.84 ± 1.12
	20	63.72 ± 2.16
	30	71.94 ± 2.84
	40	109.86 ± 3.24
	50	122.92 ± 4.42
Sodium desoxycholate	10	64.45 ± 2.11
	20	70.80 ± 2.23
	30	82.64 ± 3.14
	40	128.68 ± 1.24
	50	132.44 ± 2.62

cellar and mixed micellar systems are also presented in Fig. 2. The P_{app} of AmB was increased more than 12-fold ($3.342 \pm 0.215 \times 10^{-5}$ cm sec^{-1}) in the system (MMS-DA1) composed of sodium desoxycholate and oleic acid relative to the nonmicellar system ($0.246 \pm 0.082 \times 10^{-5}$ cm sec^{-1}). The increase in P_{app} of AmB was also noted in the systems MS-DA ($3.204 \pm 0.604 \times 10^{-5}$ cm sec^{-1}) and MMS-DA2 ($3.208 \pm 0.424 \times 10^{-5}$ cm sec^{-1}) prepared with sodium desoxycholate and sodium desoxycholate with monoolein, respectively, as shown in Table 3. The increase in the P_{app} of AmB in mixed micellar systems was consistent with increase in paracellular membrane permeabilities.

The rate of absorption of AmB was increased by more than 20-fold ($39.764 \pm 5.126 \times 10^{-5}$ mg sec^{-1}) in the system MMS-DA3 composed of sodium desoxycholate with soya lecithin as compared to the nonmicellar system ($1.662 \pm 0.242 \times 10^{-5}$ mg sec^{-1}).

The enhanced gastrointestinal membrane permeability of AmB in the presence of bile salt–fatty acid mixed

Table 2

Concentration of Amphotericin-B in Various Mixed Micellar Systems After 1 hr at 37°C

System Code	Composition	Concentration μg/ml (±SD)
C	Control	62.84 ± 1.42
MMS-TA1	Sodium taurocholate + oleic acid	154.92 ± 3.42
MMS-TA2	Sodium taurocholate + monoolein	166.97 ± 4.62
MMS-TA3	Sodium taurocholate + soya lecithin	148.83 ± 2.64
MMS-CA1	Sodium cholate + oleic acid	182.40 ± 8.24
MMS-CA2	Sodium cholate + monoolein	204.60 ± 4.16
MMS-CA3	Sodium cholate + soya lecithin	191.30 ± 3.24
MMS-DA1	Sodium desoxycholate + oleic acid	314.80 ± 4.56
MMS-DA2	Sodium desoxycholate + monoolein	416.92 ± 6.22
MMS-DA3	Sodium desoxycholate + soya lecithin	340.10 ± 5.14

All systems were prepared in phosphate buffer solution without sodium chloride at pH 7.4. The concentration of each component in the system was 40 mM.

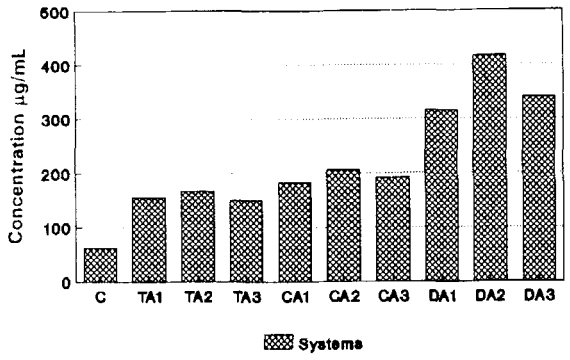

Figure 1. Solubility of AmB in mixed micellar systems after 1 hr at 37°C.

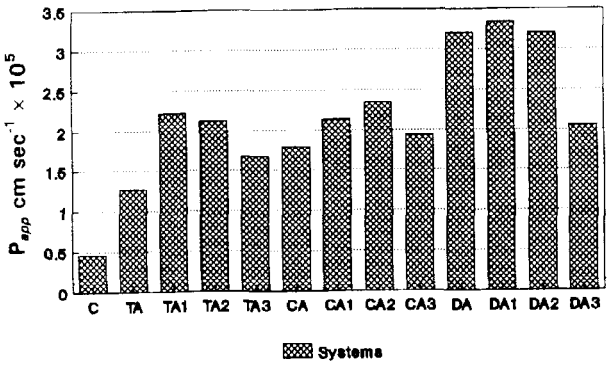

Figure 2. Apparent permeability coefficient (P_{app}) of AmB with mixed micellar systems.

micelles may be attributed to a combined effect of mixed micelles on diffusion through the membrane, as well as to the decreased barrier potential of the GIT in the presence of mixed-micellar-forming constituents.

CONCLUSION

The deep rooted micelles of fungal infestation necessitate antifungal therapy for a prolonged period. The large dose and frequent administration may lead to contraindicative manifestation.

The present study revealed that the bile salt combined with lipid increased the permeability of the intestinal membrane and enhanced the absorption of AmB across the membrane. The enhanced absorbability of AmB via mixed micellar system could bring about a considerable reduction in dose and could successfully produce the effective drug level; thus, AmB may be considered a therapeutic mainstay.

These data provided evidence that the P_{app} of AmB significantly improved in the presence of mixed micellar systems composed of sodium desoxycholate with monoolein/oleic acid, which represent a novel drug delivery system.

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Table 3

Apparent Permeability Coefficient (P_{app}) and Rate of Absorption (R) of Amphotericin-B with Mixed Micellar Systems

System Code	Composition	P_{app} (cm sec^{-1} × 10^5 ± SD)	R (mg sec^{-1} × 10^5 ± SD)
C	Control	0.46 ± 0.082	1.662 ± 0.242
MMS-TA	Sodium taurocholate	1.274 ± 0.148	10.768 ± 1.896
MMS-TA1	Sodium taurocholate + oleic acid	2.216 ± 0.248	19.428 ± 3.116
MMS-TA2	Sodium taurocholate + monoolein	2.118 ± 0.316	20.326 ± 2.484
MMS-TA3	Sodium taurocholate + soya lecithin	1.678 ± 0.184	16.148 ± 3.112
MMS-CA	Sodium cholate	1.794 ± 0.062	12.464 ± 2.248
MMS-CA1	Sodium cholate + oleic acid	2.136 ± 0.088	22.426 ± 4.628
MMS-CA2	Sodium cholate + monoolein	2.342 ± 0.084	26.208 ± 3.484
MMS-CA3	Sodium cholate + soya lecithin	1.938 ± 0.046	22.549 ± 4.626
MMS-DA	Sodium desoxycholate	3.204 ± 0.604	17.942 ± 2.682
MMS-DA1	Sodium desoxycholate + oleic acid	3.342 ± 0.216	35.542 ± 4.408
MMS-DA2	Sodium desoxycholate + monoolein	3.208 ± 0.424	39.764 ± 5.126
MMS-DA3	Sodium desoxycholate + soya lecithin	2.056 ± 0.118	25.942 ± 3.428

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