DRUG DEVELOPMENT AND INDUSTRIAL PHARMACY, 21(17), 2021-2027 (1995)

EFFECT OF VARIOUS LIPID-BILE SALT MIXED MICELLES ON TRANSFER OF AMPHOTERICIN-B ACROSS THE EVERTED RAT INTESTINE

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ABSTRACT

The effect of various lipid-bile salt mixed micelles on the transfer of Amphotericin B (AmB) across the everted rat intestine were investigated.The results indicated that both bile salt solutions and mixed micellar solutions alter membrane permeability and thereby significantly improved transfer of AmB across the membrane. Mixed micelles composed of mono olein and oleic acid with sodium desoxycholate markedly enhanced the intestinal transfer of AmB. On the other hand, Glyceryl monosterate with bile salts caused a small alteration of the permeability. The present study revealed that the therapeutic efficacy of poorly absorbable drugs can be improved by delivering the drug with mixed micelles based microcarrier systems.

INTRODUCTION

The conventional micellar systems are known to enhance the solubility of poorly absorbable drugs, resulting into improved bioavailability. The problem of solubility can be solved by dissolving the drug in micelles. In general, this is a poor solution because micelles are very unstable upon dilution and interact with various blood components after intravenous administration. Mixed micelles (micro-carriers) are now recognized as an absorption potentiator for poorly absorbable drugs[1]. A significant increase in absorptionof heparin, a poorly absorbable macro molecules has been reported when administered entrapped in mixed micellar systems consisted of bile salts-mono olein/oleic acid[2]. Mucosal membrane permeability

was noted to be increased markedly in the presence of fusogenic lipids i.e. mono olein or oleic acid[3]. The bile salts were found to increase the membrane permeability above the critical micelle concentration[4]. The critical micelle concentration of many surfactants is lowered by mixed micelle formation with other molecules[5]. Intestinal absorption of Carboxyfluorescein is much small with Liposomes than with the lipid-surfactant mixed micelle[6]. Diffusion of vit K_1 solubilized by phosphatidylcholine-sodium deoxycholate mixed micelle through porous membranes having various pore characteristics was examined[7]. The Pharmacokinetics and precoagulant activity of a new mixed micellar preparation of Vit K_1 was investigated[8]. According to Borgstrom's theory the breakdown product of fat digestion (i.e. fatty acids and mono-glycerides) are absorbed across the intestinal membrane from a mixed micellar solutions[9]. The mechanism of the enhancement of intestinal absorption of sulfaguanidine by bile salts was investigated together with the poor absorbability of the drug itself[10].

Amphotericin B (AmB) a lipophilic polyene antibiotic is the drug of choice in the treatment of disseminated fungal infections occuring as a result of reduced immunocompetence seen in chemotherapy and AIDS patients and are frequently fatal[11]. Because of its poor therapeutic index, it is currently used only to treat severe infections. Chemical modifications of AmB has so far proven unsuccessful[12].

Therefore, recent attempts have focused to improve the absorption of AmB, a poorly absorbable drug by incorporating it in to various lipid-bile salt mixed micellar systems (MMS). In the present studies, the various mixed micellar systems were prepared and their effects on the transfer of AmB across the everted rat small intestine were investigated.

MATERIALS AND METHODS

Materials

Sodium taurocholate and sodium desoxycholate were obtained from Loba Chemical Pvt. Ltd., India, Glyceryl mono-stearate, oleic acid and mono olein from Fluka, India Ltd.,

Methods

The mixed micellar solutions were prepared by mixing 40 mM lipids and 40 mM bile salts solutions containing AmB in excess and by sonicating the mixture with Soniweld Imeco Ultrasonic for 3 minutes at 37^{o} [13].

Albino Male rats weighing 200 to 250 g (fasted 24 hr.) were anesthetized with ether. After a midline abdominal incision, the small

intestine was removed and the rat was sacrificed. The intestine was rinsed with cold normal saline and cut 15 cm from the pylorus. The 14 cm portion was discarded and the intestine was everted on a glass rod[14]. The segments each of 5 cms in length were obtained. One end of the segment was tied and 2 ml of Krebs-Phosphate buffer (KPB) solution was placed in the intestinal sac is immersed in 20 ml of mixed micellar solution containing AmB at pH 7.4. The flask and its contents are then oxygenated and agitated continuously with Yorco water bath shaker at 37^0 for 1-hr period[15]. The serosal solution was collected and filtered through 0.45 µm millipore filter. The filtered solution was then analyzed for AmB with the Beckman DB-G Grating Spectrophotometer at 382 nm. The amount of AmB in each sample was calculated by means of standard curve. Neither phosphate buffer nor KPB interfered with the assay procedures.

RESULTS AND DISCUSSION

The critical micellar concentration (cmc) values are determined for the sodium taurocholate and sodium desoxycholate from the solubilization of AmB at 37^0 . The solubilization curves for AmB in varying concentration of sodium taurocholate and sodium desoxycholate solution are shown in Figure 1.

It can be observed from the curve (Fig.1) that there in an increase in the solubility of AmB with the increase in the concentration of bile salts. The enhanced solubilization may be attributed to the formation of micelles after a certain minimum concentration of bile salts has been exceeded i.e. CMC. The CMC values for bile salts determined from the solubilization curve are between 30 to 40 mM,

In the study performed by Bates at el it has been found that the bile salts are inherently present in the concentration of 40 mM in the intestine during fat absorption[16]. Hence the same concentration i.e. 40 mM was chosen in the present study.

The various lipid-bile salt mixed micelles solutions wer prepared using phosphate buffer (KH_2PO_4-Na_2HPO_4) without sodium chloride at pH 7.4. Sodium chloride was avoided as it precipitates the AmB from solution and pH 7.4 was chosen to provide maximum stability to the drug.

The composition of the mixed micellar systems and the concentration of AmB in serosal solution after 1 Hr. period are presented in Table 1.

The MMS composed of sodium desoxycholate and mono olein/oleic acid caused an about 4-5 fold increased the transfer of

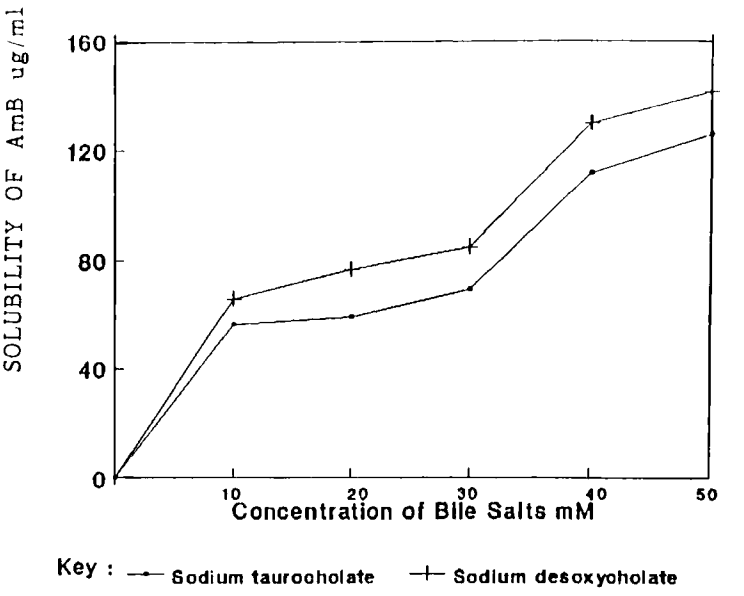

FIGURE 1

Solubility of AmB as a function of bile salts concentration

TABLE 1

Concentration of Amphotericin-B in serosal fluid after incubation for 1 Hr. at 37^0

Systems	Code	Composition[a]	Concentration[b] of AmB µg/ml.
-		Control	52.28
MS-I		Sodium taurocholate	102.04
MS-II		Sodium desoxycholate	122.12
MMS-I		Sodium taurocholate + oleic acid	112.64
MMS-II		Sodium taurocholate + mono olein	118.00
MMS-III		Sodium taurocholate + glycerylmonosterate	110.12
MMS-IV		Sodium desoxycholate + oleic acid	172.06
MMS-V		Sodium desoxycholate + mono olein	182.08
MMS-VI		Sodium desoxycholate +glycerylmonostearate	114.06

a. All systems are prepared in phosphate buffer solution without sodium chloride at pH 7.4. The concentration of each component in the systems (Lipids and bile salt) : 40 mM.

b. Five intestinal segments were used for each study.

AmB across the everted rat small intestine. The enhancement in the drug transport across the membrane may be accounted by three possible mechanism; first, the micelle complex found may have an absorption rate constant different from that of free drug itself to the loss of thermodynamic activity of a drug in the medium, second, bile salts may affect the absorption rate of a drug by a local concentration build up effect such as an accumulation on the absorptive surface and third, it may have a direct effect on the permeability characteristics of the intestinal mucosa there by enhancing the absorption of a poorly absorbable drugs[18]. MMS composed of bile salts along with lipids increased the transfer of AmB across the everted rat intestine are shown in Figure 2.

In the present paper demonstrated that the incorporation of monoolein and oleic acid with bile salts enhanced the permeability of intestinal membrane and such change was mainly because of polar heads present in the fatty acids. It was reported that the fatty acids which do not have polar heads did not increase the permeability of intestinal membrane, although the lipids which have polar heads enhanced the permeability[19].

In biological membranes however, free fatty acids and monoglycerides are considered to play an important role in regulating many physiological functions. It has been reported that free fatty acids and monoglyerides are 2.6% and 0.5% of the total lipid content of rat small intestinal micro villus membranes[20].

The micellar systems composed of bile salt, sodium desoxycholate and sodium taurocholate have also been found to produce 2-3 fold increase in the transfer of AmB. Thus, these systems are also helpful in improving the drug transport to a lesser extent in comparison to the mixed micellar systems.

CONCLUSION

The purpose of the present work was to contrast the effect of lipid-bile salt mixed micelles on the transfer of an antifungal drug AmB across the everted rat small intestine and explored the possible commonality of effects of micellar and mixed micellar systems.

The present studies revealed that the bile salts, (sodium desoxycholate and sodium taurocholate) alongwith lipids (mono olein and oleic acid) increased the permeability of intestinal membrane and enhanced the transfer of AmB across the membrane.

Thus, it may be concluded that mixed micellar systems can be used as vehicle for designing novel drug delivery systems for poorly absorbable drug(s).

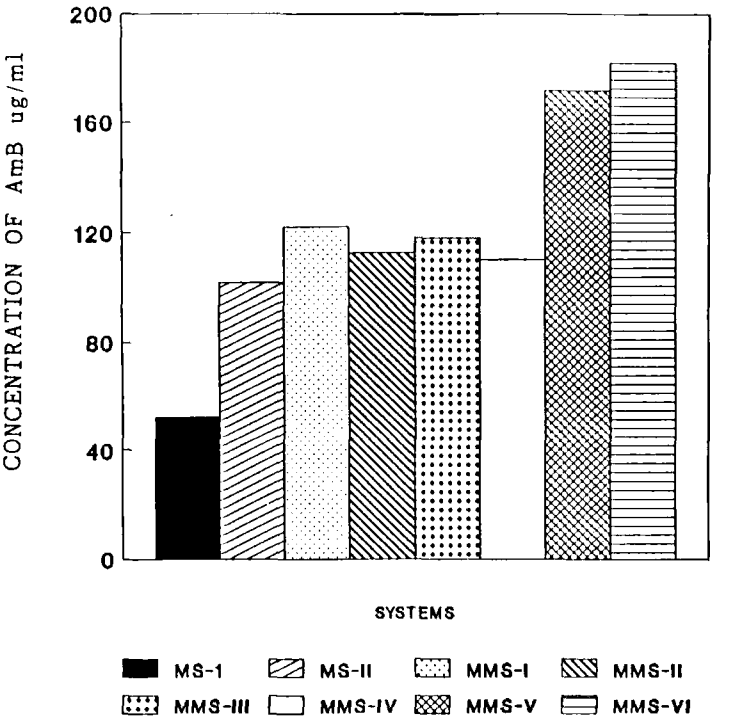

FIGURE 2

Effect of various micellar and mixed micellar based systems on transfer of AmB across the everted rat Intestine

Such studies will significantly contribute to a greater understanding of the complex process of drug transfer by microcarriers across the membrane and additional studies are in progress to further elucidate the observation presented in this communication.

ACKNOWLEDGEMENTS

The authors are grateful to Dr.B.N.Tiwari, Director, Central Instrumental Laboratories, Doctor H.S. Gour Vishwavidyalaya, Sagar for his excellent technical help provided and also wish to thank to Dr. P.N. Raval, Manager, Synbiotics Limited, Baroda for providing gift sample of Amphotericin-B.

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