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IMPROVEMENT OF ABSOLUTE BIOAVAILABILITY OF NORMALLY POORLY ABSORBED DRUGS: INDUCEMENT OF THE INTESTINAL ABSORPTION OF STREPTOMYCIN AND GENTAMYCIN BY LIPID–BILE SALT MIXED MICELLES IN RAT AND RABBIT

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SUMMARY

The effect of lipid–bile salt mixed micelles on the intestinal absorption of streptomycin and gentamycin was investigated in the loop of small and large intestine of rat. While bile salts micellar solutions did not enhance the absorption of aminoglycosides, their mixed micellar solutions including monoolein, oleic or lauric acid markedly enhanced their absorption. However, lecithin–bile salt mixed micellar solution did not affect the absorption. Pretreatment with mixed micellar solutions showed neither a direct action on the mucosal membrane nor a transient increase in permeability to aminoglycosides.

Improvement of bioavailability in the rabbit was evaluated using various formulations and different routes of administration. In the rectal administration, not only mixed micellar solutions but also lyophilized mixed micelles powder improved bioavailability. The duodenal administration of mixed micellar solution, however, was not effective, indicating that enhanced absorption of drugs by mixed micelles may be more pronounced with rectal administration.

INTRODUCTION

Administration of very poorly absorbable drugs which are water-soluble has been limited mostly to parenteral route. Some of them (gentamycin, cefazolin, etc.) have been administered by intramuscular injection. Some reports showed a causal relationship between quadriceps contracture and intramuscular injection (Gunn, 1964; Saunders et al., 1965), and also in Japan frequent injections caused muscular atrophy (Moritani, 1977). To avoid the risk and inconvenience of frequent injections, it is highly desirable to render them absorbable from the intestine so that they can be administered orally or rectally.

It is well known that ingested fats, mainly triglycerides of long-chain fatty acid, are emulusified in the gut lumen and subsequently hydrolyzed by pancreatic lipase to water-

insoluble monoglyceride and fatty acid. These insoluble products are solubilized by bile salts, which form water-soluble 'mixed micelles' and are easily absorbed (Carey et al., 1970; Isselbacher and Ockner, 1974; Dietschy and Westergaard, 1976). Also, it has been demonstrated that the absorption of a macromolecular substance, heparin, may be increased by the addition of monoolein mixed micelles (Muranishi et al., 1977).

In the present investigation, streptomycin and gentamycin were chosen as poorly absorbable small molecular drugs. These aminoglycosides are ordinarily not absorbed from the gastrointestinal tract to any significant degree, and when parenterally administered, the total urinary excretions are about 85% of administered gentamycin in 24 h and about 65% of administered streptomycin in 12 h (Regamey et al. 1973; Adcock and Hittig, 1946). Any increase in absorption of these almost totally unabsorbed drugs would therefore be easier to demonstrate and be of greater practical interest than the enhanced absorption of an already well-absorbed drug.

The present work was undertaken in order to induce the absorption of the aminoglycosides in the presence of mixed micellar solutions.

MATERIALS AND METHODS

Materials. Streptomycin sulfate and gentamycin sulfate were supplied from Takeda Chemical Industries and Shionogi Co. respectively. Monoolein of high purity grade (Nikko Chemicals) was used. Sodium glycocholate and sodium taurocholate were synthesized according to the method of Norman (Norman, 1955). The purity of both bile salts was checked by thin layer chromatography and infrared spectroscopy. All other chemicals were of reagents grade.

Preparation of test solutions. Mixed micellar solutions were prepared by dissolving lipid in pH 65. isotonic phosphate buffer containing bile salt and drug. A clear solution was obtained upon sonicating the mixture at 37°C for 3 min with Ohtake sonicator model 5202.

Preparation of lyophilized mixed micelle powders. Drug was dissolved in 160 mM mixed micellar solution prepared with distilled water. The solution was lyophilized with ATMO-VAC freeze dryer No. 5003 and kept in desiccator.

Procedure of in situ absorption experiment. Male Wistar albino rats weighing 200–250 g were anesthetized with intraperitoneal pentobarbital sodium, 8 mg/200 g body weight. The intestine was exposed through midline incision, then a closed loop of the entire small or large intestine was prepared by ligation at proximal and distal ends. The bile duct was not ligated. The test solution was introduced into the intestinal loop in the volume of 4 ml to the small intestine or 2 ml to the large intestine per 200 g body weight.

Pretreatment experiment with mixed micellar solution was performed according to the following procedure. The mixed micellar solution, not containing drug, was first introduced into the loop. After 1 h pretreatment the entire solution in the intestine was forced out by the aid of syringe air followed with saline. After this procedure, drug dissolved in pH 6.5 isotonic buffer solution was infused into the loop as usual experiment.

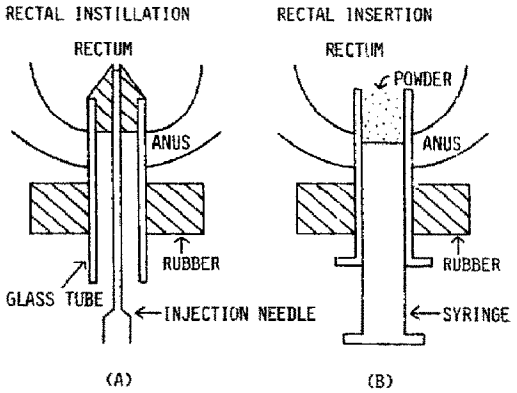

Fig. 1. The implements for rectal administration to rabbit. These were tied to the loins of rabbit with string during the experiment.

Procedure of in vivo absorption experiment. Male rabbits weighing 2–2.5 kg were used in the cross-over experiment to compare the bioavailabilities following the intestinal administration of various formulations. For the duodenal administration a catheter was introduced into the duodenum through the stomach and the test solution containing gentamycin was instilled in the volume of 10 ml/2.5 kg body weight. Rectal administration was done by the aid of implements made of syringe and rubber, etc., as shown in Fig. 1. The drug solutions were instilled through the injection needle in a volume of 5 ml/2.5 kg as depicted in Fig. 1A. Lyophilized mixed micelle powders were administered into the rectum through the syringe as represented in Fig. 1B. After administration blood samples were collected from ear vein and plasma concentration was determined.

Analytical method. The antimicrobial activities of aminoglycosides in plasma were determined by the disc plate method using *Bacillus subtilis* PCI 219 as the test organism.

RESULTS

Absorption of aminoglycosides from the intestinal loop of rat

The following mixed micelle adjuvants were selected in this study to promote the absorption of aminoglycosides: sodium glycocholate (Na-GC) or sodium taurocholate (Na-TC) as surfactant, and monoolein, oleic acid, lauric acid or lecithin as lipid. Plasma concentration–time profile of streptomycin after administration to the small and large intestinal loop is shown in Fig. 2. Administered doses were 8 mg and 4 mg to the small and large intestine respectively per 200 g body weight. The plasma peak is seen at 2–3 h after the administration of 40 mM monoolein–taurocholic acid mixed micellar solution to the small intestine and at 15 min after the administration of 10 mM mixed micellar solution to the large intestine. Therefore, plasma concentrations 1 h (increasing portion) and 15 min after administration to the small and large intestine respectively were chosen to compare various formulations.

The effect of the concentrations of taurocholic acid and monoolein on the absorption of streptomycin is shown in Fig. 3. An enhancement of absorption to effective degree

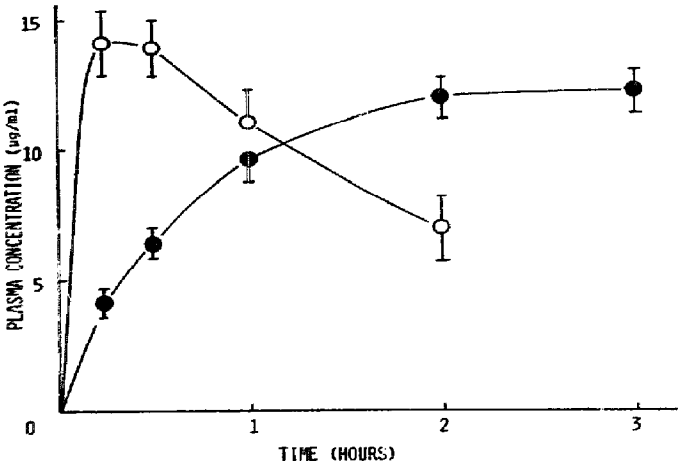

Fig. 2. Plasma concentration–time profile of streptomycin after administration to the small and large intestinal loop. Streptomycin was administered to the small intestine as 40 mM mixed micellar solution in the dose of 8 mg/200 g body weight and to the large intestine as 10 mM mixed micellar solution in the dose of 4 mg/200 g body weight. Each value is the mean ± S.E.M. for 4 animals. ○, large intestine; ●, small intestine.

occurred above 40 mM and 10 mM in the small and large intestine respectively. Therefore, the absorption experiments below were performed using 40 mM and 10 mM mixed micellar solution to the small and large intestine respectively.

Table 1 shows 1 h plasma concentration of streptomycin after administration to the small intestine in various formulations. Plasma concentration was negligible with no adjuvant in buffer solution and a small increase of plasma concentration was seen in the case of 40 mM bile salt micellar solution. But a significant increase in the 1 h sample was elicited after administration of 40 mM lipid–bile salt mixed micellar solutions except

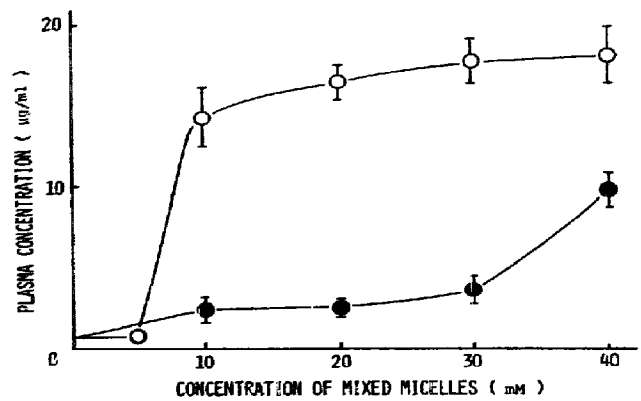

Fig. 3. Effect of the concentrations of taurocholic acid and monoolein on the absorption of streptomycin. The plots are 1 h and 15 min plasma concentrations after administration to the small and large intestine respectively. Administered dose is the same as Fig. 2. Each value is the mean ± S.E.M. for 4 animals. ○, large intestine; ●, small intestine.

TABLE 1

PLASMA CONCENTRATION OF STREPTOMYCIN AT 1 H AFTER ADMINISTRATION OF VARIOUS FORMULATIONS INTO THE SMALL INTESTINE

Composition [a]	μg/ml ± S.E.M. [b]	(1 h)
None	<1.5	(5) [c]
40 mM Na-GC	3.8 ± 0.2	(4)
40 mM Na-TC	3.8 ± 0.2	(5)
40 mM Na-GC + 40 mM monoolein	9.8 ± 0.3	(4)
40 mM Na-TC + 40 mM monoolein	11.6 ± 0.6	(4)
40 mM Na-GC + 40 mM oleic acid	17.6 ± 0.5	(6)
40 mM Na-TC + 40 mM oleic acid	11.9 ± 1.6	(7)
40 mM Na-GC + 40 mM lauric acid	11.7 ± 2.7	(4)
20 mM Na-TC + 40 mM monoolein	2.4 ± 0.3	(5)
30 mM Na-TC + 40 mM monoolein	2.5 ± 0.3	(5)
40 mM Na-GC + 40 mM lecithin	<1.5	(3)

[a] All formulations were prepared in pH 6.5 isotonic phosphate buffer. Administered dose of streptomycin was 8 mg/200 g rat.
[b] Figures represent the mean ± standard errors.
[c] Figures in parentheses refer to the number of animals.

when lecithin was used as lipid. The mixed micellar solutions gave 3 times higher plasma concentration than the micellar solutions. When concentration of bile salt was decreased to 20 or 30 mM, the enhancing effect of mixed micellar solution almost disappeared.

Table 2 shows the 1 h plasma concentration of gentamycin after small intestinal administrations at a dose of 4 mg/200 g body weight. A similar increase of plasma concentration to that of streptomycin by 40 mM micellar and mixed micellar solution shown in Table 1 was observed.

The effect of mixed micelles on the absorption of aminoglycosides in the large intestine including the rectum was also examined. Administered doses were 4 mg for streptomycin and 2 mg for gentamycin per 200 g body weight. As shown in Table 3, they were not absorbed from buffer solution and there was no increase of plasma concentra-

TABLE 2

PLASMA CONCENTRATION OF GENTAMYCIN AFTER ADMINISTRATION TO THE SMALL INTESTINE

Administered dose of gentamycin was 4 mg/200 g rat.

Composition	μg/ml ± S.E.M.	(1 h)
None	1.2 ± 0.1	(4)
40 mM Na-GC	4.7 ± 0.8	(4)
40 mM Na-GC + 40 mM monoolein	8.4 ± 0.6	(5)
40 mM Na-GC + 40 mM oleic acid	8.9 ± 1.4	(4)

TABLE 3

PLASMA CONCENTRATION OF AMINOGLYCOSIDES AFTER ADMINISTRATION TO THE LARGE INTESTINE

Administered doses were 4 mg for streptomycing and 2 mg for gentamycin per 200 g rat respectively.

	Composition	μg/ml ± S.E.M.	(15 min)
Streptomycin	None	<1.5	(4)
	10 mM Na-GC	<1.5	(4)
	10 mM Na-GC + 10 mM oleic acid	14.9 ± 1.1	(4)
Gentamycin	None	0.4 ± 0.0	(4)
	10 mM Na-GC	0.4 ± 0.0	(4)
	10 mM Na-GC + 10 mM monoolein	11.5 ± 0.7	(4)

tion after administration of 10 mM bile salt micellar solution. But a significant enhancement in the 15 min concentration was observed after administration of 10 mM oleic acid– or monoolein–bile salt mixed micellar solution. Although the concentrations of bile salt and lipid were a quarter of those in the small intestine, the enhancement of absorption was clearly elicited.

Pretreatment experiment with mixed micellar solution

To observe the permeability change by mixed micelles, several pretreatments with mixed micellar solutions were performed. Plasma concentrations of aminoglycosides after 1 h pretreatment in the small or large intestine are shown in Table 4. It is noted that none of the pretreatments resulted in an increase of plasma concentration. Therefore, it is suggested that the enhancement of absorption may not be caused by mucosal damage, and even if the mucosal permeability was accelerated the effect would not last longer.

TABLE 4

EFFECT OF PRETREATMENT WITH MIXED MICELLAR SOLUTIONS ON THE ABSORPTION OF AMINOGLYCOSIDES FROM THE INTESTINE

After 1 h pretreatment with mixed micellar solution, pH 6.5 buffer, solution containing drug was administered. Administered doses were 8 mg for streptomycin and 2 mg for gentamycin per 200 g rat respectively.

Composition of pretreatment solution	μg/ml ± S.E.M.	
Small intestine	Streptomycin	(1 h)
None	<1.5	(5)
40 mM Na-TC + 40 mM monoolein	<1.5	(4)
40 mM Na-GC + 40 mM oleic acid	<1.5	(4)
Large intestine	Gentamycin	(15 min)
None	0.4 ± 0.0	(4)
10 mM Na-GC + 10 mM monoolein	0.4 ± 0.1	(4)

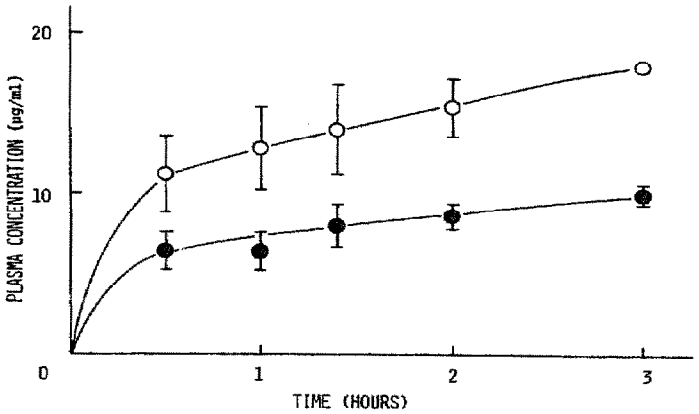

Fig. 4. Comparison of gentamycin absorption from the mixed micellar solution between closed and distal-free loop of the duodenum. Administered dose of gentamycin was 4 mg/200 g rat. Each value is the mean ± S.E.M. for 4 animals. ○, closed loop of duodenum; ●, distal-free of duodenum.

Comparison of gentamycin absorption between closed loop and distal-free loop of rat duodenum

Since the closed loop in the above experiments was prepared by ligation at the proximal and distal ends of the small intestine, the test solution did not move. In the physiological state, however, there is ordinary transit in the gastrointestinal tract. Therefore the absorption of gentamycin from closed loop and distal-free loop was compared.

Gentamycin, 4 mg/200 g body weight of rat, was administered as 40 mM monoolein–taurocholic acid mixed micellar solution into the closed loop of the duodenum or the distal-free duodenum in the volume of 1 ml. Plasma concentration in the case of distal-free loop was about one-half lower than that of closed loop as shown in Fig. 4. It is suggested that the enhancement effect in the small intestine by mixed micelles in vivo would be smaller than expected from the closed loop experiment, because the solution introduced moves through the intestinal tract in vivo.

Effect of mixed micelles and their lyophilized powder in vivo on the absorption of gentamycin in rabbit

In order to know whether mixed micelles increase the intestinal absorption of aminoglycoside in rabbit as in rat, gentamycin was administered in vivo to rabbit with monoolein–taurocholic acid mixed micelles. Gentamycing 10 mg/2.5 kg body weight was administered intravenously and plasma concentration measured periodically to obtain standard AUC. Gentamycin was administered in the dose of 30 mg/2.5 kg body weight into the duodenum or the rectum in cross over fashion.

Fig. 5 shows plasma concentration of gentamycin after intravenous injection and duodenal instillation. After intravenous injection plasma concentration was decreased monoexponentially. Duodenal instillation of 10 ml of 40 mM mixed micellar solution did not show remarkable increase in plasma concentration. The control experiment which is duodenal instillation of its buffer solution showed only a little absorption, although it was not shown in the figure.

Plasma concentration after the rectal administration of 5 ml of 10 mM or 20 mM

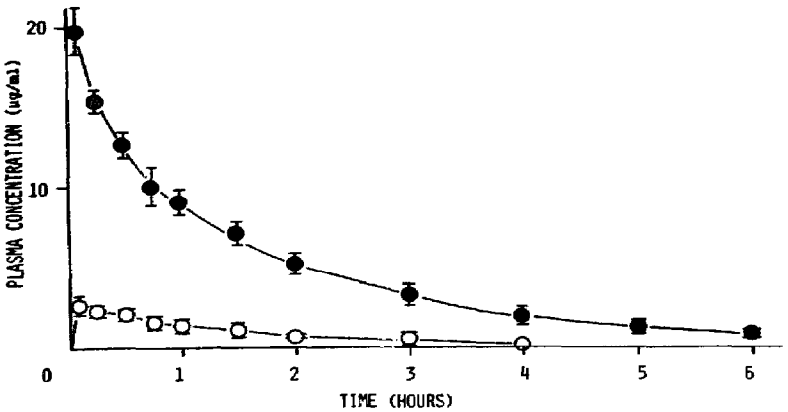

Fig. 5. Plasma concentration of gentamycin after intravenous injection and duodenal instillation. Each value is the mean ± S.E.M. for 3–6 animals. •, intravenous injection, gentamycin 10 mg/2.5 kg; ○, duodenal instillation, gentamycin 30 mg/2.5 kg as 40 mM mixed micellar solution.

mixed micellar solution is shown in Fig. 6. Contrary to the duodenal instillation, plasma concentration was markedly increased with increase of mixed micelles concentration, and their peaks of plasma concentration were already present within 15–30 min after administration.

For the pharmaceutical application of mixed micelles, we tried to dry these solutions to make powder by lyophilization. The white fine powder obtained could be easily dispersed in buffer solution. The results obtained from administration of the powder are shown together in Fig. 6. The administered dose of gentamycin was 15 mg/2.5 kg body weight, one-half of the above dose, and the amount of lyophilized mixed micelles corre-

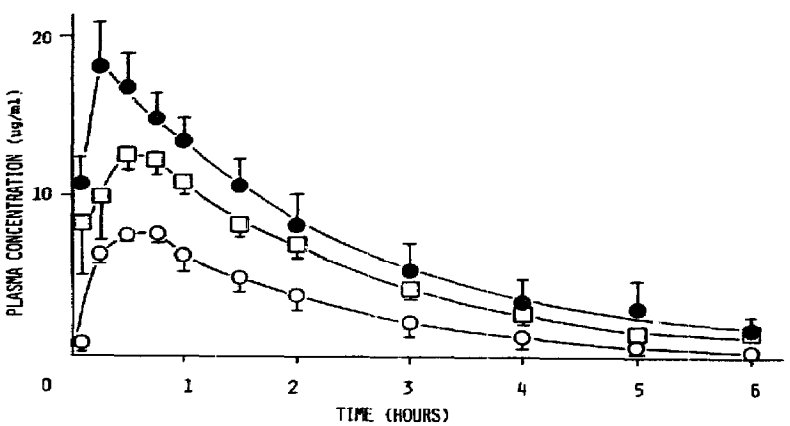

Fig. 6. Plasma concentration of gentamycin after rectal administration. Each value is the mean ± S.E.M. for 3 animals. Rectal instillation: ○, gentamycin 30 mg/2.5 kg rabbit as 10 mM mixed micellar solution; •, gentamiycin 30 mg/2.5 kg rabbit as 20 mM mixed micellar solution. Rectal insertion: □, gentamycin 15 mg/2.5 kg rabbit with powdered mixed micelles.

TABLE 5

BIOAVAILABILITY OF GENTAMYCIN IN VARIOUS PREPARATIONS AND ROUTES OF ADMINISTRATION

Preparation	Bioavailability (% ± S.E.M.) from AUC	
Intravenous injection	100	
Duodenal instillation		
None	4.1 ± 1.6	(3)
40 mM mixed micellar solution	6.8 ± 0.5	(3)
Rectal instillation		
None	0.1 ± 0.1	(3)
10 mM mixed micellar solution	18.4 ± 3.4	(3)
20 mM mixed micellar solution	44.6 ± 4.9	(3)
Rectal insertion		
Powdered mixed micelles	58.1 ± 8.2	(3)

sponds to that of 5 ml of 10 mM mixed micellar solution. Results with this powder showed the most increased plasma concentration of gentamycin.

The area under the curve of plasma concentration (AUC) was determined by trapezoidal approximation to extrapolate to infinity. Results of the bioavailability with AUC of intravenous injection as 100% are summarized in Table 5. Rectal administration of mixed micellar solution potentiated the absorption of gentamycin much more than the duodenal instillation, and the absolute bioavailability of rectal administration as 20 mM mixed micellar solution reached about 45%. Such enhancement of absorption was more pronounced in the case of lyophilized mixed micelles which indicated about 58% bioavailability of the aminoglycoside.

DISCUSSION

It has been recognized that bile salts are essential substances in the absorption of lipids (Isselbacher and Ockner, 1974; Dietschy and Westergaard, 1976). The effect of bile salts on drug absorption has been investigated (Gibaldi, 1970; Gibaldi and Feldman, 1970a, b; Kakemi et al., 1970a, b). Bile salts, sodium glycocholate and sodium taurocholate somewhat enhanced the absorption of phenol red (Kakemi et al., 1970a), which is a poorly absorbable compound. This enhancing effect is probably same as the effect of polysorbate 80 on the absorption of micelle-free drugs (Kaneda et al., 1974). In this investigation bile salts also caused an enhanced absorption of aminoglycosides to small degree. On the other hand, monoolein– or oleic acid–bile salt mixed micelles much more enhanced the absorption of aminoglycosides than bile salt alone. This observation is similar to the enhanced absorption of heparin in the presence of mixed micelles (Muranishi et al., 1977).

The enhancing effect was not caused by 1 h pretreatment with mixed micellar solutions. Considering the enhancing effect of bile salts on the absorption of phenol red in the previous report (Kakemi et al., 1970a), it is suggested that mixed micelles may not have a strong direct action on the mucosal membrane and their action on the membrane

may be easily reversible. Sodium glycocholate and taurocholate themselves are known to be extremely less harmful to the intestinal tract contrary to dihydroxy bile salts such as deoxycholate and chenodeoxycholate (Martin and Phillips, 1972). Our microscopic observation supports there being no apparent damage to the mucosal cells from these mixed micelles (to be published). These findings seem to be important because mixed micelles do not cause much damage to intestinal mucosa and the combinations of bile salt and certain lipids can induce a remarkable increase in the absorption of aminoglycosides.

In the in vivo experiment with rabbit, gentamycin was unexpectedly not well absorbed when mixed micellar solution was administered into the duodenum. From Fig. 3 it is considered that the concentration of mixed micelles need to be kept at high concentrations, such as 40 mM, within the small intestinal lumen. Mixed micelles would be, however, diluted during transit in the intestinal lumen, and therefore the enhancing effect is supposed to be decreased. On the other hand, the rectal instillation of mixed micellar solution to rabbit caused a remarkable increase in the absorption of gentamycin. Consequently it is considered that rectal administration with mixed micelles is more advantageous for potential absorption of poorly absorbable drugs than oral administration for the following two reasons: first, the enhanced absorption by mixed micelles is essentially more effective in the large intestine than the small intestine. Their effect in the large intestine of rat can be caused at one-fourth of their concentration in the small intestine. Second, the influence of the large intestinal motility on the movement of rectal contents is small, and in addition fluid which is secreted at the large intestine is considerably small; therefore, mixed micelles are not expected to be diluted in the large intestinal lumen.

For the pharmaceutical application, such a solution is not always practically convenient to administer, and mixed micelles will be rather unstable. Therefore lyophilization of mixed micellar solutions was attempted to satisfy these requirement, and mixed micelle powder was obtained. This powder was very easily dispersed in water and reproduced mixed micellar-like solution. Rectal administration of mixed micelle powder including gentamycin resulted in obviously marked increase of absorption. In conclusion, the rectum is a suitable place in which administration of mixed micelles can induce the absorption of normally poorly absorbable drugs.

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