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Bile Salt-Acylcarnitine Mixed Micelles as Potential Absorption Enhancer on Intestinal and Nasal Drug Delivery

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1. INTRODUCTION

Many oral, rectal or nasal formulations of poorly absorbable drugs require absorption enhancing agents to generate therapeutically effective systemic plasma levels. Of these absorption promoters bile salts, surfactants, chelating agents, fatty acids, phospholipids, enamines, mixed micelles, fusidic acid derivatives, medium chain fatty acid salts, and fatty acid derivatives of carnitines seemed to be candidates for further studies, due to their effectiveness and very mild mucosal damage potential[1] (Table I). Such absorption enhancing agents have been utilized extensively to increase mucosal permeability or to lower the physical and enzymatic barrier function of the nasal and intestinal mucosa. Although the precise mechanism of action of absorption enhancers is not known, it is thought to be based on reduction of mucus viscosity, expanding the dimension of the paracellular pathway to solute transport, inhibition of proteolytic enzymes, enhancement of membrane fluidity and reverse micelle formation in the cell membrane, creating transient pores. Therapeutic agents like antibiotics, peptides and proteins exhibit poor bioavailability following oral and nasal administration because of ionic charge, hydrophilic properties, high molecular weight, poor penetration of the intestinal and nasal mucosa and poor resistance against proteolytic enzymes.[2,3] As a consequence, these therapeutic entities are most commonly administered by parenteral routes. Although parenteral administration may be acceptable in acute situations, it is inconvenient for self-administration and undesirable for chronic administration. Consequently, investigation of alternative noninvasive methods, such as intranasal, pulmonary, oral, transdermal, vaginal or rectal routes of delivery for such drugs have received increasing attention.[4] However, in the absence of an absorption enhancer, the alternative routes are much less efficacious than parenteral route.

Among absorption enhancing adjuvants, acylcarnitines were known as potential absorption enhancing agents for drugs that are poorly absorbed from the gastrointestinal (GI) tract. Particularily, palmitoyl-DL-carnitine chloride (PCC) has been reported to be the most effective absorption promoting adjuvant following oral and rectal administration. PCC was found to significantly promote duodenal absorption of somatostatin analogue. PCC at a concentration of 10 mg/m*l*, also significantly enhanced the rectal absorption of cefoxitin, gentamicin, cytarabine, somatostatin analogue and α-methyldopa which may be partly attributable to slight loss of epithelial cells.[5] Further PCC was a potent absorption enhancer for gentamicin when administered vaginally to rats[6] and for human growth hormone (hGH) when administered by the nasal route.[7] But PCC alone did not substantially influence the absorption of ^{3}H-dihydroergotoxine (HDHE) from the rat intestine and did not increase HDHE activity in plasma.[8] Peroral coadministration of PCC and cefoxitin also provided very little impro-

Table I—Classes of Enhancers of Intestinal Drug Absorption and Some of Their Representatives

1. Non-steroidal anti-inflammatory drugs and derivatives
   - sodium salicylate
   - sodium 5-methoxysalicylate
   - indomethacin
   - diclofenac
2. Surfactants
   - nonionic: polyoxyethylene ethers
   - anionic: sodium laurylsulfate
   - cationic: quaternary ammonium compounds
3. Bile salts
   - dihydroxy bile salts: sodium deoxycholate
   - trihydroxy bile salts: sodium cholate
4. Sodium tauro-24,25-dihydrofusidate (STDHF)
5. Medium-chain fatty acids
   - octanoic acid
   - decanoic acid
6. Medium-chain glycerides
   - glyceryl-1-monooctanoate
   - glyceryl-1-decanoate
7. Enamines
   - DL-phenylalanine ethylacetoacetate enamine
8. Mixed micelles
   - glyceryl monooleate + sodium taurocholate
   - linoleic acid + HCO 60
9. Calcium binding agents
   - EDTA
10. Phenothiazines
    - chlorpromazine
11. Liposomes
12. Azone
13. Fatty acid derivatives of carnitine and peptides
    - palmitoyl-DL-carnitine
    - N-myristoyl-L-propyl-L-glycinate
14. Saponins
15. Concanavalin A
16. Phosphate and phosphonate derivatives
    - DL-α-glycerophosphate
    - 3-amino-1-hydroxypropylidene-1,1-diphosphonate (APD)
17. Polyacrylic acid
18. Diethyl maleate (DEM) and diethylethoxymethylene malonate (DEEMM)
19. Methylxanthine: caffeine

vement in cefoxitin bioavailability in dogs. These results appeared to indicate that the extent of enhancing absorption in response to PCC may be drug dependent and site dependent. Among the adjuvants used in the nasal and oral formulations of drug, bile salts, partly because of their natural occurrences, have been investigated extensively as potentially safe absorption promoters. The absorption of sulfaguanidine and phenol red in the small intestine was enhanced by bile salts at or above their critical micellar concentration (CMC).[9] When sodium glycocholate (NaGC) was added at a concentration of 1% w/v to the insulin solution at pH 7.4, the absorption of insulin through the nasal mucosa was enhanced.[10] At 0.5% w/v concentration the toxicity of NaGC to the nasal mucosa was much less than that observed for other surfactants, however this concentration resulted in only 7-8% Met-hGH nasal bioavailability.[11] Consequently formulations containing adjuvant combinations are often necessary to improve the extent of drug absorption. By using mixed micellar solutions containing 0.2-2% w/v of a fusogenic lipid, solubilized by the addition of a surfactant such as NaGC, absorption enhancement of cefmetazole, cyclosporine A and streptomycin can be achieved in the colon,[12] rectum[13] and small intestine.[14] Unsaturated fatty acid-bile salt mixed micelles, particularly linoleic acid-NaGC mixed micelles, also enhanced the *in situ* nasal absorption of [D-Arg^2]kyotorphan[15] and *in vivo* nasal absorption of insulin with a mild to moderate morphological alteration.[16]

In the present study, we therefore initially investigated the site dependence of absorption of azidothymidine (AZT) and acyclovir in the absence of adjuvants, the effect of various acylcarnitines, NaGC and their mixed micelles on the nasal and intestinal absorption of acyclovir and examined specificity, site dependence, concentration dependence and reversibility of absorption promoting actions by using an *in situ* recirculating perfusion method. Acyclovir, AZT and phenol red

were selected as model compounds. Acyclovir is an acyclic analogue of guanosine that is incompletely absorbed after oral administration with about 20% bioavailability in humans[17] and about 6% bioavailability in rats.[18] Poor mucosal absorption of acyclovir might be due to poor water solubility (1.3 mg/ml) as well as low lipid solubility (octanol/water partition coefficient 0.018).[19] Recently the drug has been introduced for treating herpes simplex and varicella zoster infections.[20] Secondly, the colonic absorption enhancement of AZT, which has a moderate water and lipid solubility (solubility in water of 30 mg/ml and octanol/water partition coefficient of 1.05),[21] was investigated by using PCC and PCC-NaGC mixed micelles. AZT was designated as an orphan drug by the Food and Drug Administration for use in the management of human immunodeficiency virus (HIV) infection although other agents (e.g., dideoxyinosine, dideoxycytidine) are currently in phase II evaluation and may be introduced to lower the mortality and frequency of opportunistic infections in a selected group of individuals with AIDS and/or AIDS related complex.[22-24] Thirdly, in order to determine the mechanism of absorption enhancement of PCC-NaGC mixed micelles, phenol red was used to model a high water soluble, poorly absorbed drug. The final objective was to develop formulations to improve the intestinal and nasal absorption of antiviral agents, which could result in increased bioavailability and decreased frequency of dosing or dose size.

2. EXPERIMENTAL METHODS

2-1. Preparation of Mixed Micellar Solution

Acylcarnitines were added to isotonic phosphate buffer solution (IPBS) consisting of 0.033 M $NaH_2PO_4 \cdot H_2O$, 0.033 M Na_2HPO_4 and 0.08 M NaCl adjusted to pH 6.5 with H_3PO_4, which contained 10 mM NaGC and drug. This solution was stirred continuously and finally sonicated at room temperature for 5 min with a Branson sonicator (model 3200, Branson Co., Shelton, CT). A mixed micellar solution containing 10 mM NaGC, a concentration above its critical micellar concentration (about 9 mM in this buffer), formed a clear solution. All the solutions were used immediately after preparation and maintained at 37± 0.5℃.

2-2. *In situ* Intestinal Perfusion Method

Male Sprague-Dawley rats weighing 220 to 300g were used to measure the intestinal absorption of acyclovir in the presence of selected absorption enhancers. Three rats were used for absorption study in each intestinal segment, each rat being used for one segment and experiment only. The rats were fasted for about 18 hr prior to the experiments but water was allowed *ad libitum*. Rats were anesthetized by an intraperitoneal injection of 30 mg/kg sodium pentobarbital. The *in situ* perfusion technique was conducted by modification of method described Farra, *et al.*[25] Details of this experimental method were as followings. The intra-abdominal temperature was maintained at 37℃ by irradiation with a 100-W lamp (tungsten). A segment of the intestine was cannulated proximally and distally so that perfusate entering the proximal cannula traversed the intestinal segment and left via the distal cannula. The proximal and distal cannulas were made of polyethylene tubing, PE-160 and PE-220, respectively. The cannula was tied in place with a loop of silk suture placed tightly around the intestine, forming a seal that prevented perfusate from leaking through the cannula junctions. The distal cannula had a relatively large internal diameter to allow a relatively high rate of perfusion with minimal back pressure in the lumen. The cannulated intestinal segment was placed in the peritoneal cavity such that it was not kinked or twisted and the midline abdominal incision was covered with gauze pads, which was moistened

frequently with isotonic phosphate buffer solution (IPBS) to maintain the tissue in a reasonable state of hydration. The four segments of a rat intestine were cannulated proximally and distally and were defined as duodenum (D, pyloric sphincter to the ligament of Treitz), upper jejunum (UJ, the next 15 cm segment following the ligament of Treitz), the combined lower jejunum and ileum (LJ, 15 cm segment ending ileo-cecal junction) and colon (C, from the cecal-colonic junction to the rectum). Drug solution was placed in a reservoir which was water-jacketed at 37± 0.5℃ via a circulating water-bath. A magnetic stir bar used to keep the contents of the reservoir well mixed. The cannulated segment was first flushed by the IPBS (37℃) to remove traces of gut contents until the perfusate was clear and clean and subsequently perfused with 10 m*l* of drug solution (also 37℃) by single pass perfusion in order to displace the remaining IPBS solution in the GI tract. The flushing process performed manually by a syringe attached to the proximal cannula, was implemented slowly so as not to expand the intestine and to cause damage due to hydrostatic pressure. The remainder of drug solution in the loop was then expelled by air from the attached syringe. The tubings attached to the inflow and outflow cannula were then transferred to a beaker containing 20 m*l* of fresh drug solution (37℃) and the perfusing fluid was perfused with 20 m*l* of drug solution containing selected absorption promoter for 2 hr by means of a peristaltic pump (Harvard Apparatus, Millis, MA) at a flow rate of 2.0 m*l* per min. The perfusate was very stable in the intestine and was sampled by removing 0.2 m*l* solution at 15 min intervals for up to 120 min.

2-3. *In situ* Nasal Perfusion Method

The rat *in situ* experimental model that was developed by Hirai, *et al.*[26] and Huang *et al.*[27] was used to study the effects of potential absorption enhancers on the nasal absorption of acyclovir. The surgical pretreatment for *in situ* nasal (N: nasal cavity) absorption study was the same as described previously in the *in situ* intestinal perfusion method. After an incision was made in the neck, the trachea was cannulated with a polyethylene tube (PE-200, Intramedic, Clay Admas, NY) to maintain respiration. Another PE-200 tube was inserted through the esophagus toward the posterior part of the nasal cavity. The passage of the nasopalatine tract is sealed with an adhesive agent (instant jet, Carl Goldberg Models Inc., Chicago, IL) to prevent the drainage of the drug solution from the nasal cavity into the mouth. The cannula served to deliver the drug solution to the nasal cavity. During the perfusion study, a funnel was provided between the nose and reservoir to minimize the loss of drug solution. The drug solution to be evaluated is placed in the reservoir, which is maintained at 37± 0.5 ℃, and circulated through the nasal cavity of the rat by means of a peristaltic pump at a flow rate of 2.0 m*l* per min. A constant volume (20 m*l*) of drug solution containing selected absorption promoter was stirred constantly and was sampled at 15 min intervals for 2 hr.

3. EVALUATION OF INTESTINAL AND NASAL ABSORPTION

In order to compare the intrinsic absorptivity of drug in four different GI segments and nasal cavity, the percent absorbed (% absorbed), apparent permeability (P_{app}) and apparent first order rate constant (K_{obs}) were used. The amount of drug absorbed (% absorbed) was determined by measuring the drug concentration remaining in the perfusate for 2 hr. The k_{obs} was calculated from the slope of first order plots of the amount of drug remanining in the perfusing solution versus time since the loss of drug from the perfusate appeared to follow first order kinetics. The P_{app} of drug in various intestinal segme-

nts and nasal cavity was calculated from eq. (1).

$$P_{app} = -\frac{Q}{2\pi rl}\ln(1-F.A.) \quad (1)$$

Q is the perfusion flow rate. 2 πrl denotes diffusional cross section and F.A. is the fraction absorbed. The intestinal length (l) was measured by excising each segment at the end of the experiment and the average radius of each intestinal segment was obtained from the literature.[28] With these data, the surface area ($2\pi rl$) of various rat intestinal segments could be calculated. The average surface area of rat nasal cavity weighing 250 g is also known[29] and summarized in Table III. P_{app} can provide meaningful comparison of the magnitude of permeation enhancement across various mucosal membranes.

4. SITE DEPENDENCE OF DRUG ABSORPTION

4-1. Site Dependence of Acyclovir Absorption without Adjuvants

Acyclovir was reported to exhibit poor and variable absorption from GI tract following oral administration. The oral bioavailability of acyclovir was low and species dependent.[19] The reason for such variable absorption is poorly understood, but is thought to be related to the low water and lipid solubility of the compound. The existence of an absorption window, due to a site specific absorption process, has been postulated to explain incomplete absorption of hydrophilic hydrochlorthiazide[30] and insulin which is high molecular weight compound.[31] The present study was undertaken to investigate and elucidate the reason(s) for poor acyclovir absorption in the GI tract and additionally to examine the possibility of acyclovir systemic absorption through the nasal cavity of rats in order to develop alternative routes of delivery. Table II showed that acyclovir was well absorbed in the D with an apparent permeability (P_{app}) of 3.69×10^{-4} cm/sec which was nearly the same as that of AZT (3.66×10^{-4} cm/sec) in the D. Comparatively lower permeability of absorption was noted in the UJ with P_{app} (0.82×10^{-4} cm/sec), which was significantly lower than the P_{app} of AZT (2.44×10^{-4} cm /sec) in the UJ. However, acyclovir absorption was virtually absent in the LJ, C and N of rats. Acyclovir was not degraded in the stomach because the difference between AUC_{0-3} in the case of oral and intraduodenal administration was not significantly different. But the drug upon doudenal administration generated maximum plasma concentration (C_{max}, 5.1 μg/ml) within 5-10 min compared to the C_{max} (1.8 μg/ml) of 40-60 min in the case of oral administration.[18] In spite of greater P_{app} of acyclovir and higher C_{max} in the D, poor absorption in UJ and no absorption in LJ and C probably caused lower bioavailability following oral administration. The total lack of absorption of acyclovir in LJ, C, and N might be due to the combination of its poor physicochemical properties and the barrier presented by lower portion of GI tract and nasal mucosal membrane, i.e., lack of adequate microvilli for absorption.

Table II—Apparent Permeability and Percent Absorbed of AZT and Acyclovir in the Different Intestinal Segments and Nasal Cavity in Rats

Compound	Route	Percent Absorbed (%)	Apparent Permeability (cm/sec×10^4)
AZT	D	14.7(0.80)	3.66(0.14)
	UJ	26.5(2.05)	2.44(0.16)
	LJ	10.1(0.57)	0.89(0.04)
	C	5.14(0.35)	0.45(0.02)
Acyclovir	D	14.8(3.24)	3.69(0.75)
	UJ	10.1(0.77)	0.82(0.06)
	LJ	0	0
	C	0	0
	N	0	0

4-2. Site Dependence of AZT Absorption without Adjuvants

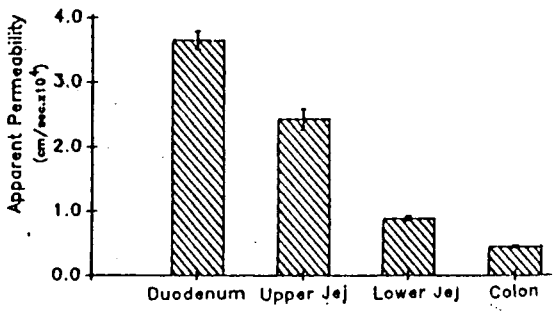


Figure 1—Apparent permeabilities of AZT in the four segments of the rat intestine. Each value is mean± S. E. (n=9).

Table II and Fig. 1 showed different apparent permeabilities in the four segments of the rat intestine. P_{app} in the D (3.66×10^{-4} cm/sec) was significantly greater than in the C (0.45×10^{-4} cm/sec) where very little AZT absorption took place. Similar decrease in P_{app} values was noticed in the D and the UJ followed by the LJ and finally the C, which indicated that intrinsic absorptivity was greater in the upper GI tract than in the lower portion possibly due to the difference in surface area for absorption due to the lower concentration of villi and microvilli in lower GI tract and colon.[32] For example, in the dog jejunum, compared with the ileum, the villi and the microvilli are much longer and wider and the enterocytes are more numerous per unit weight of tissue, resulting in a greater surface area for absorbtion per unit length.[33] Such a vast available surface area increases the efficiency of the absorption of hydrophilic small drug molecules in the upper GI tract. Such higher intrinsic absorption in the upper GI tract will be required to develop sustained release AZT absorption delivery and targeting delivery to the stomach as duration of AZT release needs to be longer than the 3-4 hr taken for transit through the small intestine (enzyme-digestible swelling hydrogel, mucosal adhesives). Alternatively, optimization of the formulations (prodrug) and alternative routes of delivery (intranasal, pulmonary, rectal and transdermal drug delivery) may be necessary to maintain constant plasma drug levels for the optimal therapeutic benefit.

5. EFFECT OF ABSORPTION PROMOTERS ON THE INTESTINALAND NASAL ABSORPTION OF ACYCLOVIR

5-1. Site Dependence of Promoting Actions of Absorption Enhancers

Due to extremely poor absorption characteristics of acyclovir in the LJ, C, and N, it was necessary to enhance the absorption of acyclovir and to compare the effects of absorption promoters at various mucosal absorption sites. We therefore studied the effects of sodium glycocholate, acylcarnitines and their mixed micelles on acyclovir absorption in the different GI segments, and nasal cavity of rats. Since these absorption promoters presumably have different mechanism of increasing membrane permeability and the morphologies of the intestinal and nasal mucosal membranes differ, the magnitudes of absorption enhancing effects might be expected to vary from site to site. For example, acyclovir was found to be absorbed to the extent of 3 to 9% after oral and duodenal administration, but after rectal administration in the abence of absorption promoter, the bioavailability of acyclovir was 37%. Its rectal administration with 4% sodium caprate resulted in 81% bioavailability.[18] Cefoxitin without PCC was appreciably absorbed 31% in the duodenum (D) vs less than 7% in the jejunum (J), ileum (I) and colon (C). When evaluated in the presence of PCC, bioavailability for cefoxitin improved by 0 (D), 22 (J), 16 (I), and > 32 fold (C).[34] On the other hand, the effect of NaGC for improving fosfomycin absorption was essentially the same in the C and J, but that of fusogenic lipid-sodium taurocholate mixed micelles was greater in the J than C.[35] On the basis of these results, absorption enhancing effects of drug appeared to be

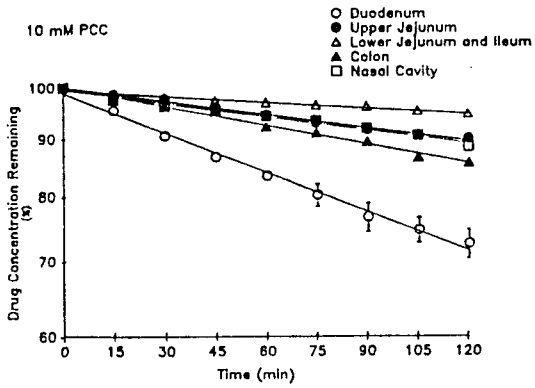


Figure 2—Effect of 10 mM PCC on the disappearance of acyclovir in the different GI segments and nasal cavity of rats. Each value is the mean± S.E. (n=3).

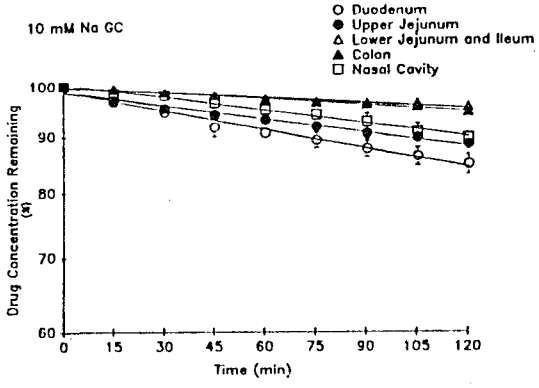


Figure 3—Effect of 10 mM NaGC on the disappearance of acyclovir in the different GI segments and nasal cavity of rats. Each value is the mean± S.E. (n=3).

site-dependent, but the effects of bile salt-acylcarnitine mixed micelles at various mucosal absorption sites have not been observed.

Fig. 2, 3 and 4 depicted the semilog plots of the remaining percent of acyclovir in the perfusate in the different GI segments and nasal cavity of rats in the presence of 10 mM PCC, 10 mM NaGC, and 10 mM PCC-10 mM NaGC mixed micelles during *in situ* recirculating perfusion for 2 hr. These figures illustrated that the disappearance of acyclovir from the perfusate followed first order kinetics for the various GI segments and nasal cavity of rats. With these semilog plots

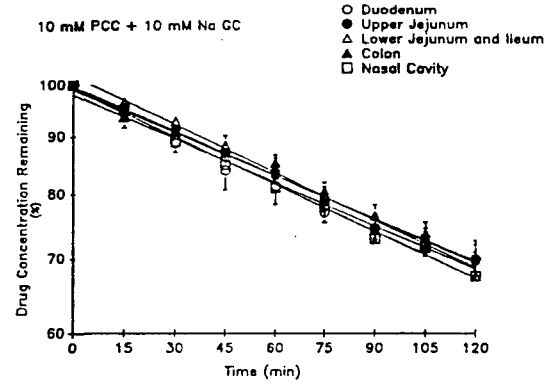


Figure 4—Effect of 10 mM PCC-10 mM NaGC mixed micelles on the disappearance of acyclovir in different GI segments and nasal cavity of rats. Each value is the mean± S.E. (n=3).

of percent remaining, the apparent first order rate constant (K_{obs}) could be obtained (Table III). These results showed that the K_{obs} of acyclovir in the presence of 10 mM PCC or 10 mM NaGC was significantly different at site and was greater in the D than that in the other absorption sites (Fig. 2 and 3) ($p<0.05$). The absorption enhancing action of 10 mM PCC or 10 mM NaGC was site-dependent but that of 10 mM PCC-10 mM NaGC mixed micelles was not. The mixed micelles produced relatively same absorption rates in the different GI segment and the nasal cavity of rats (Fig. 4).

As shown in Fig. 5, the P_{app} of acyclovir was greatest in the N and was increased significantly in four segments of rat intestine by addition of 10 mM PCC into 10 mM NaGC solution. The apparent acyclovir permeability (P_{app}), corrected for surface area of absorption, was in the following order: N>D>LJ>C>UJ. The highest P_{app} seen for the N is attractive for the nasal delivery of acyclovir in the presence of this absorption promoter. August *et al* reported that the rank order of insulin efficacy was also increased as followings; nasal>rectal>buccal>subling-ual route when NaGC was used as absorption promoting agent.[36]

The ratios of P_{app} of acyclovir in the prese-

Table III—Mucosal Absorption Parameters of Acyclovir in Various Intestinal Segments and Nasal Cavity of of Rats

Route	Adjuvant	Surface Area (cm^2)	Percent Absorbed (%)	Apparent Permeability ($cm/sec \times 10^4$)	Apparent First Order Rate Constant ($min \times 10^4$)
Duodenum	None	14.91 (0.30)	14.8 (3.24)	3.69 (0.75)	11.14 (1.99)
	10 mM PCC	14.62 (0.23)	27.4 (2.18)	7.21 (0.65)	27.03 (2.82)
	10 mM NaGC	14.51 (0.36)	15.1 (1.79)	3.74 (0.39)	13.0 (1.69)
	10 mM PCC+10 mM NaGC	14.62 (0.25)	30.3 (1.85)	8.24 (0.29)	29.91 (1.31)
Upper Jejunum	None	43.05 (0.34)	10.1 (0.77)	0.82 (0.06)	8.23 (0.39)
	10 mM PCC	42.95 (0.98)	10.0 (1.00)	0.82 (0.07)	9.08 (1.17)
	10 mM NaGC	42.77 (1.05)	11.5 (0.46)	0.95 (0.02)	9.35 (0.83)
	10 mM PCC+10 mM NaGC	42.95 (0.56)	30.1 (2.92)	2.78 (0.29)	29.65 (2.87)
Lower Jejunum & Ileum	None	37.53 (0.75)	0	0	0
	10 mM PCC	37.68 (1.04)	5.3 (1.02)	0.47 (0.83)	4.03 (0.92)
	10 mM NaGC	37.85 (0.80)	4.4 (0.95)	0.40 (0.79)	3.73 (0.93)
	10 mM PCC+10 mM NaGC	37.68 (0.29)	32.5 (0.65)	3.48 (0.06)	32.74 (0.93)
Colon	None	38.15 (0.78)	0	0	0
	10 mM PCC	38.01 (0.89)	14.3 (1.24)	1.40 (0.05)	12.80 (1.57)
	10 mM NaGC	38.35 (0.44)	5.4 (0.53)	0.48 (0.04)	4.43 (0.04)
	10 mM PCC+10 mM NaGC	38.52 (0.33)	29.8 (1.95)	3.07 (0.22)	29.41 (1.66)
Nasal Cavity	None	10.4	0	0	0
	10 mM PCC	10.4	11.4 (0.23)	3.88 (0.08)	9.21 (0.58)
	10 mM NaGC	10.4	10.3 (1.20)	3.49 (0.43)	8.89 (0.10)
	10 mM PCC+10 mM NaGC	10.4	32.5 (0.84)	12.59 (0.40)	32.88 (1.94)

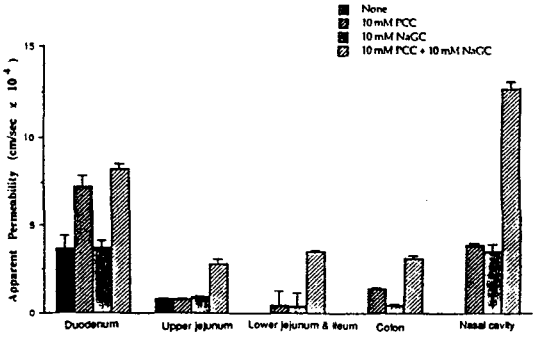


Figure 5—Apparent permeabilities of acyclovir in the different GI segments and nasal cavity in the presence and absence of various adjuvants. Each value is the mean± S.E. (n=3).

nce of 10 mM PCC-10 mM NaGC mixed micelles to that in the presence of 10 mM PCC or 10 mM NaGC were 1.14 and 2.2 for D, 3.3 and 2.02 for UJ, 7.4 and 8.7 for LJ, 2.64 and 6.4 for C and 3.24 and 3.6 for N (Table IV). These results showed that the synergistic effect of mixed micellar solution of 10 mM NaGC with 10 mM PCC at various absorption sites was highest in LJ and was low in the upper GI tract. The prmeation enhancement of acyclovir by mixed micelles in part was due to solubilization of PCC by NaGC. The presence of 10 mM NaGC or 10 mM PCC produced a small enhancement of acyclovir absorption compared to mixed micelles of 10 mM PCC with 10 mM NaGC. The data from Table IV and Fig. 5 clearly show that acylcarnitines need to be solubilized in the mixed micelles for significant absorption

Table IV—Ratio of Apparent Permeability with PCC-NaGC Mixed Micelles to That of PCC and NaGC

Route	$P_{app} \times 10^4$	Ratio	
	Mixed Micelles	PCC	NaGC
Duodenum	8.24± 0.29	1.14	3.2
Upper jejunum	2.78± 0.29	3.3	2.0
Lower jejunum and Ilueum	3.48± 0.06	7.4	8.7
Colon	3.07± 0.22	2.6	6.4
Nasal cavity	12.59± 0.4	3.24	3.6

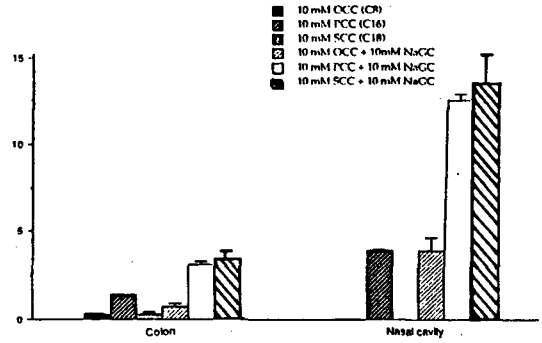


Figure 6—Effect of acyl chain length of acylcarnitines and acylcarnitine-NaGC mixed micelles on acyclovir absorption in the colon and nasal cavity of rats. Each value is the mean± S.E. (n=3).

enhancement to occur in the different GI tracts and nasal cavity of rats. The promoting effect of the mixed micelles appeared to be synergistic and was much greater than that with single adjuvants due probably to micellar solubilization of acylcarnitines by NaGC.

5-2. Effects of Carbon Chain Length in Acylcarnitines

L-carnitine has been extensively studied as an acyl acceptor in the mitochondrial acyltransferase system. The compound is involved in fatty acid utilization and apparently served as an intramembrane carrier molecule to transport fatty acids to the mitochondrial interior.[37] However L-carnitine was ineffective in enhancing absorption of drug in the GI tract of rat. Long chain carnitine esters are present in bile and consequently in the intestinal contents.[38] In order to examine the effect of carbon chain length on absorption enhancing effect, acylcarnitines, i.e. 10 mM DL-octanoylcarnitine chloride (OCC), 10 mM palmitoyl-DL-carnitine chloride (PCC) and 10 mM DL-stearoylcarnitine chloride (SCC) and their mixed micellar solutions containing 10 mM NaGC were tested for acyclovir absorption promotion in the C and N of rats. Fig. 6 clearly illustrated that the enhancing effect was significantly dependent on the carbon chain length of acyl moiety in the mixed micellar solution. PCC was effective in enhancing absorption of acyclovir in the C and N of rats, but OCC and SCC were totally ineffective in the N. While, NaGC-OCC mixed micelles slightly increased the absorption of acyclovir in the C and N of rats, the mixed micellar solution of NaGC with PCC or SCC could significantly increase the mucosal membrane permeability of acyclovir by both routes. These results indicated that PCC was the most effective absorption-promoting adjuvant among the acylcarnitines tested. These observations also lead to the hypothesis that solubilization of acylcarnitines by surfactants such as NaGC would allow more lipid molecules to be available at the nasal and/or intestinal membrane mucosal interfaces for subsequent absorption and/or interaction with membrane components, resulting in increased membrane permeability. Relatively high enhancing effect of PCC probably suggests a paracellular absorption enhancing mechanism. PCC because of its increased hydrophobicity could be taken up by mucosal cell lipid bilayer where it can act as translocator of acyclo guanosine analogues such as acyclovir.

5-3. Effect of Various PCC Concentration in the Mixed Micellar Solution Containing 10 mM NaGC

PCC prefered to leave the bulk phase to segregate readily at the water/air interphase. So PCC did not distribute uniformly in solutions. This phenomenon requires the solvent

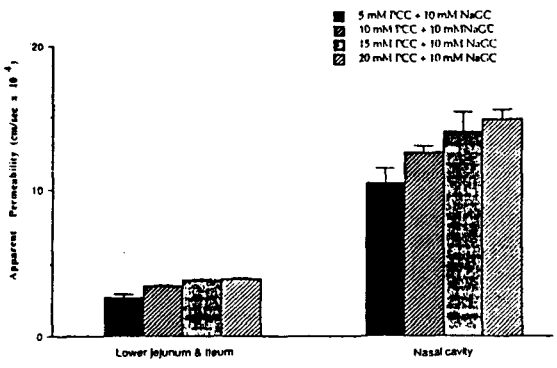


Figure 7—Effect of PCC concentration in acylcarnitine-NaGC mixed micelles on acyclovir absorption in the combined lower jejunum and ileum and nasal cavity of rats. Each value is the mean± S.E. (n=3).

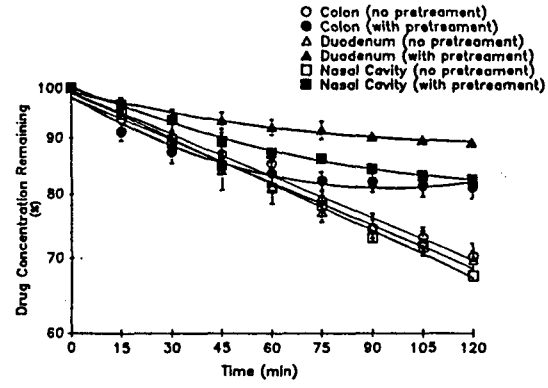


Figure 8—Reversibility of absorption enhancing effect with 10 mM PCC-10 mM NaGC mixed micelles in the presence and absence of pretreatment for 1 hr in the duodenum, colon and nasal cavity of rats. Each value is the mean± S.E. (n=3).

of appropriate polarity compatible with the amphiphilic nature of PCC molecules in order to distribute uniformly PCC in solution.[39] The concentration dependence of PCC in the mixed micellar solution containing 10 mM NaGC was investigated in order to determine the minimum effective concentration of PCC needed to enhance intestinal and nasal absorption. Fig. 7 clearly demonstrated that the extent of acyclovir absorption enhancement was independent of PCC concentration (5-20 mM) in the mixed micellar solution at both absorption sites (LJ and N). PCC and NaGC alone at 10 mM concentration did not appear to disrupt the mucosal membrane. This result demonstrated that optimum concentration of PCC in mixed micelles containing 10 mM NaGC was 10 mM as an acyclovir absorption promoter without damage of mucosal membrane.

5-4. Reversibility of Intestinal and Nasal Mucosal Permeability

Reversibility was examined following adjuvant pretreatment and subsequent washing with adjuvant-free buffer solution containing drug. In order to roughly estimate the damaging effect on the intestinal and nasal mucosa by mixed micellar solution, we compared the apparent first order rate constant (K_{obs}) in the D, C and N of rats with and without

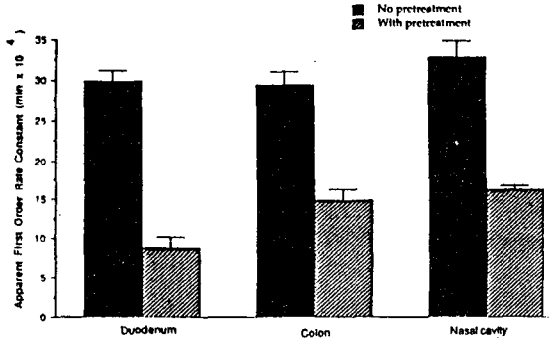


Figure 9—Comparison of apparent first order absorption rate constants of acyclovir with and without 10 mM PCC-10 mM NaGC mixed micellar pretreatment for 1 hr in the duodenum, colon and nasal cavity of rats. Each value is means± S.E. (n=3).

mixed micellar solution pretreatment for 1 hr. The rat intestinal tract and nasal cavity were perfused with 10 mM PCC-10 mM NaGC mixed micellar solution containing acyclovir for 1 hr and then were flushed with isotonic phosphate buffer solution for 10 min. The remainder of buffer solution in the loop was then expelled by air from the attached syringe. The perfusion was then restarted using 0.1 mM acyclovir solution without any adjuvants. Fig. 8. showed that the transient effect could be reversed within 60-120 min after removal of the adjuvants. The first or-

der rate constants associated with no pretreatment with mixed micelles were greater than those of pretreatment with mixed micelles (Fig. 9), suggesting absence of long lasting mucosal damage. However, histological studies are needed to be performed to justify their clinical use.

6. ENHANCEMENT OF COLONIC AZT ABSORPTION BY PCC-NaGC MIXED MICELLES

The colorectum, which can serve as the site for either drug absorption or drug administration, is more capable of maintaining the promoter concentration above the effective level than the small intestine because of longer retention and decreased dilution effect. Due to the lower P_{app}(0.422×10^{-4} cm/sec) of AZT in the C (Table II), we investigated the enhancement of AZT colonic absorption by using PCC and its mixed micellar solution with NaGC. Fig. 10 depicted the semilog plots of the remaining percentage of AZT in the C of rat in the absence and presence of absorption promoters and these results showed that bile salt-acylcarnitine mixed micelles promoted the colonic absorption of AZT. The use of mixed micelles significantly increased the P_{app} of AZT in the C by a factor of 5.4 times that seen without any adjuvant. The P_{app} of AZT in the presence of PCC-NaGC mixed micelles was increased 1.5 times greater than that in the presence of PCC

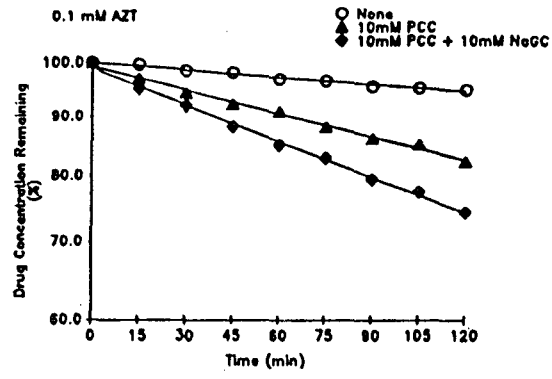


Figure 10—Semilog plots of the percentage of AZT remaining versus time in the rat colon in the presence of various adjuvants. Each value is the mean± S. E. (n=3).

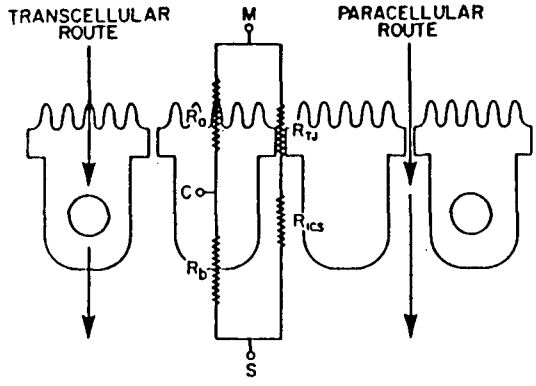
(Table V). The enhancing effect with mixed micelles is synergistic and much greater than with single adjuvant (PCC). This finding indicates the possibility of common permeation route in the colonic absorption of AZT.

7. ENHANCEMENT OF COLONIC PHENOL RED ABSORPTION BY PCC-NaGC MIXED MICELLES

There are two permeation routes in transepithelial drug transport by passive diffusion, the transcellular route through the lipoidal cell membrane and the paracellular route from the tight junction to the lateral intercellular space. (Fig. 11) Tight junctions are regions of close contact between apical

Table V—Comparison of the Apparent Permeabilities and Percents Absorbed Observed in the Colon of Rats with Different Adjuvants

Compound	Composition	Percent Absorbed (%)	Apparent Permeability (cm/sec×10^4)
AZT	None	5.0(0.29)	0.48(0.04)
	10 mM PCC	17.6(0.97)	1.69(0.1)
	10 mM PCC+10 mM NaGC	25.7(0.2)	2.59(0.02)
Phenol red	None	–	–
	10 mM PCC	3.4(0.62)	0.30(0.06)
	10 mM PCC+10 mM NaGC	21.5(0.47)	2.11(0.05)



$$R_c = R_a + R_b$$

$$R_s = R_{TJ} + R_{ICS}$$

$$R_T = \frac{(R_c)(R_s)}{R_c + R_s}$$

Figure 11—Two permeation routes across epithelia.
R_c: transcellular resistance
R_s: resistance of paracellular shunt path
R_a and R_b: resistance of apical and basolateral cell membrane
R_t and R_{ics}: resistance of tight junction proper and intercellular space

ends of epithelial cells and are potential barriers for intestinal drug absorption.[41] The small intestine contains leaky epithelium. However intestinal permeability decreases along the distal direction because of increasing tightness of cell junctions.[42] In paracellular transport, drug molecules can pass through tight junctions. This means that adjuvants which cause the dilation of tight junctions may enhance the entry of the hydrophilic and/or ionic molecules.[43] Fig. 12 depicted the semilog plots of the remaining percentage of phenol red in the C of rat with and without absorption enhancers. PCC-NaGC mixed micelles used to enhance the absorption of phenol red in the C increased by a factor of 7, comparing with PCC (Fig. 13). Absorption for the very water soluble dye, phenol red was also increased by mixed micelles which indicates that it possibly permeates exclusively through water filled channels, i.e., the paracellular route. Thus, the enhancement of membrane permeability through paracellular routes in the colon by bile

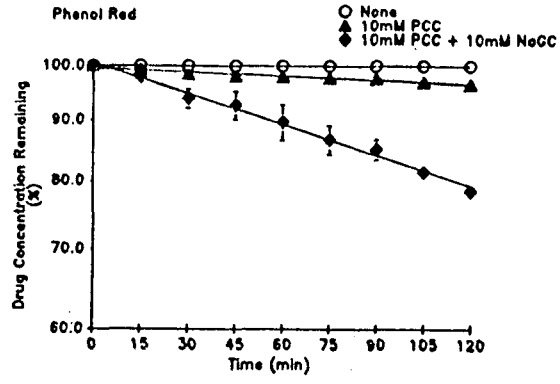


Figure 12—Semilog plots of the percentage of phenol red remaining versus time in the rat colon in the presence of various adjuvants. Each value is the mean± S.E. (n=3).

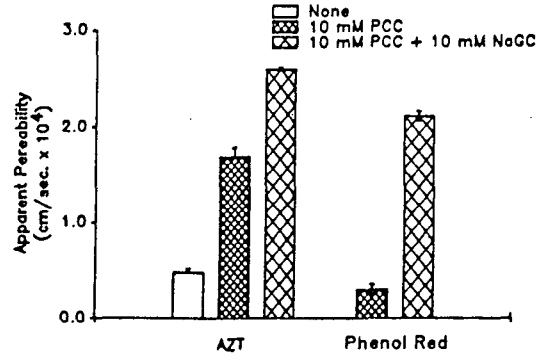


Figure 13—Apparent permeabilities of AZT and phenol red in the rat colon in the presence of various adjuvants. Each value is the mean± S.E. (n=3).

salt-acylcarnitine mixed micelles may provide greater absorption of water-soluble drugs.

8. CONCLUSION

The present study employed rat *in situ* nasal and intestinal perfusion techniques and utilized sodium glycocholate (NaGC) (surfactant), three different acylcarnitines (amphiphiles) as well as their mixed micelles as potential nasal and intestinal absorption promoters of drugs. Acylcarnitines used were DL-octanoylcarnitine chloride (OCC), palmitoyl-DL-carnitine chloride (PCC) and DL-stearoylcarnitine chloride (SCC). Summarizing the present study, it can be concluded

as followings.

1) All acylcarnitines and NaGC by themselves produced negligible enhancement of acyclovir absorption in the rat intestine and either OCC or SCC alone was totally ineffective in the nasal cavity. However, the mixed micellar solutions of NaGC with PCC or SCC could significantly increase the mucosal membrane permeability of acyclovir in the C and N. On the other hand, NaGC-OCC mixed micelles slightly increased the absorption of acyclovir by both routes.

2) When mixed micellar solution of NaGC with PCC was used, the rank order of apparent acyclovir permeability (P_{app}), cm/sec, corrected for surface area of absorption, was N ($12.59\pm 0.40\times10^{-4}$)>D ($8.24\pm 0.29\times10^{-4}$) >LJ ($3.48\pm 0.06\times10^{-4}$)>C ($3.07\pm 0.22\times10^{-4}$) >UJ ($2.78\pm 0.29\times10^{-4}$). In contrast the P_{app} rank order for acyclovir without any absorption promoter was D ($3.69\pm 0.75\times10^{-4}$)>UJ ($0.82\pm 0.06\times10^{-4}$)>LJ>C and N (O).

3) The magnitude of absorption promotion was dependent on the hydrophobicity, i.e., carbon chain length of the acylcarnitines but independent of acylcarnitine concentration (5-20 mM) in the mixed micellar solution. The effect could be reversed within 60-120 min after removal of the adjuvant from the D, C and N.

4) Corrected for the length of each segment, the apparent permeability (P_{app}) of AZT without adjuvants was $3.66\pm 0.14\times10^{-4}$ cm /sec. (mean± S.E.) in the D, $2.44\pm 0.16\times10^{-4}$ cm/sec in the UJ, $0.893\pm 0.04\times10^{-4}$ cm/sec in the LJ, and $0.453\pm 0.02\times10^{-4}$ cm/sec in the C. Bile salt-acylcarnitine mixed micelles appeared to be an effective adjuvant in promoting colonic absorptions of AZT. The use of mixed micelles significantly increased the apparent permeabilities of AZT in the colon by a factor of 5.4.

5) The addition of PCC-NaGC mixed micelles significantly increased the P_{app} of phenol red in the C by a factor of 7, comparing with PCC. Since the absorption of phenol red was enhanced by mixed micelles, a paracellular transport pathway may be involved. The effect of mixed micellar solution of PCC with NaGC was synergistic and was much greater than that with single adjuvants probably due to micellar solubilization of acylcarnitines by NaGC.

6) These results suggest that bile salt-acylcarnitine mixed micelles, in particular, PCC-NaGC mixed micelles can be used as potential intestinal or nasal mucosal absorption promoter of poorly permeable agents.

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