Acyclovir Permeation Enhancement Across Intestinal and Nasal Mucosae by Bile Salt–Acylcarnitine Mixed Micelles

Gee-Bae Park,[1] Zezhi Shao,[1] and Ashim K. Mitra[1,2]

Received August 17, 1991; accepted March 19, 1992

The purpose of this study was to investigate the absorption enhancement of acyclovir, an antiviral agent, by means of bile salt–acylcarnitine mixed micelles. The specificity, site dependence, palmitoyl-DL-carnitine chloride (PCC) concentration dependence, and effects of absorption promoters on acyclovir absorption via the nasal cavity (N) and four different intestinal segments of the rat, i.e., duodenum (D), upper jejunum (UJ), combined lower jejunum and ileum (LJ), and colon (C) were evaluated. The present study employed the rat *in situ* nasal and intestinal perfusion techniques and utilized sodium glycocholate (NaGC), three acylcarnitines, and their mixed micelles as potential nasal and intestinal absorption promoters. Acylcarnitines used were DL-octanoylcarnitine chloride (OCC), palmitoyl-DL-carnitine chloride (PCC), and DL-stearoylcarnitine chloride (SCC). All acylcarnitines and NaGC by themselves produced negligible enhancement of acyclovir absorption in the rat intestine, while OCC and SCC were 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 colon and nasal cavity. On the other hand, NaGC–OCC mixed micelles slightly increased the absorption of acyclovir by both routes. When a 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 ($10.54 \pm 0.62 \times 10^{-5}$) > D ($6.82 \pm 0.30 \times 10^{-5}$) > LJ ($2.90 \pm 0.08 \times 10^{-5}$) > C ($2.54 \pm 0.14 \times 10^{-5}$) > UJ ($2.30 \pm 0.22 \times 10^{-5}$). In contrast, the P_{app} rank order for acyclovir without any absorption promoter was D ($2.49 \pm 0.44 \times 10^{-5}$) > UJ ($0.64 \pm 0.03 \times 10^{-5}$) > LJ, C, and N (0). The effect of mixed micellar solutions was synergistic and was much greater than that with single adjuvants probably because of micellar solubilization of acylcarnitines by NaGC. The magnitude of absorption promotion was dependent on the hydrophobicity, i.e., carbon-chain length of the acylcarnitines. The enhanced permeability could be reversed within 60–120 min after removal of the adjuvant from the duodenum, colon, and nasal cavity. These results suggest that bile salt–acylcarnitine mixed micelles can be used as intestinal or nasal mucosal absorption promoters of poorly permeable agents.

KEY WORDS: acyclovir; nasal permeation; intestinal permeation; bile salt; acylcarnitine; mixed micelles; permeation enhancement.

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 (1,2), surfactants (3), fusidic acid derivatives (4), medium-chain fatty acid salts (5), and fatty acid derivatives of carnitines (6) seemed to be candidates for further mechanistic studies. Such absorption enhancing agents have been utilized extensively to increase mucosal permeability or to alter the physical and enzymatic barrier function of the nasal and intestinal mucosa. Some therapeutic agents such as peptides and proteins exhibit a poor bioavailability following oral and nasal administration because of ionic charge, high molecular weight, and high susceptibility to proteolytic enzymes (7,8). Therefore, these therapeutic entities are 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, investigations of alternative noninvasive methods, such as intranasal, pulmonary, oral, or rectal routes, of delivery for such compounds have received increasing attention (9).

In our previous article (10), 10 m*M* palmitoyl-DL-carnitine chloride (PCC) and its mixed micelles with 10 m*M* sodium glycocholate (NaGC) were reported to promote significantly colonic absorption of AZT and phenol red, mainly by what appears to be a paracellular route. Previous results also appeared to indicate that the extent of absorption enhancement in response to PCC may be drug dependent and site dependent (11). In the present study, we therefore investigated 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 effects of absorption promoting actions. Acyclovir was selected as a model compound due to its poor absorption characteristics (12–15).

MATERIALS AND METHODS

Materials

Acyclovir [9-(2-hydroxyethoxymethyl) guanine] was a gift from Burroughs Wellcome Company (Research Triangle Park, NC). DL-Octanoylcarnitine chloride, palmitoyl-DL-carnitine chloride, DL-stearoylcarnitine chloride, thymine, and sodium glycocholate were obtained from Sigma Chemical Company (St. Louis, MO). Heptanesulfonic acid, sodium salt, was obtained from Aldrich Chemical Company (Milwaukee, WI). Other reagents were of analytical grade. All solvents used were of HPLC grade. All the solutions were freshly prepared and filtered prior to use.

Methods

Preparation of Mixed Micellar Solutions

Acylcarnitines were dissolved in mixed micellar isotonic phosphate buffer solution (IPBS). The buffer solution was prepared from 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. It also contained 0.1 m*M* acyclovir. The solution was stirred continuously and, finally, sonicated at room temperature for

[1] Department of Industrial and Physical Pharmacy, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, Indiana 47907.

[2] To whom correspondence should be addressed.

0724-8741/92/1000-1262$06.50/0

5 min with a Branson Sonicator (Model 3200, Branson Co., Shelton, CT). A micellar solution containing 10 m*M* NaGC, a concentration above its critical micellar concentration (about 9 m*M* in this buffer), formed an optically clear solution. All the solutions were used immediately after preparation and brought to 37 ± 0.5°C prior to the initiation of a study.

In Situ *Intestinal Perfusion Method*

Male Sprague–Dawley rats weighing 220 to 300 g were used to measure the intestinal absorption of acyclovir in the presence of selected absorption enhancers. Three rats were used for each intestinal segment, each rat being used for one segment and experiment only. The animals were fasted for about 18 hr prior to the experiments but water was allowed ad libitum. They were anesthetized by an intraperitoneal injection of 30 mg/kg sodium pentobarbital. The *in situ* perfusion technique was conducted according to Farraj *et al.* (16) with slight modification. Details of this experimental method have been described in our previous report (10). The four segments of a rat intestine were cannulated proximally and distally and were defined as duodenum (pyloric sphincter to the ligament of Treitz), upper jejunum (the next 15-cm segment following the ligament of Treitz), combined lower jejunum and ileum (15-cm segment ending at the ileocecal junction), and colon (from the cecal–colonic junction to the rectum). The segments were perfused with 20 ml of drug solution containing a known concentration of a selected absorption promoter for 2 hr by means of a peristaltic pump (Harvard Apparatus, Millis, MA) at a flow rate of 2 ml/min. The drug was very stable in the intestinal perfusate, which was periodically sampled (0.2 ml) at 15-min intervals for up to 120 min.

In Situ *Nasal Perfusion Method*

The rat *in situ* experimental model developed by Hirai *et al.* (17) and Huang *et al.* (18) was utilized to study the effects of potential absorption enhancers on the nasal absorption of acyclovir. The surgical pretreatment for *in situ* nasal 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 Adams, 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 was sealed with an adhesive agent (Instant Jet, Carl Goldberg Models Inc., Chicago, IL) to prevent 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 the reservoir to minimize the loss of drug solution. The drug solution to be evaluated was placed in the reservoir and was maintained at 37 ± 0.5°C. The perfusate was circulated through the nasal cavity of the rat by means of a peristaltic pump. A constant volume (20 ml) of drug solution containing selected absorption promoter was perfused continuously at a constant flow rate of 2 ml/min and the perfusate was sampled at 15-min intervals for 2 hr.

Analytical Procedure

The concentrations of acyclovir remaining in the nasal and intestinal perfusates were determined by a modification of the HPLC assay method reported previously by Land and Bye (19). An aliquot of the perfusate (0.2 ml) was withdrawn periodically and immediately mixed with 0.2 ml of internal standard solution (10 μg/ml thymine). The samples were vigorously vortexed and centrifuged at 7000 rpm for 10 min prior to injection onto the column. The HPLC system comprised a Waters Model 6000A solvent delivery system equipped with a Waters U6K injector, a Waters Model 440 absorbance detector, and a Fisher Recordal Series 5000 strip-chart recorder. Samples (20 μl) were injected on to an Alltech Direct-Connect cartridge guard column comprising an adsorbosphere C18, 10-mm cartridge attached to an analytical Waters Resolve 5-μm spherical C18 reversed-phase analytical column (3.9 × 150 mm). The mobile phase contained 1% (v/v) acetonitrile in 10 m*M* ammonium acetate buffer at pH 5.0 containing 1.0 m*M* sodium heptanesulfonate. The wavelength of detection was 254 nm. The eluent was pumped at a rate of 1.0 ml/min. Temperature was ambient. Acyclovir was stable in the mixed micellar solution during the time course of an experiment.

Determination of Absorption Characteristics of Acyclovir

In order to compare the intrinsic absorptivity of acyclovir in four GI segments and the nasal cavity, the percentage absorbed (% absorbed), apparent first-order rate constant (K_{obs}), and apparent permeability (P_{app}) were determined. 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 remaining in the perfusing solution versus time since the loss of acyclovir from the perfusate appeared to follow first-order kinetics. P_{app} was calculated from Eq. (1).

$$P_{app} = k_{obs} \cdot \frac{V}{S} \quad (1)$$

The terms k_{obs}, V, and S denote the observed apparent first-order rate constant, volume of perfusion medium (20 ml), and absorptive surface area, respectively. In the case of the intestinal segments, S was calculated from $2\pi rl$. 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 a previous study (10). The average surface area of a 250-g rat nasal cavity including the sinus is also known (20). Therefore, P_{app} provides a limited but meaningful comparison of the magnitude of permeation enhancement across various mucosal membranes.

RESULTS AND DISCUSSION

In Situ Absorption of Acyclovir Without Adjuvants

Acyclovir is known to exhibit poor and variable absorption from the GI tract following oral administration. The oral bioavailability of acyclovir is low and species dependent (14). The reason for such variable absorption is poorly un-

derstood but is thought to be related to the low aqueous 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 some hydrophilic (21) and high molecular weight compounds (22). The present study was initiated to investigate and elucidate the reason(s) for poor acyclovir absorption across the GI tract and nasal mucosae. Data presented in Table I show that acyclovir was well absorbed in the duodenum (D), with an apparent permeability (P_{app}) of 2.49×10^{-5} cm/sec. A comparatively lower rate of absorption was observed in the upper jejunum (UJ), with a P_{app} of 0.64×10^{-5} cm/sec. Further, acyclovir absorption was virtually absent in the LJ, colon (C), and nasal cavity (N). Nevertheless, maximum plasma concentration (C_{max} = 5.1 μg/ml) was obtained upon duodenal administration with a peak time of 5–10 min, as compared to the C_{max} of 1.8 μg/ml with a late peak time of 40–60 min in the case of per oral administration (13). In spite of the greater P_{app} of acyclovir and higher C_{max} in the duodenum, the lack of absorption in LJ and C probably caused the 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 transport barrier present in the lower portion of GI tract and nasal mucosal membrane, i.e., reduced folds and microvilli.

Site-Dependent Absorption Enhancement of Acyclovir

Due to extremely poor absorption characteristics of acyclovir in the LJ, C, and N, experiments were designed to study the absorption enhancement of acyclovir and to compare the effects of absorption promoters at various mucosal absorption sites. We had, therefore, studied the effects of sodium glycocholate, acylcarnitines, and their mixed micelles on acyclovir absorption in different GI segments and the nasal cavity of rats. Since these absorption promoters presumably have different mechanisms of membrane permeability enhancement and the morphologies of the intestinal and nasal mucosal membranes differ, the magnitudes of absorption enhancing effects are expected to vary from site to site.

Figures 1–3 depict the semilogarithmic plots of the remaining percentage of acyclovir in the perfusate in the different GI segments and nasal cavity of rats in the presence of 10 m*M* PCC, 10 m*M* NaGC, and 10 m*M* PCC–10 m*M* NaGC mixed micelles during *in situ* recirculating perfusion as a function of time. These figures demonstrate that the disappearance of acyclovir from the perfusate follows first-order kinetics in the various GI segments as well as in the nasal cavity. From these plots of percentage remaining versus time, the apparent first-order rate constants (k_{obs}) were obtained and are listed in Table I. The absorption enhancing action of 10 m*M* PCC or 10 m*M* NaGC was found to be site dependent but that of 10 m*M* PCC–10 m*M* NaGC mixed micelles was not. Mixed micelles produced comparatively the same absorption rates in the different GI segments and the nasal cavity. The apparent acyclovir permeability (P_{app}) in the presence of mixed micelles, corrected for surface area of absorption, was in the following order: N > D > LJ > C > UJ. The ratios of the P_{app} of acyclovir in the presence of 10 m*M* PCC–10 m*M* NaGC mixed micelles to that in the presence of 10 m*M* PCC or 10 m*M* NaGC alone were 1.11 and 2.28 for D, 3.28 and 3.15 for UJ, 8.05 and 8.79 for LJ, 2.27 and 6.68 for C, and 3.57 and 3.70 for N (Table I). These results showed that the synergistic effect of mixed micellar

Table I. Apparent First-Order Absorption Rate Constants and Permeabilities of Acyclovir in Various Intestinal Segments and Nasal Cavity of the Rat

Route	Adjuvant	Apparent first-order rate constant ($min^{-1} \times 10^4$)	Apparent permeability ($cm/sec \times 10^5$)
Duodenum	None	11.14 (1.99)[a]	2.49 (0.44)
	10 m*M* PCC	27.03 (2.82)	6.16 (0.64)
	10 m*M* NaGC	13.00 (1.69)	2.99 (0.39)
	10 m*M* PCC + 10 m*M* NaGC	29.91 (1.31)	6.82 (0.30)
Upper jejunum	None	8.23 (0.39)	0.64 (0.03)
	10 m*M* PCC	9.08 (1.17)	0.70 (0.09)
	10 m*M* NaGC	9.35 (0.83)	0.73 (0.06)
	10 m*M* PCC + 10 m*M* NaGC	29.65 (2.87)	2.30 (0.22)
Lower jejunum & ileum	None	0	0
	10 m*M* PCC	4.03 (0.92)	0.36 (0.08)
	10 m*M* NaGC	3.73 (0.93)	0.33 (0.08)
	10 m*M* PCC + 10 m*M* NaGC	32.74 (0.93)	2.90 (0.08)
Colon	None	0	0
	10 m*M* PCC	12.80 (1.57)	1.12 (0.14)
	10 m*M* NaGC	4.43 (0.04)	0.38 (0.01)
	10 m*M* PCC + 10 m*M* NaGC	29.41 (1.66)	2.54 (0.14)
Nasal cavity	None	0	0
	10 mM PCC	9.21 (0.58)	2.95 (0.18)
	10 m*M* *NaGC*	8.89 (0.10)	2.85 (0.03)
	10 m*M* PCC + 10 m*M* NaGC	32.88 (1.94)	10.54 (0.62)

[a] Numbers in parentheses denote standard errors (n = 3).

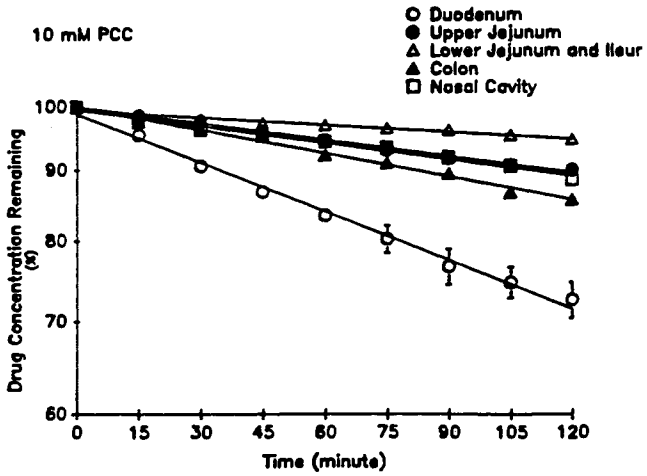

Fig. 1. Effect of 10 m*M* PCC on the disappearance of acyclovir in different GI segments and nasal cavity of rats. Each value is plotted as the mean ± SE (n = 3).

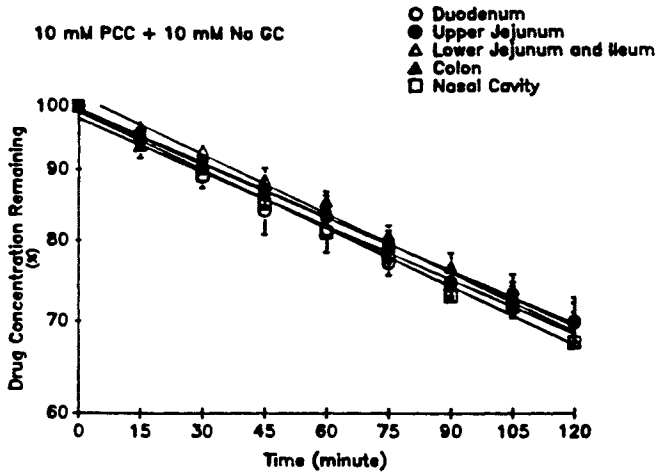

Fig. 3. Effect of 10 m*M* PCC–10 m*M* NaGC mixed micelles on the disappearance of acyclovir in different GI segments and nasal cavity of rats. Each value is plotted as the mean ± SE (n = 3).

solution of 10 m*M* NaGC with 10 m*M* PCC at various absorption sites was maximum in the LJ and was comparatively lower in the upper GI tract.

Effects of Acyl-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 serves as an intramembrane carrier molecule to transport fatty acids to the mitochondrial interior (23). However, L-carnitine by itself was ineffective in enhancing absorption of compounds in the rat GI tract (6). Long-chain carnitine esters are present in bile and, consequently, in the intestinal contents (24). In order to examine the effect of carbon-chain length of carnitine esters on the absorption enhancing effect, acylcarnitines, i.e., 10 m*M* DL-octanoylcarnitine chloride (OCC), 10 m*M* palmitoyl-DL-carnitine chloride (PCC), and 10 m*M* DL-stearoylcarnitine chloride (SCC), and their mixed micellar solutions containing 10 m*M* NaGC were examined for acyclovir absorption enhancement in the rat colon and nasal cavity. Results presented in Table II clearly demonstrate that the enhancing effect is significantly dependent on the carbon-chain length of the acyl moiety. PCC was effective in enhancing absorption of acyclovir in both colon and nasal cavity, but OCC and SCC were totally ineffective in the nasal cavity. NaGC–OCC mixed micelles slightly increased the absorption of acyclovir in colon and nasal cavity, whereas the mixed micellar solution of NaGC with PCC or SCC could significantly increase the mucosal transport of acyclovir at both absorption sites. These results indicate that PCC is probably the most effective absorption promoting adjuvant among the acylcarnitines tested.

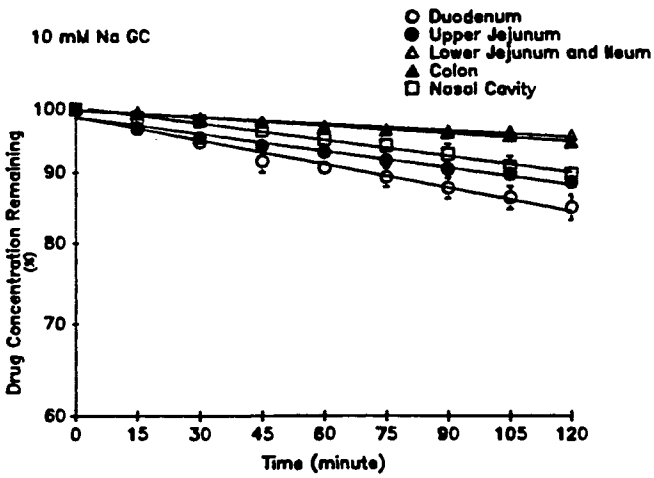

Fig. 2. Effect of 10 m*M* NaGC on the disappearance of acyclovir in different GI segments and nasal cavity of rats. Each value is plotted as the mean ± SE (n = 3).

Effect of PCC Concentration in the Mixed Micellar Solution Containing 10 m*M* NaGC on Acyclovir Absorption

The enhancement effect by NaGC alone was not noticeable in the GI mucosa. However, bile salt–acylcarnitine mixed micelles enhanced the absorption of acyclovir to a much greater extent than NaGC alone except in the duodenum. It is likely that acylcarnitines are essential for absorption enhancement of acyclovir, while NaGC contributes mainly to solubilization of the hydrophobic acylcarnitines. The concentration effect of PCC in the mixed micellar solution with 10 m*M* NaGC on acyclovir transport was investigated. Table III summarizes the effect of 0–20 m*M* PCC on acyclovir absorption in the lower jejunum/ileum and nasal cavity in the presence of 10 m*M* NaGC. The trend of enhancement (apparent permeability increases) at both absorptive sites exhibited similar patterns. Initial inclusion of PCC significantly increased acyclovir permeability. This increasing trend slowed down above 10 m*M* and plateaued at 15 m*M* PCC concentrations. Therefore the enhancing effect depends on the concentration of PCC in mixed micelles and appears to be a saturable process as well. Further, the incorporation of PCC did not significantly alter the thermodynamic activity of acyclovir as evidenced by the low binding

Table II. Effect of Acyl-Chain Length on Acylcarnitine and Acylcarnitine–NaGC Mixed Micelle Enhanced Acyclovir Absorption in the Colon and Nasal Cavity of the Rat

Route	Adjuvant	Apparent first-order rate constant ($min^{-1} \times 10^4$)	Apparent permeability ($cm/sec \times 10^5$)
Colon	10 m*M* OCC	2.27 (0.80)[a]	0.20 (0.07)
	10 m*M* PCC	12.80 (1.57)	1.12 (0.14)
	10 m*M* SCC	2.46 (2.46)	0.22 (0.12)
	10 m*M* OCC + 10 m*M* NaGC	6.80 (1.45)	0.60 (0.13)
	10 m*M* PCC + 10 m*M* NaGC	29.41 (1.66)	2.54 (0.14)
	10 m*M* SCC + 10 m*M* NaGC	32.87 (5.22)	2.91 (0.46)
Nasal cavity	10 m*M* OCC	0	0
	10 m*M* PCC	9.21 (0.58)	2.95 (0.19)
	10 m*M* SCC	0	0
	10 m*M* OCC + 10 m*M* NaGC	10.37 (1.87)	3.32 (0.60)
	10 m*M* PCC + 10 m*M* NaGC	32.88 (1.94)	10.54 (0.62)
	10 m*M* SCC + 10 m*M* NaGC	36.84 (5.61)	11.81 (1.80)

[a] Numbers in parentheses denote standard errors ($n = 3$).

constant of acyclovir to PCC mixed micelles (data not shown).

Reversibility of Intestinal and Nasal Mucosal Permeability

Reversibility was examined following adjuvant pretreatment and subsequent washing with adjuvant-free buffer solution containing acyclovir. In order to estimate roughly the damaging effect on the intestinal and nasal mucosa of mixed micellar solutions, the rat intestinal tract and nasal cavity were perfused with a 10 m*M* PCC–10 m*M* NaGC mixed micellar solution containing acyclovir for 1 hr and subsequently flushed with an isotonic phosphate buffer solution for 10 min. The perfusion was then repeated using a 0.1 m*M* acyclovir solution without any adjuvants. Figure 4 shows that the transient effect could be reversed within 60–120 min after removal of the adjuvants, suggesting the absence of long-lasting mucosal damaging effect. The morphological changes of rat intestinal and rectal tissues exposed to 0–10 mg/ml (0–23 m*M*) PCC have been evaluated by Fix *et al.* (6). At a 10 mg/ml concentration, only slight alterations in intestinal tissues were observed such as a thin epithelium, microvillus tip extrusion, and an increased amount of mucin in the lumen. Further, all changes appeared to be restricted in the epithelial membrane, while the integrity of the cytoplasm remained intact. Due to the large number of intestinal sites as well as the nasal cavity studied in this investigation, morphological examinations were not conducted. However, our reversible acyclovir permeability experiments provided some indication of the relative safety of such absorption enhancers. Nevertheless, further mechanistic investigations are needed to define the pathways by which bile salt–acylcarnitine mixed micelles influence the biological membranes. Long-term toxicity of acylcarnitines must also be assessed.

In conclusion, the promoting effect of acylcarnitines and bile salt–acylcarnitine mixed micelles on the transport of a hydrophilic model compound, acyclovir, was significant. Although the underlying mechanism of such enhancement is still unknown, the effect appears to be a general one with respect to both intestinal and nasal mucosae. Due to the natural occurrence of acylcarnitine in nearly all animal species, their use as absorption promoters may prove to be safe and effective.

Table III. Effect of PCC Concentration in Mixed Micellar Solutions Containing 10 m*M* NaGC on Acyclovir Absorption in the Combined Lower Jejunum/Ileum and Nasal Cavity of the Rat

Route	Concentration (m*M*)	Apparent first-order rate constant ($min^{-1} \times 10^4$)	Apparent permeability ($cm/sec \times 10^5$)
Lower jejunum & ileum	0	3.73 (0.93)[a]	0.33 (0.08)
	5	27.52 (2.85)	2.39 (0.25)
	10	32.74 (0.93)	2.90 (0.08)
	15	36.78 (2.60)	3.24 (0.23)
	20	37.02 (1.77)	3.25 (0.16)
Nasal cavity	0	8.89 (0.10)	2.85 (0.03)
	5	28.68 (2.28)	9.19 (0.73)
	10	32.88 (1.94)	10.54 (0.62)
	15	36.38 (4.29)	11.66 (1.38)
	20	36.89 (2.30)	11.82 (0.74)

[a] Numbers in parentheses denote standard errors ($n = 3$).

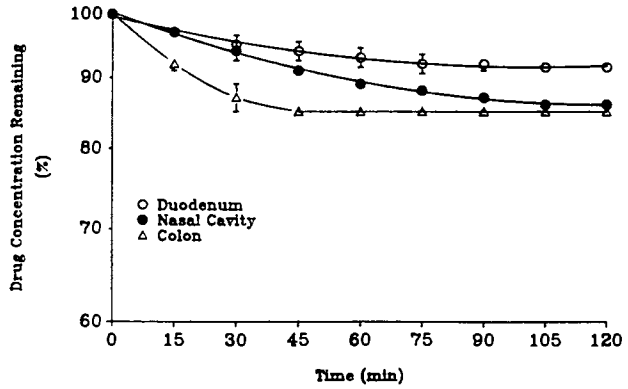

Fig. 4. Disappearance of acyclovir after removal of the mixed micelles from duodenum, colon, and nasal cavity of rats. Membrane permeability returned to its original impermeable state within 60–120 min. Each value is plotted as the mean ± SE (n = 3).

ACKNOWLEDGMENTS

This study was supported in part by an AAPS Young Investigator Award (A.K.M.) and in part by NIH Grant NS 25284. Z.S. is a David Ross Research Fellow.

REFERENCES

1. P. Tengamnuay and A. K. Mitra. Bile salt-fatty acid mixed micelles as nasal absorption promoters of peptides. II. In vivo nasal absorption of insulin in rats and effect of mixed micelles on the morphological integrity of the nasal mucosa. *Pharm. Res.* 7:370–375 (1990).
2. G. S. Gordon, A. C. Moses, R. D. Silver, J. S. Flier, and M. C. Carrey. Nasal absorption of insulin—Enhancement by hydrophobic bile salts. *Proc. Natl. Acad. Sci. USA* 82:7419–7423 (1983).
3. S. Hirai, R. Yashiki, and H. Mima. Effects of surfactants on the absorption of insulin in rats. *Int. J. Pharm.* 9:165–172 (1981).
4. J. P. Longenecker, A. C. Moses, J. S. Flier, R. D. Silver, M. C. Carey, and E. J. Dubovi. Effects of sodium taurodihydrofusidate on nasal absorption of insulin in sheep. *J. Pharm. Sci.* 76:351–355 (1987).
5. M. Mishima, U. Wakita, and M. Nakano. Studies on the promoting effects of medium chain fatty acid salts on the nasal absorption of insulin in rats. *J. Pharmacobiodyn.* 10:624–631 (1987).
6. J. A. Fix, K. Engle, P. A. Porter, P. S. Leppert, S. J. Selk, C. R. Cardner, and J. Alexander. Acylcarnitines: Drug absorption-enhancing agents in the gastrointestinal tract. *Am. J. Physiol.* 251:G332–G340 (1986).
7. V. H. L. Lee. Enzymatic barriers to peptide and protein absorption and the use of penetration enhancers to modify absorption. In S. S. Davis, L. Illum, and E. Tomlinson (eds.), *Delivery Systems for Peptide Drugs,* Plenum Press, New York, 1986, pp. 87–104.
8. E. J. van Hoogdalem, A. G. de Boer, and D. D. Breimer. Intestinal drug absorption enhancement: An overview. *Pharm. Ther.* 44:407–443 (1989).
9. D. A. Epstein and J. P. Longenecker. Alternative delivery systems for peptides and proteins as drugs. *CRC Crit. Rev. Ther. Drug Carr. Syst.* 5:99–139 (1988).
10. G. B. Park and A. K. Mitra. Mechanism and site dependency of intestinal mucosal transport and metabolism of thymidine analogues. *Pharm. Res.* 9:322–327 (1992).
11. J. Martinkova, I. Tilser, V. Krejcik, J. Parizek, and K. Volene. Effect of pamitoyl-DL-carnitine on the ^{3}H-dihydroergotoxine intestinal absorption in rat. *Arzneim-Forsch/Drug Res.* 40(I): 145–149 (1990).
12. P. de Miranda and M. R. Blum. Pharmocokinetics of acyclovir intravenous and oral administration. *Antimicrob. Chemother.* 12 (Suppl. B):29–37 (1983).
13. M. Yamazaki, S. Itoh, M. Sawanoi, M. Kobayashi, S. Suzuki, T. Komatsu, and K. Tanabe. The effect of fatty acids on the rectal absorption of acyclovir in rats. *J. Pharm. Pharmacol.* 42:441–443 (1990).
14. P. de Miranda, H. C. Krasny, D. A. Page, and G. B. Elion. The disposition of acyclovir in different species. *J. Pharmacol. Exp. Ther.* 219:309–315 (1981).
15. O. L. Laskin. Clinical pharmacokinetics. *Clin. Pharmacokin.* 8:187–201 (1983).
16. N. F. Farraj, S. S. Davis, G. D. Parr, and H. N. E. Stevens. Absorption of progabide from aqueous solutions in a modified recirculating rat intestinal perfusion system. *Int. J. Pharm.* 43:93–100 (1988).
17. S. Hirai, T. Yashiki, and H. Mima. Absorption of drugs from the nasal mucosa of rat. *Int. J. Pharm.* 7:317–325 (1981).
18. C. H. Huang, R. Kimura, R. B. Nassar, and A. A. Hussain. Mechanism of nasal absorption of drug. I. Physicochemical parameters influencing the rate of *in situ* nasal absorption of drugs in rats. *J. Pharm. Sci.* 74:608–611 (1985).
19. G. Land and A. Bye. Simple high-performance liquid chromatographic method for the analysis of 9-(2-hydroxyethoxymethyl) guanine (acyclovir) in human plasma and urine. *J. Chromatogr.* 224:51–58 (1981).
20. Y. W. Chien, K. S. E. Su, and S. F. Chang. *Nasal Systemic Drug Delivery,* Marcel Dekker, New York, 1989, p. 31.
21. J. Lynch, R. E. Pownall, and D. C. Taylor. Site-specific absorption of hydrochlorthiazide in the rat intestine. *J. Pharm. Pharmaco.* 39 (Suppl.):55p (1987).
22. R. J. Schilling and A. K. Mitra. Intestinal mucosal transport of insulin. *Int. J. Pharm.* 62:53–64 (1990).
23. J. Bremer. Carnitine-metabolism and functions. *Physiol. Rev.* 63:1420–1480 (1983).
24. J. J. Hamilton and P. Hahn. Carnitine and Carnitine esters in rat bile and human duodenal fluid. *Can. J. Physiol. Pharmacol.* 65:1816–1820 (1987).