Mechanism and Site Dependency of Intestinal Mucosal Transport and Metabolism of Thymidine Analogues

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This study has been undertaken to investigate the mechanisms of intestinal mucosal transport and metabolism of thymidine analogues and to identify any optimal site(s) of the rat intestine particularly involved in the absorption of thymidine analogues. The intestinal absorption of 3′-azido-3′-deoxythymidine (AZT) was studied at three initial concentrations in four segments of the rat intestine using an *in situ* recirculating perfusion technique. Disappearance of AZT followed first-order kinetics throughout the gastrointestinal (GI) tract at all tested concentrations. The apparent first-order rate constants were found to be relatively invariant over a broad range of concentrations from 0.01 to 1.0 m*M*. Corrected for the length of each segment, the apparent permeability (P_{app}) of AZT was 3.01 ± 0.32 × 10^{-5} cm/sec (mean ± SE) in the duodenum, 2.06 ± 0.24 × 10^{-5} cm/sec in the upper jejunum, 0.76 ± 0.13 × 10^{-5} cm/sec in the combined lower jejunum and ileum, and 0.32 ± 0.10 × 10^{-5} cm/sec in the colon, which indicated that intrinsic absorptivity was greater in the upper GI tract than in the lower portions possibly due to the differences in surface area for absorption. No AZT metabolite appeared in any part of the GI tract. On the other hand, thymidine and other analogues, i.e., 5-iodo-2′-deoxyuridine and 2′-deoxyuridine, were rapidly metabolized into nucleobase and sugar in the upper GI tract, whereas in the colon no metabolite appeared. A free 3′-OH group appears to be necessary for the metabolism (catabolism) of thymidine analogues in the rat intestine mainly by pyrimidine nucleoside phosphorylase. Finally, bile salt–acylcarnitine mixed micelles appeared to be an effective adjuvant in promoting colonic absorptions of AZT and phenol red. The use of mixed micelles increased the apparent permeabilities of AZT in the colon by a factor of 5.4, and for phenol red the permeability increased from a negligible value to 1.76 × 10^{-5} cm/sec. Since the absorptions of both AZT and phenol red were enhanced by mixed micelles, a paracellular transport pathway may be involved.

KEY WORDS: thymidine analogues; intestinal absorption; mechanism; metabolism; colonic absorption; enhancement; mixed micelle.

INTRODUCTION

Thymidine analogues have shown potential in antiviral therapy, including recent applications as anti-acquired immunodeficiency syndrome (anti-AIDS) drugs. Particularly, 3′-azido-3′-deoxythymidine (AZT) was designated an orphan drug by the Food and Drug Administration for use in the management of human immunodeficiency virus infection. Further agents (e.g., dideoxyinosine, dideoxycytidine) are currently in phase III clinical trial and may be introduced in the near-future to lower the mortality and frequency of opportunistic infections in a selected group of individuals with AIDS and/or AIDS-related complex (1–3).

AZT is a thymidine analogue in which the 3′-hydroxy group is replaced by an azido group that imparts a low water and moderate lipid solubility to the compound (solubility in water of 30 mg/ml and octanol/water partition coefficient of 1.05) (4). The absorption of AZT from the GI tract appeared to be both rapid and complete. However, a short biological half-life (1.1 hr) and low oral bioavailability (60%) due to hepatic first-pass metabolism resulted in frequent administration (200 mg every 4 hr) (5–7). An adequate AZT concentration in the body must be maintained to achieve the anticipated anti-AIDS effect. The required dosage regimen may cause severe hematologic side effects, which may be attributable to an excessive plasma concentration of AZT (8). Therefore it is desirable to design sustained-release oral formulations or to develop alternative routes of administration which will enable zero-order delivery of AZT. An acceptable and reproducible systemic availability from sustained-release dosage forms can be achieved only if drug absorption is relatively uniform over most of the length of intestinal tract (9,10). So if AZT has a similar intrinsic absorptivity in the different regions of the GI tract, we can predict and facilitate the development of new approaches to the controlled-release delivery systems for AZT.

Among the thymidine analogues, idoxuridine (5-iodo-2′-deoxyuridine) was used topically in the treatment of herpes simplex infections of the cornea (11). Thymidine was also used as a means of preventing or reversing the toxic effects of methotrexate (12). The disadvantageous pharmacokinetic properties of thymidine and idoxuridine from a clinical standpoint were their short plasma half-lives and rapid metabolism (catabolic breakdown) in the tissues (liver, brain) (13,14). Acylcarnitines were tested as potential absorption enhancing agents for gastrointestinal drug delivery. Particularly, palmitoyl-DL-carnitine chloride (PCC) has been reported to be the most effective absorption promoting adjuvant following oral administration (15). Further, PCC was a potent absorption enhancer for gentamicin when administered vaginally to rats (16) and for human growth hormone (hGH) when administered by the nasal route (17).

We initially investigated the intestinal absorption characteristics of AZT at three initial concentrations (0.01, 0.1, and 1.0 m*M*) in four segments of the rat intestine (duodenum, upper jejunum, combined lower jejunum and ileum, and colon) using an *in situ* recirculating perfusion technique. Subsequently, we studied the metabolism of thymidine analogues in the rat intestine to understand the substrate structural requirements and enzyme systems responsible for such breakdown. Finally, in order to determine the effect of adjuvants on the intestinal absorption of AZT and phenol red, absorption promoters such as palmitoyl-DL-carnitine chloride (PCC) and its mixed micellar solution with sodium glycocholate (NaGC) were used to enhance colonic absorption. Overall this report describes intestinal mucosal transport and metabolism of thymidine and three of its analogues, i.e., 3′-azido-3′-deoxythymidine (AZT), 5-iodo-2′-deoxyuridine

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(IDU), and 2'-deoxyuridine. The chemical structures of these compounds are depicted in Fig. 1.

MATERIALS AND METHODS

Materials

Zidovudine (azidothymidine; AZT) was kindly donated by Burroughs Wellcome Company (Research Triangle Park, N.C.). 5-Iodo-2'-deoxyuridine (idoxuridine), thymidine, 2'-deoxyuridine, β-hydroxyethyltheophylline, 5'5'-dithio-bis(2-nitrobenzoic acid), palmitoyl-DL-carnitine chloride, and sodium glycocholate were obtained from Sigma Chemical Company (St. Louis, MO). Heptane sulfonic acid, sodium salt, was obtained from Aldrich Chemical Company (Milwaukee, Wis.). The isotonic phosphate buffer solution consisted 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. The buffer solutions were prepared with analytical reagent-grade chemicals. All solvents used were of HPLC grade.

Methods

Intestinal Absorption Characteristics of AZT

In situ absorption measurements were carried out by a modification of a method described previously (18). The intestinal absorption of thymidine analogues was determined by measuring the concentration of the drug remaining in the perfusate. Male Sprague–Dawley rats (220–300 g) were fasted for about 18 hr prior to the experiments but water was allowed ad libitum. Animals were anesthetized by an intraperitoneal injection of a 30 mg/kg sodium pentobarbital and the peritoneal cavity was opened by a midline incision. The intraabdominal temperature was maintained by laying the animal on a warmer wooden platform, with the temperature maintained at 37°C 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.

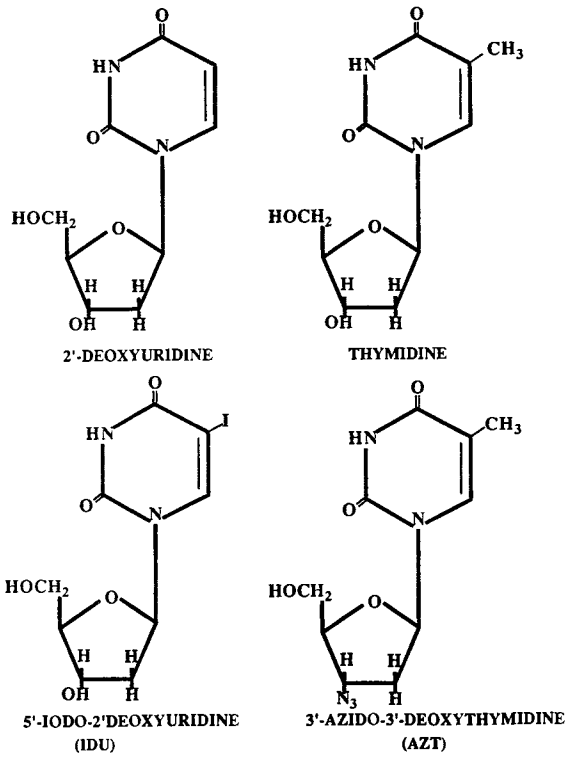

Fig. 1. Structures of thymidine analogues studied.

The proximal and distal cannulas were made of polyethylene tubing, PE160 and PE 220, respectively. They were 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 for a relatively high rate of perfusion with minimal back pressure to 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 solutions (IPBS) to maintain the tissue in a reasonable state of hydration. The first segment was defined as the duodenum (pyloric sphincter to the ligament of Treitz). The next 15-cm portion of the tract following the ligament of Treitz was the upper jejunum. The combined lower jejunum and ileum comprised the next 15 cm that ended at the ileocecal junction. The length of each gut segment was measured using a standard 15-cm silk thread. Finally, the colon continued from the cecal–colonic junction to the rectum. Absorption measurement in each region was made in triplicate, one rat being used for only one segment in a given experiment.

Drugs were dissolved in isotonic phosphate buffer solution. The drug concentrations used in the perfusing solutions were 0.01, 0.1, and 1.0 m*M*. The perfusate also contained phenol red as a nonabsorbable marker in order to indicate the change in volume due to the absorption of water as reported by Schanker *et al.* (19). Only 2% phenol red disappeared from a 10-cm intestinal segment within 2 hr, which suggests only a 0.2% water loss/cm of intestine. Such a small volume of fluid loss (<0.5% water loss/cm of segment) can be considered negligible and can be ignored when calculating intestinal permeabilities (19). Drug solution was placed in a reservoir which was water-jacketed at 37 ± 0.5°C via a circulating water bath. A magnetic stir bar was used to keep the contents of the reservoir well mixed. The cannulated segment was first flushed by the IPBS (37°C) to remove traces of gut contents until the perfusate became clear and was subsequently reperfused by a single-pass perfusion with 10 ml of drug solution (also 37°C). This procedure was adopted to displace the IPBS solution remaining in the intestinal segment. The flushing procedure was performed manually through a syringe attached to the proximal cannula and the syringe tip was placed very carefully so as not to expand the intestinal lumen or cause any mucosal damage due to change in hydrostatic pressure. The remainder of drug solution inside the loop was then expelled by forcing air through the attached syringe. The tubings attached to the inflow and outflow cannula were transferred to a beaker containing 20 ml of fresh drug solution (37°C) and the perfusing fluid was then circulated through the intestine for 2 hr by means of a peristaltic pump at a flow rate of 2.0 ml/min. The perfusate

was sampled by removing 0.2 ml of solution at 15-min intervals up to 120 min. The percentage of drug remaining was calculated from the decrease in drug concentration as a function of time. Each gut segment was excised and the length measured at the end of the experiment.

Analytical Procedure

An HPLC assay method described previously for AZT determination in biological fluids has been adopted in this study where direct injection of perfusate onto the HPLC column was possible because of extremely low protein or other endogenous contents of the intestinal perfusate (20). The thymidine analogues remaining in the intestinal perfusate were quantitated by a reversed-phase HPLC. The withdrawn samples were diluted with 0.2 ml of internal standard solution (β-hydroxyethyltheophylline). The HPLC system comprised of 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 onto the Alltech Direct-Connect cartridge guard column containing the adsorbosphere C18, 10-mm cartridge attached to an analytical Waters Resolve 5-μm spherical C18 reversed-phase column (3.9 × 150 mm). The mobile phases were pH 5.0, 10 m*M* ammonium acetate in 15% (v/v) acetonitrile for AZT and phenol red and pH 5.0, 1–3% (v/v) acetonitrile solution containing 10 m*M* ammonium acetate and 1.0 m*M* heptane sulfonic acid, sodium salt, for other thymidine analogues. The wavelength of detection was 254 nm. The eluent was pumped at a rate of 1.0 ml/min. Temperature was ambient. There were no interfering endogenous peaks.

The peak height ratios of analyte to internal standard were linear with the analyte concentration within the range of 0.001 to 0.1 m*M*, with correlation coefficients of 0.999. The interday coefficients of variation were 1.75 and 4.82% at 0.1 and 0.001 m*M* concentrations, respectively.

Metabolism of Thymidine Analogues in the Rat Intestine

Metabolism study of the thymidine analogues was also carried out using an *in situ* recirculating perfusion technique. IDU was selected initially to explore the site dependency of intestinal metabolism. Its intestinal catabolism was investigated in the four segments of rat intestine (duodenum, upper jejunum, combined lower jejunum–ileum, and colon). Finally, metabolic disappearance of thymidine analogues was studied with 3′-azido-3′-deoxythymidine (AZT), thymidine, and 2′-deoxyuridine in the upper jejunum of rat. HPLC chromatograms were scrutinized thoroughly for the appearance of all metabolites including thymine, 5′-glucuronide, and 3′-amino-3′-deoxythymidine. The HPLC assay was sensitive enough to detect any appearance of these metabolites. An enzyme inhibition experiment was also performed in the upper jejunum of the rat to identify the enzyme class involved in the cleavage of IDU in the absence and presence of a specific enzyme (pyrimidine nucleoside phosphorylase) inhibitor [5′5′-dithio-bis(2-nitrobenzoic acid)].

Investigation of AZT Colonic Absorption Enhancement

The technique used in this investigation once again involved *in situ* recirculating perfusion. A mixed micellar solution was prepared by the following procedure. Palmitoyl-DL-carnitine chloride (10 m*M*) was prepared in 20 ml of pH 6.5 isotonic phosphate buffer solution (IPBS) containing a mixture of bile salt (10 m*M* NaGC), 0.1 m*M* AZT, and phenol red. The solution was stirred continuously and finally sonicated at room temperature for 5 min with a Branson sonicator. The above solution was perfused through the colon for 2 hr. The concentration of AZT and phenol red remaining in the colonic perfusate was quantitated by reversed-phase HPLC using the conditions described under

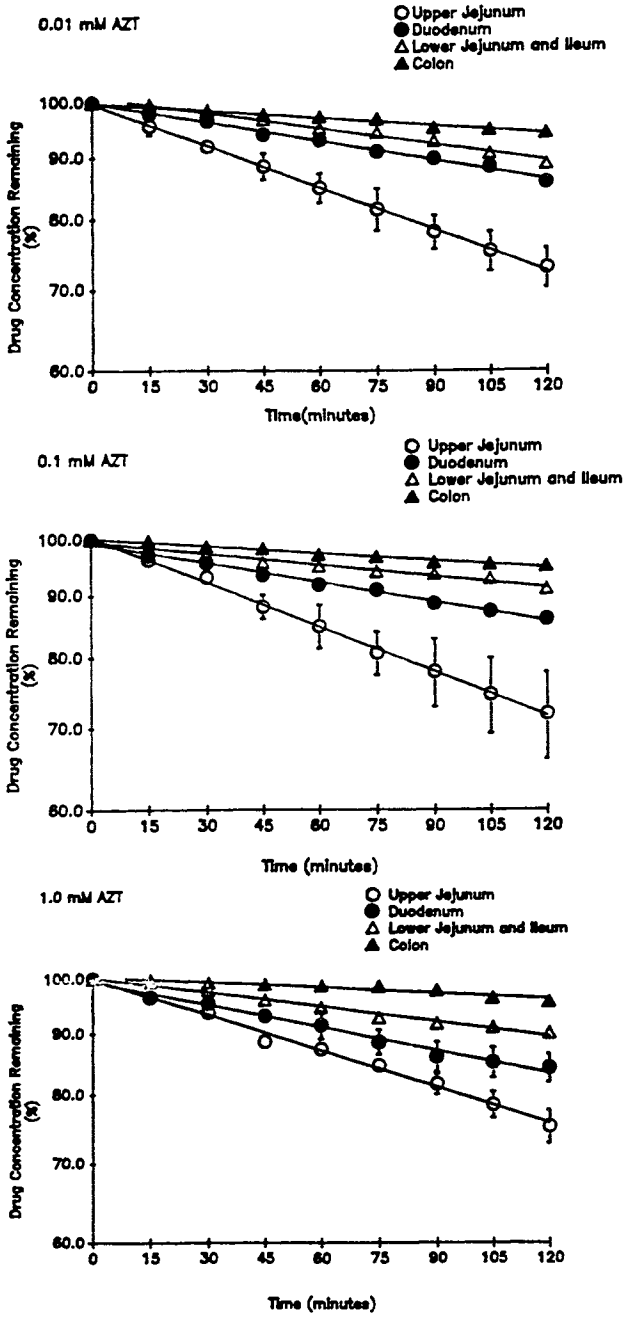

Fig. 2. Semilogarithmic plots of the percentage of AZT remaining versus time in the four segments of the rat intestine as a function of initial AZT concentration.

Table I. Apparent Permeabilities and Percentage of AZT Absorbed in the Different GI Segments of Rats

Intestinal segment	Intestinal radius (cm)	Intestinal length (cm)	Percentage of AZT absorbed	Apparent permeability (cm/sec × 10^5)
Duodenum	0.275	8.4 (0.21)[a]	14.7 (0.8)[a]	3.01 (0.32)[a]
Upper jejunum	0.45	14.9 (0.43)	26.5 (2.05)	2.06 (0.24)
Lower jejunum and ileum	0.4	15.4 (0.23)	10.1 (0.57)	0.76 (0.13)
Colon	0.8	7.6 (0.22)	5.14 (0.35)	0.32 (0.10)

[a] Numbers in parentheses denote standard errors (n = 9).

Analytical Procedure. The HPLC analyses have shown that AZT and phenol red were stable in the mixed micellar solution for at least 3 hr.

Calculation of the Apparent Permeabilities of AZT

Apparent intestinal permeability per unit length was calculated in order to compare the intrinsic absorption potential of each drug in different regions of rat GI tract. A simple relationship relating first-order absorption rate constant and permeability coefficient could be used to calculate apparent permeabilities of AZT as shown in Eq. (1).

$$\text{Apparent permeability (cm} \cdot \text{sec}^{-1}) = \text{first-order absorption rate constant (sec}^{-1}) \times \frac{\text{volume of perfusate (cm}^3)}{\text{diffusional area of intestinal lumen (cm}^2)} \quad (1)$$

RESULTS AND DISCUSSION

Mechanism of AZT Intestinal Absorption

This study was performed to investigate the effects of two parameters on AZT absorption—the location of the intestinal segment (duodenum, upper jejunum, combined lower jejunum and ileum, and colon) and the concentration of AZT in the recirculating perfusing solution. Concentration studies with the *in situ* intestinal recirculating perfusion generated linear AZT disappearance rates on semilogarithmic plots at concentrations of 0.01, 0.1, and 1.0 m*M* and showed no evidence of saturation kinetics in any part of the rat intestine. Typical data are presented in Fig. 2. With these semilogarithmic plots of percentage drug remaining till 2 hr, the apparent first-order rate constants could be obtained. Such rate constants were found to be relatively invariant over a broad range of concentrations from 0.01 to 1.0 m*M* in all four segments of the rat intestine. The apparent permeabilities (P_{app}) of AZT in various rat intestinal segments were calculated from an effective radius of the intestine (21) and measured intestinal length utilizing Eq. (1) and are summarized in Table I. The apparent permeabilities within the concentration range of 0.01 to 1.0 m*M* remained fairly constant because the fraction of drug absorbed was found to be independent of concentration. No overall decrease in apparent intestinal permeability was observed with an increase in initial perfusate drug concentration (Fig. 3). Based on the data from these *in situ* recirculating perfusion studies, it is apparent that the major absorption mechanism of AZT within the therapeutic concentration range in four segments of the rat intestine is passive diffusional transport.

Site Dependence of AZT Intestinal Absorption

The apparent permeability can provide meaningful comparisons of intrinsic absorption characteristics of a compound and, when corrected for the length of each intestinal segment of rat, can predict the site dependency of drug intestinal absorption. Table I summarizes apparent permeabilities of AZT in the four segments of the rat intestine. P_{app} in the duodenum (3.01×10^{-5} cm/sec) was significantly greater than in the colon (0.32×10^{-5} cm/sec), where very little AZT absorption took place. A similar gradual decrease in P_{app} values was observed, beginning with the duodenum and the upper jejunum, followed by the combined lower jejunum–ileum and the colon. Regional differences in absorption rate constants of thymidine analogues may not be due to differences in aqueous diffusion layer resistances because, at a recirculating flow rate of 2.0 ml/min, the aqueous layer thickness will be minimal. Resistance offered by such layers

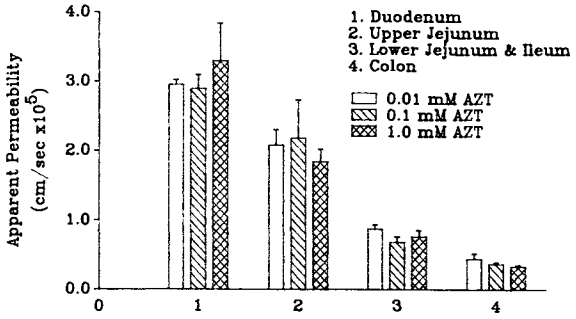

Fig. 3. Apparent permeabilities of AZT in four segments of the rat intestine as a function of concentration. Each value is the mean ± SE (n = 3).

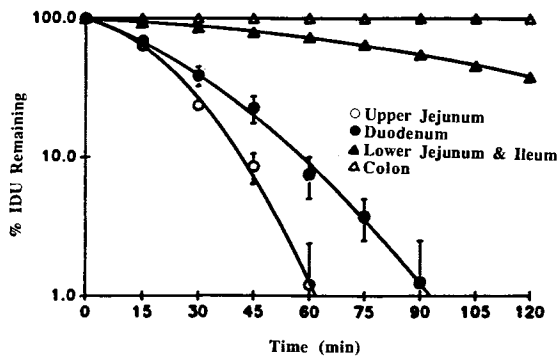

Fig. 4. Disappearance of IDU in four segments of the rat intestine.

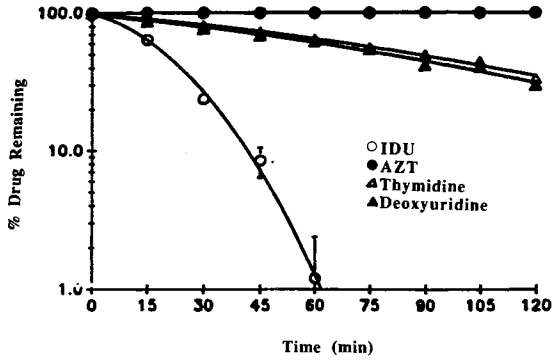

Fig. 5. Disappearance of thymidine analogues in the upper jejunum of rat.

Table II. Comparison of the Apparent Permeabilities and Percent Absorbed in the Colon of Rats with Different Adjuvants

Compound	Composition	Percentage absorbed	Apparent permeability (cm/sec × 10^{-5})
AZT	None	5.0 (0.29)	0.40 (0.02)
	10 m*M* PCC	17.6 (0.97)	1.41 (0.08)
	10 m*M* PCC + 10 m*M* NaGC	25.7 (0.2)	2.16 (0.02)
Phenol red	None	—	—
	10 m*M* PCC	3.4 (0.62)	0.25 (0.05)
	10 m*M* PCC + 10 m*M* NaGC	21.5 (0.47)	1.76 (0.04)

to fairly hydrophilic molecules will be negligible compared to the membrane resistance. Such progressive lowering of apparent permeabilities indicated that intrinsic absorptivity was greater in the upper GI tract than in the lower portions possibly due to differences in surface area for absorption. Such differences may result from less folding of villi and microvilli in lower portions GI tract and colon (23). 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 absorption per unit length (23). Such a vast available surface area increases the efficiency of the absorption of hydrophilic small drug molecules in the upper GI tract. Since AZT absorption takes place more readily from upper GI tract, a delivery device which will hold the AZT dose in the upper GI tract will prolong its therapeutic blood levels.

Intestinal Metabolism of Thymidine Analogues

The metabolism of thymidine analogues was also determined by analyzing *in situ* intestinal perfusate. IDU was completely metabolized by the upper jejunum perfusate within 1 hr, which suggests that the enzyme responsible for metabolism of IDU is rich in the upper portions of the rat intestine. Such IDU catabolism was absent in the colon (Fig. 4). IDU was catabolized into 5-iodouracil, which was determined by HPLC. Figure 5 depicts the disappearance of thymidine analogues in the upper jejunum of rat, and these results showed that no AZT metabolism took place but thymidine, IDU, and 2′-deoxyuridine were all rapidly metabolized into nucleobase and sugar. In order to identify the enzyme involved, a study was undertaken in the absence and presence of the pyrimidine nucleoside phosphorylase inhibitor [5′5′-dithio-bis(2-nitrobenzoic acid)]. As depicted in Fig. 6, near-complete inhibition of IDU catabolism was observed in the upper jejunum of the rat. These results demonstrated that the ineffectiveness of an oral delivery system for IDU could be attributed to its rapid catabolism by pyrimidine nucleoside phosphorylase. It appears that the structural requirement of a free 3′-OH group is necessary for the metabolism (catabolism) of thymidine analogues by pyrimidine nucleoside phosphorylase and any modification of that group (like in AZT) may hinder base-sugar cleavage.

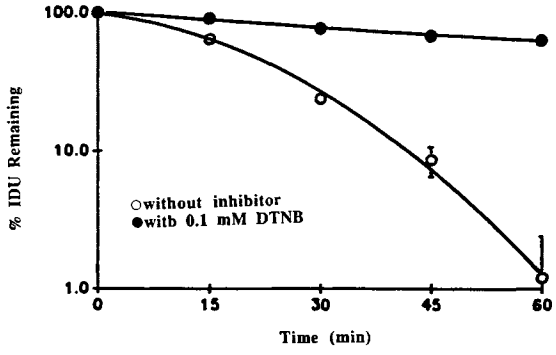

Fig. 6. Effect of pyrimidine nucleoside phosphorylase inhibitor, 5′5′-dithio-bis(2-nitrobenzoic acid) (DTNB), on the hydrolysis of IDU in the upper jejunum of rat.

Colonic Absorption Enhancement of AZT

The colorectum, which can serve as the site for either drug absorption or drug administration, is more suitable for maintaining the promoter concentration above the effective level than the small intestine because of longer retention and a decreased dilution effect. Due to the lower P_{app} (0.32 × 10^{-5} cm/sec) of AZT in the colon, we investigated the enhancement of AZT colonic absorption by using palmitoyl-DL-carnitine chloride (PCC) and its mixed micellar solution with sodium glycocholate (NaGC). The results summarized in Table II show that bile salt–acylcarnitine mixed micelles promoted the colonic absorption of AZT and phenol red to a significant extent. The use of mixed micelles significantly

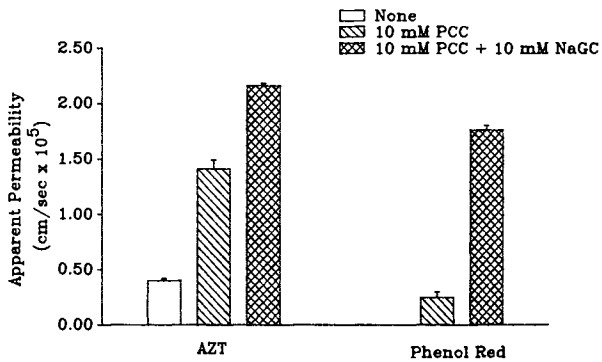

Fig. 7. Apparent permeabilities of AZT and phenol red in the rat colon in the presence of various adjuvants.

increased the P_{app} of AZT in the colon by a factor of 5.4, and for phenol red the apparent permeability increased from a negligible value to 1.76×10^{-5} cm/sec. The enhancing effect is synergistic and much greater than with a single adjuvant (PCC) (Fig. 7). This finding indicates the possibility of a common permeation route in the colonic absorption of AZT and phenol red. Since the absorption of a highly water-soluble anionic dye, phenol red, was increased by mixed micelles, it is possible that transport might have occurred exclusively through water-filled channels, i.e., the paracellular route. Tight junctions are regions of close contact between apical ends of epithelial cells and are potential barriers for intestinal drug absorption (24). The small intestine contains leaky epithelium. As a result, permeation enhancement of thymidine analogues (small hydrophilic molecules) by mixed micelles was very limited. However, intestinal permeability decreases along the distal direction because of increasing tightness of cell junctions (25). Adjuvants which cause the dilation of tight junctions may enhance the entry of the hydrophilic and/or ionic molecules (26). Thus, enhancement of membrane permeability through paracellular routes in the colon by bile salt–acylcarnitine mixed micelles may provide greater absorption of water-soluble drugs.

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REFERENCES

1. M. A. Fischl, D. D. Richman, J. H. Grieco, M. S. Gottlieb, P. A. Volberding, O. L. Laskin, J. M. Leedom, J. E. Groopman, D. Mildvan, R. T. Schooley, G. G. Jackson, D. T. Durack, and D. King. The efficacy of azidothymidine (AZT) in the treatment of patients with AIDS and AIDS-related complex. *N. Engl. J. Med.* 317:185–191 (1987).
2. J. M. Collins and J. D. Unadkat. Clinical pharmacokinetics of zidovudine: An overview of current data. *Clin. Pharmacokin.* 17:1–9 (1989).
3. G. D. Morse, J. L. Lechner, J. A. Santora, and S. L. Rozek. Zidovudine upadate: 1990. *Drug Int. Clin. Pharm.* 24:754–760 (1990).
4. T. Seki, T. Kawaguchi, and K. Juni. Enhanced delivery of zidovudine through rat and human skin via ester prodrugs. *Pharm. Res.* 7:948–952 (1990).
5. R. Yarchoan, R. W. Klecker, K. J. Weinhold, P. D. Markham, H. K. Lyerly, D. T. Durack, E. Gelmann, S. N. Lehrman, R. M. Blum, D. W. Barry, G. M. Shearer, M. A. Fishl, H. Mitsuta, R. C. Gallo, J. M. Collins, D. P. Bolognesi, C. E. Myers, and S. Broder. Administration of 3'azido-3'-deoxythymidine, an inhibitor of HTLV-III/LAV replication, to patients with AIDS or AIDS-related complex. *Lancet* 1:575–580 (1986).
6. R. W. Klecker, J. M. Collins, R. Yarchoan, R. Thomas, J. F. Jenkins, S. Broder, and C. E. Myers. Plasma and cerebrospinal fluid pharmacokinetics of 3'-azido-3'-deoxythymidine: A novel pyrimidine analog with potential application for the treatment of patients with AIDS and related diseases. *Clin. Pharmacol. Ther.* 41:407–412 (1987).
7. M. R. Blum, S. H. T. Lioa, S. S. Good, and P. Demiranda. Pharmacokinetics and bioavailability of zidovudine in humans. *Am. J. Med.* 85 (Suppl. 2A):189–194 (1988).
8. R. Yarchoan, H. Mitsuya, C. E. Myers, and S. Broder. Clinical pharmacology of 3'-azido-2'3'-dideoxythymidine (zidovudine) and related dideoxynucleosides. *N. Engl. J. Med.* 321:726–738 (1989).
9. D. C. Taylor and R. Grundy. Propranolol absorption in different regions of the rat gastrointestinal tract in situ: Implications for sustained release formulations. *J. Pharm. Pharmacol.* 32:499–500 (1980).
10. D. C. Taylor. Sites and mechanisms of intestinal drug absorption. *Pharm. Int.* 6:179–183 (1986).
11. H. E. Kaufman. Clinical cure of herpes simplex keratitis by 5-iodo-2'-deoxyuridine. *Proc. Soc. Exp. Biol. Med.* 109:251–252 (1962).
12. W. D. Ensminger and E. Frei. The prevention of methotrexate toxicity by thymidine infusions in humans. *Cancer Res.* 37:1857–1863 (1977).
13. D. S. Zaharko, B. J. Bolten, D. Chiuten, and P. H. Wiernik. Pharmacokinetic studies during phase I trials of high-dose thymidine infusions. *Cancer Res.* 39:4777–4781 (1979).
14. W. W. Oppelt, D. R. Clarkson, and S. L. Melvin. Metabolism of 5-iodo-2'-deoxyuridine (IUdR) in brain and liver of dogs. *Res. Commun. Chem. Pathol. Pharmacol.* 2:553–566 (1971).
15. J. A. Fix, K. Engle, P. A. Porter, P. S. Leppert, S. J. Selk, C. R. Gardner, and J. Alexander. Acylcarnitines: Drug absorption-enhancing agents in the gastrointestinal tract. *Am. J. Physiol.* 251:G332–G340 (1986).
16. J. L. Richardson, P. S. Minhas, N. W. Thomas, and L. Illum. Vaginal administration of gentamicin to rats. Pharmaceutical and morphological studies using absorption enhancers. *Int. J. Pharm.* 56:29–35 (1989).
17. D. T. O'Hagan, H. Critchley, N. F. Farraj, A. N. Fisher, B. R. Johansen, S. S. Davis, and L. Illum. Nasal absorption enhancers for biosynthetic human growth hormone in rats. *Pharm. Res.* 7:772–776 (1990).
18. 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).
19. L. S. Schanker, D. J. Tocco, B. B. Brodie, and C. A. M. Hogben. Absorption of drugs from the rat small intestine. *J. Pharmacol. Exp. Ther.* 123:81–88 (1958).
20. M. A. Hedaya and R. J. Sawchuk. Sensitive liquid-chromatographic method for determination of 3'-azido-3'-deoxythymidine (AZT) in plasma and urine. *Clin. Chem.* 34:1565–1568 (1988).
21. R. Hebel and M. W. Stromberg. Digestive system. In *Anatomy of the Laboratory Rat,* Williams and Wilkins, Baltimore, 1976, pp. 43–54.
22. K. E. Carra and P. G. Toner. Morphology of the intestinal mucosa. In T. Z. Csaky (ed.), *Pharmacology of the Intestinal Permeation I,* Springer-Verlag, Berlin, 1984, Chap. 1.
23. J. W. L. Robinson, H. Menge, F. V. Sepulveda, and V. Mirkovitch. Functional and structural characteristics of the jejunum and ileum in the dog and the rat. *Digestion* 15:188 (1977).
24. M. J. Jackson. Drug transport across gastrointestinal epithelia. In L. R. Johnson (ed.), *Physiology of the Gastrointestinal Tract,* 2nd ed., Raven Press, New York, 1987, pp. 1597–1621.
25. L. Luciano, E. Reale, G. Rechkemmer, and W. VonEngelhardt. Structure of zonula occludentes and the permeability of the epithelium to short-chain fatty acids in the proximal and the distal colon of guinea pig. *J. Membr. Biol.* 82:145–156 (1984).
26. M. Tomita, M. Shiga, M. Hayashi, and S. Awazu. Enhancement of colonic drug absorption by the paracellular permeation route. *Pharm. Res.* 5:341–346 (1988).