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

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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 vitro 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 mM. Corrected for the length of each segment, the apparent permeability (Papp) of AZT was 3.01 ± 0.32 × 10^-5 cm/sec (mean ± SE) in the duodenum, 2.06 ± 0.24 × 10^-2 cm/sec in the upper jejunum, 0.76 ± 0.13 × 10^-3 cm/sec in the combined lower jejunum and ileum, and 0.32 ± 0.10 × 10^-3 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-ido-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^-3 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., didexoyinosine, didexoyctidine) 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-ido-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). Acycercarnitines 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 mM) in four segments of the rat intestine (duodenum, upper jejunum, combined lower jejunum and ileum, and colon) using an in vitro 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-ido-2'-deoxyuridine

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(1DU), 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-hydroxyethylthiophylline, 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 NaH₂PO₄ · H₂O, 0.033 M Na₂HPO₄, and 0.08 M NaCl adjusted to pH 6.5 with H₃PO₄. 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.

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 mM. 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

![Fig. 1. Structures of thymidine analogues studied.](image-url)