Mechanism of Intestinal Absorption of Ranitidine and Ondansetron: Transport Across Caco-2 Cell Monolayers

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We have investigated the transport of ranitidine and ondansetron across the Caco-2 cell monolayers. The apparent permeability coefficients (P_{app}) were unchanged throughout the concentration range studied, indicating a passive diffusion pathway across intestinal mucosa. No metabolism was observed for ranitidine and ondansetron during the incubation with Caco-2 cell monolayers. P_{app} values for ranitidine and ondansetron (bioavailability of 50 and ~100% in humans, respectively) were $1.03 \pm 0.17 \times 10^{-7}$ and $1.83 \pm 0.055 \times 10^{-5}$ cm/sec, respectively. The P_{app} value for ranitidine was increased by 15- to 20-fold in a calcium-free medium or in the transport medium containing EDTA, whereas no significant change occurred with ondansetron, indicating that paracellular passive diffusion is not rate determining for ondansetron. Uptake of ondansetron by Caco-2 cell monolayers was 20- and 5-fold higher than that of ranitidine when the uptake study was carried out under sink conditions and at steady state. These results suggest that ranitidine and ondansetron are transported across Caco-2 cell monolayers predominantly via paracellular and transcellular pathways, respectively.

KEY WORDS: ranitidine; ondansetron; Caco-2; intestinal absorption; *in vitro* model.

INTRODUCTION

The Caco-2 cell line, a well-differentiated human intestinal cell line derived from colorectal carcinoma, has been investigated as a potential *in vitro* model for drug absorption and metabolism studies (1–6). Caco-2 cells undergo enterocytic differentiation after reaching confluency in culture (7–11). Several active transport systems that are located in the small intestinal cells (e.g., transporters for sugars, amino acids, dipeptides, bile acids, and cobalamine intrinsic factor) are also expressed in Caco-2 cells (3,12–14). Transepithelial electric resistance (TEER) of Caco-2 cells, however, is similar to that of colonic cells and higher than that of small intestinal cells indicating the formation of tighter epithelia (2,15). Hence, Caco-2 cell culture model can serve as a reasonably good *in vitro* model to elucidate the transport mechanisms of drug molecules including the contribution of paracellular diffusion pathway to the transport of drug molecules across intestinal mucosa.

A good correlation between oral drug absorption in humans and apparent drug permeability coefficients (P_{app}) in Caco-2 cell culture model has been reported by Artursson and Karlsson (16). Drugs that are completely absorbed in humans have P_{app} values $>1 \times 10^{-6}$ cm/sec, and drugs that are absorbed 50–60% have P_{app} values between 1.5 and 2.5 $\times 10^{-7}$ cm/sec. Such a correlation can be more meaningful if the underlying mechanisms associated with the difference in the permeability of the drug molecules are elucidated.

Although exceptions exist, it is generally thought that the epithelial cells of the intestinal mucosa are more permeable to the lipophilic compounds than to the hydrophilic compounds because of a higher propensity of the lipophilic compounds to partition into the cell membranes. Transcellular and paracellular pathways are, therefore, postulated to be the predominant routes for the transport of lipophilic and hydrophilic compounds, respectively. Testosterone [$P_{app} = 5.1 \times 10^{-5}$ cm/sec (16)] is transported predominantly by transcellular mechanism, whereas mannitol [$P_{app} = 1.8 \times 10^{-7}$ cm/sec (16)] is transported predominantly via the paracellular pathway. It is not known whether the drug molecules that have intermediate P_{app} values are transported via both pathways or via only one pathway with an intermediate transport rate.

Results of clinical studies indicated that absorption of ondansetron (cf. Fig. 1), a 5-HT_3 receptor antagonist for the treatment of chemotherapy- and radiotherapy-induced emesis (17,18), is nearly complete when given orally to adult humans (19,20). Absolute bioavailability was measured to be approximately 70% because of the first-pass metabolism (21). Results of clinical studies on adult humans also indicated that the bioavailability of ranitidine (cf. Fig. 1), the H_2 antagonist for the treatment of chronic peptic ulcers, was ~50%, with no evidence of liver extraction.

These studies showed that ranitidine was absorbed mainly by the small intestine, and not significantly by the large intestine (22,23). In the present study, the epithelial transport rates of these two drugs as well as the mechanism of their transport are investigated using the Caco-2 cell culture model. We show that ranitidine and ondansetron are transported across Caco-2 cell monolayers predominantly via paracellular and transcellular passive diffusion pathways, respectively.

MATERIALS AND METHODS

Chemicals

D-[1-^{14}C]Mannitol (radiochemical purity, 99%; sp act, 55 mCi/mmol) was purchased from NEN Research Products, Boston, MA. [4-^{14}C]Testosterone (radiochemical purity, 95%; sp act, 54.5 mCi/mmol) and *N*-[methyl-^{3}H]ranitidine (radiochemical purity, 99%; sp act, 6.5 Ci/mmol) were purchased from Amersham Corp., Arlington Heights, IL. [2(im)-^{14}C]Ondansetron (radiochemical purity, 99%; sp act, 53.5 mCi/mmol) was prepared by Glaxo Group Research, Ware, UK. Unlabeled ondansetron and ranitidine were obtained from Glaxo Inc. Research Institute, Research Triangle Park, NC, and unlabeled mannitol and testosterone were purchased from Sigma Chemical Co., St. Louis, MO.

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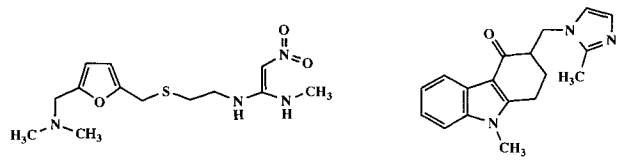

Ranitidine Ondansetron

Fig. 1. Structures of ranitidine and ondansetron.

Incubation Media

Eagle's minimum essential medium (mod.) 1× (with Earle's salts and L-glutamine) was obtained from Fisher Scientific, Pittsburgh, PA. Fetal bovine serum (FBS), nonessential amino acids (NEAA), Hank's balanced salt solution (HBSS), 0.05% trypsin and 0.02% EDTA in HBSS, glucose, *N*-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES), ethylenediaminetetraacetic acid (EDTA), and phosphate-buffered saline (PBS) were purchased from Sigma Chemical Co.

Caco-2 Cell Culture Model

Caco-2 cells were maintained at 37°C in minimum essential medium, containing 10% FBS and 1% NEAA in an atmosphere of 5% CO_2 and 90% relative humidity (1,7). Cells grown in 75-cm^2 T flasks (Costar, Cambridge, MA) were supplied in 25 mL of culture medium. Cells were passaged every 3–4 days at a split ratio of 1 to 5 (1.0 mL of 0.05% trypsin and 0.02% EDTA in HBSS per flask was used for trypsinization) and confluency was reached within 4–5 days. For transport and cellular uptake studies, cells were seeded at a density of 300,000 cells/well onto a 4.71-cm^2 polycarbonate membrane of Transwell (24.5-mm i.d., 3.0 μm pore size; Costar) to late confluency (20–25 days, transmembrane resistance reaches ~300 Ω · cm^2 after 10 days). Media were changed every 2–3 days after seeding and replaced with transport media (HBSS containing 25 m*M* glucose and 10 m*M* HEPES buffer, pH 7.4) 1 hr before the experiment. Calcium-free transport medium and transport medium that contains both EDTA and calcium were used as appropriate.

Cellular Metabolism Studies

[^{14}C] Ondansetron (60 μ*M*) was added to both the upper chamber (apical side; AP) and the lower chamber (basolateral side; BL) of Transwells, and AP and BL samples from the 1-hr incubation study were analyzed by a HPLC system with a radiochemical detector and a UV detector at 302 nm. A Spherisorb CN column (100 × 4.6 mm) and an isocratic mobile phase (65% acetonitrile and 35% 25 m*M* ammonium acetate, pH 4.0) were used. The retention time of ondansetron was 4.1 min. AP and BL samples from the 2-hr incubation of cell monolayers with [^{3}H]ranitidine (0.9 m*M* on both sides of Transwells) were analyzed by HPLC with a radiochemical detector and a UV detector at 320 nm. A Spherisorb ODS column (250 × 4.6 mm, 5 μm) and an isocratic mobile phase of 35% 50 m*M* phosphate buffer, pH 6.0, 60% methanol, and 5% tetrahydrofuran were used. The retention time of ranitidine was 6.2 min.

Cellular Transport Studies

Transport experiments were initiated by replacing medium on the AP side of Transwells with 1.5 mL transport medium containing either ondansetron or ranitidine. Transport rates were monitored at various time points by measuring the amount of drug present on the BL side (2.6 mL) of Transwells. The amount of radiolabeled drug transported to the BL side of cell monolayers was quantitated by liquid scintillation counting in a Beckman LS-5801 spectrophotometer. All transport experiments were carried out under sink conditions as the concentrations of drugs in the BL chamber remained at least 10-fold lower than those in the AP chamber throughout the experiments. Two measurements were taken for each time point. Drug concentrations used in the transport studies were 0.142 μ*M* to 14.25 m*M* for [^{3}H]ranitidine (0.15 μCi/mL) and 3.48 to 111.4 μ*M* for [^{14}C]ondansetron (0.03 μCi/mL).

Cellular Uptake Studies

Uptake experiments were initiated by adding radiolabeled drugs (2.5–2.7 μ*M*) in transport medium to either the AP side or both the AP and the BL sides of Transwells (i.e., for compounds such as ranitidine, transport studies were conducted either under sink conditions or at a steady state). Cell monolayers were incubated for 3 hr, washed twice with transport medium containing a severalfold excess of unlabeled drug, and incubated with tissue solubilizer overnight at room temperature. Cellular uptake of drug was quantitated by liquid scintillation counting in a Beckman LS-5801 spectrophotometer. Mannitol and testosterone, compounds that are transported across Caco-2 monolayers via paracellular and transcellular passive diffusion pathway, respectively, were chosen as controls in these studies.

Data Analysis

The apparent permeability coefficients (P_{app}) were calculated from the following equation: $P_{app} = (dQ/dt)/C_o/A$ (cm/sec), where dQ/dt is the permeability rate (mol/sec), C_o is the initial concentration on the AP side of cell monolayers (mol/mL), and A is the surface area of the porous membrane (4.71 cm^2). Permeability rates (dQ/dt) were calculated by plotting the amounts of drug transported to the BL side vs time and determining the slope of these plots. The permeability rates (dQ/dt) were then plotted vs the initial concentrations (C_o) to obtain the value of the slope, $(dQ/dt)/C_o$. The correlation coefficients (r^2) obtained from the least-squares linear regression analysis were in the range of 0.97–1.00.

RESULTS AND DISCUSSION

Cellular Metabolism

Under HPLC conditions, no metabolites were detected in either AP or BL samples from the cellular metabolism studies of ondansetron and ranitidine (data not shown). Since the presence of cytochrome P450IIIA activities, the major cytochrome P450 found in the intestine, has not yet been fully characterized in the Caco-2 cell line, the absence of ondansetron and ranitidine metabolites may not necessarily imply that intestine is not the site of metabolism. Never-

theless, the absence of metabolite(s) enabled us to measure the rate of transport of ondansetron and ranitidine using the total amount of radioactivity in the samples.

Transport of Ranitidine and Ondansetron Across Caco-2 Cells

Linear transport was observed for ranitidine and ondansetron (cf. Fig. 1) over 120 and 60 min, respectively. Apparent permeability coefficients (P_{app}) for the transport of these compounds were found to be unchanged throughout the concentrations studied, indicating a simple passive diffusion pathway for the transport of these compounds across Caco-2 monolayers (Fig. 2). P_{app} values for ranitidine and ondansetron (bioavailability of 50 and 100% in humans, respectively) were 1.03 ± 10^{-7} and $1.83 \pm 0.055 \times 10^{-5}$ cm/sec, respectively.

Effect of Extracellular Calcium

It has been reported that the integrity of the tight junctions can be modulated by varying the calcium concentrations in the media (15,24). To determine the relative contribution of transcellular and paracellular transport for ondansetron and ranitidine, the transport of [^{3}H]ranitidine (0.03 μM) and [^{14}C]ondansetron (0.05 μM) was studied in calcium-free medium or in the transport medium containing EDTA, respectively. The result shown in Fig. 3 indicates that a more than 15-fold increase in P_{app} (from 1.03×10^{-7} to 1.86×10^{-6} cm/sec) for ranitidine was observed when calcium was removed from the transport medium. A more than 20-fold increase in P_{app} (from 1.03×10^{-7} to 2.5×10^{-6} cm/sec) for ranitidine was also caused by the presence of 1.25 or 2.5 mM EDTA in the transport media (data not shown). A concomitant increase in [^{14}C]mannitol (paracellular leakage marker) transport was also observed in the above studies, indicating the increase in junctional pore size. Together these results suggest that paracellular passive diffusion is the major pathway for the transport of ranitidine across Caco-2 cell monolayers. Extracellular calcium concentrations, on the other hand, had no effect on the P_{app} value (from 1.83×10^{-5} to 1.95×10^{-5} cm/sec) for ondansetron (Fig. 3), indicating that paracellular passive diffusion is not the rate-determining step for the transport of ondansetron across Caco-2 cell monolayers. A similar approach has been used for elucidating the paracellular transport mechanism for the β-blocker, atenolol (15). Breimer and co-workers also reported the effect of AP and/or BL EDTA on the permeability of hydrophilic fluorescein-Na and fluorescein isothiocyanate dextrans (FITC-dextrans) (24). It was found that EDTA increased the transport of FITC-dextrans across Caco-2 cell monolayers compared to nontreated cell monolayers.

Cellular Uptake

While the effects of extracellular calcium on the transport of ranitidine across Caco-2 cells provide good circumstantial evidence for the paracellular transport of this drug, definitive evidence for the transport mechanism was provided by cellular uptake studies. The experiments were initiated by adding radiolabeled drugs in transport medium to both the AP side and the BL side of the cell monolayers, and the cellular uptake was measured under steady-state condi-

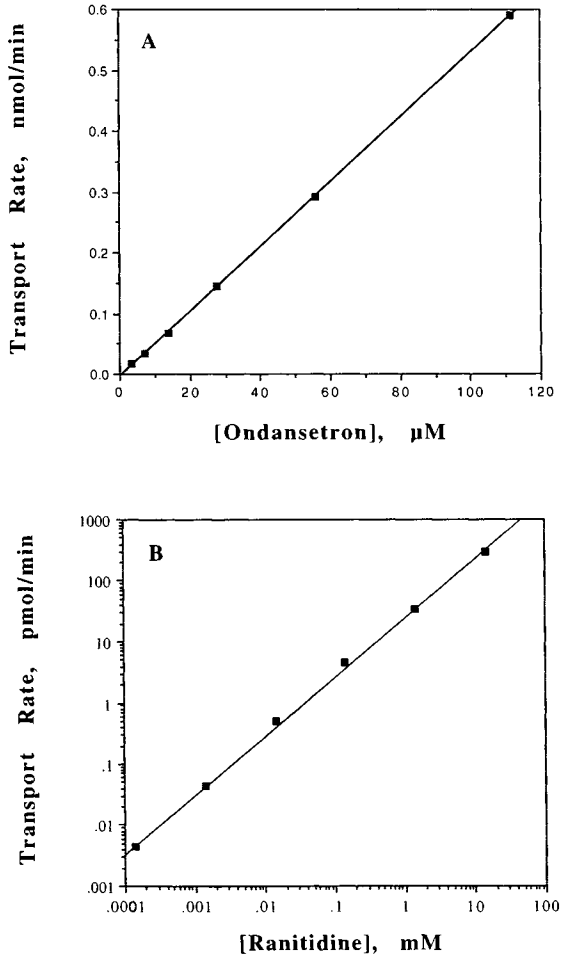

Fig. 2. Rates of transport of [^{14}C]ondansetron (A) and [^{3}H]ranitidine (B) (log–log plot) as a function of concentration. A linear relationship between transport rate and concentration was observed. Correlation coefficients (r^2) were 0.99 and 1.0 for ranitidine and ondansetron, respectively.

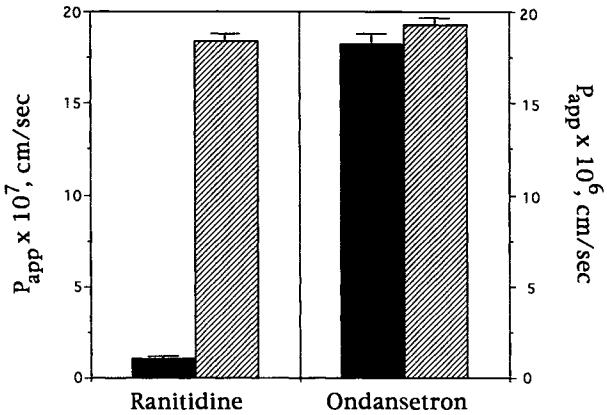

Fig. 3. Effect of extracellular calcium concentration on the apparent permeability coefficient of [^{3}H]ranitidine and [^{14}C]ondansetron. (■) Transport experiment conducted at an extracellular calcium concentration of 1.26 mM. (▨) Transport experiment conducted in the calcium-free medium.

Table I. Cellular Uptake of Ranitidine, Ondansetron, Mannitol, and Testosterone by Caco-2 Cell Monolayers

Compound	Uptake (pmol)[a]	Uptake (pmol)[b]
Ranitidine	17.8 ± 1.7	3.75 ± 0.24
Ondansetron	99.3 ± 16.8	76.8 ± 7.1
Mannitol	5.0 ± 1.0	0.83 ± 0.16
Testosterone	76.9 ± 8.7	30.3 ± 4.3

[a] Radiolabeled drugs were added to both the BL and the AP sides of monolayers.
[b] Radiolabeled drugs were added to the AP side of monolayers.

tions. It was expected that ranitidine, which appears to be transported across intestinal mucosa via the intercellular junctions, should not be taken up into the cells very effectively. The results in Table I, indeed, show that ranitidine is taken up into the cells far less efficiently than is ondansetron, the drug that traverses the intestinal mucosa by a transcellular mechanism. When the uptake study was carried out under sink conditions by adding radiolabeled drugs in the transport medium to the AP side of cell monolayers, a more representative condition for the transport of drugs, the cellular uptake of ranitidine was 20-fold lower than that of ondansetron. In similar experiments, mannitol, a compound that does not traverse across the Caco-2 monolayers, was taken up 15- to 30-fold less efficiently than the transcellularly transported compound, testosterone. These studies clearly demonstrate the advantage of using the Caco-2 cell culture model to study the mechanism of drug transport and, specifically, to evaluate the contribution of the paracellular vs the transcellular pathway to the transport of drug molecules across intestinal mucosa.

In summary, we have observed that lipophilic compounds with high P_{app} values, testosterone and ondansetron, are transported by a transcellular route. In contrast, polar compounds such as ranitidine have low P_{app} values because of their inability to permeate cell membranes. The primary route for the transport of polar compounds is via the paracellular pathway. One can expect gradients of transparacellular transport as one goes from lipophilic to hydrophilic compounds. The Caco-2 cell culture model should prove to be an excellent tool for dissecting these transparacellular components.

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