TAXOL TRANSPORT BY HUMAN INTESTINAL EPITHELIAL CACO-2 CELLS

U. KRISTINA WALLE AND THOMAS WALLE

Department of Cell and Molecular Pharmacology and Experimental Therapeutics, Medical University of South Carolina

(Received August 27, 1997; accepted December 11, 1997)

This paper is available online at http://www.dmd.org

ABSTRACT:

Taxol (paclitaxel) belongs to a new class of antimicrotubule antitumor drugs with clinical activity against common solid tumors and acute leukemias. Preclinical studies have suggested that taxol is not absorbed after oral doses. However, whether the observed low oral bioavailability is the result of poor absorption or extensive presystemic hepatic metabolism is not clear. For this reason, we studied the transepithelial flux of taxol, using the human colonic cell line Caco-2 as a model. The cells were grown to confluence on permeable polycarbonate membrane inserts, to permit flux experiments after loading of [3H]taxol on either the apical or basolateral side. The flux of taxol across the Caco-2 cell layer was linear with time for up to 3 hr. The flux from the basolateral to the apical side was 4–10 times greater than that from the apical to the basolateral side. Whereas the absorptive transport appeared linearly related to the taxol concentration (0.5–20 μM), the efflux was saturable. The apparent KM of the active efflux component was 16.5 μM. Verapamil (50 μM) significantly decreased the active transport component. These data support the conclusion that rapid passive diffusion of taxol through the intestinal epithelium is partially counteracted by the action of an outwardly directed efflux pump, presumably P-glycoprotein. However, the relatively high apparent permeability coefficient for the apical to basolateral taxol transport (4.4 ± 0.4 × 10−6 cm/s; N = 17) suggests that the drug may still be effectively absorbed in the intestinal tract.

Materials and Methods

Materials. Generally labeled [3H]taxol (15–30 Ci/mmol, 1 μCi/μl ethanol) and [3H]vinblastine sulfate (12 Ci/mmol, 0.25 μCi/μl methanol) were purchased from Moravek Biochemicals (Brea, CA). Taxol, fetal calf serum and other cell culture medium components, HBSS,1 and (±)-verapamil hydrochloride were purchased from Sigma Chemical Co. (St. Louis, MO). [14C]Mannitol (50–62 mCi/mmol) was obtained from Amersham Corp. (Arlington Heights, IL).

Caco-2 Cell Culture. The human colon adenocarcinoma cell line Caco-2 (American Type Culture Collection, Rockville, MD) was grown as monolayers, according to procedures recommended by the American Type Culture Collection, in Eagle’s minimum essential medium with Earle’s salts, 10% fetal calf serum, 1% nonessential amino acids, 100 units/ml penicillin, and 0.1 mg/ml streptomycin, in a humidified 37°C incubator with 5% carbon dioxide. Stock cultures were grown in 75-cm² flasks and split 1:6 or 1:12 at 80% confluency, using trypsin-EDTA (Augustijns et al., 1993). For transport studies, the Caco-2 cells were seeded at a density of 100,000 cells/insert in Transwell, 1-cm², permeable polycarbonate inserts (0.4-μm pore size; Corning Costar Corp., Cambridge, MA) in 12-well plates. The inserts were fed every 2 days for the first 2 weeks and then daily until they were used for experiments 20–30 days after seeding (Augustijns et al., 1993). The integrity of the cell monolayers was evaluated by measuring transepithelial electrical resistance values with a Millicell-ERS voltmeter (Millipore Corp., Bedford, MA). The cell inserts were used for experiments when the resistance exceeded 300 Ωcm². In each experiment, the transport of [14C]mannitol was measured in two inserts. The cell monolayers were considered tight when the mannitol transport was <0.3% of the dose/hr, corresponding to a PAp value (Augustijns, 1990) of 0.4 ± 10⁻⁶ cm/sec.

Taxol Transport. Before experiments, the cells were washed twice with warm HBSS containing 25 mM HEPES, pH 7.4 (Augustijns et al., 1993). After each wash, the plates were returned to the incubator for 30 min. Transepithelial electrical resistance values were measured after the last wash. The buffer was then replaced with fresh HBSS/HEPES buffer on one side of the cell layer and taxol in HBSS/HEPES buffer on the other side. The apical side of the cell layer (insert) contained 0.5 ml and the basolateral side (well) contained 1.5 ml. In

1 Abbreviations used are: HBSS, Hanks’ balanced salt solution; PAp, apparent permeability coefficient; HEPES, N-(2-hydroxyethyl)piperazine-N’-(2-ethanesulfonic acid); CYP or P450, cytochrome P450.
each experiment, two inserts were used for each treatment. The taxol solution (0.5–20 μM) contained 0.5 μCi/ml [3H]taxol and no more than 0.5% dimethylsulfoxide. When taxol was added on the apical side, the inserts were moved to a well with fresh buffer every 30 min. At the end of the experiment (3 hr), the radioactivity in aliquots from each well and insert was measured by liquid scintillation counting after the addition of biodegradable counting scintillant cocktail (Amersham). When taxol was added on the basolateral side, the buffer in the insert was replaced with 0.5 ml of fresh buffer every 30 min. The radioactivity in each 30-min sample and in an aliquot of the 3-hr basolateral solution was determined. In transport inhibition experiments, verapamil hydrochloride (50 μM) was added to the buffer on both sides of the cell layer. The taxol concentration used in these experiments was 10 μM.

Calculations. For each experiment, the mean transport rate was calculated from the linear portion of the plot of the total amount of taxol transported vs. time. Because there was a slight lag time before transport occurred and because the transport rate appeared to level off at 3 hr in some experiments, the 1–2.5-hr time points were used for this calculation (i.e., four time points for each experiment). The $P_{\text{app}}$ values, expressed in centimeters per second (Artursson, 1990), were calculated as $\Delta Q/\Delta t \times 1/60 \times 1/1A \times 1/C$, where $\Delta Q/\Delta t$ is the permeability rate (in micrograms per minute), $A$ is the surface area of the membrane (in square centimeters), and $C$ is the initial concentration in the donor chamber (in micrograms per milliliter). The statistical significance of differences between treatments was evaluated using two-tailed, paired, Student $t$ tests, with a significance level of $p < 0.05$.

Results

The flux of taxol across Caco-2 cell monolayers, when the drug was loaded on either the apical or basolateral side of the cells, is shown in fig. 1. As can be seen, the flux was essentially linear for up to 3 hr for all taxol concentrations studied (0.5–20 μM). The flux from the basolateral to the apical side was 4–10-fold greater than that from the apical side to the basolateral side.

To determine the molecular specificity of these measurements, some of the apical and basolateral samples were also analyzed by reverse-phase HPLC with radiometric detection, as previously described (Walle, 1996). All radioactivity corresponded to taxol itself. The recovery of taxol in all of the experiments was >90%. There was no evidence of taxol decomposition or Caco-2 cell-mediated metabolism by CYP2C8 or CYP3A4 (Walle, 1996).

The influence of the concentration of taxol on its flux across Caco-2 cell monolayers was examined (fig. 2). The range of concentrations used was limited by the lack of taxol solubility in protein-free buffer (~25 μM). Taxol flux was expressed as a rate (picomoles per hour per square centimeter). The apical to basolateral flux appeared linear with increasing taxol concentrations, although there was a slight trend toward increased flux at the highest concentration. In contrast, the basolateral to apical flux was clearly saturable (fig. 2A), indicating the presence of an active efflux mechanism. Thus, whereas the observed basolateral to apical flux is the result of passive diffusion plus active transport, the apical to basolateral flux would be the result of passive minus active transport. When the active transport rate was calculated as (basolateral transport – apical transport)/2 (Gan et al., 1996) and examined in a Lineweaver-Burk plot (fig. 2B), an apparent $K_M$ value of 16.5 μM and a $V_{\text{max}}$ of 1050 pmol/hr/cm$^2$ were obtained. Expressed in another way, the $P_{\text{app}}$, for the basolateral to apical transport decreased from 31.8 ± 5.6 × 10$^{-6}$ cm/sec (mean ± SE) at 0.5–2 μM to 22.1 ± 4.2 × 10$^{-6}$ cm/sec at 20 μM taxol, whereas the apical to basolateral transport was fairly constant (4.4 ± 0.4 × 10$^{-6}$ cm/sec; $N = 17$) throughout the concentration range studied. The $P_{\text{app}}$ values for the basolateral to apical transport were significantly higher than those for the apical to basolateral transport at each taxol concentration ($p = 0.001$–0.02).

To distinguish active transport from potential facilitated diffusion as the efflux mechanism, incubations were performed as described above but with identical concentrations (10 μM) of labeled taxol on both the apical and basolateral sides of the monolayer. The concentration of taxol on the apical side (volume, 0.5 ml) increased throughout the 3-hr experiments to 23.8 ± 0.7 μM at 180 min, compared with 10.7 ± 0.1 μM at 30 min ($p < 0.0001$; $N = 3$), consistent with active transport. A comparable amount of taxol was lost from the basolateral side (volume, 1.5 ml) (remaining concentration, 7.2 ± 0.6 μM at 3 hr).

A series of experiments used inhibition to address the nature of the basolateral to apical efflux. In these experiments, a 50 μM concentration of the P-glycoprotein inhibitor verapamil reduced the efflux by about 30% (fig. 3). Concomitant with this inhibition was a 2-fold increase in the apical to basolateral flux. In similar experiments using 10 nM vinblastine as the substrate (Hunter et al., 1993a,b), 50 μM

![Fig. 1. Transepithelial flux of taxol across the Caco-2 cell monolayer.](image)

A. Apical to basolateral flux; B, basolateral to apical flux. The taxol concentrations used were 0.5 μM (□), 1 μM (●), 2 μM (▲), 5 μM (△), 10 μM (○), and 20 μM (▪). Each point is the mean value of three to seven experiments.
verapamil reduced the vinblastine efflux by about 40% while increasing apical to basolateral flux 2.4-fold (data not shown; N = 2).

**Discussion**

The finding in this study that taxol effluxes from Caco-2 cells is consistent with the known expression of P-glycoprotein on the apical side of these human intestinal cells (Hunter *et al.*, 1993b) and the fact that taxol is a substrate of this transporter (Gupta, 1985; Horwitz *et al.*, 1986). The ability of verapamil, a well-known P-glycoprotein antagonist (Racker *et al.*, 1986), to reduce this active transport is additional evidence for the involvement of this mechanism. The apparent $K_M$ value of 16.5 $\mu$M for the saturable process for taxol was very similar to that previously reported for the P-glycoprotein substrate vinblastine (19.0 $\mu$M), also in the Caco-2 cell system (Hunter *et al.*, 1993a,b). The $V_{max}$ value of 1050 pmol/hr/cm$^2$ for taxol was also similar to that calculated for vinblastine (643 pmol/hr/cm$^2$). As the efflux mechanism becomes saturated at higher drug concentrations, the apical to basolateral flux, i.e. absorption, would be expected to increase, as it appears to do for both vinblastine (Hunter *et al.*, 1993a) and cyclosporin (Augustijns *et al.*, 1993). For taxol there was, however, only a slight trend toward an increase.

However, of greater importance as a potential predictor of oral absorption is the apical to basolateral $P_{app}$. This was $4.4 \times 10^{-6}$ cm/sec for taxol and only $1.0 \times 10^{-7}$ cm/sec for vinblastine. According to a previous study (Artursson and Karlsson, 1991), a $P_{app}$ value in Caco-2 cells of $>1 \times 10^{-6}$ cm/sec should, in general, be associated with efficient intestinal absorption in humans. Therefore, it is hypothesized that the low oral bioavailability of taxol (Eiseman *et al.*, 1994; Sonnichsen and Relling, 1994) may be more dependent on presystemic metabolism in the liver than on lack of absorption. This should be directly testable in humans.

As expected, there was no metabolism of taxol in the Caco-2 cells. CYP3A4, the major P450 isofrom in human intestine (Kolars *et al.*, 1992; Watkins *et al.*, 1987), has been indicated to be present at low levels in the Caco-2 cell line (Gan *et al.*, 1996; Schmidlin-Ren *et al.*, 1997). However, CYP3A4 catalyzes only the formation of a minor taxol metabolite (Cresteil *et al.*, 1994; Harris *et al.*, 1994; Walle, 1996), whereas the major taxol metabolite is formed by hepatic CYP2C8 (Rahman *et al.*, 1994), an isofrom that appears to be absent in the intestine (Goldstein and de Morais, 1994) and presumably also in Caco-2 cells.

For drugs like taxol and vinblastine, which are substrates for P-glycoprotein, it is uncertain to what extent we can extrapolate from Caco-2 cells to the *in vivo* human situation. Although the presence of P-glycoprotein has been established, by Western blot analysis or immunofluorescence with a monoclonal antibody, in all parts of the gastrointestinal tract (Fricker *et al.*, 1996) and in Caco-2 cells (Fricker *et al.*, 1996; Hosoya *et al.*, 1996; Hunter *et al.*, 1993b), the latter with P-glycoprotein expression increasing with culture age (Hosoya *et al.*, 1996), no direct quantitative comparison has been made. Thus, more information on the level of P-glycoprotein expression in human intestine, compared with Caco-2 cells, is needed. Other drugs that have been shown to be affected by P-glycoprotein in Caco-2 cells include cyclosporin (Augustijns *et al.*, 1993), sparfloxacin (Cormet *et al.*, 1995), digoxin (Cavet *et al.*, 1996), and talinolol (Wetterich *et al.*, 1996). To examine the potential contribution of CYP3A4 to the transport of drugs like taxol and vinblastine using the Caco-2 cell system, a novel development involving increased expression of
CYP3A4 with addition of 1α,25-dihydroxyvitamin D₃ to the growth medium should be helpful (Schmiedlin-Ren et al., 1997).

Acknowledgments. The authors are grateful to Dr. Tim Bradshaw of Glaxo Wellcome Inc. for helpful advice on starting the Caco-2 cell culture (Augustijns et al., 1993).

References


