ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Dec. 1997, p. 2693–2698 Vol. 41, No. 12
0066-4804/97/$04.00+0

Transepithelial Transport of the Fluoroquinolone Ciprofloxacin by Human Airway Epithelial Calu-3 Cells

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Received 17 March 1997/Returned for modification 28 August 1997/Accepted 8 October 1997

Although fluoroquinolone antibiotics such as ciprofloxacin are able to gain access to lung tissue and both pleural and bronchial secretions, the characteristics of transport and cellular uptake of ciprofloxacin in human epithelial lung tissue remain obscure. We have chosen human airway epithelial (Calu-3) cells, reconstituted as functional epithelial layers grown on permeable filter supports, as a model with which to assess both transepithelial transport and cellular uptake of ciprofloxacin. Transepithelial ciprofloxacin fluxes in absorptive (apical-to-basal) and secretory (basal-to-apical) directions were similar throughout the concentration range studied (1.0 μM to 3.0 mM). Transepithelial mannitol fluxes measured concurrently were substantially smaller than ciprofloxacin fluxes in Calu-3 epithelia, suggesting the existence of a mediated transcellular route in addition to a paracellular route for transepithelial permeation. Apical-to-basal ciprofloxacin flux (at 10 μM) was inhibited by a 100-fold excess of unlabelled norfloxacin, enoxacin, and ofloxacin, while secretory flux was unaffected. Cellular uptake of ciprofloxacin, determined as a cell/medium ratio, was greater from the basal compartment (2.7-fold) than apical uptake (1.39-fold) measured at 100 μM ciprofloxacin and showed no saturation up to 3 mM ciprofloxacin. Comparison of the permeation of ciprofloxacin was made with that of lipophilic substrates such as vinblastine and digoxin. There was a linear correlation between transepithelial permeability (P_{a-b}) and their oil/water partition coefficients with mannitol < ciprofloxacin < digoxin < vinblastine. Comparison of transport of ciprofloxacin across human airway Calu-3 epithelia with that across intestinal Caco-2 epithelia emphasizes the absence of a specific secretory pathway; ciprofloxacin permeation in Calu-3 epithelia appears to be mediated primarily by a transcellular route, with mediated transfer at apical and basal membranes occurring via transporters with low affinity to ciprofloxacin.

Pharmacokinetic studies demonstrate that ciprofloxacin and other fluoroquinolones penetrate well into fluids and tissue of the lung. Ciprofloxacin concentrates well into lung tissue (20). Levels of ciprofloxacin in pleural fluid, sputum, bronchial secretions, and saliva are similar and comparable to levels in serum after 12-h periods (3). Metabolite concentrations in all tissues assayed (lung, bronchial mucosa, and pleural tissue) are low compared to ciprofloxacin concentrations (20). The fluoroquinolone ofloxacin also penetrates well into human lung tissue (22), as do enoxacin (25) and lomefloxacin (2). This means that, unlike aminoglycosides and β-lactams, which are excluded from intracellular infection sites, fluoroquinolones are efficient in the treatment of respiratory infections (17), which can be caused by both intracellular and extracellular pathogens (18). Penetration of fluoroquinolones into bronchial secretions implies transepithelial permeation across the bronchial epithelium. However, the mechanisms of this transport into lung tissues and fluids have yet to be identified.

The purpose of this study was to examine the characteristics of transepithelial transport across human airway (Calu-3) epithelia and investigate the possible existence of a specific secretory pathway for ciprofloxacin in human airway epithelial cells. The cell line Calu-3 was identified in a survey of 12 cell lines derived from human cancers (for Calu-3, a bronchial adenocarcinoma) as the only cell line that formed polarized monolayers with tight junctions separating apical and basolateral domains (23). Furthermore, when grown on permeable supports, functional epithelia were formed that, when mounted in Ussing chambers, demonstrated cyclic AMP-dependent Cl^- secretion typical of native airway epithelia (10, 23). This retention of differentiation was not dependent on air interface culture, as is observed with primary cultures of bovine tracheal epithelium (16). The Calu-3 cell system provides a convenient in vitro model with which to investigate many aspects of bronchial epithelial cell biology, including transport of fluoroquinolones, such as ciprofloxacin. We have previously studied the mechanistic basis of ciprofloxacin secretion across human intestinal Caco-2 epithelia (8, 9). In this system, an active saturable secretion is responsible for blood-to-lumen ciprofloxacin transport that is identical in operational characteristics to that identified in native animal tissue. The characteristics of ciprofloxacin transport by Calu-3 cells were directly compared to those of the secretory transport exhibited by human intestinal Caco-2 cells (8, 9).

MATERIALS AND METHODS

Materials. [^{14}C]ciprofloxacin (1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-[2,3-^{14}C]piperazinyl-quinolone-3-carboxylic acid) (specific activity, 258.26 μCi · mg^{-1}) and unlabelled ciprofloxacin were generous gifts from Bayer (Wuppertal, Germany). [^{3}H]mannitol (specific activity, 30 Ci/mmol) and [^{3}H]vinblastine sulfate (specific activity, 16 Ci/mmol) were from Amersham (Little Chalfont, Buckinghamshire, United Kingdom). [^{14}C]mannitol (specific activity, 50 Ci/mmol) and [^{3}H]digoxin (specific activity, 20 Ci/mmol) were from New England Nuclear (Stevenage, Hertfordshire, United Kingdom). Cell culture media, supplements, and plastic were supplied by Life Technologies (Paisley, Strathclyde, United Kingdom). All other chemicals were supplied by BDH or Sigma (Poole, Dorset, United Kingdom).

Cell culture. Calu-3 cells were purchased from the American Type Culture Collection and used between passages 21 and 37. The cells were cultured in Eagle's minimal essential medium supplemented with nonessential amino acids (1%), L-glutamine (2 mM), fetal calf serum (10%), penicillin (50 U · ml^{-1}), and

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streptomycin (50 mg · ml^{-1}). Caco-2 cells were obtained from I. Hassan (Ciba-Geigy Pharmaceuticals, Horsham, Sussex, United Kingdom) and used between passages 95 and 114. Caco-2 cells were cultured in Dulbecco's modified Eagle's medium containing glucose (4.5 g · $liter^{-1}$) and supplemented with nonessential amino acids (1%), L-glutamine (2 mM), fetal calf serum (10%), and gentamicin (60 mg · ml^{-1}). Cell monolayers were prepared by being seeded at high density (4.4×10^5 to 5.0×10^5 cells · cm^{-2}) onto tissue culture inserts (Transwell polycarbonate filters [Costar]; 4.2-cm^2 growth area). Cell monolayers were maintained at 37°C in a humidified atmosphere of 5% CO_2 in air. The formation of functional epithelial layers was estimated by microscopy and determination of transepithelial electrical resistance with a WPI Evometer fitted with "chopstick" electrodes (World Precision Instruments, Stevenage, Hertfordshire, United Kingdom) measured at 37°C in Krebs buffer (12). Cell layers were typically used when the transepithelial resistance across the monolayer was between 300 and 400 Ω · cm^2 (Calu-3 cells) and 200 and 300 Ω · cm^2 (Caco-2 cells) (9, 13, 14). (Note that the resistance of the filter assembly was subtracted.) We verified the differentiated epithelial characteristics of Calu-3 by measurement of forskolin-stimulated short circuit current in selected layers (10, 23).

Transepithelial transport experiments. Uptake and transport experiments with ciprofloxacin were performed 14 to 21 days after seeding and 18 to 24 h after feeding. Transepithelial flux measurements were performed as described previously (24). Briefly, the cell monolayers (24.5 mm in diameter) were washed by sequential transfer through four beakers containing 500 ml of modified Krebs buffer, which contained 137 mmol of NaCl, 5.4 mmol of KCl, 2.8 mmol of $CaCl_2$, 1.0 mmol of $MgSO_4$, 0.3 mmol of NaH_2PO_4, 0.3 mmol of KH_2PO_4, 10 mmol of glucose, and 10 mmol of HEPES-Tris (pH 7.4, 37°C) per liter and placed in six-well plates, each well containing 2 ml of modified Krebs buffer. Krebs buffer (2 ml [pH 7.4]) was placed in the upper filter cup (apical solution), and the filters were incubated for 10 min at 37°C. The experimental compositions of the buffers in the apical and basal chambers were identical, except where stated otherwise. Radiolabelled [^{14}C]ciprofloxacin and [^{3}H]mannitol (0.1 mCi/ml) were added to either the apical or basolateral chamber, and in each case, an equivalent concentration of unlabelled substrate was present in the contralateral chamber. For experiments in which the unlabelled ciprofloxacin concentration was varied and other fluoroquinolones were present, equal concentrations were present in both the apical and basolateral bathing solutions as specified in the text and figure legends. Fluxes in the absorptive (apical-to-basal [$J_{a\text{-}b}$]) and secretory (basal-to apical [$J_{b\text{-}a}$]) directions were determined for 1 h (after a 20-min preincubation to establish a state of linear flux) on adjacent paired cell monolayers and were calculated as follows: $J_{a\text{-}b} = D_b \cdot M/D_t \cdot S$, $J_{b\text{-}a} = D_a \cdot M/D_t \cdot S$, and $J_{net} = J_{a\text{-}b} - J_{b\text{-}a}$, where D_b or D_a is the amount of radioactivity appearing in the contralateral (basal) compartment after 1 h, D_t is the initial radioactivity in the originating (apical or basal compartment), M is the ciprofloxacin molar content of the apical compartment, and S is the epithelial surface area. Fluxes are expressed as nanomoles or picomoles per square centimeter per hour. Net flux (J_{net}) was calculated as the difference in transepithelial bidirectional fluxes; monolayers were assigned as pairs during experimentation, allowing direct calculation of a single value of net flux for statistical analysis.

Apparent permeability was calculated for the appropriate flux as $P_{a\text{-}b} = J_{a\text{-}b}/C_a$, where P is the permeability and C is the concentration of the solute (ciprofloxacin, etc.) in the originating compartment.

The passive (paracellular) route across the epithelium was estimated by concurrent mannitol flux determinations. Mannitol flux into the contralateral chamber was typically 2% at the end of the incubation period. Although transepithelial resistance allowed rejection of nonconfluent or damaged epithelia, mannitol flux values of >5% led to rejection of the monolayer and associated ciprofloxacin flux data.

At the end of the incubation period, cell monolayers were washed by sequential transfer through four beakers containing 500-ml volumes of ice-cold Krebs buffer (pH 7.4) to remove any loosely associated extracellular radiolabel and were removed from the insert. Cell monolayer-associated radiolabel was determined by scintillation counting. The adequacy of the washing protocol was assessed by the retention of the extracellular marker, [^{3}H]mannitol; this amounted to 0.013% (apical) to 0.047% (basal) of total mannitol label present in the incubation solutions. In comparison, the amount of (intracellular) ciprofloxacin retained was 8.0-fold (apical) to 10.9-fold (basal) higher than the amount of mannitol. Cellular uptake of radiolabelled ciprofloxacin from apical or basal bathing solutions was expressed as millimolar concentration or as a cell/medium (C/M) ratio. Cell height (h) was determined by confocal microscopy (14, 23), and this value was used in the determination of intracellular volume ($\pi \cdot r^2 \cdot h = 5.1$ μl per 4.2 cm^2).

Determinations of the transepithelial transport of vinblastine sulfate and digoxin (1 μM vinblastine sulfate with [^{3}H]vinblastine sulfate as tracer, 1 μM digoxin with [^{3}H]digoxin as tracer) were made identically to those of ciprofloxacin, with [^{14}C]mannitol as the paracellular marker.

Statistics. Results are expressed as means ± standard errors of n determinations. Statistical analysis was performed with Student's unpaired t test or one-way analysis of variance with a Bonferroni's post test for multiple comparisons (Graph-Pad Instat, San Diego, Calif.).

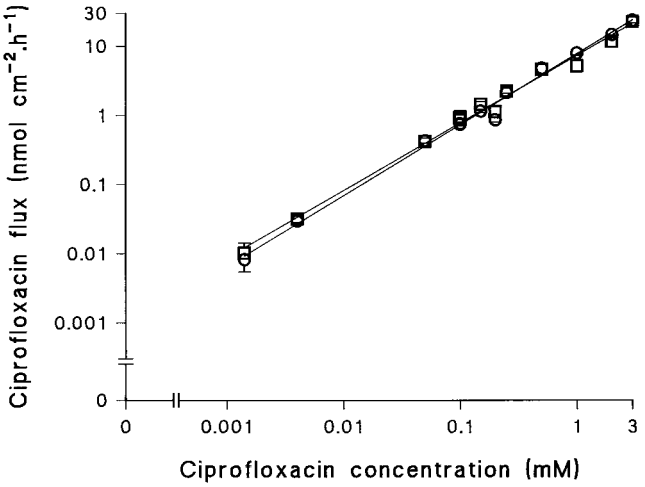

FIG. 1. Concentration dependence of ciprofloxacin transport by human airway Calu-3 cell monolayers over an extended concentration range. Transepithelial [^{14}C]ciprofloxacin fluxes (0.1 μCi · ml^{-1} [5 μM]) were determined in adjacent paired monolayers for apical-to-basal flux ($J_{a\text{-}b}$) (□) and basolateral-to-apical flux ($J_{b\text{-}a}$) (○). A net secretory flux ($J_{net} = J_{b\text{-}a} - J_{a\text{-}b}$) was not present. Increasing concentrations of unlabelled ciprofloxacin were present in both apical and basolateral bathing fluid compartments. The solid lines are regression lines for the data. n = 3 to 12 epithelia per data point.

RESULTS

Transepithelial ciprofloxacin fluxes and uptake in human airway Calu-3 epithelia. Figure 1 shows the transepithelial fluxes of ciprofloxacin across confluent monolayers of human airway Calu-3 cells over an extended concentration range (5 μM to 3 mM). Note that for convenience of visual presentation, both ordinates are plotted as logarithmic scales. Throughout, apical-to-basolateral flux ($J_{a\text{-}b}$) was similar in magnitude to basolateral-to-apical flux ($J_{b\text{-}a}$), and both were linearly dependent on concentration. At 0.1 mM ciprofloxacin, a small net absorption was observed, but this was not significantly different from zero ($J_{a\text{-}b}$ = 0.91 ± 0.10 nmol $cm^{-2} \cdot h^{-1}$, $J_{b\text{-}a}$ = 0.76 ± 0.03 nmol $cm^{-2} \cdot h^{-1}$, and J_{net} = 0.14 ± 0.09 nmol $cm^{-2} \cdot h^{-1}$, [n = 12 for all]). This compares with a significant basal-to-apical net transport (secretion) of ciprofloxacin observed in Caco-2 epithelia ($J_{a\text{-}b}$ of 1.03 ± 0.62 nmol $cm^{-2} \cdot h^{-1}$, $J_{b\text{-}a}$ of 3.29 ± 0.27 nmol $cm^{-2} \cdot h^{-1}$, J_{net} of −2.26 ± 0.35 nmol $cm^{-2} \cdot h^{-1}$ [n = 3 for all] in Caco-2 cells at 0.1 mM ciprofloxacin).

To establish whether the transepithelial fluxes of ciprofloxacin across Calu-3 epithelia were transcellular rather than paracellular, the percentage of administered ciprofloxacin transported into the contralateral chamber was compared with the percentage of administered paracellular marker mannitol transported into the contralateral chamber at the end of the 1-h incubation period in the same epithelia (Fig. 2). The magnitude of ciprofloxacin transported exceeded that of mannitol in all cases, in both apical-to-basolateral and basolateral-to-apical directions, by between 2.1- to 8.7- and 1.76- to 4.68-fold, respectively (Fig. 2a). Since mannitol (molecular weight, 180) is excluded from entering the cell and since transepithelial passage occurs via a solvated paracellular route, the higher transport of ciprofloxacin (molecular weight, 331) in Calu-3 cells cannot be accounted by paracellular transport alone. The data from Calu-3 epithelia contrast with those found for ciprofloxacin transport across human intestinal Caco-2 epithelia (Fig. 2b). In these cells, the percentage of administered ciprofloxacin dose transported in the basolateral-to-apical direction exceeded the percentage of administered mannitol by about

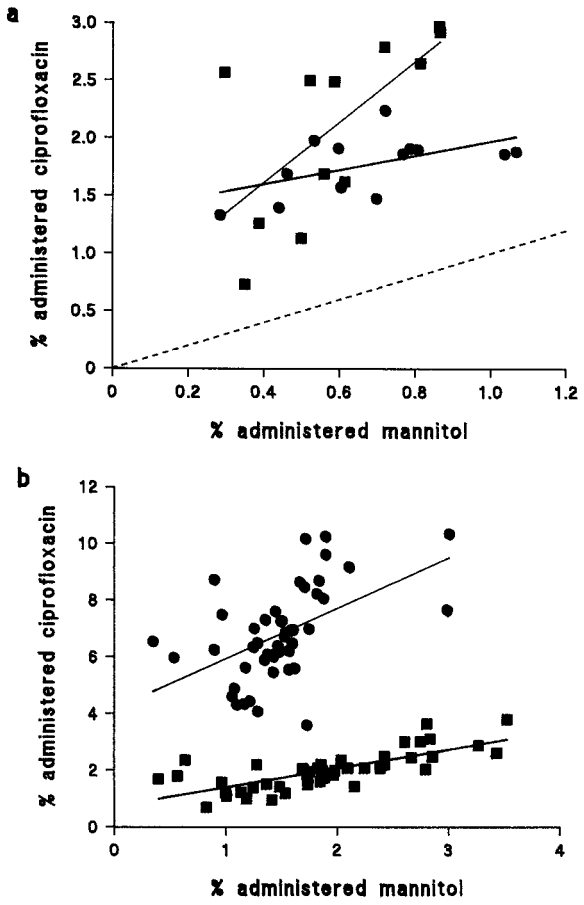

FIG. 2. Comparison of transepithelial transport of ciprofloxacin with that of the paracellular marker mannitol in human airway (Calu-3) epithelia and human intestinal (Caco-2) epithelia. (a) Transport of [^{14}C]ciprofloxacin (5 μM) and [^{3}H]mannitol (3.3 nM) in Calu-3 cells is expressed as the percentage of the administered radioisotope in the contralateral chamber for $J_{a\text{-}b}$ (■ [n = 12]) and $J_{b\text{-}a}$ (● [n = 13]). Unlabelled ciprofloxacin (0.1 mM) and mannitol (0.1 mM) were present in both compartments. The dashed line represents the percentage of administered ciprofloxacin that would be transported if transport was equivalent to that of the paracellular marker mannitol. (b) Transport of [^{14}C]ciprofloxacin (5 μM) and [^{3}H]mannitol (10 nM) in Caco-2 cells is expressed as the percentage of the administered radioisotope in the contralateral chamber for $J_{a\text{-}b}$ (■ [n = 46]) and $J_{b\text{-}a}$ (● [n = 47]). Unlabelled ciprofloxacin (0.1 mM) and mannitol (0.1 mM) were present in both compartments.

fourfold. In the apical-to-basolateral direction, however, the percentage of administered mannitol was similar to that of ciprofloxacin, suggesting this transport may be mediated partially by the paracellular route.

The cellular uptake of ciprofloxacin was determined at the apical and basolateral membrane surfaces at low (1.4 to 250 μM) and high (0.1 to 3 mM) concentration ranges (Fig. 3a and b, respectively). Uptake across the basolateral surface exceeded uptake across the apical surface at all concentrations studied; there was no saturation of cellular uptake. The apparent C/M ratios were 1.39 and 2.70 for loading from the apical and basolateral surfaces, respectively (at 0.1 mM). This indicates that there was a concentrative accumulation of ciprofloxacin from the basolateral compartment, although this was lower than that seen in Caco-2 cells (C/M ratio of 7.0 for uptake from the basolateral solution). Therefore, ciprofloxacin handling in Calu-3 cells (similar transepithelial fluxes, lack of active net secretion, and small concentrative accumulation at the basolateral membrane) differs markedly from ciprofloxacin transport in Caco-2 cells (active saturable net secretion, with a marked concentrative accumulation at the basolateral membrane [8, 9]).

Effect of other fluoroquinolones upon the transport and cellular uptake of ciprofloxacin in Calu-3 cells. The permeation of ciprofloxacin across Calu-3 cells appears to be similar in magnitude in both absorptive and secretory directions and is significantly higher than that of mannitol, suggesting that it is transported by either simple diffusion-solubility criteria or carrier-mediated (facilitated) diffusion across the cell surface. To investigate these possibilities, the effect of other fluoroquinolones on ciprofloxacin transport and cellular uptake was investigated, inhibition being an indication of competition for a common transporter. Norfloxacin, enoxacin, pefloxacin, and ofloxacin (at 1 mM) all significantly inhibited $J_{a\text{-}b}$ of ciprofloxacin, while having no effect upon $J_{b\text{-}a}$ of ciprofloxacin (Fig. 4a). Thus the small net absorption seen at this concentration of ciprofloxacin (10 μM) was reversed on addition of all four

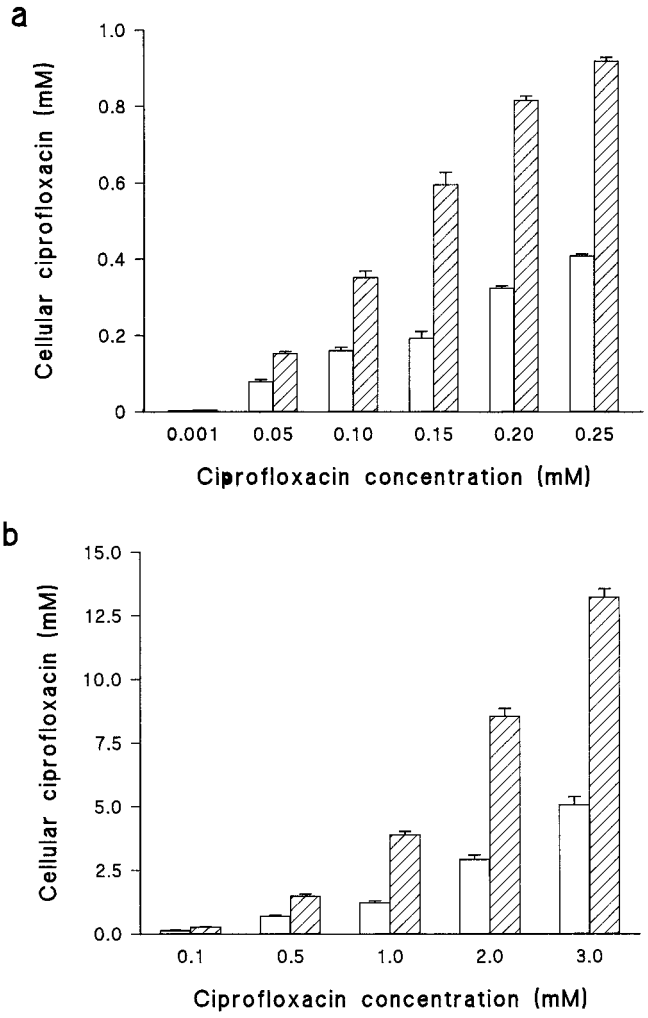

FIG. 3. Cellular uptake of ciprofloxacin in human airway (Calu-3) epithelia. Cellular uptake of ciprofloxacin after a 1-h incubation with increasing ciprofloxacin concentrations at the apical (open columns) and basolateral (hatched columns) membrane surfaces. [^{14}C]ciprofloxacin (5 μM) was present in either apical or basolateral bathing solution, and increasing concentrations of unlabelled ciprofloxacin were added. n = 3 to 12 epithelia per data point. (a) Lower concentration range to 0.25 mM external ciprofloxacin. (b) Higher concentration range up to 3.0 mM external ciprofloxacin.

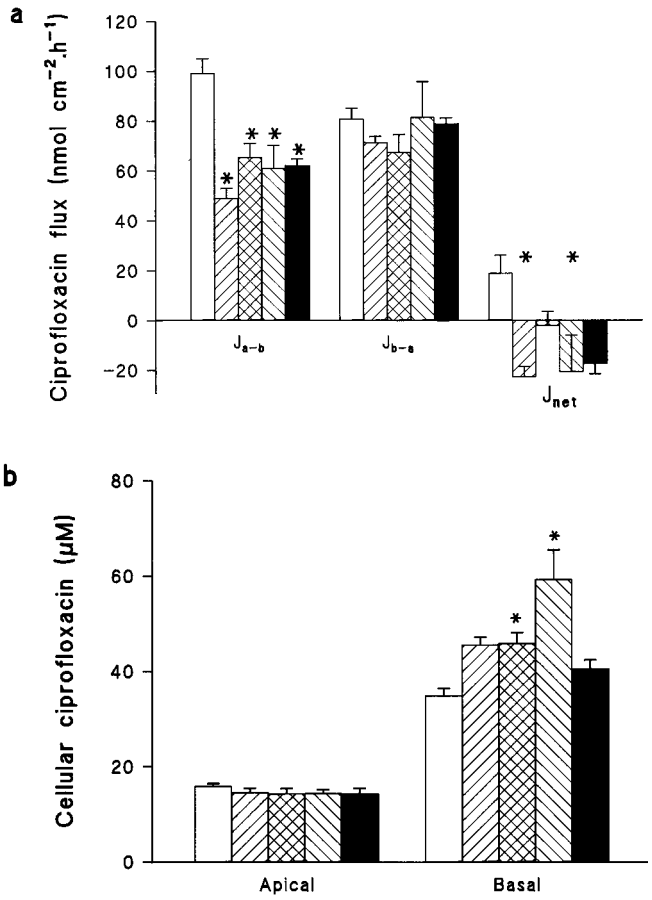

FIG. 4. Transepithelial [^{14}C]ciprofloxacin fluxes and cellular uptake in the presence of related fluoroquinolones. (a) Transepithelial absorptive (J_{a-b}), secretory (J_{b-a}), and net (J_{net}) fluxes of [^{14}C]ciprofloxacin (1 μM) alone (open columns) and in the presence of 1 mM norfloxacin (upward-sloping columns [▨]), 1 mM enoxacin (cross-hatched columns), 1 mM pefloxacin (downward-sloping columns [▧]), and 1 mM ofloxacin (solid columns). Unlabelled ciprofloxacin was present in both the apical and basolateral compartments at 10 μM. n = 3 to 4 epithelia per data point. Asterisks denote values significantly different from control values ($P < 0.05$). (b) Cellular uptake of [^{14}C]ciprofloxacin from the apical or basal solutions in the presence of related fluoroquinolones. Symbols and details are the same as those in panel a.

fluoroquinolones, and a net secretion was seen. Ciprofloxacin itself had no inhibitory effect on its own transport at the maximal concentration allowable because of the limit of solubility (Fig. 1). Two of the fluoroquinolones, enoxacin and pefloxacin, also significantly increased basolateral uptake of ciprofloxacin, while none of the fluoroquinolones had any effect upon apical uptake of ciprofloxacin (Fig. 4b).

Comparison of the apparent permeabilities of ciprofloxacin, mannitol, digoxin, and vinblastine with their respective log *D* values. The bidirectional transports of digoxin, vinblastine, ciprofloxacin, and mannitol (all at 1 μM) were measured in the same experiment in order to compare the calculated apparent permeabilities (e.g., J_{a-b}/C_a, where *a* and *b* denote the apical and basal compartments, respectively, and C_a is the external solution concentration of compartment *a*) of the substrates with their log *D* (octanol/water partition coefficient) values. This will give an indication of the relationship between the ability of the compound to permeate the epithelium and its lipophilicity. As with ciprofloxacin, there is no evidence of net secretion of either digoxin or vinblastine by Calu-3 epithelia. The absorptive permeability (P_{a-b}) slightly exceeded secretory permeability (P_{b-a}) for all substrates. Both the absorptive and secretory permeabilities correlated well with the lipophilicity (as indicated from the octanol/water partition coefficients), permeation in both directions increasing in the following order: mannitol < ciprofloxacin < digoxin < vinblastine (Fig. 5).

These data are in contrast with the relationship found in Caco-2 epithelia (Fig. 5). The apparent permeability of ciprofloxacin, digoxin, and vinblastine for basal-to-apical flux (P_{b-a}) exceeded that in the absorptive direction (P_{a-b}), indicating net secretion. Secretory transport reduces the apparent absorptive permeability, and all values are lower than that observed in Calu-3 cells (Fig. 5). Additionally, P_{a-b} did not increase upon increasing lipophilicity and was comparable to that exhibited by mannitol. Comparison of the higher secretory permeabilities (P_{b-a}) for ciprofloxacin, vinblastine, and digoxin in Caco-2 epithelia to those values seen for Calu-3 cells emphasizes the importance of specific secretory mechanisms to transepithelial permeation in intestinal epithelia.

DISCUSSION

The present study has utilized the human airway epithelial cell-line Calu-3 as a model with which to study the permeation of the fluoroquinolone antibiotic ciprofloxacin. The Calu-3 cell model is likely to represent a phenotype typical of a serous cell of the proximal airway (cartilaginous airways, third- to sixth-generation bronchial airways), since functional epithelia are clearly capable of cyclic AMP-stimulated Cl^- secretion (4, 10, 23). However, it is important to recognize the considerable cellular heterogeneity of the airways (proximal, distal, and alveolar) and the paucity of information concerning the physiological properties of fresh excised material especially from distal airways (4). Despite the considerable interest in the cellular mechanism of fluid and electrolyte transport in airway epithelia, comparatively little attention has been paid to the cellular mechanisms underlying drug permeation. As a functional epithelium, drug permeation across the Calu-3 cell system may be via a nonselective diffusion-driven paracellular route (the tight-junction, lateral interspace) or transcellular,

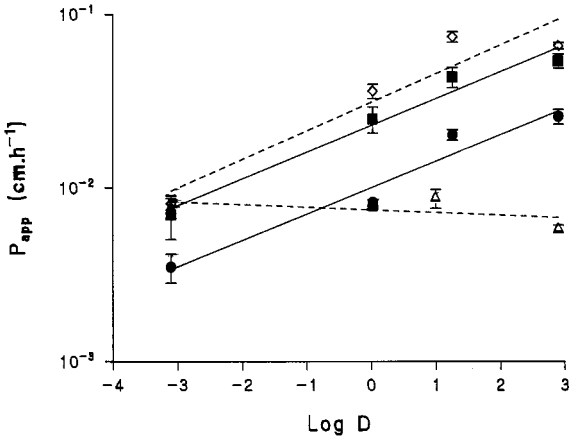

FIG. 5. Transepithelial permeabilities (P_{a-b} and P_{b-a}) of mannitol, ciprofloxacin, digoxin, and vinblastine compared with octanol/water (log *D*) partition coefficients in Calu-3 and Caco-2 cells. Apparent permeabilities (P_{app}) were calculated as described in Materials and Methods. Values for log *D* are as follows; mannitol, −3.1 (manufacturer's data sheet); ciprofloxacin, 0.025 (manufacturer's data sheet); digoxin, 1.26 (19); and vinblastine, 2.9 (6). The solid lines are linear regression lines for the Calu-3 data for J_{a-b} (■) and J_{b-a} (●). n = 4 epithelia per data point. The dashed lines are linear regression lines for the Caco-2 data for J_{a-b} (△) and J_{b-a} (◇). n = 4 to 12 epithelia per data point.

either by passive transport or mediated by specific intrinsic transporters.

The present data show that in human Calu-3 epithelia, both apical-to-basolateral and basolateral-to-apical fluxes are equivalent, and thus there is no marked net transport (absorption or secretion) of ciprofloxacin. This is in contrast with data from intestinal epithelia, such as human Caco-2 cells, in which a specific saturable secretory process exists. In intestine, secretory transporters such as MDR-1 at the apical (luminal) surface render the epithelium effectively impermeable to substrates which on a priori grounds would be considered permeant (see below). In Caco-2 epithelia, ciprofloxacin secretion is mediated by a mechanism distinct from MDR (8, 9). Two known MDR-1 substrates, vinblastine and digoxin, were tested (5, 7, 12–14) with Calu-3 epithelia and were not subject to transepithelial secretion. This lack of functional evidence for MDR-1 expression is in agreement with the inability to easily detect MDR-1 in human lung tissue and most lung cancers (7).

However, a large proportion of ciprofloxacin transport in both the absorptive and secretory directions in Calu-3 epithelia appears to be transcellular, since the magnitude of transport greatly exceeds that of the water-soluble paracellular marker mannitol. This situation is identical to the absence of net transport of the fluoroquinolone pefloxacin exhibited by human intestinal Caco-2 cells (9). Pefloxacin has $J_{a\text{-}b}$ and $J_{b\text{-}a}$ of similar magnitude and a small or no net transport. The transport of pefloxacin in both directions shows signs of saturation at 10 mM. With a three-compartment model, calculated pefloxacin permeabilities at the apical membrane showed an asymmetry ($P_{a\text{-cell}} > P_{\text{cell-}a}$) indicative of mediated (active) transport (9), while cross-inhibition of uptake by other fluoroquinolones at the basolateral membrane suggested a common transporter at this membrane face (9). Analysis of ciprofloxacin transport in Calu-3 cells with this three-compartment model shows a similar asymmetry with exit permeabilities from the cell across both cell borders being lower than entry permeabilities (9). However, there is no evidence of saturation at the maximal concentration used (3 mM [due to the limits of solubility]). Mediated transport steps at both apical and basal membranes would thus be of low affinity for ciprofloxacin.

Uptake of ciprofloxacin from the apical compartment shows a small apparent accumulation, and basolateral uptake exceeds extracellular concentrations by approximately threefold. In vivo studies show that ciprofloxacin concentrates in bronchial tissue, reaching tissue/plasma drug ratios of between 1.4 and 4.4 (20). Thus, the uptake of ciprofloxacin into Calu-3 airway epithelia is consistent with in vivo studies with humans. Ciprofloxacin is zwitterionic at physiological pH 7.4 ($pK_{a1} = 6.5$, $pK_{a2} = 8.5$, and pI = 7.4) because of a negatively charged carboxyl group and a positively charged nitrogen of the piperazine ring. The apparent cellular concentration would thus be indicative of active transport. However, preferential transport of the ionic or cationic form or sequestration of ciprofloxacin in endomembrane acidic compartments may contribute to the apparent accumulation.

Further competition studies were performed in order to establish whether transport of ciprofloxacin in Calu-3 cells was via simple or facilitated diffusion. The reduction in $J_{a\text{-}b}$ on addition of norfloxacin, pefloxacin, ofloxacin, and enoxacin (1 mM) suggests that a proportion of transport may be due to a facilitated transport mechanism. Since transepithelial transport must result from sequential transfer across two cellular membranes in series, norfloxacin inhibition of ciprofloxacin flux was analyzed with a three-compartment model (9). This shows that the pattern of reduction of $J_{a\text{-}b}$ combined with increased cellular ciprofloxacin (from the basal solution) by norfloxacin results from inhibition of ciprofloxacin transport at both the apical and basolateral borders, but the key effect is inhibition of the exit permeability from the cell across the basolateral cell border ($P_{\text{cell-}b}$).

The existence of different mechanisms of penetration of fluoroquinolones across bronchial membranes other than by simple diffusion has been suggested, based on the fact that concentrations of the antibiotics were higher in epithelial lining fluid (the fluid bathing the terminal bronchioles and alveoli) than in bronchial biopsy concentrations (11). In studies of penetration of ciprofloxacin into bronchial secretions of mechanically ventilated patients, however, Saux et al. (21) showed that the ratio of bronchial secretion to plasma for ciprofloxacin was 0.32 at peak values in plasma (approximately 10 μM). The present data showing mediated transport rather than active uphill secretion are consistent with the latter study. There is evidence from other organs of carrier-mediated transport of ciprofloxacin (e.g., from cerebrospinal fluid to blood) (15).

However, a substantial part of ciprofloxacin transport across human airway epithelia appears to be via simple diffusive solubility processes. Since ciprofloxacin transport did not show any sign of saturation at the maximal concentration used, the relationship between apparent permeability and lipophilicity was investigated. If the permeation of the four substrates in this study was due to a simple diffusion lipid solubility model, then permeation would be expected to decrease with increasing polarity, such that mannitol < ciprofloxacin < digoxin < vinblastine. There was a broad linear correlation between the apparent permeability of the four substrates and their lipophilicity, as measured from their octanol/water partition coefficients, suggesting that the majority of ciprofloxacin transport is due to passive transport. The $P_{a\text{-}b}$ of ciprofloxacin (2.49 ± 0.43 $cm \cdot h^{-1} \times 10^{-2}$) was comparable to permeability values for substrates of similar lipophilicity in Caco-2 cells (e.g., felodipine, $\log D = 3.48$, $P_{a\text{-}b} = 8.17$ $cm \cdot h^{-1} \times 10^{-2}$; hydrocortisone, $\log D = 1.53$, $P_{a\text{-}b} = 7.74$ $cm \cdot h^{-1} \times 10^{-2}$ [from reference 1]). In contrast, in Caco-2 cells when the absorptive permeabilities of vinblastine, digoxin, ciprofloxacin, and mannitol are examined, it can be seen that there is no correlation between $P_{a\text{-}b}$ and the log D values, and the epithelium is rendered relatively impermeable to digoxin, vinblastine, and ciprofloxacin at the apical membrane. This is due to an active extrusion of the substrate across the apical membrane (described above and in references 8 and 9 and 12 to 14).

Therefore, in summary, human airway epithelial Calu-3 cells transport ciprofloxacin to approximately the same level in both directions. The transport is primarily transcellular, and it appears to be dominated by simple diffusion-solubility processes. There may also be a component of transport via a facilitated mechanism, since $J_{a\text{-}b}$ was reduced by other fluoroquinolones, suggesting competitive inhibition. This transport is distinct from that occurring in human intestinal Caco-2 epithelia, where ciprofloxacin is subject to an active saturable net secretion. The utility of using cultured airway epithelia such as Calu-3 cells as a model with which to investigate drug permeation and disposition in lung tissue warrants further study.

ACKNOWLEDGMENT

M.E.C. was a BBSRC-CASE student with Glaxo Wellcome Research and Development Ltd.

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