Development and Characterization of Rabbit Tracheal Epithelial Cell Monolayer Models for Drug Transport Studies

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Purpose. The objective of this study was to investigate how culture conditions would affect the morphological, functional, and permeability characteristics of rabbit tracheal epithelial cell layers being considered for drug transport studies.

Methods. Rabbit tracheocytes were isolated by protease treatment and plated onto collagen-treated permeable supports at 1.3×10^6 cells/cm^2. After 24 hr, cell layers were cultured either air-interfaced (AIC) on their apical surface or under conventional liquid covered conditions (LCC).

Results. Scanning electron microscopy revealed mature cilia in AIC cell layers and ciliated cells denuded of cilia in LCC cell layers. Compared with LCC, AIC cell layers (n = 20) achieved a significantly higher peak equivalent short-circuit current (74.1 ± 6.5 vs. 51.6 ± 3.4 μA/cm^2), a higher potential difference (70.9 ± 2.8 vs. 60.5 ± 3.0 mV), and a lower peak transepithelial electrical resistance (1.1 ± 0.03 vs. 1.5 ± 0.02 kohms.cm^2). About 70% of the short-circuit current in AIC was amiloride-sensitive whereas <10% was furosemide-sensitive, similar to that found in native tissue. The corresponding values in LCC were 50% and 46%. The permeability of both AIC and LCC to lipophilic solutes (dexamethasone and propranolol) was similar, whereas the permeability of hydrophilic solutes (mannitol, sucrose, and albuterol) in AIC was only half that in LCC.

Conclusions. Given the striking similarity in morphological and functional characteristics of the AIC to those in the *in vivo* situation, the AIC is favored as an *in vitro* model for future drug transport studies.

KEY WORDS: tracheal epithelial cells; air-interfaced culture; ion transport; drug transport; permeability.

INTRODUCTION

The pulmonary route is an attractive non-invasive alternative to the oral route for drug delivery. This is particularly important for drugs that are chemically and metabolically unstable in the gastrointestinal tract. Interest in pulmonary drug delivery has been focused primarily on the permeability characteristics of the distal alveoli (1), for the simple reason that they comprise a very large surface area (>100 m^2) for drug absorption. Nevertheless, tracheobronchial deposition of inhaled drug entrained in particles or droplets 5-10 μm in size is significantly greater than alveolar deposition (2), it would be equally important to understand the permeability of this region to drugs to fully appreciate the overall drug transport characteristics of the respiratory tract. This study focuses on the trachea, as it is more accessible to *in vitro* experimental manipulation than are the bronchi and bronchioles.

Primary cultures of airway epithelium have been maintained traditionally under liquid-covered conditions, contrary to what exist *in vivo* in the airway lumen. The majority of these culturing conditions resulted in subconfluent cell layers that may be more adequate for the assessment of epithelial cell functions such as mucin secretion, ciliary activity, and hormonal responsiveness (3-5) than for assessing the barrier properties of the epithelium to solutes. There is growing evidence that culturing of airway epithelial cells at an air-interface through a thin film of adherent liquid more closely resembles the morphological appearance, polarity, differentiation and ion transport characteristics of native tissue *in vivo* (6,7). This has been reported in a number of species including guinea pig (3,6), rat (8), canine (7), and human (9,10) airway epithelium. However, there exists no information on how the permeability of the air-interfaced tracheal culture to low molecular weight solutes, including drugs, would differ from cultures grown under liquid submerged conditions.

Thus, the objective of this study was to investigate how liquid-covered culture conditions versus air-interfaced cultured conditions would affect the morphological, functional, and permeability characteristics of rabbit tracheal epithelial cell (RTE) layers. Rabbits were selected for this study because the yield of isolated tracheocytes (15-25 × 10^6 cells/animal) would be larger than that obtainable with the hamster, rat and guinea pig models (<5 × 10^6 cells/animal) (6). In addition, rabbit tracheas are more readily available when compared with dog and human tracheas.

MATERIALS AND METHODS

Materials

Male, New Zealand white albino rabbits (2.5-3.0 kg) were obtained from Irish Farms (Los Angeles, California). Ca^{2+}- and Mg^{2+}-free Hanks balanced salt solution (HBSS), Ca^{2+}-free minimum essential medium (S-MEM), certified fetal bovine serum (FBS), and fungizone were obtained from Life Technologies (Grand Island, New York). Pronase E (Type XIV), deoxyribonuclease I (Type IV, DNase I), propranolol hydrochloride, and albuterol sulfate were obtained from Sigma Chemical Co. (St. Louis, Missouri). PC-1 medium (a defined medium containing predetermined amounts of insulin, transferrin, fatty acids, and other growth and attachment factors in a 1:1 DME:F12) were obtained from Hycor Biomedical (Portland, Maine). Collagen-treated Transwells (0.45 μm, 12 mm O.D.) were obtained from Costar (Cambridge, Massachusetts). 1-[^{3}H(N)]-Mannitol (26.4 Ci/mmole) and 6,7-[^{3}H]-dexamethasone (43.9 Ci/mmole) were from New England Nuclear-DuPont (Boston, Massa-

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chusetts. [^{14}C]-Sucrose (0.58 Ci/mmole) was from Amersham (Arlington, Illinois).

Preparation of Rabbit Tracheal Epithelial (RTE) Cultures

A modified procedure of Robison et al. (11) was used to prepare cultured rabbit tracheocyte cell layers. Upon euthanasia, the section of the rabbit trachea between the larynx and the bifurcation into the main stem bronchi was excised and aseptically trimmed off its extraneous cartilage and connective tissues. The excised trachea was cut into three pieces, opened longitudinally, and incubated in 0.2% Pronase E in S-MEM at 37°C for 90 min. The epithelial cell layer was gently scraped off with a sterile scalpel blade (size 10), mixed in S-MEM containing 0.5 mg/ml DNase I solution and 10% FBS, and centrifuged at 210× g at room temperature for 10 min. The cells were washed twice in S-MEM containing 10% FBS, filtered through a 40 μm cell strainer, centrifuged at the same settings, and the resulting cell pellet suspended in PC-1 medium supplemented with 2 mM L-glutamine, 100 units/ml penicillin, 100 μg/ml streptomycin, 50 μg/ml gentamicin, and 1 μg/ml fungizone.

After ascertaining cell viability on the basis of exclusion of 0.2% trypan blue, 500 μl of the final cell suspension was plated onto 12 mm collagen-treated Transwell filter inserts at 1.3×10^6 cells/cm^2, and 1.5 ml of PC-1 medium added to bathe the basal side of the filter insert. After 24 hr in a humidified environment with 95% air and 5% CO_2, both media bathing the basal and cell side were removed, in that order, and the adhered cells gently washed with 0.5 ml fresh PC-1 medium preequilibrated at 37°C in a 95% air/5% CO_2 atmosphere. After gently suctioning off the washing fluid, the cell layers were cultured either in an air-interface having their apical surfaces directly exposed to air (AIC) or under conventional liquid-covered conditions (LCC). For AIC, 0.8 ml of PC-1 medium was added to the basolateral reservoir. The apical surface of the monolayer was directly exposed to ambient conditions, being spared from desiccation by the thin film of adherent liquid (~20 μl) in a humidified 5% CO_2/95% air environment. For LCC, 0.5 ml PC-1 medium was added apically and 1.5 ml basolaterally. For both culture conditions, the medium was changed daily.

Forty-eight hr after plating, the transepithelial electrical resistance (R_t) and spontaneous potential difference (PD) were measured daily using an EVOM voltohmmeter device (WPI, Sarasota, Florida), and corrected for the background due to the blank filter and bathing medium. The equivalent short-circuit current (Ieq) was estimated from the ratio PD/R_t. For AIC cell layers, 0.5 ml and 0.7 ml pre-equilibrated medium were added to the apical and basolateral reservoirs, respectively, and the cell layers were allowed to attain a steady potential for about 5 min prior to making bioelectric measurements.

Morphological Examination of RTE Monolayers by Electron Microscopy

Tracheal cell layers were processed for electron microscopy on days 0 (just after isolation) and 5. Each cell monolayer was fixed in 2.5% glutaraldehyde in PBS at 4°C for 2 hr, rinsed in ice-cold PBS five times, fixed in 1.5% osmium tetroxide in PBS at 4°C overnight, and stained with 1.5% uranyl acetate in 50% ethyl alcohol for 30 min. Stained cell layers were dehydrated in graded ethyl alcohol of 70%, 85%, 95%, and 100% for 5, 5, 5 and 10 min, respectively, and finally embedded in Spurr resin for sectioning for transmission electron microscopy with a JEOL 100C at 80 keV.

Evaluation of Ion Transport Properties of RTE Monolayers

To gain an understanding of the cellular location of various ion transporters in the tracheal epithelial cell layers, the effect of known ion transport inhibitors on the bioelectric parameters of cell layers was measured. These inhibitors and their final concentrations were: (a) ouabain, a Na^+-K^+-ATPase inhibitor, 10 μM; (b) amiloride, a Na^+ channel blocker, 10 μM; (c) benzamil, a more potent amiloride analog, 10 μM; (d) furosemide, a blocker of the Na^+-(K^+)-Cl^- cotransporter, 10 μM; (e) N-phenylanthranilic acid (NPAA), a chloride channel blocker, 0.5 mM; and (f) 4,4-diisothiocyanotostilbene-2,2-disulfonic acid (DIDS), a bicarbonate-related transport blocker, 50 μM.

Confluent cell layers with peak R_t and Ieq (days 4 through 8) were mounted in modified Ussing chambers, as described by Robison et al. (6). The cell layers were continually short-circuited except for 3 sec periods during which a constant voltage pulse (dV = 10 mV) was applied every 50 sec. The background voltage and resistance of the collagen-treated filter and the bathing medium were corrected for automatically by the voltage clamping device. Tracheal epithelial cell layers were allowed to reach a steady-state short-circuit current (Isc) (usually within 15 min) before a pharmacological agent was added to the medium in the apical or basolateral reservoir. Responses were measured as percent inhibition from control baseline values, and reported as means ± s.e.m. for $n \geq 3$.

Evaluation of Solute Transport Properties of RTE Monolayers

The model solutes were hydrophilic mannitol (5 μCi/ml), sucrose (1 μCi/ml), and albuterol (1 mM); and lipophilic dexamethasone (1 μCi/ml) and propranolol (0.1 mM). Confluent cell layers (day 4 to 8) were washed once with the preequilibrated (5% CO_2/95% air, at 37°C) transport medium and incubated for 20 min. The transport medium used was PC-1 for ^{3}H-mannitol, ^{14}C-sucrose, and ^{3}H-dexamethasone, and bicarbonate Ringer's solution (25 mM HCO_3^-, pH 7.3, 37°C) for albuterol and propranolol. Transport studies were initiated by adding 50 μl aliquot of drug stock solution to the transport medium in the apical donor compartment, while keeping apical volume the same. At 30, 60, 120, 180 and 240 min, 200 μl aliquots were withdrawn from the receiver fluid (1.5 ml) for sample analysis and were replaced with an equal volume of fresh transport medium preequilibrated in 5% CO_2 and 95% air. At the end of the 4 hr transport experiment, the R_t and PD were measured to monitor cell monolayer integrity.

Radiolabeled drug samples were mixed with 5 ml scintillation cocktail (Econosafe, Research Products International, Mount Prospect, Illinois) and counted in a liquid scintillation counter for 5 min. Samples of albuterol and propranolol were assayed using reversed phase HPLC on a Rainin Microsorb-MV C18 column (250 × 4.6 mm, 5 μm (Woburn,

Massachusetts)). For albuterol, the mobile phase was a mixture of 1% acetonitrile and 25 mM ammonium dihydrogen phosphate and 1 mM N,N-dimethyloctylamine (pH 3.0) at a flow rate of 0.8 ml/min, with UV detection at 277 nm. Under these conditions, albuterol eluted at about 5 min and atenolol (10 μg/ml, internal standard) at 8 min. For propranolol, the mobile phase was a mixture of 0.2% triethylamine hydrochloride (pH 3.0) and 20% acetonitrile during the first 10 min, increasing linearly up to 30% within 15 min, and maintained at 30% up to 25 min. With a flow rate of 1 ml/min, and UV detection at 290 nm, timolol (10 μg/ml, internal standard) eluted at about 7 min and propranolol at 23 min.

The cumulative appearance of drug in the receiver solution was plotted as a function of time. Correction for receiver compartment dilution was made by taking into account for the cumulative amount of drug sampled at the preceding time points. The steady-state flux (J) and the apparent permeability coefficient (Papp) were estimated by linear regression using the equation:

$$\text{Papp} = J/(AC_0) = (dQ/dt)/(AC_0) \quad (1)$$

where $J = dQ/dt$ is the solute flux, A is the diffusional surface area (1.13 cm^2), and C_0 is the initial drug concentration. The data is represented as means ± s.e.m. of at least 3 observations. Data was compared using the unpaired t-test, and a p value of ≤ 0.05 was considered as statistically significant.

RESULTS

Primary Cultured Monolayers of Tracheal Epithelial Cells

Approximately 15-25 × 10^6 tracheal epithelial cells were obtained from each animal, with a viability >90% as assessed by trypan blue exclusion. When plated at a density of 1.3 × 10^6 cells/cm^2, both AIC and LCC cell layers appeared to reach confluence and began to exhibit a measurable transepithelial resistance from day 3 onwards (Figure 1). Both AIC and LCC developed a peak R_t on day 7 and a peak Ieq and PD on day 5. Compared with LCC, AIC developed a higher Ieq (44%) and PD (17%) but a lower R_t (28%) (Table I).

Rabbit tracheocytes plated on a Biocoat Variety Pack (Collaborative Biomedical Products, Bedford, Massachusetts) exhibited selectivity for fibronectin and rat tail collagen (type 1), as judged by the development of a slightly lower spontaneous PD and R_t compared to those cultured on collagen-treated Transwell filters. Laminin and matrigel-coated inserts, on the other hand, did not afford comparable bioelectric properties, with matrigel being the least conducive to cell attachment (data not shown).

Morphological Examination of Tracheal Epithelial Cell Layers

Both AIC and LCC 6-day old confluent cell layers exhibited approximately 14% positive periodic acid Schiff (PAS) staining when counted under a 40× magnification with a Nikon microscope, indicating a similar population of glycoprotein-secreting cells (data not shown). Transmission electron micrographs under 4,000× magnification of isolated

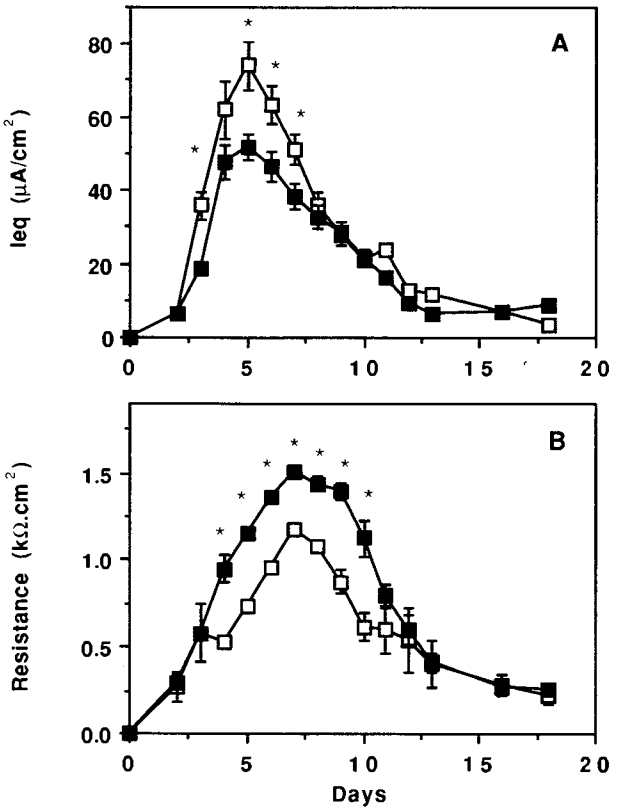

Fig. 1. Time course of equivalent short-circuit current (Ieq, plot A) and transepithelial electrical resistance (R_t, plot B) in air-interfaced (□) and liquid-covered (■) rabbit tracheal epithelial monolayers. Error bars represent s.e.m. for n = 20. Asterisk represents a significant difference between AIC and LCC, $p < 0.05$.

tracheocytes in suspension (Figure 2A) revealed a heterogeneous population of ciliated cells (c), secretory cells (s) characterized by a distinctly granular appearance, and basal cells (b) characterized by a slightly elongated cell shape. Transmission electron micrographs of confluent day 5 AIC cell layers under 4,000× magnification exhibited a pseudostratified appearance of the different cell types in a single layer with overlapping cytoplasmic regions (Figure 2B). The same pattern was seen with LCC except that the cells appeared to be flattened (data not shown). Under 30,000× magnification (Figure 2C), intercellular junctional complexes, in the location marked by an arrowhead on the micrograph, can be seen in AIC cell layers. In addition, microvilli and cilia with an abundance of apical localized mitochondria are observed in AIC cell layers. Scanning electron micrographs (SEM) under

Table I. Bioelectric Properties[a] of Rabbit Tracheal Epithelial Monolayers Cultured Under Air-Interfaced (AIC) and Liquid-Covered Conditions (LCC)

	AIC	LCC
Ieq (μA/cm^2)	74.1 ± 6.5	51.6 ± 3.5[b]
R_t (kohms.cm^2)	1.18 ± 0.03	1.51 ± 0.02[b]
PD (mV)	70.9 ± 2.8	60.5 ± 3.03[b]

[a] Means ± s.e.m. for n = 20 monolayers, see text for abbreviations.
[b] Statistical difference between AIC and LCC; $p < 0.05$

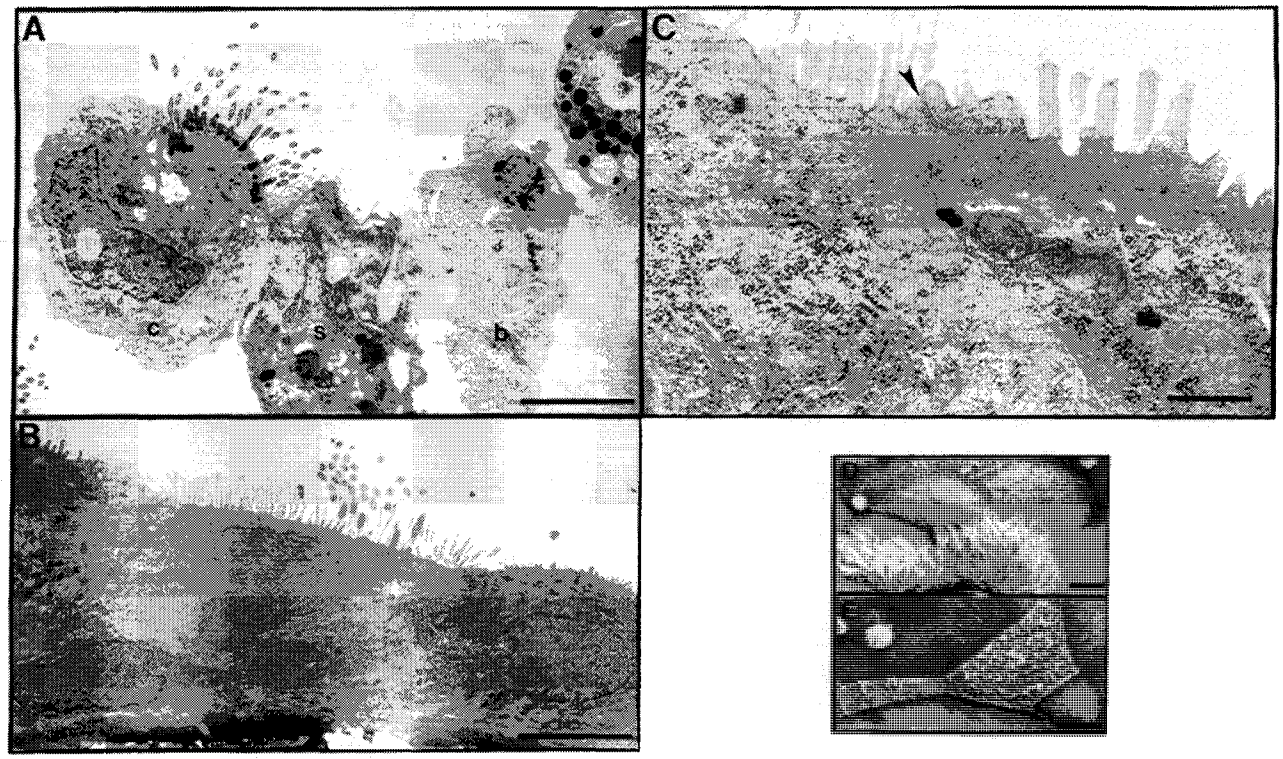

Fig. 2. Morphological appearance of rabbit tracheal epithelial cells and their monolayers under the electron microscope. Plate A displays isolated rabbit tracheal epithelial (RTE) cells in suspension (4,000× magnification), bar = 5 μm. The cell types seen are: ciliated cells (c), secretory cells (s), and basal cells (b). Plate B shows an air-interfaced RTE cell monolayer (4,000× magnification), bar = 5 μm. Plate C shows the tight junctions (indicated by an arrowhead) in an air-interfaced RTE cell monolayer (30,000× magnification), bar = 0.5 μm. Plate D shows the ciliary appearance of an air-interfaced RTE cell monolayer (1,500× magnification), bar = 5 μm. Plate E shows a scanning electron micrograph of liquid-covered RTE cell monolayer exhibiting denuded cilia on ciliated cells (2,200× magnification), bar = 5 μm.

1,500× magnification (Figure 2D) showed the presence of cilia on the apical surface of ciliated cells in AIC, and less prominent cilia or denuded ciliated cells in LCC at 2,200× magnification (Figure 2E).

Ion Transport Properties of Tracheal Epithelial Monolayers

Figure 3 summarizes the effect of ion transport inhibitors on the spontaneous Isc as a percent inhibition in the baseline values of AIC and LCC monolayers. Panel A shows the response to ion transport inhibitors that were effective on apical but not basolateral application, and panel B shows responses to inhibitors effective on basolateral but not apical application. In Figure 3A, amiloride and benzamil dramatically decreased the Isc with a half-time of monximal inhibition ($t_{1/2}$) of about 4 min; more so in AIC (~70%) than in LCC (~50%). NPAA inhibited Isc by ~30% in AIC and by ~50% in LCC (Figure 3A). In Figure 3B, basolateral treatment with ouabain reduced Isc gradually by 90% in both AIC and LCC, with a ($t_{1/2}$) of 18 min. Furosemide was more effective in LCC (~45%) than in AIC (~5%). DIDS inhibited the Isc in both AIC and LCC cell layers by ~14% (Figure 3B).

Permeability of the RTE Monolayers to Model Solutes

The flux of the hydrophilic solutes (^{3}H-mannitol, ^{14}C-sucrose, and albuterol) across both AIC (Figure 4A inset) and LCC (Figure 4B inset) cell layers was linear. By contrast, the flux of dexamethasone and propranolol showed curvilinearity (Figure 4A and 4B), probably due to the collapse of sink conditions as the percent of dose transported after 2-3 hours exceeded 10%. As shown in Table II, the Papp of the hydrophilic solutes was similar and was about two orders of magnitude smaller than that of the lipophilic solutes. While both AIC and LCC were equally permeable to lipophilic dexamethasone and propranolol (Table II), the LCC was significantly more permeable to the hydrophilic solutes than the AIC.

DISCUSSION

Morphological and Bioelectric Properties of LCC and AIC Tracheal Epithelial Monolayers

The present study demonstrates that it is possible to culture rabbit tracheal epithelial cells under air-interfaced conditions that resembles the *in vivo* situation. These conditions result in cultures with phenotypic appearance and electrophysiological characteristics more like the native tissue than do the liquid-covered conditions.

Perhaps the most striking morphological difference between the two culture models is that AIC acquires a cuboidal or cobblestone appearance (Figures 2B and 2D), whereas LCC acquires a more squamous or flattened appearance (Figure 2E), a condition also typically found in previously

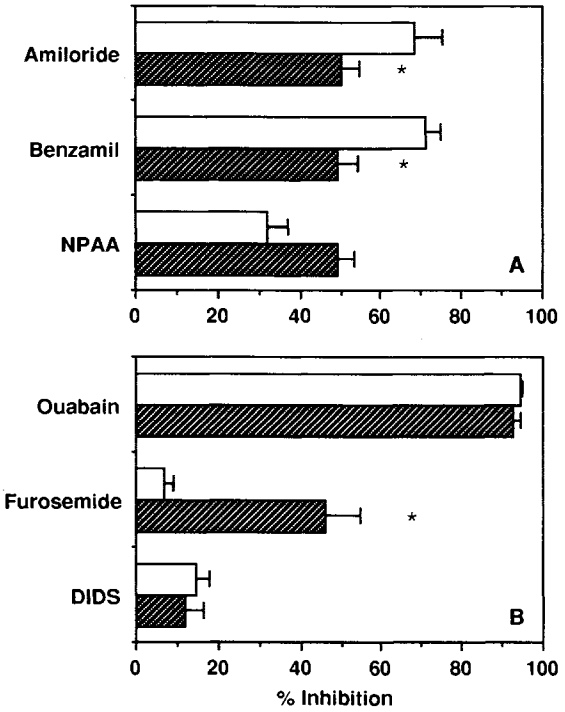

Fig. 3. Effect of various ion transport inhibitors on baseline short-circuit current of air-interfaced (▨) and liquid-covered (□) rabbit tracheal epithelial cell monolayers. Panel A shows responses to ion transport inhibitors effective only by apical treatment. Panel B shows the effect of ion transport inhibitors effective only by basolateral treatment. The concentrations were 10 μM amiloride, 10 μM benzamil, 500 μM NPAA, 10 μM ouabain, 10 μM furosemide, and 50 μM DIDS. Error bars denote s.e.m. for n ≥ 3. Asterisk indicates a significant difference in the extent of inhibition between AIC and LCC at $p < 0.05$.

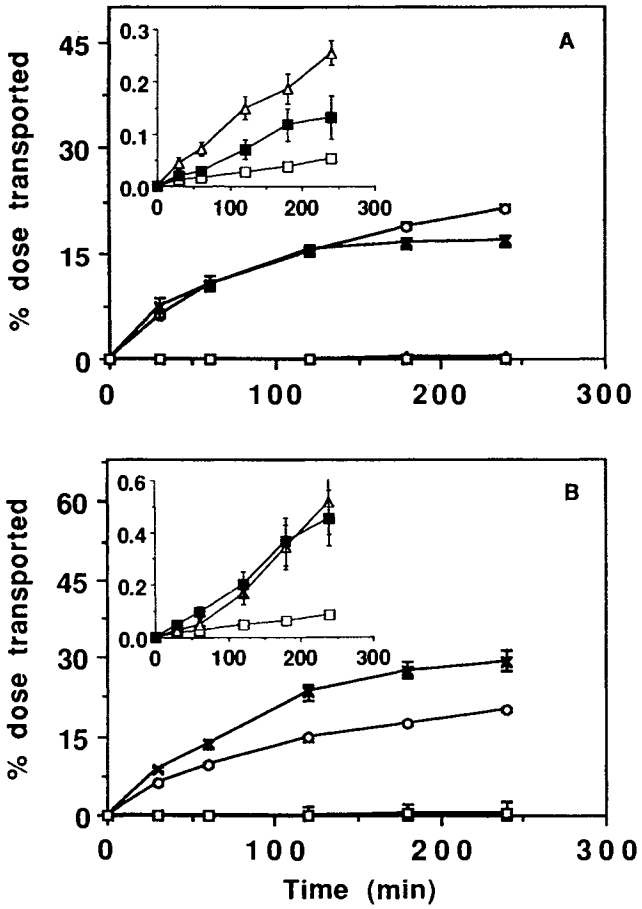

Fig. 4. Time course of solute transport across air-interfaced (plot A) and liquid-covered (plot B) rabbit tracheal epithelial monolayers. Insets enlarges the time course of hydrophilic solute transport. Error bars denote s.e.m. for n ≥ 3. Key: ■, mannitol; □, sucrose; △, albuterol; X, dexamethasone; ○, propranolol.

reported tracheal and bronchial culture models (10,12). Moreover, AIC displays the presence of cilia on the apical surface of ciliated cells (Figures 2D), whereas denuded or less prominent cilia are seen in LCC ciliated cells (Figures 2E). This finding is consistent with the observation made by de Jong *et al.* (9) in human bronchial epithelial cells in culture, that air exposure is necessary for ciliogenesis.

Epithelial cells presumably rich in glycoprotein were stained positively with PAS in both AIC and LCC models (data not shown). The approximate percentage of PAS-positive cells in both culture models were estimated to be about the same at 14%. This density of secretory cells in culture is noticeably similar to the relative abundance of secretory cells in the rabbit trachea *in vivo,* comprising of: 17.6% Clara cells and 1.3% mucus goblet cells (both known to produce mucin-like glycoproteins), 28% basal cells, 43% ciliated cells, and 9.4% unidentified cells (13).

Electrophysiologically, AIC appear to possess a significantly higher Ieq (~75 μA/cm^2) (Table I), 70% of which is attributable to an amiloride-sensitive Na^+ absorptive pathway and <10% to a furosemide-sensitive pathway. By contrast, the peak Ieq in LCC is lower (~50 μA/cm^2) (Table I), only 50% of which is attributable to a Na^+ absorptive pathway and as much as ~45% to a furosemide-sensitive pathway (Figures 3A and 3B). Thus, the presence of a fluid layer on epithelial cells results in a significant decrease in Isc and an alteration of ion transport characteristics in confluent tracheal cultures (Table I, Figures 3A and 3B). However, this difference is not thought to be dependent on the apical fluid volume, as found in guinea pig tracheal cell cultures (11).

The transepithelial resistance of 1,180 ohms·cm^2 in AIC and 1,510 ohms·cm^2 in LCC is higher than 200-700 ohms·cm^2 for native tissue (14). This difference in R_t, commonly observed in primary cell cultures, has been ascribed to a higher density of tight junctions per unit area in native tissue compared to cells in culture. This is probably due to the columnar nature of native epithelial cells, as opposed to a more cuboidal appearance for cultured epithelial cells (15).

We attribute the morphological and functional characteristics in the tracheal epithelial cell layers grown under the air-interfaced condition partly to improved delivery of oxygen across the thin film of liquid to the cells that may subsequently change cellular respiration to a more aerobic nature. In canine bronchial epithelial cells, O_2 consumption in air-exposed cell layers is increased, elevating oxidative metabolism (7). The net result is an increase in cellular ATP levels, thereby generating a more favorable electrochemical gradient for Na^+ transport. Such a possibility is supported by the elevated amiloride-sensitive Na^+-current observed in

Table II. Permeability Coefficients (Papp, $\times 10^{-6}$ cm/sec) of Model Solutes Across the AIC and LCC

Solute	log P[a]	Papp[b] AIC	LCC
Mannitol	−3.1	0.12 ± 0.03 (n = 12)	0.28 ± 0.06 (n = 15)[c]
Sucrose	−3.7	0.03 ± 0.002 (n = 3)	0.05 ± 0.003 (n = 4)[c]
Albuterol	0.11	0.22 ± 0.03 (n = 4)	0.46 ± 0.42 (n = 4)
Dexamethasone	1.83	9.8 ± 0.4 (n = 6)	8.7 ± 0.3 (n = 6)
Propranolol	3.2	31.7 ± 8.0 (n = 3)	23.8 ± 3.0 (n = 3)

[a] Log octanol/pH 7.4 buffer partition coefficient.
[b] Mean Papp ± s.e.m.
[c] Significant difference between Papp in AIC and LCC ($p < 0.05$).

the AIC model (Figure 3A). Although oxygen and nutrients may be supplied in part by the bronchial circulation *in vivo*, they may also be absorbed from the lumenal surface via apically-located transporters such as the Na^+-coupled amino acid transporter (16), and the Na^+-dependent glucose transporter (17).

Overall, the bioelectric response of the RTE cell monolayers to the exposure of various ion transport inhibitors is consistent with the notion that Na^+ enters the RTE cells by an apical conductive pathway (comprising probably of amiloride-sensitive Na^+ channels) by electrodiffusion and exits the cells via the basolaterally-located Na^+, K^+-ATPase. Cl^-, on the other hand, may enter the cell by a basolateral Na^+-(K^+)-Cl^- cotransport process and a basolateral HCO_3^--related process such as HCO_3^-/Cl^- antiport. Cl^- exits the cells via an apically-located Cl^--conductive pathway, widely known to exist in the airway epithelial cells as active Cl^- secretion (15).

Permeability of LCC and AIC Monolayers to Model Solutes

The culture conditions appear to affect the transport of hydrophilic drugs more so than lipophilic drugs (Table II). This is probably a consequence of the overriding importance of the paracellular pathway in the transport of hydrophilic drugs, including mannitol, sucrose, and albuterol. As such, the barrier properties of cell layers must be ascertained in evaluating the transport of these drugs. Conceivably, some drugs may compromise tight junctional integrity, thereby accelerating their own transport. In AIC, no alteration in monolayer integrity was observed for the model drugs studied, except for albuterol, which lowered the R_t by 12% after 4 hours. The precise mechanism responsible for this is unknown, but it may be associated with β-receptor stimulation. By contrast, in LCC, the R_t was decreased for most of the solutes, at the same concentrations as used in AIC. Propranolol (0.1 mM) elicited a 60% reduction and albuterol (1.0 mM) a 40% decrease in R_t (data not shown). Thus, for reasons not immediately obvious, LCC is more sensitive than AIC to the solutes tested. Tight junctional arrangement (e.g. number of strands, junctional length, presence of pores or channels within the junction, or length of lateral intercellular space (18)) may be widely different in AIC and LCC, although at the magnification of electron microscopy studied we cannot discern such fine differences. Nevertheless, this may partly explain the higher permeability of hydrophilic drugs (mannitol, sucrose, and albuterol) in LCC than in AIC cell layers (Table II).

The permeability coefficient of mannitol in AIC, 1.2×10^{-7} cm/sec is comparable to the value of 1.8×10^{-7} cm/sec seen in cultured rat alveolar epithelial cells (19) with an equally high R_t (>2,000 ohm·cm^2). It thus appears that, species differences aside, the conducting airway and lower respiratory epithelia are relatively tight, thereby restricting the transport of hydrophilic solutes from the lumen to the blood side. The AIC model exhibits permeability and conductance values that are more reproducible from animal to animal compared to published data on excised tissue models where the experimental setup, tissue handling and mounting technique may vary between laboratories. The 10 times higher permeability reported for mannitol and sucrose in excised tissues (14,20) is probably due to differences in experimental techniques between laboratories, as manifested by the lower R_t (~200 vs. 1,180 ohms·cm^2 for AIC), that may be associated with inadvertent edge damage to the tissue while mounting. A difference in tight junctional density between isolated tissue and cell culture models may also influence solute permeabilities and conductance values. Indeed, in our hands, a 5 times higher Papp of mannitol in isolated trachea was observed ($5.7 \pm 0.3 \times 10^{-7}$ cm/sec, n = 3) with a R_t of 445 ± 34 ohms·cm^2 (n = 6) when mounted in Ussing chambers. This permeability value is comparable to that of 3.3×10^{-7} cm/sec reported for the Papp of mannitol in the excised canine tracheal epithelium (21) and 0.45×10^{-7} cm/sec obtained in the tighter isolated intact rat lung (22).

Tight junctions between cells play a secondary role in the transport of lipophilic drugs across RTE cell layers. This is indicated by the lack of correlation between the Papp of dexamethasone and propranolol and the R_t (data not shown). Their respective Papp's are similar in both culture models (Table II). The lack of any effect by the culture condition (AIC vs. LCC) on the transport of lipophilic drugs dexamethasone and propranolol, suggests trancellular diffusion may be the major pathway. The larger surface area contributed by the transcellular when compared with the paracellular pathway is a likely factor in the 30- to 200-fold higher permeability seen in the lipophilic solutes as compared with the hydrophilic solutes used in this study (Table II).

Advantages and Limitations of the Tracheal Primary Culture

The tracheal epithelial culture model offers three major

advantages: (a) Exclusion of non-epithelial cells types and basement membranes that may complicate data interpretation, (b) Direct accessibility of the basolateral membrane to studying detailed transport mechanisms, and (c) Extended viability of epithelial cell cultures with no alteration to the barrier properties for 2-3 days of experimental duration, as compared with few hours of viability with isolated tracheal strips. Yet, tracheal epithelial cells survive for a finite period of time (10-12 days) in culture. Based on bioelectric properties, transport studies must be carried out in cultured cell layers between 4 to 8 days. Prior to day 4, the cultures appear subconfluent, and later than day 8 the barrier properties wane. A potential disadvantage of culture models, is loss of mucus secretory function over time, an integral part of the mucociliary escalator. Although, mucus production may be occurring in our culture models (via the 14% PAS positive cells), we did not estimate mucin production.

CONCLUSION

This study sets the stage for evaluating the permeability of the conducting airway to drugs of varying molecular sizes, so as to estimate the molecular cut-off for paracellular permeability; and for evaluating its permeability to drugs of varying lipophilicity, so as to estimate the optimum lipophilicity desired of a drug candidate targeted for delivery to the conducting airway of the lungs. Towards that end, we propose that the air-interfaced cultures (AIC) be preferred to the liquid-covered cultures (LCC) as an *in vitro* model for drug transport studies, for three main reasons. First, from a morphological point of view, AIC is highly differentiated having a phenotypic appearance that resembles the native tissue (7,10). Second, AIC cultures appear to retain a similar high level of active ion transport of intact tissue with a peak equivalent short circuit current, Ieq of 75 $\mu A/cm^2$ (Table 1) vs. 85 $\mu A/cm^2$ in the native tissue (14). Third, the experimental environment of AIC is more akin to rabbit tracheal epithelium *in vivo*.

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