ELSEVIER

International Journal of Pharmaceutics 208 (2000) 1–11

international journal of pharmaceutics

www.elsevier.com/locate/ijpharm

Characterization of the Calu-3 cell line as a tool to screen pulmonary drug delivery

Kimberly A. Foster [a,b], Michael L. Avery [c], Mehran Yazdanian [b], Kenneth L. Audus [c,*]

[a] *Department of Pharmaceutical Chemistry, University of Kansas, Lawrence, KS 66047, USA*
[b] *Pharmaceutical Sciences, G.D. Searle, Skokie, IL 60077, USA*
[c] *Pharmaceutics Department, Boehringer Ingelheim Pharmaceuticals, Inc. Ridgefield, CT 06877-0368, USA*

Received 21 December 1999; received in revised form 18 May 2000; accepted 19 May 2000

Abstract

The objective of this research was to examine the human sub-bronchial gland cell line, Calu-3, and assess its potential as a metabolic and transport model to study drug delivery to the respiratory epithelium. The present studies were conducted using Calu-3 cells grown in Transwells® or in multiwell cluster plates. TEER values for Calu-3 monolayers were determined using the World Precision Instrument Voltohmmeter and STX-2 electrode. The results confirmed that Calu-3 cells form tight monolayers and give appreciable TEER values in culture when grown under air-interface conditions. Permeability data for small lipophilic molecules across Calu-3 monolayers suggested that the cell line is a suitable model to examine the transport of low molecular weight substances and xenobiotics. Calu-3 cells were also found to efflux FITC-transferrin (MW 80 000) in a polarized manner. The metabolic capacity of Calu-3 cells was also examined. The P4501A1 and P4502B isozymes were determined to be functional, but not inducible, with fluorescent resorufin assays. The data indicated that the Calu-3 cell line may be useful for studying the contributions of bronchial epithelial cells to mechanisms of drug delivery at the respiratory epithelium. © 2000 Published by Elsevier Science B.V.

Keywords: Respiratory epithelium; Calu-3; P450; TEER; Transport

1. Introduction

In recent years, much attention has been focused on delivering drugs via the pulmonary route. This is due to the many advantages that pulmonary drug delivery offers including a large absorptive surface area, avoidance of the hepatic first pass effect, and the ability to deliver drugs locally to the lung in the case of pulmonary

Abbreviations: EROD, 7-ethoxyresorufin *O*-dealkylase; PBSA, phosphate buffered saline supplemented with 0.63 mM $CaCl_2$, 0.74 mM $MgSO_4$, 5.3 mM dextrose, and 0.1 mM ascorbic acid, pH 7.4; PROD, 7-pentoxyresorufin O-dealkylase; TEER, transepithelial electrical resistance; ZO-1, Zonula Occluden-1.

* Corresponding author. Present address: 236B Simons, 2095 Constant Avenue, Lawrence, KS 66047-2504, USA. Tel.: +1-785-8643609; fax: +1-785-8645736.

0378-5173/00/$ - see front matter © 2000 Published by Elsevier Science B.V.
PII: S0378-5173(00)00452-X

disease. At present, there is very little information known about the mechanisms of drug delivery to the respiratory epithelium on a cellular level. A cell culture model of the respiratory epithelium that closely mimics the transport and metabolic properties of human bronchial epithelium would be an invaluable tool to easily examine the metabolic and transport properties of the cells comprising the pulmonary barrier.

The literature cites several cell lines that are currently being used as in vitro models of respiratory epithelium. Among these are a Type II-like pulmonary epithelial cell line, A549, and the bronchiole cell lines HBE4/E6/E7 and Calu-3. The Calu-3 cell line, derived from human bronchial epithelium, is believed to have serous cell properties (Finkbeiner et al., 1993; Haws et al., 1994; Singh et al., 1997; Lee et al., 1998). This cell line has been extensively researched due to its ability to form tight monolayers in culture and its ability to express the cystic fibrosis transmembrane conductance regulator (CFTR) protein (Shen et al., 1994; Lee et al., 1998). While much information exists in the literature on the ion channel mechanisms of the Calu-3 cell line, there is little information regarding its transport and metabolic properties. The goal of this paper was to characterize the transport and metabolic properties of the Calu-3 cell line to assess its utility as a model to screen drugs and excipients intended to be delivered via the pulmonary route.

2. Material and methods

2.1. Cell maintenance and subculturing

Calu-3 cells were obtained from the ATCC (Rockville, MD) and maintained in a 1:1 mixture of Ham's F12 (Gibco) and DMEM (Sigma, St. Louis, MO), with 10% fetal bovine serum (Atlanta Biologicals) and supplemented with 100 μg/ml penicillin G (Sigma) and 100 μg/ml streptomycin sulfate (Sigma). The cells were plated in 150 cm^2 cell culture flasks and subcultured before reaching confluency using a 0.25% trypsin solution in EDTA (Sigma). The culture medium was changed every 2 days. The cells were split 1:2 during each passage. The passages used for the following experiments were 19–35.

2.2. Lucifer yellow uptake experiments

The fluid-phase marker, Lucifer yellow (MW 457), was obtained from Molecular Probes (Eugene, OR). Calu-3 cells were plated at a density of 5×10^5 cells/ cm^2 in 12-well cluster plates. Uptake experiments were conducted once the cells had formed confluent monolayers. Before each experiment, the culture media were removed and the cell monolayers were rinsed three times with PBSA and allowed to equilibrate for 1 h at either 37 or 4°C in PBSA. At various time points, an aliquot of Lucifer yellow stock solution was added to each well. At the end of the experiment, the media was removed from the cells and the monolayers were washed three times with ice-cold phosphate buffered saline (PBS). The monolayers were then digested with 0.2 N NaOH in 0.5% Triton X-100. Cellular uptake of Lucifer yellow was determined by fluorescence spectroscopy (SLM Instruments, Inc). The samples were normalized spectrophotometrically (Shimadzu UV160U) for protein content using a BCA Protein Assay Reagent Kit (Pierce). The internalization of Lucifer yellow was determined by subtracting the uptake (mg probe/mg protein) at 4°C from the values at 37°C. All timepoint values were done in triplicate.

2.3. TEER values

The TEER of Calu-3 monolayers was determined at selected day intervals in culture according to the method of Nerurkar et al. (1996), using the World Precision Instrument Voltohmmeter and STX-2 electrode. TEER was also measured and verified with an End-Ohm Chamber and Physiological Instruments Voltohmmeter VCC600 (data not shown). The authors obtained lower TEER value measurements (usually 3.5 times) when using the End-Ohm Chamber; however, the aseptic STX-2 TEER sampling method was chosen for convenience so that the cells could continue to grow in culture. TEER values for Calu-3 monolayers were measured for up to 23 days in culture (Prosser et al., 1996; Singh et al., 1997).

2.4. Transport experiments

The permeability of various radiolabeled substances and anti-asthma agents across Calu-3 monolayers was determined. Briefly, Calu-3 cells were plated at a density of 5×10^5 cells/cm^2 in collagen-coated Transwells® (12 mm diameter, 0.4 μm pore size). After the cells had attached to the Transwells® overnight, the media was removed from the apical compartment to allow the monolayer to grow at the air-interface. Air-interface conditions have been shown to improve the differentiation of primary cultures of human airway epithelia (Yamaya et al., 1992; Shen et al., 1994). Under these growth conditions, monolayer confluency could be gauged as the point at which culture medium ceased to leak from the basolateral chamber to the inside, or apical, chamber (Shen et al., 1994). Transport experiments were conducted on days 10–14 in culture. Before each experiment, the cells were washed three times with PBSA and allowed to equilibrate for 1 h at 37°C. The transport of substances across Calu-3 monolayers was monitored for 1 h. The apparent permeability coefficient for each marker was calculated using the equation:

$$P = \text{Flux}/(A^*C_d)$$

where Flux is the slope of the amount of probe transported versus time, A is the area of the Transwell® membrane, and C_d is the initial donor concentration. The permeability of substances across the cells was adjusted for the permeability of the markers across collagen-coated Transwells® in the absence of cells as detailed previously (Adson et al., 1994).

2.5. Transferrin efflux experiments

Calu-3 cells were plated at a density of 5×10^5 cells/cm^2 in collagen-coated Transwells® (12 mm diameter, 0.4 μm pore size). Asymmetric efflux experiments were conducted with confluent Calu-3 monolayers as described by Raub and Newton (1991), with some modifications. Briefly, the apical and basolateral sides of the cell monolayers were washed three times with PBSA and allowed to equilibrate in PBSA for 30 min at 37°C to deplete the cells of endogenous transferrin from the culture medium. The PBSA was removed from the cells and replaced with 1 μM fluorescein-transferrin on the apical and basolateral sides simultaneously for 1 h at 37°C. The cells were washed on both sides three times with PBSA at room temperature and fresh PBSA was added to both sides. The efflux of fluorescein-transferrin from Calu-3 monolayers was then monitored for 1 h at 37°C. The amount of fluorescein-transferrin accumulated in the apical and basolateral compartments was determined by fluorescence spectroscopy (SLM Instruments, Inc).

2.6. EROD and PROD P450 assays

7-Alkoxyresorufin *O*-dealkylase activity was measured by the fluorometric method of Burke and Mayer (1974), with some modifications. Calu-3 cells were seeded at a density of 5×10^5cells/cm^2 in six-well plates from Nunclon. Experiments were conducted when the cells formed confluent monolayers and all experiments were conducted in culture medium. The cells were washed three times with PBS and treated with 10 μM dicumerol (3,3′-Methylene-bis(4-hydroxycoumarin)) and either 8 μM 7-ethoxyresorufin (substrate for P4501A1) or 15 μM 7-pentoxyresorufin (substrate for P4502B). Control wells were also prepared and treated in an identical fashion with the exception that no substrate was added to these monolayers. The cells were incubated at 37°C for 30 min. After the incubation, a 750-μl aliquot of culture medium from each well was removed and placed in a glass test tube and 250 μl of a β-glucuronidase (15 Fishman U)/arylsulfatase (120 Roy U) solution in 0.1 M sodium acetate (pH 4.5) was added to each test tube. The test tubes were incubated for 2 h at 37°C. A 2.0-ml aliquot of 95% ethanol was then added to each test tube and the samples were centrifuged for 10 min at 3000 rpm. A 2-ml sample of the supernatant was placed in a cuvet for fluorescence detection (SLM fluorometer, excitation 530 nm, emission 590 nm). The amount of resorufin in the samples was determined using a resorufin standard curve and corrected for the volume of the original sample. A BCA protein assay (Pierce) was conducted on the

monolayers in order to determine the amount of resorufin/mg protein.

In other experiments, the cells were treated with known inducers of P450 enzymes for 24–48 h. P4501A1 activity was induced with micromolar concentrations of 3-methylcholanthrene with dexamethasone, and β-napthoflavone. P4502B activity was induced using phenobarbitol. The enzymatic activity was measured using the ethoxyresorufin *O*-dealkylase (EROD) and pentoxyresorufin *O*-dealkylase (PROD) methods as described above. These values were compared to the enzymatic activity found in monolayers, which had not been pretreated with an inducer. BCA Protein Assays (Pierce) were conducted to determine enzyme activity per mg cell protein.

2.7. Testosterone metabolism

Calu-3 cells were plated on six-well Nunc plates (approximately 5×10^5 cells/cm^2) and allowed to grow until confluency was reached. During culture, 200 μM of dexamethasone, a known P4503A4 inducer (Parkinson, 1996), was added to the wells for 48 h prior to the metabolism experiment. Metabolism experiments were conducted in serum-free DMEM without phenol red, pH 7.4, according to the method of Wortelboer et al. (1990). Briefly, dexamethasone treated Calu-3 monolayers were washed three times in serum-free DMEM (without phenol red), and allowed to equililbrate for 1 h at 37°C. After the equilibration period, 250 μM of testosterone was added to the cell monolayers. Aliquots of the culture medium were taken at various timepoints and were immediately frozen at −20°C until analysis by HPLC. The cell monolayers were washed after the end of the experiment and saved for protein analysis.

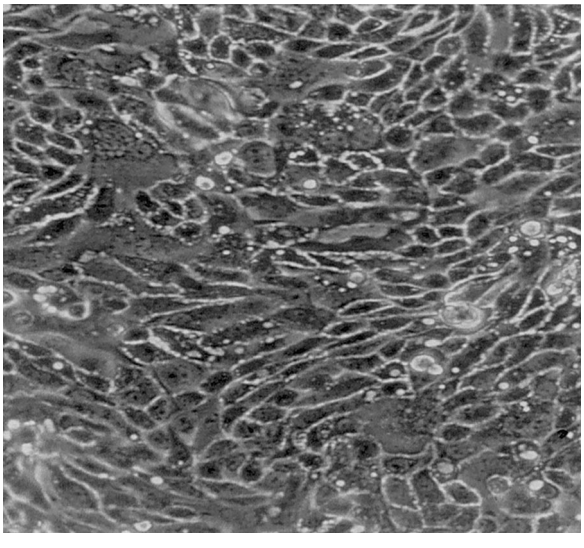

Fig. 1. Confluent Calu-3 monolayer as viewed by light microscopy (100×). Calu-3 monolayers generally consisted of cuboidal and polygonal cells, which were closely apposed.

Frozen samples were thawed and analyzed after extraction with dichloromethane. Dichloromethane was removed by evaporation under a stream of N_2 at room temperature. The residue was dissolved in 130 μl of 50% (v/v) methanol/water. The samples were analyzed at 254 nm using a Rainin Dynamax UV-1 HPLC system with a Dynamax (300 Å) C-18 column under gradient conditions. The run time for each sample was 18 min. Mobile phase A consisted of 5% acetonitrile, 95% water, and 0.1% TFA. Mobile phase B was 100% acetonitrile. The gradient was set at 100% A initially and decreased linearly to 50% A over 12 min, 0% A at 14–16 min, and then returned to 100% A at 18 min.

2.8. Statistical analysis

The data were analyzed with the Minitab® statistical software package using a Tukey's test in a one-way ANOVA ($\alpha = 0.05$). All data points indicate the average of three samples. Error bars are the standard deviations of the samples.

3. Results

3.1. Growth and morphology

Calu-3 cells which were continuously propagated, grew rapidly and consistently, and were passaged approximately once a week. Calu-3 monolayers generally consisted of cuboidal and polygonal cells, which were closely apposed (Fig. 1). Transmission electron micrographs of Calu-3

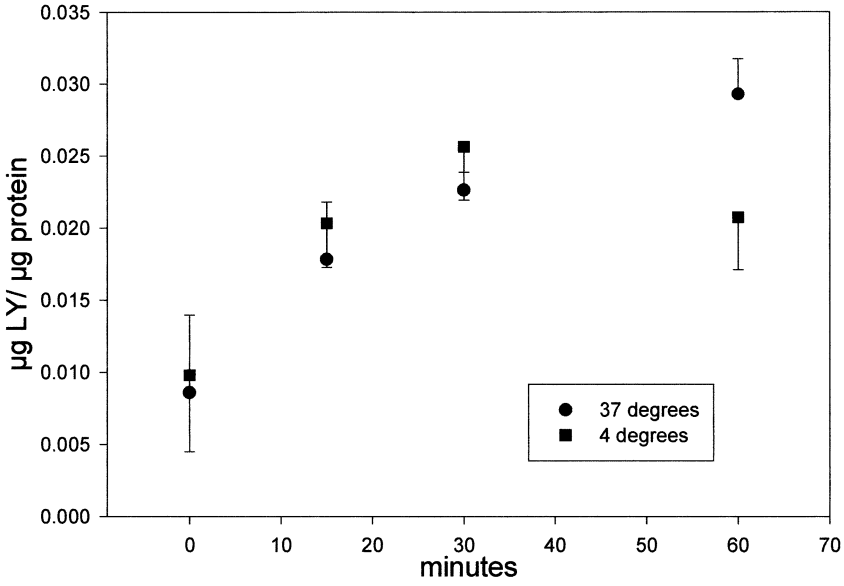

Fig. 2. Uptake of fluid-phase marker, Lucifer yellow, in Calu-3 monolayers versus time at 37 and 4°C. Calu-3 cells were grown to confluency in 12-well cluster plates. The cells were washed 3 × with PBSA and equilibrated in PBSA at 37 or 4°C for 1 h prior to the start of the experiment. Lucifer yellow was added to each well for a specified time period. Uptake was determined using fluorescence spectroscopy and normalized for cellular protein.

monolayers revealed microvilli on the apical surface and electron dense areas near the upper portions of the intercellular junctions where tight junctions would be expected (data not shown). Confluent monolayers were generally formed after about 3 days post-seeding when plated at 1×10^5 cells/cm^2 in 12-well cluster plates. Histological staining of cross sections revealed that Calu-3 cells, when plated at 5×10^5 cells/cm^2, retained a predominant monolayer condition in Transwells® with a few patches of multiple layers for up to day 23 of culture; however, very little difference was seen in the permeability of the monolayers after day 14 in culture (data not shown).

3.2. Lucifer yellow uptake, flux experiments and TEER measurements

Uptake experiments conducted with the fluid-phase endocytic marker, Lucifer yellow, showed that the dye associated with the cell monolayers in a temperature-independent fashion (Fig. 2). There was no statistical difference between Lucifer yellow Calu-3 uptake at 37°C (i.e. dye bound and internalized) and 4°C (i.e. only bound dye). Therefore, Lucifer yellow did not appear to be internalized by Calu-3 cells during the duration of the experiment. Although longer timepoints may have revealed internalization of Lucifer yellow by Calu-3 cells, the data indicated that Lucifer yellow transport across Calu-3 monolayers grown in Transwells® would occur primarily through a paracellular route in the time-frame of the transport studies. Lucifer yellow was chosen as a paracellular marker to measure monolayer 'tightness' in subsequent experiments.

The TEER was also used as a measure of monolayer 'tightness'. Monolayer resistance measurements indicate that Calu-3 cells, when seeded at 5×10^5 cells/cm^2, generate measurable TEER after 6 days in culture (Fig. 3). The resistance values appear to increase with days in culture until a plateau is reached (usually between day 10 and day 14 of culture). Further experiments were conducted on monolayers grown in culture for 10–14 days to determine the relationship between TEER and flux of the paracellular marker, Lucifer yellow (Fig. 4). These data show that Lucifer

yellow flux changes very little after the monolayers reach resistance readings above 300 Ω*cm^2.

3.3. Transport experiments

The permeation of various radiolabeled small molecular weight substances across Calu-3 monolayers was determined as described above and is shown in Fig. 5. The permeability of the anti-asthma agent, albuterol sulfate, was determined by HPLC analysis. Excluding sucrose, the data indicated that there was a linear relationship between the log of the permeability of the drug substances examined and the log of their octanol/water partition coefficient.

In further studies, sucrose [^{14}C] permeability was determined simultaneously with the permeability of the [^{3}H] small molecules to assess monolayer integrity. It should be stated that the permeability of sucrose across monolayers exposed to cocaine, for instance, appeared to increase approximately four-fold as compared to sucrose permeability determined during transport experiments with the other drugs included in this study (data not shown). On a parallel note, studies conducted in our laboratory showed that cocaine exposure to Calu-3 monolayers resulted in decreased TEER values of treated monolayers. This may have resulted from cocaine-induced permeability changes in the Calu-3 monolayer and demonstrate the possibility of using the Calu-3 cell line as a model to examine drug-induced permeability changes in the lower respiratory epithelium.

Fluorescein-transferrin efflux experiments indicated that Calu-3 monolayers efflux intracellular transferrin preferentially out the basolateral side to a greater extent than the apical side (Fig. 6). Transferrin efflux rates for both directions appeared to level off after 45 min.

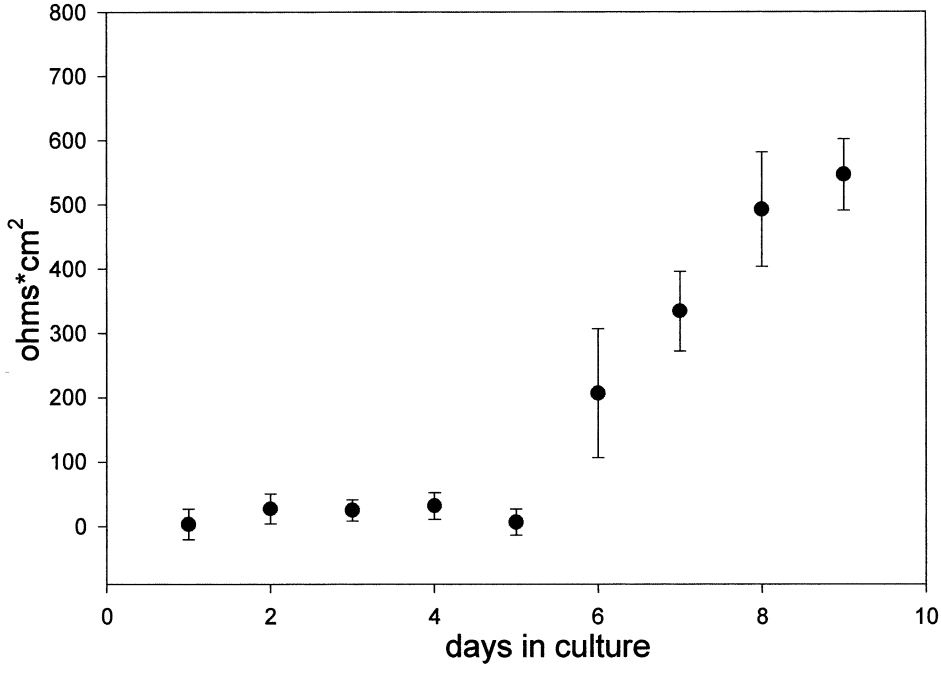

Fig. 3. TEER values of Calu-3 monolayers grown in 12-well Transwells® versus days in culture. The TEER values were determined in culture medium after rehydrating the apical membrane for 30 min using an STX-2 electrode and World Precision Instrument Voltohmmeter. The monolayer TEER was corrected for the resistance of the filter support alone.

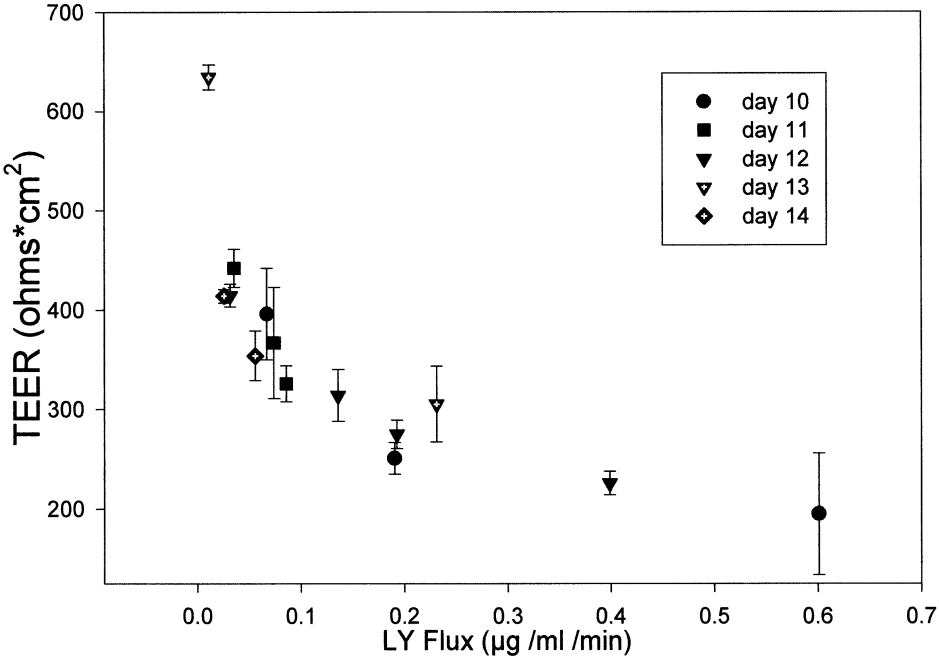

Fig. 4. Calu-3 monolayer TEER versus flux of the paracellular marker, Lucifer yellow, versus days in culture. Calu-3 cells were plated in 12-well Transwells® at a density of 5×10^5 cells/cm^2. The TEER was determined for each monolayer after a 30-min equilibration period in PBSA prior to the start of the flux experiment. The Flux of Lucifer yellow (apical → basolateral) was determined by fluorescence spectroscopy (SLM Instruments, Inc.).

3.4. Calu-3 P450 EROD and PROD assays, testosterone metabolism

Western Blots of Calu-3 microsomes revealed that the cells express CYP1A1, CYP2B6, CYP3A4, and CYP2E1 (data not shown). EROD and PROD assays, however, indicated that CYP1A1 and CYP2B6 were not inducible under the conditions employed in our studies (Fig. 7). The inability of dexamethasone-treated Calu-3 monolayers to metabolize testosterone to the 6-β hydroxytestosterone metabolite also implied that CYP3A4 is not inducible in these cells.

4. Discussion

4.1. TEER and Lucifer yellow flux

Winton et al. (1998), recently reported the presence of the tight junction associated protein ZO-1 and the adherin protein E-cadherin in Calu-3 cell monolayers. The present study confirms previous reports that the Calu-3 cell line is capable of forming tight monolayers in culture which generate significant TEER, a characteristic of bronchiole epithelium in vivo. Other groups reporting TEER values for Calu-3 monolayers grown on filter supports show values ranging between 700 and 2500 Ω*cm^2 (Loman et al., 1997). Variability was also observed by the present authors depending on the technique employed (End-Ohm Chamber or STX-2 electrode) to achieve the measurements. This demonstrates the importance for researchers to be cautious when comparing reported TEER values across labs. The data indicated that there was an inverse relationship between TEER and Lucifer yellow flux; however, once the monolayers achieve an appreciable TEER (> 300 Ω*cm^2), there is very little change in Lucifer yellow flux across monolayers with higher TEER values. This trend has been shown

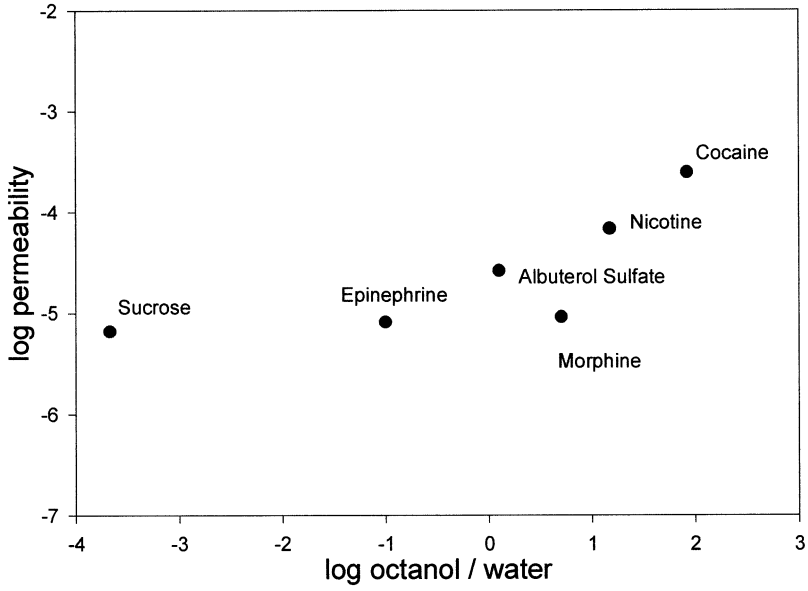

Fig. 5. Log permeability coefficients for the passage of small molecules across Calu-3 monolayers grown in Transwells® versus log of the molecule's respective octanol/H_2O partition coefficient. The correlation between log permeability and log octanol/H_2O suggests that the small lipophilic molecules are crossing the monolayers by a simple diffusion mechanism. The K_{ow} were obtained from literature values (Leo et al., 1971).

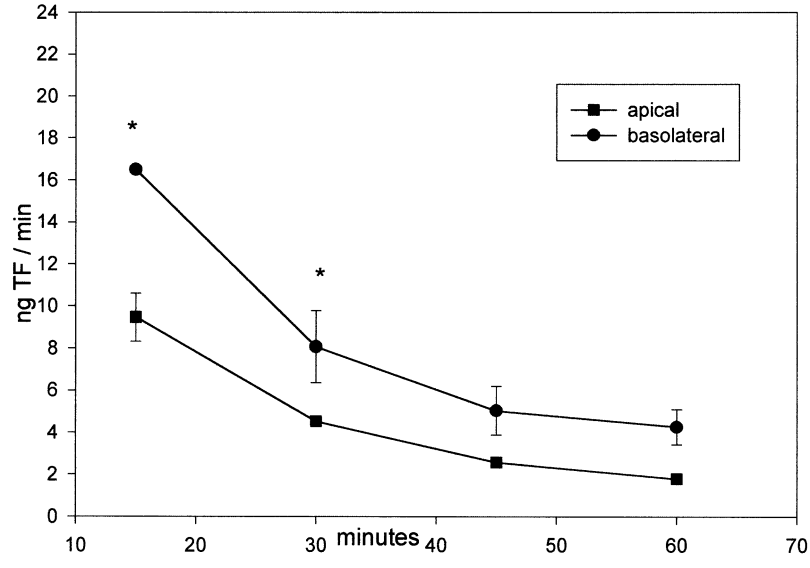

Fig. 6. Efflux of intracellular fluorescein-transferrin out of Calu-3 monolayers. Calu-3 cells grown in Transwells® were washed 3 × with PBSA and equilibrated in PBSA for 30 min to deplete the cells of endogeneous transferrin. Monolayers were then saturated simultaneously on the apical and basolateral sides with 1 μM fluorescein-transferrin for 1 h at 37°C. The cells were then washed on both sides 3 × with PBSA and the bidirectional efflux was determined at indicated timepoints using fluorescence spectroscopy. (Note: *denotes significance at 95% confidence level).

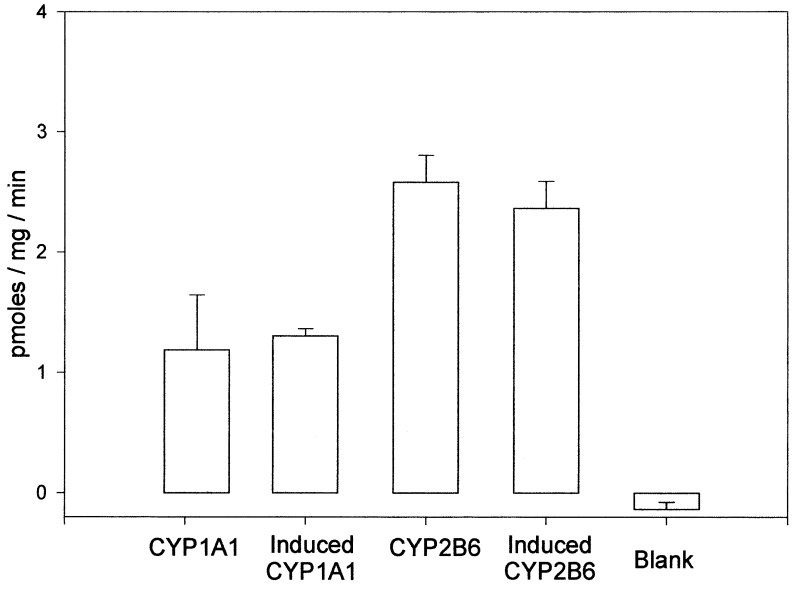

Fig. 7. Calu-3 P450 activity as determined by EROD (CYP1A1) and PROD (CYP2B) assays. Calu-3 cells were plated in six-well Nunc plates and allowed to grow to confluency. The CYP1A1 and CYP2B6 activity of the cells was then measured and compared to activity measured for cells, which had been pretreated with inducers. Induced cells were treated for 48 h with 100 μM 3-MC plus 1 μM dexamethasone (CYP1A1 induction) or 500 μM phenobarbitol (CYP2B induction). The results indicate that the cells were not induced by the treatment.

previously for primary cultures of rat Type II pulmonary epithelial cells and is believed to be dependent on the size and charge of the marker (Adson et al., 1994). Fig. 4 also indicates that there was some variability between monolayer TEER from plate to plate; therefore, it was important to check the TEER of monolayers before conducting transport experiments to insure that it was well above the TEER value for the Transwell® support alone (approximately 115 Ω*cm^2).

4.2. Transport experiments

Fig. 5 suggested that small lipophilic substances crossed Calu-3 monolayers by a simple transcellular diffusion mechanism. The data for sucrose permeability does not fit the linear relationship shown by the drug substances and was indicative of the reliance of this molecule on predominantly the paracellular mechanism for permeation across cell monolayers (Adson et al., 1994).

The results obtained for fluorescein-transferrin efflux experiments were in accordance with a typical recycling mechanism for transferrin (Raub and Newton, 1991) on the basolateral side, the surface representing the blood side of the bronchial epithelium in vivo. This was consistent with transferrin efflux data determined for brain microvessel endothelial cells, BBMECs (Raub and Newton, 1991), and the Type II pulmonary epithelium cell line, A549 (Foster et al., 1998). This polarizability of Calu-3 monolayers was also in agreement with results previously published (Loman et al., 1997) which showed the vectoral transport of dimeric IgA across Calu-3 monolayers.

4.3. Metabolism experiments

The P450 mono-oxygenase systems in the lungs are involved in the metabolism of endogenous compounds such as fatty acids and steroids and lipid-soluble xenobiotics that enter through the circulation or by inhalation (Ma et al., 1996). The cytochrome P450 enzymes of the lung are considered to be concentrated to specific cell categories (Serabibjit-Singh et al., 1988; Raub and Newton,

1991) with the majority of P450 activity in the lung attributed to Clara cells, Type II pulmonary epithelial cells, and alveolar macrophages (Devereux et al., 1989). There is limited information in the literature about the P450 activity of lung serous cells, the presumed origin for the Calu-3 cell line.

Two of the major P450 enzymes associated with the lung include the cytochromes CYP1A1 and CYP2B (Ito et al., 1992). In rabbit lungs, CYP1A1 accounts for 1–3% of the total CYP450 activity while CYP2B1 and CYP4B1 collectively account for over 90% of the total CYP450 activity (Ma et al., 1996). These isozymes are significant due to their possible role in bioactivation of procarcinogens, which may lead to lung cancer.

The data for deethylation of 7-ethoxyresorufin in Calu-3 cells (1.19 ± 0.46 pmol/mg per min) was comparable to the values reported for the Type II pulmonary epithelial cell line, A549, (2.48 ± 0.91 pmol/mg per min) and was consistent with values found for adult human lung microsomes (4.2 ± 5.1 pmol/mg per min; Minchin and Boyd, 1983). The reported enzymatic activity for CYP2B1 from microsomes isolated from whole rabbit lungs was 910 ± 240 pmol/min per mg protein (Devereux et al., 1989) was substantially greater than the CYP2B activity determined for Calu-3 cells in this work (2.58 ± 0.22 pmol/mg per min). Neither CYP1A1 nor CYP2B isozyme activities in Calu-3 cells were inducible by pretreatment with typical polycyclic aromatic hydrocarbons (Fig. 7). Data obtained from testosterone metabolism experiments indicated that Calu-3 cells do not possess functional CYP3A4 due to lack of formation of the 6β-hydroxytestosterone metabolite (data not shown). Raunio et al. (1998), previously reported that the CYP3A4 isozyme was present in only 20% of the human pulmonary epithelial samples examined. Therefore, while the Calu-3 cells exhibited functional expression of representative P450s, CYP1A1 and CYP2B, the inducibility of the enzymes could not be demonstrated under the conditions of our studies. However, while the P450 activity reported in this paper for Calu-3 cells is lower than the activity reported for Type II pulmonary epithelial cells and microsomes isolated from whole lung tissue, the results still suggest a capacity for oxidative metabolism by Calu-3 cells.

The present study has shown that the Calu-3 cell line forms tight polarized monolayer in culture. Other commercially available cell lines derived from the respiratory epithelium such as the A549 cell line (Foster et al., 1998) lack the ability to generate high TEER in culture. The Calu-3 cell line appears to be a potential model to screen potential drug candidates and formulations to be delivered to the respiratory epithelium. Future work will examine the effect of drugs and excipients on Calu-3 monolayer TEER values and P4501A1 and P4502B activity.

Acknowledgements

This work was supported by Boehringer Ingelheim and an NIGMS predoctoral training program in biotechnology (K.A. Foster). The authors gratefully acknowledge the support of Corning Costar for the Cellular and Molecular Biopharmaceutics Handling Laboratory at the University of Kansas.

References

Adson, A., Raub, T., Burton, P., Barsuhn, C., Audus, K., Ho, N., 1994. Quantitative approaches to delineate paracellular diffusion in cultured epithelial cell monolayers. J. Pharm. Sci. 83, 1529–1536.

Burke, M., Mayer, R., 1974. Ethoxyresorufin: direct fluorimetric assay of a microsomal *O*-dealkylation which is preferentially inducible by 3-methylcholanthrene. Drug Metab. Dispos. 2, 583–588.

Devereux, T., Domin, B., Philpot, R., 1989. Xenobiotic metabolism by isolated pulmonary cells. Pharm. Ther. 41, 243–256.

Finkbeiner, W., Carrier, S., Teresi, C., 1993. Reverse tranc-scription-polymerase chain reaction (RT-PCR) phenotypic analysis of cell cultures of human tracheal epithelium, tracheobronchial glands, and lung carcinomas. Am. J. Respir. Cell Mol. Biol. 9, 547–556.

Foster, K., Oster, C., Mayer, M., Avery, M., Audus, K., 1998. Characterization of the A549 cell line as a type II pulmonary epithelial cell model for drug metabolism. Exp. Cell Res. 243, 359–366.

Haws, C., Finkbeiner, W., Widdicombe, J., Wine, J., 1994. CFTR in Calu-3 human airway cells: channel properties and role in cAMP-activated Cl^- conductance. Am. J. Physiol. 266, L502–L512.

Lee, M., Penland, C., Widdicombe, J., Wine, J., 1998. Evidence that Calu-3 human airway cells secrete bicarbonate. Am. J. Physiol. 274, L450–L453.

Loman, S., Radl, J., Jansen, H., Out, T., Lutter, R., 1997. Vectorial transcytosis of dimeric IgA by the Calu-3 human lung epithelial cell line: upregulation by IFN-γ. Am. J. Physiol. 272, L951–L958.

Ma, J., Bhat, M., Rojanasakul, Y., 1996. Drug metabolism and enzyme kinetics in the lung. In: Hickey, A. (Ed.), Inhalation Aerosols. Marcel Dekker, New York, pp. 155–195.

Minchin, R., Boyd, M., 1983. Localization of metabolic activation and deactivation systems in the lung: significance to the pulmonary toxicity of xenobiotics. Annu. Rev. Pharmacol. Toxicol. 23, 217–238.

Nerurkar, M., Burton, P., Borchardt, R., 1996. The use of surfactants to enhance the permeability of peptides and proteins through Caco-2 cells by inhibition of an apically polarized efflux system. Pharm. Res. 13, 528–534.

Parkinson, A. Toxicology by Casarett & Doull, 1996.

Raub, T., Newton, C., 1991. Recycling kinetics and trancytosis of transferrin in primary cultures of bovine brain microvessel endothelial cells. J. Cell. Physiol. 149, 141–151.

Raunio, H., Hakkola, J., Hukkanen, J., Pelkonen, O., Edwards, R., Boobis, A., Anttila, S., 1998. Expression of xenobiotic-metabolizing cytochrome P450s in human pulmonary tissues. Arch. Toxicol. Suppl. 20, 465–469.

Serabibjit-Singh, C., Nishio, S., Philpot, R., Plopper, C., 1988. The distribution of cytochrome P-450 monooxygenase in cells of the rabbit lung: an ultrastructural immunocytochemical characterization. Mol. Pharmacol. 33, 279–289.

Shen, B., Finkbeiner, W., Wine, J., Mrsny, R., Widdicombe, J., 1994. Calu-3: a human airway epithelial cell line that shows cAMP-dependent Cl^- secretion. Am. J. Physiol. 266, L493–L501.

Singh, M., Krouse, M., Moon, S., Wine, J., 1997. Most basal I_{sc} in Calu-3 human airway cells is bicarbonate-dependent Cl^- secretion. Am. J. Physiol. 272, L690–L698.

Winton, H., Wan, H., Cannell, M., Gruenbert, D., Thompson, P., Garrod, D., Stewart, G., Roginson, C., 1998. Cell lines of pulmonary and non-pulmonary origin as tools to study the effects of house dust mite proteinases on the regulation of epithelial permeability. Clin. Exp. Allergy 28, 1273–1285.

Wortelboer, H., De Kruif, C., Van Iersel, A., Falke, H., Noordhoek, J., Blaauboer, B., 1990. The isoenzyme pattern of cytochrome P450 in rat hepatocytes in primary culture, comparing different enzyme activities in microsomal incubations and in intact monolayers. Biochem. Pharmacol. 40, 2525–2534.