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Research Paper

Expression and functional activity of P-glycoprotein in passaged primary human nasal epithelial cell monolayers cultured by the air–liquid interface method for nasal drug transport study

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Abstract

Objectives P-glycoprotein (P-gp) is an efflux transporter encoded by the multidrug resistance gene (MDR1), which is also known as the human *ABCB1* gene (ATP-binding cassette, subfamily-B). The objectives of this study were to investigate the expression of P-gp in passaged primary human nasal epithelial (HNE) cell monolayer, cultured by the air–liquid interface (ALI) method, and to evaluate its feasibility as an in-vitro model for cellular uptake and transport studies of P-gp substrates.
Methods Reverse transcriptase-polymerase chain reaction (RT-PCR) was performed to verify the expression of the MDR1 gene. Transport and cellular uptake studies with P-gp substrate (rhodamine123) and P-gp inhibitors (verapamil and cyclosporin A) were conducted to assess the functional activity of P-gp in HNE cell monolayers cultured by the ALI method.
Key findings MDR1 gene expression in primary HNE cell monolayers cultured by ALI method was confirmed by RT-PCR. The apparent permeability coefficient (P_{app}) of the P-gp substrate (rhodamine123) in the basolateral to apical (B to A) direction was 6.9 times higher than that in the apical to basolateral (A to B) direction. B to A transport was saturated at high rhodamine123 concentration, and the treatment of P-gp inhibitors increased cellular uptake of rhodamine123 in a time- and concentration-dependent manner.
Conclusions These results support the MDR1 gene expression and the functional activity of P-gp in primary HNE cell monolayers cultured by the ALI method.
Keywords P-glycoprotein; human nasal epithelial cell monolayer; in-vitro models; nasal drug delivery

Introduction

P-glycoprotein (P-gp), an ATP-binding cassette (ABC) transporter, consists of 1276–1280 amino acids (170 kDa molecular weight). It is of a tandemly duplicated structure, where each one has a nucleotide binding domain and six transmembrane domains.[1] It is mainly expressed in the apical side of the cell membrane and distributed in the intestine, liver, kidney, blood–brain barrier, blood–testis barrier and adrenal gland.[2] P-gp is well known for its function as an efflux pump for xenobiotic substances with broad substrate specificity. More specifically, P-gp is responsible for the multi-drug resistance (MDR) of cancer cells to chemotherapeutic agents.[3] In addition, it is assumed that the role of P-gp located in the respiratory tract is related to the efflux of xenobiotic compounds. The expression of P-gp has been identified in the respiratory epithelium and its derived cell culture systems, which include the human bronchus,[4] human bronchial epithelial cell line (16HBE14o-),[5] human broncho-tracheal epithelial cell line (Calu-3),[6,7] normal human bronchial epithelial (NHBE) cell monolayer cultured by the air–liquid interface (ALI) method[8] and normal human nasal respiratory mucosa.[9]

Recently, nasal drug delivery has become a research topic of interest to pharmaceutical scientists as an alternative to intravenous and oral administration. It has several advantages, including avoidance of drug degradation resulting from the first-pass effect in the liver, direct

brain targeting, alternative route of biomacromolecule delivery, and rapid absorption and onset of action.[10–13] Although excised animal nasal tissues have been used for in-vitro transport studies,[14] specific differences in enzymatic activities and cell differentiation have been observed. Moreover, the pharmacokinetic patterns of intranasally administered drugs in animal models are not completely consistent with those of humans. Since the human nasal epithelial cell line (RPMI 2650) does not express tight junctions, it is not suitable for transport studies. Thus these limitations have prompted research into primary culture of human nasal epithelial cells. Recent studies on the primary culture of nasal epithelial cells from a variety of species, including human, have provided valuable in-vitro models for the study of nasal physiology in healthy and disease states.[14,15] However, problems, including the limited amount of available cells and epithelial cell differentiation, still remain to be overcome.

One way to solve these problems was to develop a primary HNE cell monolayer system focusing on the formation of tight junction and cilia for drug transport studies.[16,17] The HNE cell monolayer system cultured by the ALI method by our research team exhibited improved efficiency compared to those cultured by the traditional liquid-covered culture (LCC) system in terms of forming better tight junction and cilia formation.[18,19] However, the expression and function of any drug transporters in HNE cell monolayers cultured by the ALI method were not verified. To understand the transport mechanism and process of each drug compound across primary HNE cell monolayers, the expression levels of transporters that participate in the drug transport process need to be elucidated. In previous reports, whether this system is able to mimic the in-vivo system in terms of P-gp expression also has not been clarified. In this report, the expression of P-gp, one of the most typical efflux transporters of the ABC family, which is involved in the transport process of many structurally divergent substances, was determined in passage cultured primary HNE cell monolayers by the RT-PCR assay of the MDR1 gene. Moreover, the transport and the cellular uptake of rhodamine123, a model P-gp substrate, were studied as an indication of its functional activity. We report on the suitability of the passage cultured primary HNE cell monolayer cultured by the ALI method as an in-vitro cell system for nasal drug transport and uptake studies where the efflux system by P-gp is functional.

Materials and Methods

Materials

Verapamil hydrochloride, rhodamine123, Hank's balanced salt solution (HBSS), sodium azide (NaN_3), 4-(2-hydroxyethyl) piperazine-1-ethanesulfonic acid (HEPES) and D-(+)-glucose were purchased from Sigma Chemical Co. (St Louis, MO). Cyclosporin A was a gift from Chong Kun Dang Pharmaceutical Co. (Seoul, South Korea). BEGM bullet kit was purchased from Cambrex Bio Science Inc. (Walkersville, MD). Other cell culture reagents and supplies were obtained from Invitrogen Co. (Grand Island, NY). Transwells® (0.4 μm pore size, 12 mm diameter, 1.12 cm^2 surface area per insert, polyester) were obtained from Costar Co. (Cambridge, MA).

ALI culture of HNE cell monolayers

The isolation and expansion of HNE cells used in this investigation has been previously reported.[16] In brief, the HNE cells were enzymatically dissociated overnight at 4°C in culture medium containing 1.0% (w/v) Pronase (type XIV protease, Sigma, St Louis, MO). Detached epithelial cells were washed and suspended in Dulbecco's Modified Eagle Medium, nutrient mixture F12 [Ham] 1 : 1 (DMEM/F12), containing 10% fetal bovine serum. Suspended epithelial cells were frozen using dimethylsulphoxide and stored in a liquid nitrogen tank. Culturing primary HNE cell monolayers by the ALI method has also been reported in our previous studies.[18,19] When human nasal epithelial cells of passage 1 and 2 attained about 70–80% confluency, the cells were trypsinized and seeded on Transwell® inserts (12-well) at densities of 1.5×10^5 cells/cm^2. The apical side (0.5 ml) and basolateral side (1.5 ml) were filled with BEGM : DMEM/F12 (50 : 50) supplemented with BEGM® SingleQuots® (all supplied by Cambrex Bio Science Inc., Walkersville, MD). The apical side was exposed to the air condition on day 3 for ALI culture, after which the medium in the basolateral side was changed every 2 days. The incubator for culturing cells was maintained at 37°C in an atmosphere of 5% CO_2 and 95% relative humidity.

Reverse transcriptase-polymerase chain reaction

Total RNAs of HNE cells grown in monolayers for 7, 10, 14 and 21 days were purified and obtained using RNeasy® Plus Mini kit (#74134) (QIAGEN Inc., Valencia, CA). Primers for the detection of the MDR1 gene (accession number AF016535) as well as for the housekeeping enzyme, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene, were purchased from Dyne Bio Inc. (Sungnam, South Korea). Reverse transcription was carried out using an AccuPower RT premix (Bioneer Co., Daejeon, South Korea) at 70°C for 5 min (incubation), 42°C for 60 min (cDNA synthesis) and 94°C for 5 min (inactivation of reverse transcriptase). PCR amplification was performed with mixtures, including cDNA template, 50 pmol of each primer, water treated by diethyl pyrocarbonate (DEPC) and HotStarTaq® DNA Polymerase (QIAGEN, Valencia, CA). The PCR procedure was carried out in the MJ Mini Thermal Cycler (Bio-Rad Laboratories, Inc. Hercules, CA). Amplification conditions were as follows: 15 min at 95°C for activating Taq polymerase, 33 cycles of 50 s at 94°C, 50 s at 55°C, 1 min at 72°C, followed by 7 min at 72°C. PCR products were subjected to gel electrophoresis (1.5% agarose gels) and GelRed™ (Biotium, Inc., Hayward, CA) was used to stain PCR products. MDR1 mRNA expression level was normalized to that of the GAPDH gene and each relative expression level was compared by using the Image J program (Version 1.43r).

Transport studies of rhodamine123 across the ALI HNE cell monolayers

For the transport study, HNE cell monolayers of passage 1 and 2 were cultured for 10–12 days by the ALI method. The transport study was performed in HNE cell monolayers when the transepithelial electrical resistance (TEER) value was higher than 500 $\Omega \times cm^2$. First of all, the cell culture medium

was eliminated and the cell monolayers were incubated in transport medium (HBSS supplemented with 10 mM HEPES and 10 mM D-(+)-glucose), for 20 min at 37°C. TEER value was measured by using the EVOM voltohmmeter (WPI, Sarasota, FL) after acquiring a steady potential. After eliminating the transport medium, P-gp inhibitors (10 μM cyclosporin A or 500 μM verapamil) solubilized in culture medium were added to the apical (0.4 ml) and basolateral (1.0 ml) sides. Then the monolayers were pre-incubated for 1 h before the transport study of rhodamine123. To carry out the A to B transport studies, 0.4 ml of transport medium containing rhodamine123 (molecular weight: 380.8, 5 μM) was added to the apical compartment and 1.0 ml of transport medium was added to the basolateral compartment. Equal volumes (1.0 ml) of samples were collected from the basolateral chamber at predetermined times (15, 30, 45, 60, 90, and 120 min) and replaced with the same volume of fresh transport medium. For the B to A transport studies, 0.4 ml of transport medium was added in the apical side and 1.0 ml of transport medium including rhodamine123 was applied to the basolateral side. Samples (0.3 ml) were collected from the apical side and replaced with the same volume of fresh transport medium.

The effect of the ATPases inhibitor (sodium azide) on the B to A transport of rhodamine123 (5 μM) was determined, where sodium azide (0.1%) in culture medium was pre-incubated for 2 h before the transport study. The B to A transport study of rhodamine123 (5 μM) was also observed at 4°C for 2 h to observe the effect of temperature. Moreover, the effect of rhodamine123 concentration on the B to A transport was determined with 1–250 μM of rhodamine123 without P-gp inhibitors.

HPLC analysis and data analysis

The samples collected from the transport studies were analysed by a Waters HPLC system equipped with a pump (Waters 515 HPLC pump, Milford, MA), an automatic injector (Waters 717 plus autosampler, Milford, MA) and a fluorescence detector (Series 200, PerkinElmer Instrument, Norwalk, CT) set at the wavelengths of 485 nm for excitation and 525 nm for emission. A reversed-phase C-18 column (XBridge™, RP-18, 250 × 4.6 mm, 5 μM, Waters Co., Milford, MA) was used. The composition of the mobile phase for rhodamine123 was 50 mM phosphate buffer (pH 3.0 adjusted with phosphoric acid) and acetonitrile (65 : 35, v/v), with the flow rate set at 1.0 ml/min.

Cellular uptake study of rhodamine123 with P-gp inhibitors

For cellular uptake experiments of rhodamine123, HNE cells were seeded on Transwell® inserts (12-well) and grown to confluency for determined periods (7, 14, and 21 days). Prior to the uptake study, the cell monolayers were incubated in culture medium with P-gp inhibitors, verapamil (500 μM) or cyclosporin A (10 μM), at 37°C for 1 h. After aspirating off the medium, 10 μM of rhodamine123 was added to each well and incubated for 30 min at 37°C. Then the medium was eliminated and the cell monolayers were rinsed at least three times with ice-cold phosphate buffered saline (PBS, pH 7.4). The cell monolayers were solubilized with 0.2% (v/v) of Triton X-100 in 0.2 N sodium hydroxide solution for 4 h. The content of rhodamine123 in each cell lysate was determined by fluorescence spectrometer FP-6500 (JASCO Co., Tokyo, Japan). The wavelength of excitation was 485 nm and the wavelength of emission was 530 nm. The protein contents of cell lysates were measured by a BCA protein assay kit (Pierce, Rockford, IL) to normalize the amounts of protein in each sample. The change in rhodamine123 uptake with the addition of inhibitors was expressed relative to that without P-gp inhibitors. Moreover, the cellular uptake due to the P-gp inhibitor was examined in HNE cell monolayers, which were cultured for 10–14 days with rhodamine123 added in concentration ranges of 0.5 to 100 μM.

Data analysis

After plotting the cumulative amount of rhodamine123 permeated through the HNE cell monolayers as a function of time, P_{app} (cm/s) was calculated from the following equation:

$$P_{app} = \frac{dQ}{dt}\frac{1}{A \times C_0} \qquad (1)$$

where dQ/dt is the linear appearance rate of rhodamine123 in the receiver chamber, transported during the sink condition, A is the surface area of the membrane insert (cm^2), and C_0 is the initial rhodamine123 concentration in the donor side.

WinNonlin® (Version 3.1, Pharsight Co., Mountainview, CA) was used to attain kinetic parameters with the Michaelis–Menten equation. These parameters were calculated by the following formula:

$$V = \frac{V_{max} \times [C]}{K_m + [C]} \qquad (2)$$

where V is the rate (transport or uptake) at given concentrations of rhodamine123, V_{max} is the maximum rate, [C] is the initial concentration of rhodamine123, and K_m is the Michaelis–Menten constant.

All the experiments in the study were performed at least three times and the data were expressed as the mean ± standard deviation (SD). The SPSS software package (ver. 18.0, SPSS, Chicago, IL) was used for the statistical analysis. Statistical analysis was performed by Kruskal–Wallis test. Statistical significance was set at $P < 0.05$.

Results and Discussion

RT-PCR assay of MDR1 mRNA expressed in the HNE cell monolayers

MDR1 is the representative gene to encode P-gp in humans. The sequences of forward and reverse primers for MDR1 and GAPDH genes are presented in Table 1.[20] Total RNA was extracted from HNE cell monolayers on days 7, 10, 14, and 21 of ALI culture. In order to confirm the expression of the P-gp in the HNE cell monolayers, the relative levels of MDR1 mRNA expression were measured by RT-PCR analysis (Figure 1). The existence of the GAPDH gene, used as the

Table 1 Primer sequences of MDR1 and GAPDH gene for RT-PCR assay

Primer		Sequence	Size
MDR1	Forward	5′-CACCTGCATTGTGATTGCTC-3′	174 bp
	Reverse	5′-AGAGTTCACTGGCGCTTTGT-3′	
GAPDH	Forward	5′-TGGTATCGTGGAAGGACTCATGAC-3′	190 bp
	Reverse	5′-ATGCCAGTGAGCTTCCCGTTCAGC-3′	

Primer sequences for MDR1 gene were taken from Endter *et al*.[20]

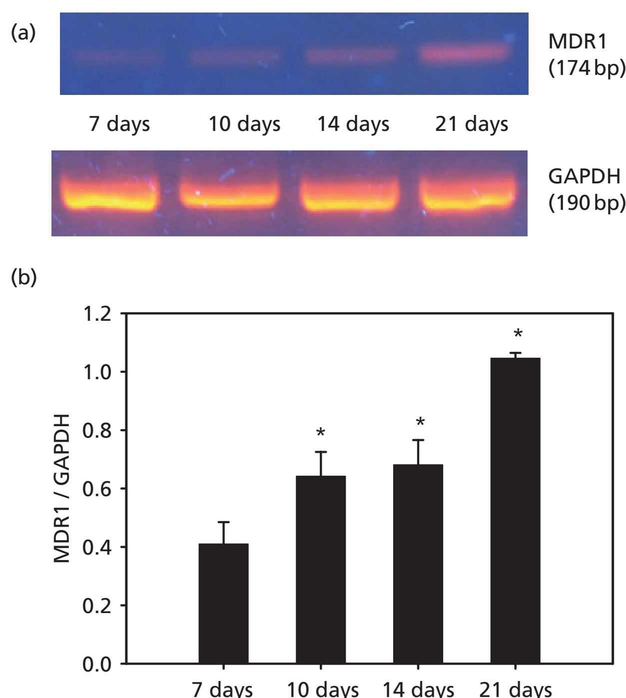

Figure 1 MDR1 mRNA expression level in the passaged primary HNE cell monolayers after 7, 10, 14 and 21 days of ALI culture analysed by (a) RT-PCR and (b) relative MDR1 gene expression level (MDR1/GAPDH ratio). Each experiment was done in triplicate. *$P < 0.05$, compared to the band of 7 days.

denominator for comparison of samples, was recognized at the location of 190 bp. As shown in Figure 1a, MDR1 mRNA band (174 bp) was present in the day 7, 10, 14 and 21 samples. An increasing trend for the MDR1 mRNA expression level was observed from days 7 to 21. There was statistically significant difference ($P < 0.05$) among the band intensities of whole groups (days 7 to 21). In our previous study, the expression level of MDR1 mRNA obtained from NHBE cell monolayers also increased with time, but the expression level was not as strong.[8] As previously reported,[18] the HNE cell monolayer seems to better mimic the physiological conditions of the human nasal mucosa, and thus is more suitable as an in-vitro model for drug transport studies. The HNE cell monolayers cultured by the ALI method in this study continuously expressed P-gp over the 21 days. Therefore, based on these RT-PCR study results and our previous report,[18] in which a maximum TEER value was observed on day 5 and maintained for at least 10 days, 10–14 days after seeding seemed to be the suitable period for culturing primary HNE cell monolayers by the ALI method for the transport study of P-gp substrates. In addition, differentiation of epithelial cells and the development of cilia in the HNE cell monolayer system were critical factors along with attaining optimal TEER values. We previously reported that the ALI culturing method can enhance differentiation and development of cilia on the apical surface compared to the LCC method.[18]

Transport studies with rhodamine123 and P-gp inhibitors

To verify the functional expression of P-gp in ALI-cultured HNE cell monolayers, the permeability of rhodamine123 in A to B and B to A directions was measured with or without P-gp inhibitors. Rhodamine123 is a representative P-gp substrate that is widely used in other cell culture models, such as Caco-2 cell monolayers[21,22] and LLC-PK1 cell monolayers.[23] In the transport study using 5 μM rhodamine123 without P-gp inhibitors (Table 2), the P_{app} value of the B to A direction at $(9.70 \pm 0.86) \times 10^{-7}$ cm/s was 6.9-fold higher than that of the A to B direction at $(1.41 \pm 0.32) \times 10^{-7}$ cm/s ($P < 0.05$). The efflux ratio of rhodamine123 without P-gp inhibitor treatment in the HNE cell monolayers (6.9) was consistent with MDR1 gene expression as detected by RT-PCR assay (Figure 1). In addition, when the HNE cell monolayers were treated with P-gp inhibitors such as verapamil and cyclosporin A, the P_{app} value of B to A significantly decreased compared to the control (without P-gp inhibitor treatment) ($P < 0.05$), while the P_{app} value of the A to B direction significantly increased ($P < 0.05$). This result was in accordance with that of our previous report on NHBE cell monolayers,[8] where the efflux ratio of rhodamine123 decreased from 2.95 to 1.65 on treatment with verapamil. Thus P-gp is undoubtedly expressed in passaged primary HNE cell monolayer and is functioning in rhodamine123 transport.

Effect of sodium azide and temperature on the efflux of rhodamine123

The function of P-gp transporter on the efflux of rhodamine123 in HNE cell monolayers was further investigated by observing the effect of ATPases and temperature. P-gp, an ABC transporter, has two membrane domains, which form pathways of transported substrates across the cell membrane, and two nucleotide-binding domains, responsible for the binding and hydrolysis of ATP.[24] Treatment with an ATPases inhibitor, such as sodium azide (NaN_3), would interrupt the binding and hydrolysis of ATP, thereby influencing the transport activity. As shown in Figure 2, pre-treatment with 0.1% sodium azide decreased the amount of

Table 2 Apparent permeability coefficients of rhodamine123 with P-gp inhibitors in the absorptive (A to B) and secretory (B to A) directions across the passage cultured HNE cell monolayers

Rhodamine123 concentration (μM)	P-gp inhibitor	P_{app} ($\times10^{-7}$ cm/s)		Efflux ratio (B to A)/(A to B)
		A to B	B to A	
5	–	1.41 ± 0.32	9.70 ± 0.86*	6.9
	Verapamil 500 μM	3.14 ± 0.22*	7.48 ± 0.34**	2.4
	Cyclosporin A 10 μM	4.39 ± 0.03*	6.45 ± 0.56**	1.5

P_{app}, permeability coefficients. Each datum represents the mean ± SD ($n \geq 3$). *$P < 0.05$, compared to A to B transport without P-gp inhibitors. **$P < 0.05$, compared to B to A transport without P-gp inhibitors.

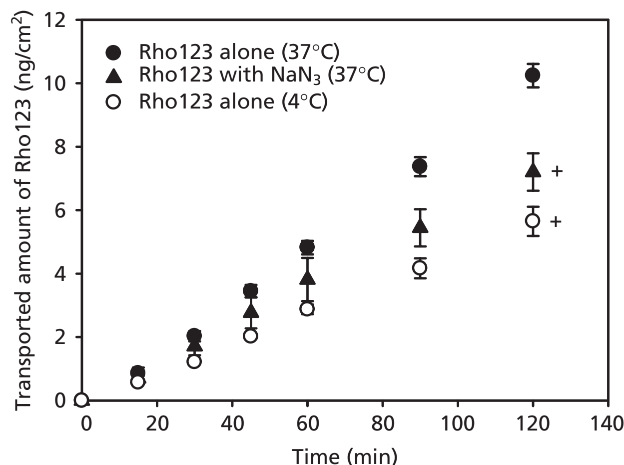

Figure 2 B to A (efflux) transport of rhodamine123 (Rho123, 5 μM) across the passaged primary HNE cell monolayers at 37°C (●) or 4°C (○), and the effect of sodium azide (0.1%) at 37°C (▲). Data are shown as mean ± SD ($n = 3$). $^{+}P < 0.05$, compared to the data without sodium azide at 37°C.

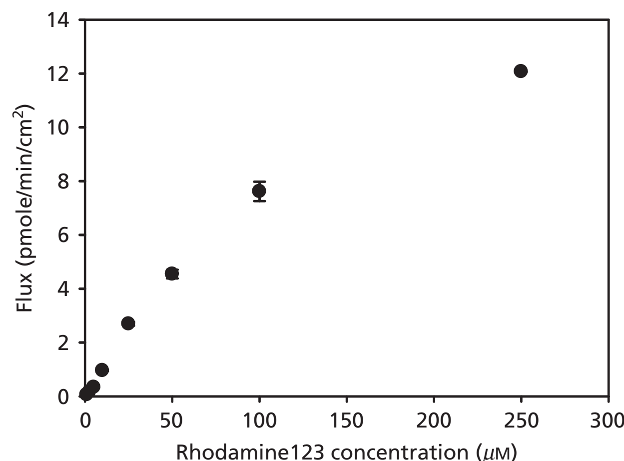

Figure 3 The effect of rhodamine123 concentration (1–250 μM) on the B to A flux across the passaged primary HNE cell monolayers at 37°C. Each point represents the mean ± SD ($n = 3$).

rhodamine123 transported for 2 h in the B to A direction (7.20 ± 0.59 ng/cm^2) by 29.7%, compared to that of rhodamine123 alone (without NaN_3) (10.24 ± 0.37 ng/cm^2) ($P < 0.05$). Moreover, conducting the 2 h transport study at 4°C resulted in a 44.9% decrease of the amount of rhodamine123 transported (5.65 ± 0.46 ng/cm^2), compared to that at 37°C ($P < 0.05$). This temperature-dependent decrease of rhodamine123 transport in the B to A direction further supports the P-gp-mediated transport of rhodamine123. However, it is interesting to note that the decrease in rhodamine123 transport at low temperature (4°C) compared to that at 37°C was greater than the decrease caused by pre-treatment with sodium azide. Considering that sodium azide specifically inhibits ATPase activity but temperature changes can influence the drug diffusivity, membrane fluidity, transporter activity and intracellular trafficking,[25] the difference in the magnitude of transport reduction between sodium azide treatment (▲; 29.7% reduction) and low temperature (○; 44.9% reduction) may be explained by the decrease in drug diffusivity and membrane fluidity.

Rhodamine123 concentration-dependent efflux transport study

The effect of rhodamine123 concentration on the B to A transport across the HNE cell monolayers was investigated over the concentration range of 1–250 μM (Figure 3). As the concentration of rhodamine123 increased, the flux showed a saturation pattern that resulted in a K_m value of 171.60 ± 10.99 μM and V_{max} value of 20.41 ± 0.70 pmole/min/cm^2 from Equation 2.

Consistent with our study, significantly greater secretary transport of rhodamine123 has been reported in respiratory epithelial cell monolayer systems, such as hAEpC, 16HBE14o-, Calu-3, and human bronchial epithelial cell, and this asymmetry was removed by the presence of P-gp inhibitors such as verapamil and cyclosporin A, although the kinetic parameters were not described.[5,8,20,26] According to the report of Troutman and Thakker,[27] kinetic parameters calculated from the transport study of rhodamine123 in Caco-2 cell monolayers yielded a K_m value of 173 ± 26.3 μM and J_{max} value of 988 ± 85.9 pmole/min/mg protein. This K_m value of rhodamine123 was comparable to the K_m value obtained from our system.

Effect of P-gp inhibitors on the cellular uptake of rhodamine123

The cellular uptake of rhodamine123 was examined after applying 10 μM, with or without P-gp inhibitor (500 μM verapamil or 10 μM cyclosporin A), to HNE cell monolayers after 7, 14 and 21 days of ALI culture. Rhodamine123 is a typical fluorescent substance used for cellular uptake experiments to

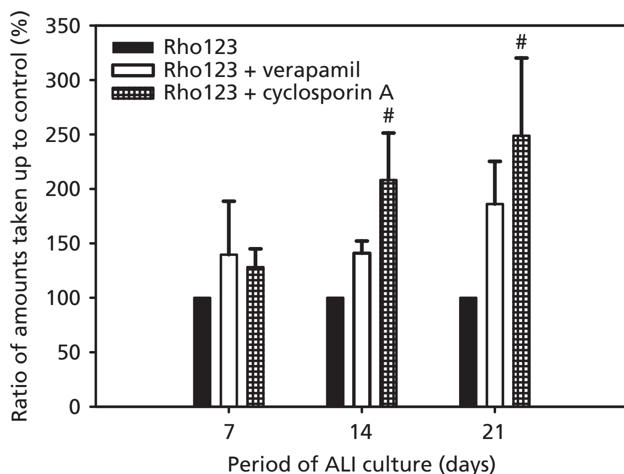

Figure 4 The effect of verapamil (500 μM) and cyclosporin A (10 μM) on the cellular uptake of 10 μM rhodamine123 (Rho123) on the passaged primary HNE cell monolayers cultured for 7, 14 or 21 days. Data represent the mean ± SD ($n \geq 3$). $^{\#}P < 0.05$, compared to the control (without P-gp inhibitor).

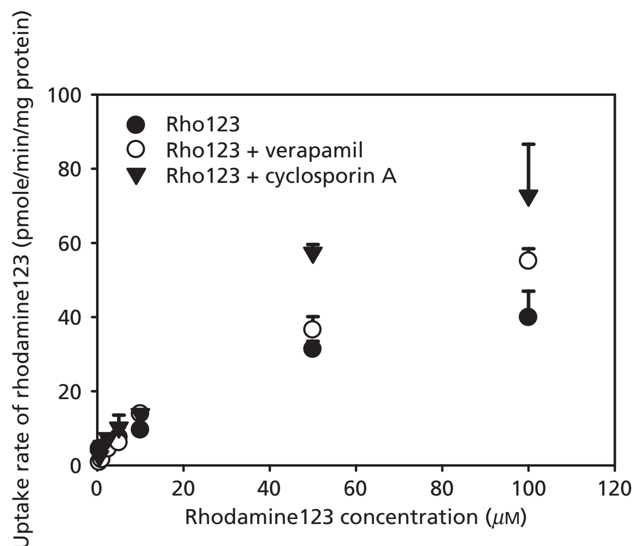

Figure 5 The effect of verapamil (500 μM) and cyclosporin A (10 μM) on the cellular uptake of various concentrations (0.5–100 μM) of rhodamine123 on the passaged primary HNE cell monolayers cultured for 10–12 days. Data represents the mean ± SD ($n \geq 3$).

evaluate the function of P-gp.[21,22] As shown in Figure 4, a slight increase in rhodamine123 uptake with P-gp inhibitor treatment, although not statistically significant, was observed in HNE cell monolayers cultured for 7 days. On day 14 of the ALI culture, when the HNE cell monolayers were pre-treated with verapamil or cyclosporin A, the average amount of rhodamine123 uptake increased compared to the control ($P < 0.05$). On day 21, the mean value of the rhodamine123 uptake amount was also increased ($P < 0.05$). The increasing pattern of the rhodamine 123 uptake with P-gp inhibitors compared to the control was related with the inhibition of efflux function. These results were also consistent with the increasing pattern of the MDR1 gene expression (Figure 1).

The dependency of rhodamine123 concentration on the cellular uptake in passaged primary HNE cell monolayers was also determined in the presence of verapamil or cyclosporin A. As shown in Figure 5, the kinetic profile showed that the uptake of rhodamine123 across the HNE cell monolayers is a saturable process. The V_{max} values calculated from the non-linear regression process are shown in Table 3. When rhodamine123 was applied alone, the V_{max} value was 54 ± 7 pmole/min/mg protein, while with verapamil and cyclosporin A treatment, values increased to 90 ± 8 pmole/min/mg protein and 113 ± 12 pmole/min/mg protein, respectively. It is of interest that the transport of rhodamine123 still shows a saturable process even though P-gp function was blocked by the presence of verapamil or cyclosporin A, which might indicate the involvement of other carrier-mediated processes of rhodamine123.

Mallants *et al*. have previously reported an absence of P-gp efflux system in their nasal cell culture system.[28] This may be due to the difference in types of membrane insert used for their transport study, as well as the variation in culture media and absence of ALI culture conditions. It is noteworthy that in our study the MDR1 gene was confirmed by RT-PCR and that its function was verified in a transport as well as a cellular uptake study. Moreover, the MDR1 gene or P-gp expression

Table 3 Kinetic parameters of rhodamine123 concentration-dependent cellular uptake study, with or without P-gp inhibitors

Group	V_{max} (pmole/min/mg protein)	K_m (μM)
Rhodamine123 alone	54 ± 7	36 ± 12
Rhodamine123 + verapamil	90 ± 8*	67 ± 13
Rhodamine123 + cyclosporin A	113 ± 12*	53 ± 12

Each datum represents the mean ± SD ($n \geq 3$). $^{*}P < 0.05$, compared to rhodamine123 alone.

have already been reported in human nasal respiratory mucosa, bronchus and various kinds of cell lines,[4,6,8,9] supporting the likelihood of the existence of the MDR1 gene in the primary HNE cell monolayers cultured by the ALI method.

Human nasal epithelium is composed of respiratory epithelium and olfactory epithelium. The nasal epithelium is used not just to deliver drugs to the systemic circulation, but also to directly deliver drugs via the olfactory epithelium to the brain, bypassing the blood–brain barrier. The existence of P-gp in the nose–brain barrier and its role in drug uptake into the brain have already been reported.[29–31]

Conclusions

The MDR1 gene expression in passage cultured primary HNE cell monolayers by the ALI method was identified by RT-PCR. Studies with rhodamine123 have shown that these passaged cell monolayers, cultured for 10–14 days, exhibited functional activity with P-gp substrates. Therefore, primary HNE monolayers cultured by the ALI method could serve as an efficient in-vitro model for P-gp-mediated drug transport and cellular uptake studies in nasal drug delivery.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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