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Evaluation of HO-1-u-1 cell line as an in vitro model for sublingual drug delivery involving passive diffusion—Initial validation studies

Yanfeng Wang [a], Zhong Zuo [a], Kenneth K.H. Lee [b], Moses S.S. Chow [a,*]

[a] School of Pharmacy and Drug Development Centre, Faculty of Medicine, The Chinese University of Hong Kong, Shatin, NT, Hong Kong SAR
[b] Department of Anatomy, Faculty of Medicine, The Chinese University of Hong Kong, Shatin, NT, Hong Kong SAR

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Abstract

The aim of this study was to provide preliminary validation of a new sublingual mucosal cell line (HO-1-u-1) for use as in vitro sublingual drug delivery screening of compounds involving passive diffusion. HO-1-u-1 cells were seeded on cell culture inserts. The ultrastructure and integrity of cell layers, inter-passage variation and directionality of drug transport, and apparent permeability coefficient (P_{app}) of eight β-blockers (representing compounds involving passive diffusion) were determined. HO-1-u-1 cells grown on inserts formed stratified and epithelial-like structure and maintained the typical histological features of normal human sublingual epithelium. The maximal integrity of the cell layer was reached in 23 days. No significant inter-passage variation was found at the passages ranging from 2 to 11 when measured by radiolabeled transcellular and paracellular markers (testosterone and mannitol, respectively). Bidirectional transport studies confirmed the passive diffusion as the mechanism of transport for these markers. The P_{app} of eight β-blockers across HO-1-u-1 cell culture ranged from 2.89 ± 0.17 to $6.37 \pm 0.37 \times 10^{-6}$ cm/s and correlated well to the P_{app} obtained from porcine sublingual mucosa ($r^2 = 0.647$ and 0.83 when excluding propranolol). The above results indicate that the HO-1-u-1 cells grown on inserts may offer as a potentially in vitro model for screening sublingual drug permeation involving passive diffusion.

Keywords: Sublingual; HO-1-u-1; Permeability; β-Blocker

1. Introduction

At present, peroral administration is the most common route of drug administration. However, there are several potential disadvantages associated with this route of administration. These include: (i) enzyme degradation in the gastrointestinal tract, (ii) first-pass metabolism through liver resulting in low bioavailability (Shojaei, 1998) and (iii) relatively slow onset of action.

Sublingual administration can offer an attractive alternative route of administration. The advantage of the sublingual drug delivery is that the drug can be directly absorbed into systemic circulation bypassing enzyme degradation in the gut and liver (Squier and Hall, 1985). In addition, the thin sublingual mucosa (about 190 μm compared to 500–800 μm of the buccal mucosa) and the abundance of blood supply at the sublingual region, allow excellent drug penetration (absorption) to achieve high plasma drug concentration with a rapid onset of action. A well-established example is nitroglycerin, which is used for the treatment of acute angina attacks (Bogaert, 1994).

Despite the above advantages of sublingual drug delivery, the number of drugs, which have been developed for sublingual administration is very limited. One reason is the lack of a simple and reliable model, which can provide rapid screening of lead compounds. The existing in vivo or in vitro models (animal or excised sublingual tissue) are inconvenient, time consuming and relatively expensive.

Recently, a human tumor cell line HO-1-u-1 (Ueda-1) was isolated from the squamous cell carcinoma of the floor of the mouth (sublingual mucosa) of a 72-year-old Japanese male. This cell line has a population doubling time of approximately 23 h and its karyotype manifests hyperduploidy. Phase contrast microscope reveals formation of epithelial-like cell layers when grown on cell culture flasks (Miyauchi et al., 1985). Myoken et al. (1987) also found the cell line to be well differentiated with filament and cytokeratins to indicate its epithelial cell origin. In view of the similar characteristics to human sublingual

* Corresponding author. Tel.: +852 26096860; fax: +852 26035295.
E-mail address: msschow@cuhk.edu.hk (M.S.S. Chow).

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Nomenclature

HBSS	Hank's balanced salt solution
log *P*	octanol-buffer partition coefficient
P_{app}	apparent permeability coefficients
TEER	transepithelial electrical resistance
TEM	transmission electron microscopy

epithelium, this cell line may provide a convenient alternative for screening of sublingual drug delivery due to its unlimited source (from the perpetual growth of the cell line). The aim of the present study is to investigate the suitability and performance of this cell line for in vitro sublingual drug delivery of compounds that undergo passive diffusion, a common mechanism for which many drugs are involved.

2. Materials and methods

To investigate the suitability of the HO-1-u-1 cell line for drug permeability studies, the cellular morphology was first identified under normal culture conditions to ensure that these cells resemble normal squamous epithelial cells of the oral cavity. Then the optimal cell culture conditions and consistency of performance were determined through monitoring of the integrity of cell layers at different passages and measuring the bi-directional transport of radiolabeled transcellular and paracellular markers. Finally, the permeabilities of the eight β-blockers (representing passive diffusion mechanism) via this cell line were determined and compared to that from porcine sublingual tissue studies. All studies were approved by Animal Ethics Committee of the Chinese University of Hong Kong.

2.1. Materials

Dulbecco's modified Eagle medium (with L-glutamine), Ham' F12 nutrient mixture (with L-glutamine), Hank's balanced salt solution (HBSS), trypsin-EDTA (0.25%, 1 mM EDTA), penicillin–streptomycin (100 IU/ml), gentamicin (10 mg/ml), fetal bovine serum and other cell culture mediums were purchased from Invitrogen Life Sciences (Hong Kong). BD-Falcon cell culture inserts (polyethylene terephthalate, 0.45 μm pore size) and BD-Falcon six-well culture plates were obtained from Becton Dickinson Labware (NJ, USA). Tissue culture flasks (75 cm^2, TC-treated) and other cell culture consumables were supplied by IWAKI (Tokyo, JP). Glutaraldehyde (25%, EM Grade), osmium tetroxide and uranyl acetate were purchased from Electron Microscopy Sciences (Washington, USA). Poly/Bed 812 was supplied by Polysciences Inc. (Warrington, PA, USA). [^{3}H]-testosterone (250 μCi) and [^{14}C]-mannitol (50 μCi) were ordered from Amersham Biosciences (Buckinghamshire, UK). OptiPhase HiSafe 3 scintillation cocktail were supplied by Perkin-Elmer Life Sciences (Turku, Finland). CellTiter96® AQ_{ueous} one solution cell proliferation assay (MTS/PES assay) was provided by Promega Co. (Madison, WI, USA). Acebutolol hydrochloride, alprenolol hydrochloride, atenolol, labetalol hydrochloride, (±)-metoprolol tartrate salt, nadolol, (±)-propranolol hydrochloride, timolol maleate salt, 1-octanol and phosphate buffered saline tablets were obtained from Sigma Chemical Co. (St. Louis, MO, USA).

2.2. Cell cultures and ultra-morphology

The HO-1-u-1 cell line was provided by Health Science Research Bank (Tokyo, Japan). The cell culture was performed according to the protocol previously described (Miyauchi et al., 1985). Briefly, the cells were cultured in a 75 cm^2 T-flask and incubated under 37 °C in 90% relative humidity atmosphere of 5% CO_2 and 95% air. The culture medium consisted of Dulbecco's modified Eagle's medium—F12 medium (1:1 mix) supplemented with 10% fetal bovine serum, 50 μg/ml gentamicin, 100 IU/ml penicillin and 100 μg/ml streptomycin. When 70% confluence was reached, the cells were subcultured by 0.25% trypsin-EDTA, followed by seeding in the BD-Falcon filter inserts. The cells in these inserts were cultured at 37 °C. The culture medium was changed three to four times a week.

The optimal initial seeding density were evaluated by daily visual examination under an inverted microscope for 30 days at densities of 7.5×10^4, 1.5×10^5 and 3.0×10^5 cells/well.

The ultra-morphology of the HO-1-u-1 cells from the filter inserts were examined by transmission electron microscopy (TEM) on the 23rd day. The cell culture inserts ($n = 3$) were fixed with 2% glutaraldehyde (pH 7.4) at 4 °C, followed by postfixing with 1% osmium tetroxide. Then the filter membrane was sequentially dehydrated in 70–100% ethanol solutions and 100% propylene oxide. The dehydrated samples were transferred into Poly/Bed 812 and polymerized in an oven at 60 °C for 48 h. The sections were produced by microtome at 80 nm and stained with 3% aqueous uranyl acetate, then by lead citrate. Each section was then examined with transmission electron microscopy.

2.3. Integrity, inter-passage variation, bi-directional transport, filter membrane binding and cell uptake

The integrity of the cell layers grown on the filter was monitored by measuring the transepithelial electrical resistance (TEER). All measurements were carried out from 8 to 29 days in culture with the resistance of cell free filter served as the background. The inter-passage variation of the HO-1-u-1 cell culture was then determined by measuring TEER and the apparent permeability coefficient of [^{14}C]-mannitol and [^{3}H]-testosterone across the cell culture.

The bi-directional transport of [^{14}C]-mannitol and [^{3}H]-testosterone was performed by analyzing the apparent permeability coefficient (P_{app}) from apical to basolateral chamber and from basolateral to apical chamber of filter inserts (BD-Falcon). After adding the loading solution with a concentration of 200 nCi/ml [^{14}C]-mannitol or 500 nCi/ml [^{3}H]-testosterone, a 200 μl sample was withdrawn from the receiver chamber at predetermined time intervals. The volume withdrawn was replaced with pre-warmed phosphate buffer immediately afterwards and the sample obtained was then mixed with a 2 ml scintillation cocktail. The radioactivity was determined by TRI-CARB

2900TR liquid scintillation analyzer (Perkin-Elmer, Wellesley, MA, USA). P_{app} of the markers was expressed as:

$$P_{app} = \frac{dQ/dt}{AC_0} \quad (1)$$

where dQ/dt (nCi/ml) is the rate at which the testing compound appeared in the receiver chamber, A (cm^2) the surface area of the cell layers and C_0 (μg/ml) is the initial concentration in the donor chamber.

At the end of the permeability study, cells grown on the filter were carefully rinsed and removed by a cell scraper. The filter inserts were then cut into small pieces and soaked in a 2 ml scintillation cocktail for determination of the radioactivity. The fraction of compound binding on filter (X_f) was calculated from the amount bound to filter divided by the initial amount in the loading solution.

At the end of sampling period, the percentage of recovery (X_{re}) was determined as the amount in the donor plus receiver chambers divided by the amount in the loading solution.

The fraction of cellular uptake of the test compound (X_c) was calculated as:

$$X_c = 1 - X_{re} - X_f \quad (2)$$

2.4. Octanolol-buffer partition coefficient and permeability of β-blockers

Prior to the permeability study, the toxic effect of the β-blockers on the cell culture was determined. The HO-1-u-1cells were firstly seeded in a 96-well culture plate (at a density of 1×10^4 cells/well) for 48 h, the culture medium was then replaced with 100 μl solution containing β-blockers (at a concentration of 1×10^{-4} M) and incubated for 5 h. Afterwards, the solutions were removed and the culture rinsed with HBSS. Then 20 μl MTS/PES reagent and 100 μl HBSS were added to each well followed by incubation for 4 h. The optical density (OD) at 490 nm was measured and the cellular viability, expressed by the "relative cellular activity" determined:

$$\text{relative cellular activity (\%)} = \frac{OD_{test} - OD_{blank}}{OD_{control} - OD_{blank}} \times 100\% \quad (3)$$

where OD_{test} is the optical density of wells with cells, test solution and MTS/PES reagent; $OD_{control}$ is the optical density of wells with cells, HBSS and MTS/PES reagent; OD_{blank} is the optical density of wells with HBSS and MTS/PES reagent (without cells).

The octanol-buffer partition coefficients of the eight β-blockers were determined according to a standard method (Shojaei et al., 1999). The concentrations of the β-blockers were analyzed by an HPLC method (see below). Each experiment was repeated four times. The partition coefficient was calculated according to Eq. (4):

$$\log P = \log \left[\frac{(C_0 - C_b)}{C_0} \frac{V_b}{V_{oct}} \right] \quad (4)$$

Table 1
HPLC analysis for the selected β-blockers

Compounds	UV detector wavelength (nm)	% Acetonitrile	Limit of detection (ng/ml)
Acebutolol (Ace)	260	20	17
Alprenolol (Alp)	271	35	206
Atenolol (Ate)	275	8	80
Labetalol (Lab)	210	28	57
Metoprolol (Met)	222	22	61
Nadolol (Nad)	210	16	83
Propranolol (Pro)	213	35	55
Timolol (Tim)	295	20	19

where C_0 is the initial concentration of the test substance in buffer, C_b the concentration in buffer after equilibration, and V_b and V_{oct} are the volume of buffer and octanol, respectively.

The concentration of each β-blocker was measured using a Waters HPLC system consisting of a 600 controller, a 717_{plus} auto-sampler, a 2487 dual λ absorbance detector and a Thermo Hypersil-Keystone Column (250 mm × 4.6 mm, 5 μm Hypersil BDS C_{18}). The mobile phase consisted of acetonitrile and 25 mM PBS (pH 4.0, containing 0.5% triethylamine). The UV wavelength and % acetonitrile for each β-blockers are shown in Table 1. The detection limits of these compounds ranged from 17 to 206 ng/ml (Table 1). The calibration curves for β-blockers showed excellent inter-day linearity ($r^2 > 0.99$) in the ranges commonly used in the permeability studies. The intra-day precision (R.S.D.) and accuracy (% difference) of all analytes were within 10%. These results indicated that the HPLC methods developed were sensitive and accurate enough for the detection of analytes from samples obtained in current permeability studies.

The permeability study of the β-blockers was carried out using concentration of 1×10^{-4} M (in HBSS at pH 7.4) in apical chamber of the insert. Initially, medium was removed and the insert rinsed twice by HBSS and equilibrated at 37 °C for 20 min. Then, HBSS was removed and the pre-warmed (37 °C) β-blocker solution was added to the donor chamber. Serial samples (200 μl each) were taken from the receiver chamber every 15 min for 2 h. Equal volume of HBSS was replaced after each sampling. The concentrations of the testing compounds were analyzed by the HPLC method as described below. All experiments were performed in triplicate and P_{app} of β-blockers calculated according to Eq. (1).

2.5. Permeability studies using porcine sublingual tissue

The permeability study using porcine sublingual mucosa were conducted according to the method described by Chen et al. (1999) and Chetty et al. (2001). Briefly, fresh sublingual tissues (floor of mouth) were excised from white pigs (male, 50–100 kg), which were procured from a local slaughterhouse. The tissues were stored in the 0.15 M isotonic phosphate buffer at 4 °C immediately upon collection. All tissues were used within 2 h of procurement. The epithelial layer was mechanically separated from the underlying connective tissue using

surgical scissors. The integrity of porcine sublingual mucosa was examined by measuring the permeability of four markers, namely [^{14}C]-mannitol, [^{3}H]-testosterone, [^{14}C]-PEG4000 and [^{3}H]-water using the method described below. The permeation study was conducted using a Side-Bi-Side diffusion chamber system (Perme Gear Co., PA, USA). The diffusion area was 0.196 cm^2 and the volume for each chamber was 4 ml. The temperature of the system was maintained at 37 °C by continuous circulation of heated water in water jackets surrounding the chambers. The solution in each chamber was stirred with magnetic bars. Each tissue was mounted between the donor and receiver chambers of the diffusion cells with the surface of mucosa facing the donor chamber. After equilibration with isotonic phosphate buffer in both chambers at 37 °C for 30 min, the receiver chamber and donor chamber were filled with 4 ml pre-warmed phosphate buffer and testing solution, respectively. Samples of 200 μl were withdrawn from the receiver chamber at pre-determined time intervals and concentration determined by the HPLC method as described above. Triplicate experiments were carried out for each testing compound and the P_{app} values were calculated according to Eq. (1).

2.6. Statistical analysis

All data were reported as mean ± S.D. Students *t*-test was used to analyze the data between two groups. ANOVA was applied to analyze the data among three or more groups. Linear regression was used to determine the correlation of the P_{app} across HO-1-u-1 cell culture model and sublingual tissue model. A $p < 0.05$ was considered significant for all tests.

3. Results

3.1. Ultra-morphology of HO-1-u-1 cells grown on filter inserts

After 23 days of cell growth on the cell culture insert, the HO-1-u-1 cells formed two to three layers. The cells from the apical site were flat and tightly connected, with short microvilli-like processes on the surface and desmosomes connected between cells. The cells from the basal site, however, were round and loose, with well-developed nucleus. No tight junction was found in the cell layers (Fig. 1). Meanwhile, desmosomes and filaments were extensively expressed between all cells, which resemble the characteristics of normal squamous epithelial cells in oral cavity (Fig. 2). The polarized structure of HO-1-u-1 cell layers is also similar to that from the normal sublingual mucosa (Wertz and Squier, 1991).

3.2. Integrity, inter-passage variation, bi-directional transport, filter membrane binding and cell uptake

When the TEER of confluent cell layers was determined from 8 to 30 days in culture (Fig. 3a), the results showed that the TEER increased continuously from 71.4 ± 9.8 Ω cm^2 to a maximum of 326.3 ± 41.3 Ω cm^2 (Day 17), then slowly decreased to 219.1 ± 28.8 Ω cm^2 over the remaining period.

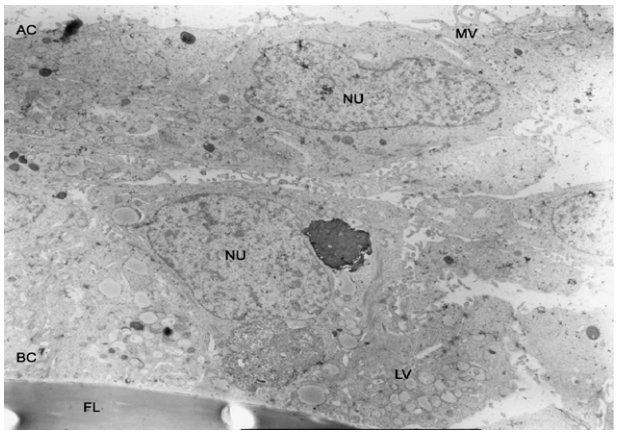

Fig. 1. Transmission electron micrograph of HO-1-u-1 cells (passage 6) on the 23rd day in culture. Cells grown on PET filter (FL) showed microvilli-like processes (MV) on the surface of apical cells (AC), and nucleus (NU) in both apical and basal cells (BC), lipid vesicles (LV) were abundant in BC.

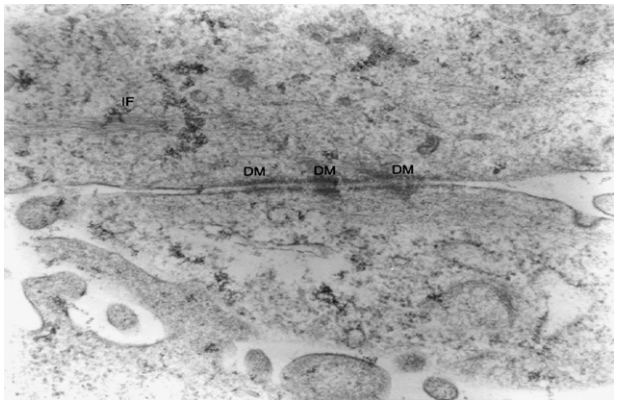

Fig. 2. Transmission electron micrograph of apical HO-1-u-1 cells on the 23rd day in culture. Note the intermediate filaments (IF) and desmosomes (DM) between cells under high resolution.

The paracellular and the transcellular transport of cell layers were evaluated by the hydrophilic marker [^{14}C]-mannitol and lipophilic marker [^{3}H]-testosterone, respectively (Fig. 3b). The P_{app} of [^{14}C]-mannitol decreased from 3.64 ± 0.28 × 10^{-6} (15 day) to 2.04 ± 0.17 × 10^{-6} cm/s (26 day), then slightly increased to 2.42 ± 0.29 × 10^{-6} cm/s (29 day). The P_{app} of [^{3}H]-testosterone, however, exhibited no significant change until the 29th day in culture (Fig. 4). The results showed that the maximal

Table 2
P_{app} values of [^{14}C]-mannitol in bi-directional flux (mean ± S.D., $n = 3$)

Markers	P_{app} (×10^{-6} cm/s)		*p*-Value[c]
	A–B[a]	B–A[b]	
[^{14}C]-mannitol	2.78 ± 0.17	2.81 ± 0.35	0.067
[^{3}H]-testosterone	4.44 ± 0.35	4.53 ± 0.40	0.690

[a] From apical chamber to basolateral chamber.
[b] From basolateral chamber to apical chamber.
[c] Difference between P_{app} were examined by unpaired Student's *t*-test and considered statistically difference if $p < 0.05$.

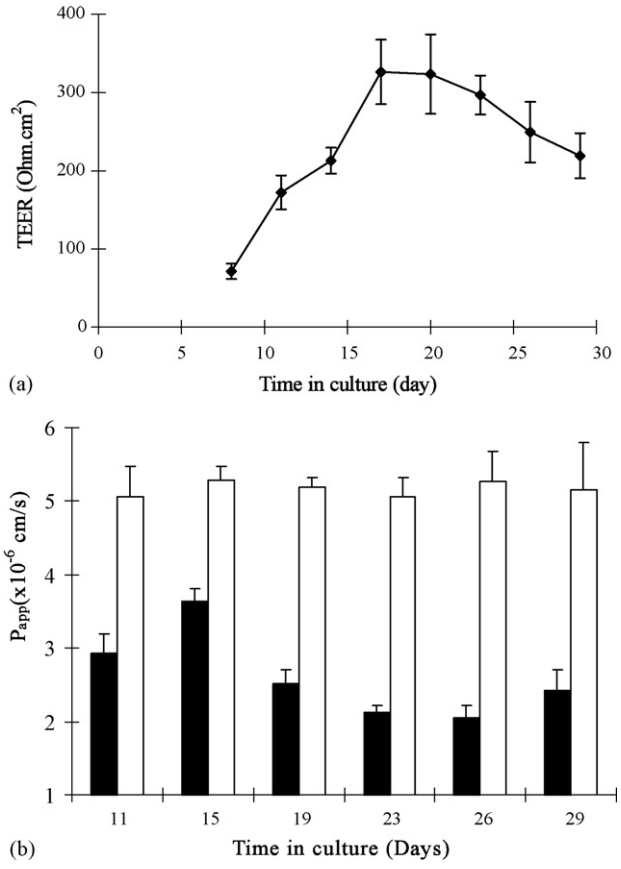

Fig. 3. TEER and P_{app} of [^{14}C]-mannitol (■) and [^{3}H]-testosterone (□) across HO-1-u-1 cell layers at different times in culture. All values are mean ± S.D., $n = 3$.

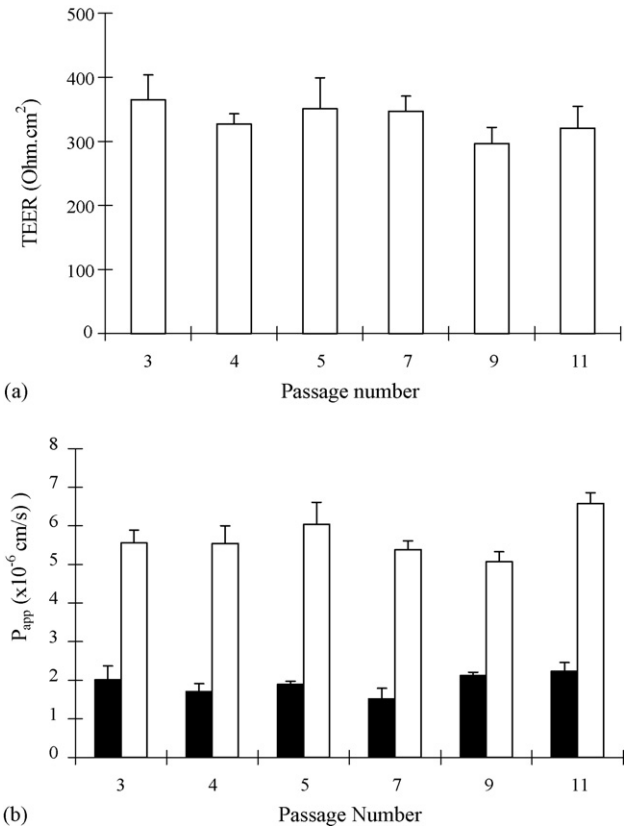

Fig. 4. TEER and P_{app} of [^{14}C]-mannitol (■) and [^{3}H]-testosterone (□) across HO-1-u-1 cell layers at different passage numbers. All values are mean ± S.D., $n = 3$.

integrity of cell layer was reached after 23–26 days in culture, whereas the thickness of cell layer did not continuously increase after reaching confluence (about 8–10 days in culture).

TEER and P_{app} observed at different passage numbers were not significantly different (Fig. 4), also the P_{app} of [^{14}C]-mannitol and [^{3}H]-testosterone from the bidirectional study showed no statistical significant difference between the two directions (Table 2).

The recoveries of compounds were found to be lipophilicity-dependant (Table 3). The lipophilic marker [^{3}H]-testosterone had much higher cell uptake (21.98%) than the hydrophilic marker [^{14}C]-mannitol (2.37%). In addition, the percentage of filter binding was also found to be lipophilicity-dependant. However, the filter-binding for both markers was very low (less than 1%).

Table 3
Recovery, filter binding and cell uptake of [^{14}C]-mannitol and [^{3}H]-testosterone ($n = 3$) by HO-1-u-1 cell layers

Markers	Recovery (%)	Filter binding (%)	Cell uptake (%)
[^{14}C]-mannitol	97.59 ± 0.49	0.03 ± 0.01	2.37 ± 0.49
[^{3}H]-testosterone	77.20 ± 5.10	0.60 ± 0.07	21.98 ± 5.17

3.3. Permeability of β-blockers across HO-1-u-1 cell line

3.3.1. Effect of β-blockers on the HO-1-u-1 cell line

The IC_{10} and IC_{50} values of the β-blockers are listed in Table 4. These results indicated that the loading concentration (1×10^{-4} M) used for the β-blockers in the permeability study was far below IC_{10} and thus should not significantly affect the integrity of HO-1-u-1 cells during the permeability studies.

3.3.2. Permeability of β-blockers and comparison to log P

The permeability values of the four markers, namely [^{14}C]-mannitol, [^{3}H]-testosterone, [^{14}C]-PEG4000 and [^{3}H]-water are presented in Table 5. As expected from their properties, [^{14}C]-

Table 4
IC_{10} and IC_{50} of selected β-blockers on HO-1-u-1 cells determined by MTS/PES assay ($n = 4$)

Compounds	IC_{10} (M)	IC_{50} (M)
Acebutolol	N.I	N.I
Alprenolol	1.9×10^{-3}	5.6×10^{-3}
Atenolol	1.8×10^{-3}	5.7×10^{-3}
Labetalol	1.8×10^{-3}	5.9×10^{-3}
Metoprolol	N.I	N.I
Propranolol	1.4×10^{-3}	5.6×10^{-3}
Nadolol	1.9×10^{-3}	5.5×10^{-3}
Timolol	3.7×10^{-3}	1.0×10^{-2}

N.I.: No inhibition at 1.0×10^{-2} M.

Table 5
Apparent permeability coefficients (P_{app}) of PEG4000, mannitol, testosterone and water across porcine sublingual mucosa (mean ± S.D., $n = 3$)

Compounds	P_{app} (10^{-7} cm/s)	Recovery (%)
[^{14}C]-PEG4000	1.28 ± 0.70	98.34 ± 0.73
[^{14}C]-mannitol	3.45 ± 1.87	92.96 ± 3.65
[^{3}H]-testosterone	40.33 ± 14.79	86.21 ± 7.07
[^{3}H]-water	100.3 ± 8.16	95.92 ± 1.92

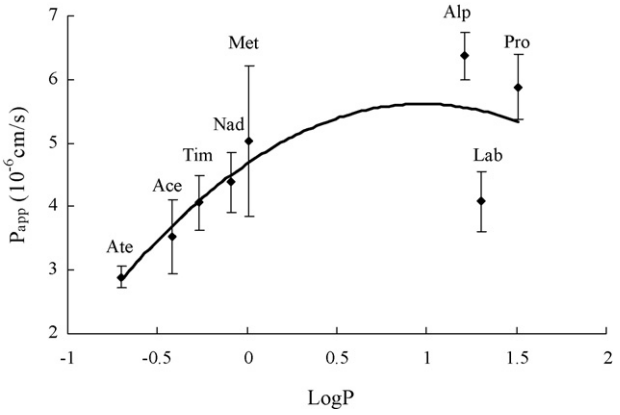

Fig. 5. Relationship between log P and P_{app} of β-blockers across HO-1-u-1 cell culture model. Each point represents the mean ± S.D., $n = 3$. Abbreviations of drug names are listed in Table 1.

PEG4000 has the lowest P_{app}, whereas [^{3}H]-water has the highest P_{app}. These results indicated that porcine sublingual mucosa was intact during the study period.

The P_{app} of the β-blockers across HO-1-u-1 cell layers ranged from $2.89 \pm 0.17 \times 10^{-6}$ (atenolol) to $6.37 \pm 0.37 \times 10^{-6}$ cm/s (alprenolol).

The P_{app} of these drugs across porcine sublingual mucosa ranged from $2.05 \pm 0.11 \times 10^{-6}$ to $49.65 \pm 13.56 \times 10^{-6}$ cm/s (Table 6). The recovery was greater than 90% for most β-blockers. Propranolol, the most lipophilic β-blocker, had the highest P_{app} among all the testing compounds.

Fig. 5 illustrates the relationship between log P and P_{app} of β-blockers across HO-1-u-1 cell culture model. A linear trend was observed for hydrophilic and moderate lipophilic compounds. For high lipophilic compounds, such as alprenolol, labetalol and propranolol, a flattened P_{app} trend was found. Of note, was the high cellular uptake and binding observed with lipophilic compounds, which could account for the flattened P_{app}.

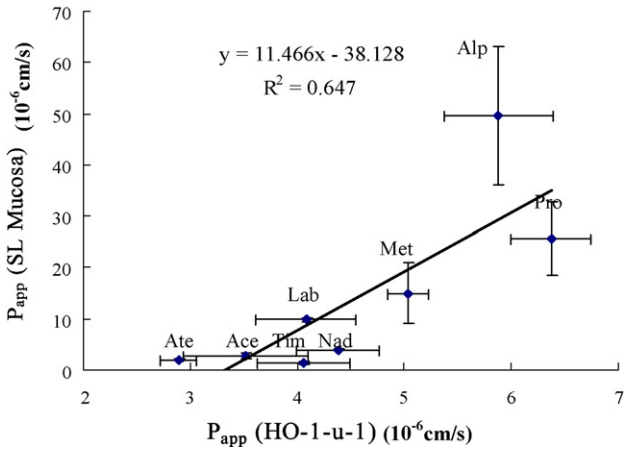

Fig. 6. Correlation between the P_{app} of β-blockers across HO-1-u-1 cell culture model and porcine sublingual mucosa. Each point represents the mean ± S.D., $n = 3$. Abbreviations of drug names are listed in Table 1.

The P_{app} values of β-blockers across the porcine sublingual mucosa correlated well to that from the HO-1-u-1 cell culture ($R^2 = 0.647$) (Fig. 6). The cell culture appeared to be more permeable than the sublingual tissue.

4. Discussion

The results of our study provided key initial validation of HO-1-u-1 cell line for sublingual drug delivery screening involving passive diffusion. Structurally, this cell line resembled histological features of the human sublingual mucosa with the presence of desmosomes, filaments and surface microvilli-like processes. From the TEM micrograph, tight junctions, which are frequently present in human colon carcinoma cell line Caco-2 (Anderberg and Artursson, 1993) and canine kidney cell line MDCK (Meza et al., 1982), were not found in HO-1-u-1 cell culture. The absence of tight junction is in agreement with the conclusion that the desmosomes are the predominant form of junction between squamous epithelial cells in oral mucosa (Swartzendruber et al., 1995). The absence of tight junction is also supported by the low TEER value for HO-1-u-1 cell layers ($326.3 \pm 41.3\ \Omega\,cm^2$) in

Table 6
P_{app} of β-blockers across HO-1-u-1 cell layers and porcine sublingual mucosa

Compounds	Mw	log P ($n = 4$)	HO-1-u-1		Sublingual mucosa	
			P_{app} (10^{-6} cm/s)	Recovery (%)	P_{app} (10^{-6} cm/s)	Recovery (%)
Acebutolol	336	−0.42	3.52 ± 0.58	92.6 ± 1.9	2.82 ± 0.48±	94.3 ± 3.6
Alprenolol	286	1.21	6.37 ± 0.37	83.7 ± 7.3	25.64 ± 7.09±	98.1 ± 1.3
Atenolol	266	−0.70	2.89 ± 0.17	101.1 ± 1.7	2.05 ± 0.11±	96.6 ± 2.0
Labetalol	328	1.24	4.08 ± 0.47	80.1 ± 3.12	9.81 ± 0.32±	95.2 ± 2.4
Metoprolol	267	0.01	5.03 ± 1.19	99.6 ± 4.7	14.91 ± 5.91±	98.7 ± 1.7
Nadolol	309	−0.09	4.38 ± 0.39	97.6 ± 3.4	3.97 ± 0.09±	94.5 ± 1.9
Propranolol	259	1.51	5.88 ± 0.51	74.1 ± 0.6	49.65 ± 13.56	98.3 ± 4.7
Timolol	316	−0.27	4.06 ± 0.43	85.1 ± 2.9	1.32 ± 0.22±	88.6 ± 5.0

comparison to that from Caco-2 monolayer (450–500 $\Omega\,cm^2$) and MDCK multi-layers (over 2000 $\Omega\,cm^2$) (Jacoben et al., 1995; Taub et al., 2002; Merrett et al., 1997).

In this study, the drug loss includes both cellular drug uptake (drug inside the cell) and non-specific drug binding to filter inserts. Since the non-specific drug binding are negligible for both hydrophilic and lipophilic markers (0.03% for [^{14}C]-mannitol and 0.6% for [^{3}H]-testosterone), the drug loss can be easily estimated by subtracting the total amount of drug in both donor and receiver chambers at the end of the experiment from the initial loading amount in the donor chamber.

The P_{app} values of β-blockers across the porcine sublingual mucosa correlated well to that from the HO-1-u-1 cell culture ($R^2 = 0.647$). This correlation ($r^2 = 0.831$) can be improved if P_{app} of propranolol is excluded (due to its high cellular uptake). The P_{app} values of the testing compounds across porcine sublingual mucosa are generally much lower than that obtained from HO-1-u-1 cell culture model. This can be explained by the loose intercellular structure of HO-1-u-1 cell layers in comparison to the sublingual tissue. These findings are also consistent with observations from the buccal carcinoma TR146 cell line when compared to the buccal tissue studies (Nielsen and Rassing, 2000).

The P_{app} range of β-blockers across cell culture model, however, was only about 3-fold as compared to over 20-fold in porcine sublingual mucosa model (Table 5). In comparing the P_{app} values of these two models, the major difference lies in the lipophilic compounds. The reason for a much lower P_{app} value for these compounds when using the HO-1-u-1 cell line is the high fraction of cellular uptake of the lipophilic compounds (estimated about 26% uptake for propranolol), which most likely resulted in a significantly decreased amount of molecules transported to the receiver chamber. This is also consistent with testosterone, which has a relatively low P_{app} value across HO-1-u-1 cell culture model (in comparison to other models) due to cellular uptake. The cellular uptake may be a unique property associated with the HO-1-u-1 model and may provide a distinctive feature among compounds undergoing passive diffusion.

Although passive diffusion is the main mechanism of transport across the oral mucosa (Beckett and Triggs, 1967; Kurosaki et al., 1986), active transporter P-glycoprotein (P-gp) was found in both normal oral mucosa (Jain et al., 1997) and oral squamous cell carcinomas (Lo Muzio et al., 2000). Since HO-1-u-1 cell line was derived from the oral squamous carcinoma, it is possible that P-gp and other active transporters may exist in these cells. Our current studies concentrated on paracellular and transcellular transport by [^{14}C]-mannitol and [^{3}H]-testosterone, which are not P-gp substrates. Interestingly, labetalol was reported to be a P-gp substrate (Mahar Doan et al., 2002), therefore, its unexpected low P_{app} observed in the present study may be caused by the P-gp efflux. When labetalol is deleted from Fig. 5, a much better correlation is observed between P_{app} of the β-blockers and log P observed. Further studies are needed to evaluate the presence of P-gp or other transporters in this cell culture model.

Sublingual drug delivery screening may potentially be carried out by using other human mucosa cell line, such as TR146 cell line. The TR146 cells grown on filters have been found to show similar characteristics as the HO-1-u-1 cell culture, such as polarized and stratified structure, frequent expression of desmosomes, intermediate filaments and microvilli-like processes. However, the TR146 cell line formed four to seven layers after 23 days in culture, in comparison to two to three layers for HO-1-u-1 cells. (This difference in the thickness appears to reflect the difference between human buccal mucosa and sublingual mucosa.) Moreover, after 23 days, granules are present in the TR146 cells grown on filter inserts, which are considered to be equivalent to the membrane coating granules in normal buccal epithelium (Jacoben et al., 1995). In the HO-1-u-1 cells, these granules are rarely found until 23 days, even though many lipids are present in the cytoplasm. These differences indicate that HO-1-u-1 cell line should be more suitable for sublingual drug delivery screening. Additionally, analysis also confirmed that the P_{app} of β-blockers from HO-1-u-1 cell line correlated well to P_{app} from porcine sublingual tissue, but not P_{app} from TR146 (unpublished data).

On the other hand, there are also several advantages for HO-1-u-1 cell culture model over excised porcine tissue: firstly, compared to the large variations observed with excised porcine tissue, the HO-1-u-1 cell culture model is highly stable and reproducible. Secondly, excluding the cell culture time, the total time for the transport study is only 3 h, as compared to over 10 h when using excised tissue (include preparation time for immediate use of the tissue). Thirdly, the cell culture model can be applied to carry out drug metabolism and drug efflux studies, which are less feasible when using excised tissue. On the other hand, the major limitation, similar to other cell culture model (i.e. Caco-2, TR146), is its relatively long culture time before reaching confluency and maximal integrity. Generally, the cell culture model for screening sublingual drug delivery is more efficient than the excised tissue, when sufficiently large number of compounds are tested.

5. Conclusion

HO-1-u-1 cells grown on cell culture inserts formed epithelial-like structure resembling the normal human sublingual epithelium. Good correlation can be obtained from the P_{app} of β-blockers (representing passive diffusion mechanism) across the HO-1-u-1 cell culture model and porcine sublingual mucosa. These results indicate that HO-1-u-1 cells grown on cell culture inserts can potentially be a suitable in vitro model for studying sublingual permeation of drugs by passive diffusion.

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