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International Journal of Pharmaceutics 194 (2000) 155–167

international journal of pharmaceutics

www.elsevier.com/locate/ijpharm

TR146 cells grown on filters as a model of human buccal epithelium: IV. Permeability of water, mannitol, testosterone and β-adrenoceptor antagonists. Comparison to human, monkey and porcine buccal mucosa

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Received 30 April 1999; received in revised form 12 September 1999; accepted 14 October 1999

Abstract

The objective of the present study was to evaluate the TR146 cell culture model as an in vitro model of human buccal epithelium. For this purpose, the permeability of water, mannitol and testosterone across the TR146 cell culture model was compared to the permeability across human, monkey and porcine buccal mucosa. Further, the permeability rates of ten β-adrenoceptor antagonists (acebutolol, alprenolol, atenolol, labetalol, metoprolol, oxprenolol, pindolol, propranolol, timolol and tertatolol) across the TR146 cell culture model and porcine buccal mucosa were related to their lipophilicity (log $D_{oct;\ 7.4}$) and capacity factor (k') and to their polar water accessible surface area (PWASA). For water, mannitol, testosterone and some of the β-adrenoceptor antagonists, the permeability enhancement across the TR146 cell culture model in the presence of sodium glycocholate (GC) was determined. The mannitol and testosterone permeability across the TR146 cell culture model could be related to the permeability across porcine and human buccal mucosa. The permeability of the β-adrenoceptor antagonists across the TR146 cell culture model varied between 2.2×10^{-6} cm/s (atenolol) and 165×10^{-6} cm/s (metoprolol). For propranolol the cellular permeability value (P_c) was lower than expected, probably due to accumulation in the TR146 cell layers. Limited correlation of permeability with k' was observed both for the TR146 cell culture model and the porcine buccal mucosa, although the porcine permeability values were ≈ 100 times less than the values determined with the TR146 cell culture model. The permeability values were also found to decrease with increasing PWASA. The PWASA value seemed to be more predictable for permeability than k'. The presence of 12.5 mM GC increased the permeability only for the hydrophilic atenolol, which may help explain the mechanism for GC-induced enhancement. The present results indicate that the TR146 cell culture model can be used as an in vitro model for permeability studies and mechanistic studies of human buccal drug delivery of drugs with different lipophilicity.

Keywords: TR146 cell culture model; Buccal permeability; Human; Monkey; Porcine; Lipophilicity; Permeability enhancer

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0378-5173/00/$ - see front matter
PII: S0378-5173(99)00368-3

1. Introduction

Buccal drug delivery is an attractive alternative to oral delivery, as presystemic loss and degradation promoted by the acidic and enzymatic environment in the stomach and intestine are avoided. Examples of drugs that undergo extensive metabolism after oral administration are propranolol (Borgström et al., 1981) and other β-adrenoceptor antagonists (Wood, 1983). Apart from increased bioavailability, the significant inter and intra subject variability in the metabolism of e.g. propranolol (Buch and Barr, 1998) would probably be less, and improved therapy might be gained by use of a buccal drug delivery system. Formulations of adhesive propranolol buccal drug delivery systems have been tested in vitro (Chen and Hwang, 1992), and an adhesive tablet containing propranolol was investigated in a human in vivo study (Taylan et al., 1996).

By the buccal absorption test (Beckett and Triggs, 1967), β-adrenoceptor antagonists have been shown to disappear from the human oral cavity (Schürmann and Turner, 1978; Randhawa and Turner, 1988). The disappearance of propranolol was shown to increase with increasing pH value of the buffer (Hitoglou-Makedou et al., 1989) comparable to the results of studies performed with hamster cheek pouch (Kurosaki et al., 1988). However, accumulation in the mucosa was indicated as up to $\approx 60\%$ of propranolol was recovered from rinsing buffers, within 30 min after removal of the test solution (Henry et al., 1980). This may be one of the reasons that human in vivo testing of a buccal tablet with propranolol only resulted in $\approx 42\%$ bioavailability relative to the oral bioavailability of a conventional tablet (Taylan et al., 1996). However, the buccal experiments were terminated after 4 h, due to serious irritation. Systemic absorption from the buccal tablets was slow, and t_{max} had not been reached at 4 h. In contrast, systemic delivery of propranolol from hamster cheek pouch showed a bioavailability of $\approx 90\%$ (Kurosaki et al., 1988). The difference may be explained by the fact that Kurosaki et al. (1988) determined the amount of propranolol remaining at the site of administration, together with the amount that had been swallowed, instead of collecting blood samples. Also, species differences may be a reason for the difference observed.

β-adrenoceptor antagonists span a large range in lipophilicity and have been used as test substances in studies of the influence of lipophilicity on the permeability across porcine buccal mucosa (Le Brun et al., 1989; Bland et al., 1991; de Vries et al., 1991) and across a primary cell culture of hamster buccal epithelium (Tavakoli-Saberi and Audus, 1989). The permeability of propranolol across porcine buccal mucosa in vitro was successfully enhanced by the use of oleic acid (Manganaro and Wertz, 1996), and the buccal permeability of some of the less permeable hydrophilic β-adrenoceptor antagonists may also be increased by the use of an absorption enhancer. The apparent distribution coefficient ($\log D$) between a lipophilic phase and an aqueous phase has been shown to correlate with the permeability across the Caco-2 cell culture model, i.e. to qualitatively estimate the passive permeability of drugs with different lipophilicity (Camenisch et al., 1998). Log D, the logarithm of the apparent distribution coefficient, is often determined experimentally using octanol and an aqueous buffer of pH 7.4 ($\log D_{oct;\ 7.4}$) (Schürmann and Turner, 1978; Tavakoli-Saberi and Audus, 1989; Camenisch et al., 1998). A number of procedures for predicting $\log D$ (at a specified pH) and $\log P$, (the logarithm of the partitioning coefficient) have also been used and compared (Moriguchi et al., 1994). However, it has been shown that the permeability correlates inversely with the polar van der Waals surface area (PvdW) (Palm et al., 1996) and inversely with the polar water accessible surface area (PWASA) (Palm et al., 1996; Krarup et al., 1998) of the drugs, thus allowing the permeability to be predicted from molecular surface properties.

In general, the cell culture approach provides some advantages compared to other in vitro models (Audus, 1996) and the TR146 cell culture model has previously been proposed as a model of the human buccal epithelium (Jacobsen et al., 1995). The cells originate from a human buccal metastasis, (Rupniak et al., 1985) and have been shown to grow on permeable inserts forming an

epithelium resembling the stratified buccal epithelium (Jacobsen et al., 1995). Differentiation patterns indicate that layers of human non-keratinized epithelium are formed (Jacobsen et al., 1999). Recently, the permeability rates and permeability pathways of hydrophilic test substances (fluorescein-labelled dextrans) across the TR146 cell culture model have been investigated. As in the studies with the porcine buccal mucosa, the permeability rates decreased linearly with increasing molecular weight (Nielsen et al., 1999). Likewise, the permeability enhancing effect of the bile salt, sodium glycocholate (GC) has been found comparable in the TR146 cell culture model and in the porcine in vitro model (Nielsen and Rassing, 1999). Studies with test substances with different characteristics such as lipophilicity should be performed to substantiate the use of the TR146 cell culture model as an in vitro model of the buccal epithelium. The permeability of the TR146 cell culture model should preferably be compared to the permeability of human buccal mucosa and not just to the permeability of animal buccal mucosa, believed to resemble the human buccal mucosa.

The primary objective of the present study was to compare the permeability of the TR146 cell culture model and buccal mucosa from human, monkey and pig, to water, mannitol and testosterone. Secondly, the permeability of β-adrenoceptor antagonists across the TR146 cell culture model and porcine buccal mucosa was compared. The n-octanol/buffer distribution coefficient ($\log D$) and the capacity factor (k') of the test substances were measured, the PWASA was calculated and these values were related to the permeability. Finally, the enhancement effect of GC on the permeability across the TR146 cell culture model was related to the lipophilicity of the β-adrenoceptor antagonists.

2. Materials and methods

2.1. Materials

Tertatolol hydrochloride was a generous gift from Institut de Recherches Internationales Servier (Courbevoie Cedex, France). Acebutolol hydrochloride, alprenolol hydrochloride, atenolol, labetalol hydrochloride, (±)-metoprolol tatrate, oxprenolol hydrochloride, pindolol, DL-propranolol hydrochloride, timolol maleate, sodium glycocholate (GC) and [^{3}H]-water were purchased from Sigma Chemical Co. (St Louis, MO). [1-^{14}C]-mannitol, [1,2,6,7-^{3}H(N)]- testosterone and L-[4-^{3}H]-propranolol were from DuPont NEN® (Boston, MA). Glucose-Ringer (GR) was prepared in Milli-Q water with chemicals from Merck (Darmstadt, Germany). Hanks Balanced Salt Solution (HBSS), Dulbecco's modified Eagels medium (DMEM), penicillin and streptomycin were purchased from Gibco BRL (Paisley, UK). Foetal calf serum (FCS) was from Harlan Sera-Lab (Belton, UK). Acetonitrile (AcCN) (Sigma-Aldrich, Dorset, UK), o-phosphoric acid (Struers Kebolab A/S, Albertslund, Denmark) and triethylamine (TEA) (Merck, Darmstadt, Germany) were used for the chromatographic analysis. 1-Octanol was purchased from Fluka Chemie AG (Neu-Ulm, Switzerland) and Ultima Gold™ MV scintillation cocktail from Packard Instrument BV (Groningen, the Netherlands). All chemicals were of analytical grade and used without further purification.

2.2. Methods

2.2.1. Cell culture

The TR146 cells were kindly provided by Imperial Cancer Research Technology (London, UK), and cultured as described previously (Jacobsen et al., 1995; Nielsen and Rassing, 1999). The cells were cultured on permeable 4.2 cm^2 Falcon® terephthalate inserts with a pore size of 0.4 μm for 28–30 days. The eight passages used were tested for mycoplasma by the PCR technique (Statens Serumlaboratorium, Copenhagen, Denmark), and found free of mycoplasma.

2.2.2. Human, monkey and porcine buccal mucosa

Biopsies of buccal mucosa (≈ 1 cm^2) were removed from healthy human volunteers (non-smokers) under local lidocaine-noradrenaline anaesthesia by otolaryngological surgeons (approved by the local ethic committee, project no.

(KF) 01-328/96, Denmark). Biopsies of buccal mucosa were obtained from cynomolgus monkeys (*Macaca fascicularis*) (Danish National Experimental Animal Inspectorate, license 1992-101-414) in anaesthesia with tiletamine/zolazepam and isoflurane. In the monkeys, the site of the biopsy was infiltrated submucosally with lidocaine-adrenaline. Buccal mucosa from pigs was isolated immediately after sacrificing the animal. All of the buccal tissue samples were kept in ice cold GR of pH 7.4 and used within 2 h. Isolation of the tissue was done mechanically by scissors and a tissue slicer (Thomas Scientific®, Swedesboro, NJ). This technique provided a tissue sample of epithelium, basal lamina and a minimum of submucosa determined by visual inspection.

2.2.3. Analytical procedures

Samples (20–100 μl) of radioactive labelled test substance were mixed with 2 ml scintillation cocktail and quantified on a Tri-Carb® liquid scintillation analyser (Packard Instrument, Meriden, CT). Quantitative reversed-phase HPLC (RP-HPLC) of the β-adrenoceptor antagonists was performed using a Merck Hitachi system consisting of a L-7100 pump, a 655A variable wavelength UV monitor and a 655A-40 autosampler. The D-7000 HPLC software system was used for data collection. A Waters Spherisorb S5ODS2 column (250 × 4.6 mm; 5 μm) (HiChrom Ltd., Berkshire, UK) was used. The flow rate was 1.0 ml/min and the injected volume was 20 μl. The mobile phase consisted of AcCN in 0.02 mM phosphate buffer and 5×10^{-3} M TEA in Milli-Q water adjusted to pH 4. For the limit of detection, a signal-to-noise ratio of 4 was considered acceptable ($n = 7$). The repeatability of the assays was determined by injection of 10^{-5} M β-adrenoceptor antagonist ($n = 7$). In Table 1, the analytical parameters for analysis of the test substances are presented.

2.2.4. Lipophilicity of β-adrenoceptor antagonists

For the β-adrenoceptor antagonists, the apparent distribution coefficient (log $D_{oct;\ 7.4}$) between octanol and either HBSS or GR (pH 7.4) was determined at room temperature (20–25°C) ($n = 4$). The octanol and the buffers were mutually saturated by shaking for 24 h. After separation of the phases, the test substance was dissolved in the aqueous buffer phase and continuously mixed with the organic octanol phase (40 rpm) using a rotamate. After 24 h the phases were left to separate, and the concentration of β-adrenoceptor antagonist in the buffer was determined by RP-HPLC. The added amount of test substance was chosen so the concentration in the buffer was between 10^{-6} and 10^{-5} M, after distribution between the phases. The volume of each phase and the pH value of the buffer phase were not changed during the experiment. Analysis of samples taken after 48 h showed the same distribution between the phases, as after 24 h. The relative lipophilicity of the β-adrenoceptor antagonists was also evaluated from the capacity factors, k', determined by RP-HPLC ($n = 4$). The mobile phase consisted of 40% (v/v) AcCN, the flow rate was 0.5 ml/min, and the wavelength was set at 210 nm, t_M was 1.99 min.

2.2.5. Boltzmann-averaged PWASA of β-adrenoceptor antagonists

The Boltzmann-averaged PWASA was based on 1000 different conformations and calculated by a recently described method (Krarup et al., 1998).

2.2.6. MTS/PMS assay

The sensitivity of the TR146 cells was measured according to the MTS/PMS assay optimized for the TR146 cell line (Jacobsen et al., 1996) and used previously (Nielsen and Rassing, 1999; Nielsen et al., 1999). The β-adrenoceptor antagonists were tested in eight concentrations between 1 μM and 0.1 M ($n = 8$).

2.2.7. Permeability studies with the TR146 cell culture model

The TR146 cell culture permeability experiments were performed at 37°C and a stirring rate of 150 rpm using a temperated horizontal shaker. The donor solution consisting of test substance in HBSS was applied to the apical side of the cell layers, and immediately hereafter samples of 100 μl were taken from both the receptor solution (2.5 ml HBSS) and the donor solution (2.5 ml). Receptor samples were withdrawn and replaced with the

Table 1
Identification (ID codes) and RP-HPLC data for β-adrenoceptor antagonists

β-adrenoceptor antagonist	ID code	Wave-length (nm)	Acetonitrile in mobile phase (% v/v)	Retention (min)	Limit of detection (M)	Repeatability ($n = 7$) (%)
Acebutolol	Ace	234	40	4.2	1×10^{-7}	±1.8
Alprenolol	Alp	214	60	4.9	2.5×10^{-6}	±1.8
Atenolol	Ate	224	15	4.8	2.5×10^{-6}	±2.1
Labetalol	Lab	210	52	4.4	1×10^{-7}	±1.8
Metoprolol	Met	222	40	4.8	2.5×10^{-7}	±0.8
Oxprenolol	Oxp	222	52	5.3	5×10^{-7}	±0.1
Pindolol	Pin	216	40	4.5	5×10^{-7}	±1.2
Propranolol	Pro	216	60	4.8	2.5×10^{-7}	±2.7
Timolol	Tim	294	40	4.2	1×10^{-6}	±1.9
Tertatolol	Ter	254	60	5.0	2.5×10^{-6}	±1.0

used buffer every 15 min for 1 h, followed by sampling every 30 min for another 3 h. In the permeability experiments, using inserts without TR146 cells, sampling was done every 10 min for 1 h. At the beginning and the end of the experiments, donor solution samples were collected. In the studies with radioactive labelled substance, the amount of radioactivity in the cells at the end of the study was determined by liquid scintigraphy, to calculate the total recovery of test substance. The cell accumulation of the non-radioactive drugs was estimated by mass-balance from the concentration in the donor and the receptor phases at the end of the experiment. The concentration of test substance was 10^{-5} M propranolol, 10^{-4} M of the other β-adrenoceptor antagonists, 2 μCi/ml ^{3}H-water or ^{14}C-mannitol, and 10 μCi/ml ^{3}H-propranolol or ^{3}H-testosterone. In experiments with enhancer, the concentration of GC in the donor solution was 12.5 mM. Before and after each permeability experiment ($n = 3$) the cell layers were examined microscopically and the transepithelial electrical resistance (TEER) values were measured using an Endohm™ culture cup connected to an EVOM voltohmmeter (World Precision Instruments Ltd., Herts, UK). The initial TEER values were $247 \pm 70\ \Omega \times cm^2$ ($n = 42$).

2.2.8. Permeability studies with human, monkey and porcine buccal mucosa

The permeability studies with buccal mucosa were performed in Plexiglas Ussing chambers. Each half-chamber contained 1 ml medium temperated to 36–37°C by a water-jacket. A gas lift of 95% O_2/5% CO_2 was used to circulate medium and maintain pH at 7.4. The exposed surface area of epithelium was 0.5 cm^2, but in the case of small tissue samples from human and monkey the area was reduced to 0.13 cm^2 by use of custom made Plexiglas inserts. With regard to the measured electrical resistance (R) and the permeability of ^{14}C-mannitol and ^{3}H-testosterone, there was no statistical difference between use of large versus small tissue samples (data not shown). The buccal mucosa from either human ($n \geq 4$), monkey ($n \geq 2$) or pig ($n \geq 4$) equilibrated for 1 h in 37°C GR prior to application of the donor solution to the mucosal side of the buccal mucosa. Samples from the serosal side were withdrawn as described for the permeability studies with the TR146 cell culture model. The concentration of test substance was 0.01 M for alprenolol, labetalol, propranolol, timolol and tertatolol, and 0.1 M for the other β-adrenoceptor antagonists, 20 μCi/ml for ^{3}H-water and ^{14}C-mannitol, and 100 μCi/ml for ^{3}H-propranolol and ^{3}H-testosterone. The thickness of the tissue samples was estimated before and after each experiment with a modified displacement transducer (Penny and Giles Position Sensors Ltd., Dorset, UK) and inspected visually. The thickness of the buccal mucosa from human, monkey and pig was 730 ± 134 μm ($n = 8$), 489 ± 112 μm ($n = 12$) and 666 ± 98 μm ($n = 64$), respectively. Likewise, R was measured throughout the study. The initial R values were $122 \pm 33\ \Omega \times cm^2$ ($n = 4$), $420 \pm 152\ \Omega \times cm^2$ ($n = 8$), and $843 \pm 278\ \Omega \times cm^2$ ($n = 80$), respectively.

2.2.9. Data analysis

The apparent distribution coefficient, D, was determined according to Eq. (1):

$$D = (C_i - C_w)/C_w \times (V_w/V_o) \quad (1)$$

where C_i is the initial concentration of test substance in the buffer, C_w is the concentration in the buffer after the experiment, V_w the volume of the buffer phase and V_o the volume of the octanol phase.

The capacity factor, k', was determined according to Eq. (2):

$$k' = (t_R - t_M)/t_M \quad (2)$$

where t_R is retention of the test substance and t_M the retention of the solvent, using one selected RP-HPLC procedure for all ten β-adrenoceptive antagonists.

The apparent permeability coefficient (P_{app}) for permeability of the test substances across the in vitro models is calculated according to Eq. (3):

$$P_{app} = dQ/dt \times 1/(A \times C_0) \quad (3)$$

where dQ/dt (mol/s) is the steady state rate of permeability, A (cm^2) the diffusion area and C_0 (mol/l) the initial donor concentration.

The cellular permeability (P_c) is determined from Eq. (4) (Karlsson and Artursson, 1991):

$$1/P_{app} = 1/P_c + 1/P_{UWL} + 1/P_{filter} \quad (4)$$

where P_{UWL} is the permeability coefficient across the unstirred water layer and P_{filter} the permeability coefficient across the filter insert. In the present study, $1/P_{UWL} + 1/P_{filter} = 1/P_{ins}$ is measured by performing permeability experiments using inserts without TR146 cells, as these values are assumed to be the same as in the experiments with TR146 cells grown on the filters.

The enhancement ratio (ER) is estimated according to Eq. (5) (Nielsen and Rassing, 1999):

$$ER = P_c(\text{enhancer})/P_c \text{ (control)} \quad (5)$$

where P_c (enhancer) represents the permeability in the presence of GC and P_c (control) the permeability in the corresponding experiment without enhancer.

Data are presented as mean ± S.D. (n), where n is the number of replicates. The correlation of linear regression is given as R^2. For significance testing, the Student's t-test is used at 95% level.

3. Results

3.1. Permeability studies with the TR146 cell culture model and human, monkey and porcine buccal mucosa

Table 2 contains the values for the permeability of water, mannitol and testosterone across the TR146 cell culture model and across buccal mucosa from human, monkey and pig. The TR146 cell culture model is approximately ten times more permeable to mannitol and testosterone than the human buccal mucosa. With regard to mannitol and testosterone permeability, monkey buccal mucosa does not seem to be a good model for human buccal mucosa for studies of permeability. The porcine buccal mucosa seems to be the least permeable of the four buccal in vitro models, but the P_{app} values of mannitol and testosterone are not statistically different from the P_{app} values obtained with human buccal mucosa. The amount of radioactive test substance that accumulated in the TR146 cells during the 4 h permeability experiment were $< 1\%$ for water and mannitol and $< 5\%$ for testosterone. In the buccal tissue from human, monkey or pig the accumulation was $< 5\%$ for water and mannitol and $< 15\%$ for testosterone.

3.2. Permeability studies with the TR146 cell culture model and porcine buccal mucosa using test substances of different lipophilicity

The MTS/PMS assay performed with TR146 cells in the exponential growth phase did not reveal TR146 cell sensitivity towards β-adrenoceptor antagonist concentrations below 10^{-3} M except for propranolol, for which the concentration was 5×10^{-4} M (data not shown). The concentration used in the permeability studies was 10^{-5} M for propranolol and 10^{-4} M for the other test substances and was considered to be completely harmless to the TR146 cells grown on filters. In support of this, the TEER values were not different from the initial TEER values after the experiments without GC.

Table 3 shows that both P_{app} and P_c increase with increasing lipophilicity of the β-adrenoceptor

Table 2
The apparent permeability coefficients (P_{app}) for the permeability of water, mannitol and testosterone across the TR146 cell culture model and across buccal mucosa from human, monkey and pig[a]

Permeability of test substances across in vitro models, P_{app} ($\times 10^6$) (cm/s)				
Test substance	TR146	Human	Monkey	Porcine
^{3}H-Water	52 ± 2.1 (n = 3)	n.d.	24 ± 7.8 (n = 4)	8.2 ± 0.9 (n = 4)
^{14}C-Mannitol	2.2 ± 0.3 (n = 3)	0.24 ± 0.2 (n = 7)	0.31 ± 0.1 (n = 2)	0.20 ± 0.1 (n = 20)
^{3}H-Testosterone	20 ± 0.6 (n = 3)	1.8 ± 0.9 (n = 4)	19 ± 3.1 (n = 4)	1.1 ± 0.9 (n = 16)

[a] Mean ± S.D., n.d. is not determined.

Table 3
Distribution coefficients (log $D_{oct;\ 7.4}$), capacity factors (k') and permeability parameters for ten β-adrenoceptor antagonists[a]

Test substance	Log D	k'	P_{ins} ($\times 10^6$) (cm/s)	P_{app} ($\times 10^6$) (cm/s)	P_c ($\times 10^6$) (cm/s)
Atenolol	−0.80	2.65	34 ± 3.1	2.1 ± 0.20	2.2 ± 0.22
Acebutolol	−0.23	4.31	35 ± 1.2	3.5 ± 0.30	3.9 ± 0.37
Pindolol	n.d.	4.48	33 ± 7.5	31 ± 1.5	n.c.
Timolol	n.d.	4.49	39 ± 3.1	24 ± 1.0	62 ± 6.6
Metoprolol	−0.07	5.12	43 ± 4.4	34 ± 2.5	165 ± 55
Labetalol	0.52	7.2	26 ± 9.3	7.7 ± 1.1	11 ± 2.2
Oxprenolol	0.88	7.52	38 ± 3.5	40 ± 5.5	n.c.
Propranolol	1.28	10.7	24 ± 1.2	4.2 ± 0.06	5.0 ± 0.09
Alprenolol	1.18	11.51	31 ± 2.3	34 ± 1.7	n.c.
Tertatolol	n.d.	11.83	26 ± 5.0	27 ± 2.0	n.c.

[a] The permeability coefficients for permeability across the insert (P_{ins}), across the TR146 cell culture model (P_{app}) and across the cell layer (P_c) are presented. Mean ± S.D., values are expressed as 10^6 cm/s, n.d. is not determined, n.c. is not calculated as P_{ins} and P_{app} are not statistically different from each other.

antagonist, whereas P_{ins} seems to be relatively independent of the drug substance. The P_{app} value and the P_c value differ for the substances that are less hydrophilic than atenolol and acebutolol, and P_c should be considered the true permeability across the epithelial barrier. It is evident that for some of the more lipophilic drugs (alprenolol, oxprenolol, pindolol and tertatolol), the insert and the aqueous water layer are just as great permeability barrier as the TR146 cell layers, and the P_c value could not reasonably be calculated. The P_c value for propranolol is not as high as would have been predicted from the lipophilicity, which is probably due to accumulation in the cells. Studies with both radioactive labelled propranolol and unlabelled propranolol showed that ≈60% of the drug accumulated in the cells.

The correlation between the estimated log D and log k' was linear ($R^2 = 0.93$, $P < 0.01$), and the log k' value was used to estimate the relative lipophilicity of the test substances.

Fig. 1 shows the log P_c for permeability across the TR146 cell culture model and the log P_{app} for permeability across the porcine buccal mucosa as a function of log capacity factor. With both models, a sigmoid curve is obtained for k' values lower than 7. At higher k' values than 7, the permeability rates across porcine buccal mucosa decrease for the β-adrenoceptor antagonists, illustrating a more bell-shaped curve following the initial sigmoid curve. This decrease in permeability rate, is probably related to the significant accumulation of tertatolol, alprenolol and propranolol in the porcine buccal mucosa, (≈20%) as well as in the TR146 cells (≈5, 5 and 60%, respectively). Labetalol was also observed to greatly accumulate in the porcine buccal mucosa tissue (≈50%).

Fig. 2 illustrates the permeability rates related to the PWASA. With increasing PWASA values from ≈60 Å^2, a decreased permeability is observed. The curve decreases in a sigmoid manner. However, for PWASA values below 60 Å^2, i.e. for

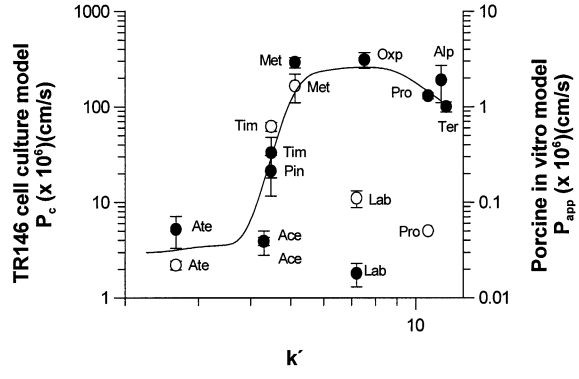

Fig. 1. Relationship between the cellular (log P_c) and apparent (log P_{app}) permeability coefficients with the capacity factor (log k') for the permeability of β-adrenoceptor antagonists across the TR146 cell culture model (○) and across the porcine buccal mucosa (●), respectively. The ID codes of the β-adrenoceptor antagonists are given in Table 1. Mean ± S.D. ($n \geq 3$).

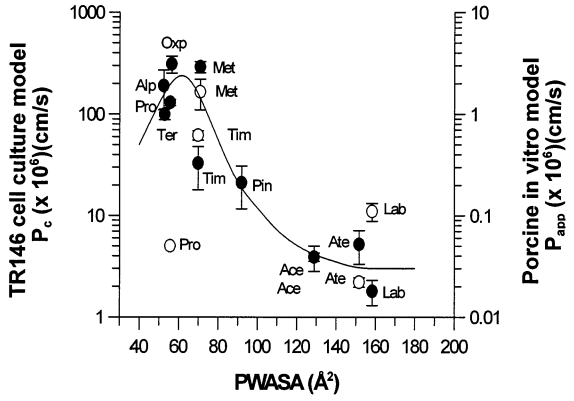

Fig. 2. Relationship between the cellular (log P_c) and apparent (log P_{app}) permeability coefficients with the average polar water accessible surface area (PWASA) for permeability of β-adrenoceptor antagonists across the TR146 cell culture model (○) and across the porcine buccal mucosa (●), respectively. The ID codes of the tested β-adrenoceptor antagonists are given in Table 1. Mean ± S.D. ($n \geq 3$).

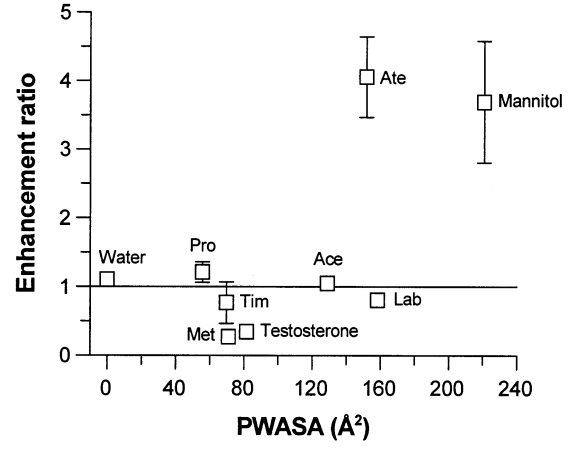

Fig. 3. Relationship of the enhancement ratio (ER) of water, mannitol, testosterone and six β-adrenoceptor antagonists permeation with the average polar water accessible surface area (PWASA) for the permeability across the TR146 cell culture model treated with 12.5 mM sodium glycocholate relative to untreated cells. The ID codes of the tested β-adrenoceptor antagonists are given in Table 1. Mean ± S.D. ($n = 3$).

tertatolol, alprenolol, and propranolol, a slight decrease in the permeability values is observed, according to increased accumulation in the tissue, as mentioned above. Interestingly, the values for labetalol fit the curve in Fig. 2 better than in Fig. 1.

3.3. The permeability enhancing effect of sodium glycocholate

In Fig. 3, the ER of the permeability across the TR146 cell culture model in the presence of GC has been depicted as a function of PWASA. Of the β-adrenoceptor antagonists, only P_c for the hydrophilic atenolol was significantly increased by the use of GC (ER 4.1 ± 0.59). The TEER values (data no shown) were decreased to 36 ± 14% ($n =$ 39) of the initial value by the use of 12.5 mM GC for 4 h.

The observed accumulation of ^{3}H-labelled propranolol in the TR146 cell culture model, which was also seen with the nonradioactive drug, decreased ≈10% in the presence of GC.

4. Discussion

4.1. Permeability studies with the TR146 cell culture model and human, monkey and porcine buccal mucosa

The permeability of water found in this study can be related to data observed in previous studies with human buccal mucosa (Lesch et al., 1989; Van der Bijl et al., 1997), buccal mucosa from dog (Galey et al., 1976), monkey (Mehta et al., 1991) and pig (Squier and Hall, 1985; Veillard et al., 1987; Squier et al., 1997). The results of some of those studies indicate that the porcine buccal mucosa is less permeable to water than the human buccal mucosa by a factor of 5–10 (Lesch et al., 1989). Water may permeate the barrier not only by passive paracellular diffusion but also by osmosis and therefore water should not be used as a paracellular marker. However, for reasons of comparison of the TR146 cell culture model with other buccal models, the permeability of water was included in these studies. The permeability of mannitol across buccal mucosa from dogs has been measured to be ≈10^{-7} cm/s which is also the permeability range observed with human buccal mucosa in this study. To the authors knowledge, data on the permeability rate of testosterone across nonkeratinized buccal mucosa are not found in literature. However, it has been demon-

strated that the bioavailability of testosterone can be successfully increased by buccal delivery from an adhesive tablet compared to delivery by an orally administered tablet (Voorspoels et al., 1996). A similar buccal formulation has successfully been used in the treatment of hypogonadal men (Dobs et al., 1998). When comparing the permeability of mannitol and testosterone, it must be kept in mind that their permeability pathways are different.

The permeability values of mannitol and testosterone across the TR146 cell culture model can be correlated to the permeability values across the human buccal mucosa by a factor of 0.1. For the porcine buccal in vitro model, this factor varies for mannitol and testosterone, and even more significant variation is seen with the monkey buccal mucosa. This indicates that the TR146 cell culture model may be a better model than monkey and porcine buccal mucosa for predicting the human buccal permeability of passively transported substances.

4.2. Permeability studies with the TR146 cell culture model and porcine buccal mucosa using test substances of different lipophilicity

The P_c values for atenolol (14.6×10^{-6} cm/s), metoprolol (1.7×10^{-6} cm/s) and propranolol (22.0×10^{-6} cm/s) across the TR146 cell culture model have previously been determined (Jacobsen et al., 1995). The P_c values determined in this study are in the same range. Yet, in this study metoprolol has a higher P_c value than atenolol, which seems reasonable with respect to the higher lipophilicity of metoprolol. The permeability of the β-adrenoceptor antagonists across the porcine buccal mucosa correspond to reported values (Le Brun et al., 1989; Bland et al., 1991; de Vries et al., 1991). The porcine buccal mucosa seems to present a much tighter barrier than the TR146 cell culture model, as the permeability of β-adrenoceptor antagonists across the latter is ≈ 100 times higher. This has also been observed with a primary culture of hamster cheek pouch (Tavakoli-Saberi and Audus, 1989).

The P_c values from studies with the TR146 cell culture model and the permeability values from studies with the porcine buccal mucosa, seem to depend on the relative lipophilicity in the same way, as seen from Figs. 1 and 2. The results obtained with the porcine buccal mucosa may correlate better to the PWASA values, than the results from the TR146 cell culture experiments. Fig. 1 indicates a sigmoid permeability relationship to $\log k'$ and Fig. 2 a sigmoid correlation between the PWASA values and the permeability values. Both figures indicate that some of the tested β-adrenoceptor antagonists are too lipophilic to give maximal permeability across the buccal tissue in the experimental setup, as indicated by the bell-shape of the curves at k' higher than 7 and at PWASA values lower than ≈ 60 Å^2. This differs from results of studies performed with a range of β-adrenoceptor antagonists in the Caco-2 cell culture model. In those studies, the correlation between the polar surface area and the permeability across the Caco-2 cell monolayers was reported to be linear (Palm et al., 1996; Krarup et al., 1998). One explanation could be that the TR146 cell culture model represents a stratified epithelium and/or that the composition of the lipophilic permeability barrier is different in the two models.

The accumulation of propranolol in the buccal tissue has previously been observed (Henry et al., 1980). It is reasonable that the significant accumulation of propranolol in the TR146 cells is not due only to its lipophilic nature as the estimated lipophilicity of testosterone is in the same range. The explanation for the apparent accumulation of propranolol could be due to degradation and accumulation of the metabolites in the cells, but this should be investigated further. The degree of ionisation of the test substances influences the permeability rates (Hicks, 1973; Schürmann and Turner, 1978; Hitoglou-Makedou et al., 1989; Coutel-Egros et al., 1992), and since most of the chosen drugs have pK_a values of ≈ 9, this is not likely the explanation of the difference between the permeability values. However, structurally labetalol is different from the other tested β-adrenoceptor antagonists. It has two benzene rings and

on one of these there is a phenolic group in the vicinity of another electron withdrawing group ($-NH_2$), which may result in a lower pK_a value than 9. Also, labetalol has the highest polar water accessible surface area, which probably contribute to the relatively low permeability rates across the two models of buccal tissue. Different MW of test substances with the same estimated lipophilicity may also cause different permeability rates (Camenisch et al., 1998; Nielsen et al., 1999). The MW of labetalol is 328, and does not vary from the other used β-adrenoceptor antagonists (MW 248–336). With regard to this and comparing Figs. 1 and 2 in general, the PWASA value more closely correlates to permeability property across buccal tissue, than the lipophilicity measured such as k' or log D. Further, use of PWASA values for estimations of lipophilicity has the advantage that the values are calculated and not determined by time consuming experiments.

4.3. The permeability enhancing effect of sodium glycocholate

GC has previously been observed to enhance the permeability of hydrophilic test substances across the TR146 cell culture model (Nielsen and Rassing, 1999; Nielsen et al., 1999). In the present study, only the permeability of mannitol and atenolol were significantly enhanced, whereas the ER values for water and the more lipophilic substances were not different from the value 1. This probably relates to the mechanism of action of GC, suggesting that it is primarily the paracellular permeability which is affected by GC. Different enhancing agents and co-solvents have been investigated for enhanced delivery of propranolol (Kurosaki et al., 1988; Coutel-Egros et al., 1992; Manganaro and Wertz, 1996), and bile salts have been shown to interact with e.g. propranolol (Grosvenor and Löfroth, 1995). In concentrations higher than the CMC, ≈ 4 mM (Nielsen and Rassing, 1999), GC has been shown to decrease the membrane permeability of propranolol (Dongowski et al., 1996) possibly due to entrapment in the micelles, which may contribute to the lack of enhancement observed in the present study.

5. Conclusion

In conclusion, the permeability barrier of the TR146 cell culture model can be considered to be similar to the barrier of human or porcine buccal mucosa in vitro with regard to the permeability of selected markers such as mannitol and testosterone. Buccal mucosa from monkey does not seem to have barrier properties similar to human buccal mucosa. It has been demonstrated that for water and a range of β-adrenoceptor antagonists with different lipophilicity, the results of the cell culture model studies can be used to predict the permeability rates across porcine buccal mucosa, and therefore possibly the permeability rates across human buccal mucosa. However, using the TR146 cell culture model, it is important to determine the P_c values, since the insert, rather than the cell layers, is the rate determining barrier for some lipophilic substances. Finally, it was observed that the enhancing effect of 12.5 mM GC was restricted to more hydrophilic test substances.

Acknowledgements

Dr Med M. Christensen and H.B.D. Olin, Department of Oto-Laryngology, Rigshospitalet, Denmark and Associate Prof. Ph.D. S. Schou, Department of Oral and Maxillofacial Surgery, School of Dentistry, Denmark are acknowledged for invaluable assistance collecting biopsies from humans and monkeys. L.H. Krarup at the Royal Danish School of Pharmacy is acknowledged for calculating and discussing the PWASA values. B. Dinitzen, L. Jespersen, N.E. Nielsen and E. Nielsen at the Royal Danish School of Pharmacy, and the staff at Roskilde Slagteriskole are acknowledged for technical assistance. PharmaBiotec III Research Center, Center for Drug Design and Transport, Erik Hørslev and wife Birgit Hørslev Foundation, and Danish Animal Welfare Society are acknowledged for economic support.

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