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International Journal of Pharmaceutics 185 (1999) 215–225

international journal of pharmaceutics

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TR146 cells grown on filters as a model of human buccal epithelium: III. Permeability enhancement by different pH values, different osmolality values, and bile salts

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Received 29 October 1998; received in revised form 7 April 1999; accepted 5 May 1999

Abstract

The aim of the present study was to evaluate the TR146 cell culture model as an in vitro model of human buccal epithelium with respect to the permeability enhancement by different pH values, different osmolality values or bile salts. For this purpose, the increase in the apparent permeability (P_{app}) of the hydrophilic marker mannitol due to exposure to solutions with pH values or osmolality values different from the physiological values was studied. As in studies with solutions of either taurocholate (TC), glycocholate (GC) or glycodeoxycholate (GDC) the results were compared to the increase in P_{app} of mannitol obtained in analog studies using porcine buccal mucosa in an Ussing chamber. The effect of the exposure on the electrical resistance of the TR146 cell culture model and the porcine buccal mucosa was measured, and the degree of protein leakage due to GC exposure was investigated in the TR146 cell culture model. The porcine buccal mucosa was approximately ten times less permeable to mannitol than the TR146 cell culture model. The P_{app} values obtained with both in vitro models were dependent on the concentration of bile salt, the degree of hydroxylation of the bile salt, and the type of conjugation to the bile salt as the enhancement effect decreased in the order GDC $\gg$ GC $>$ TC. Increased P_{app} values correlated with a decrease in the electrical resistance of the TR146 cell culture model and the porcine buccal mucosa. GC was shown to induce concentration dependent protein leakage in the TR146 cell culture but only from the site of application, and the results indicate that duration of exposure further than 120 min was of minor importance. The present results indicate that the TR146 cell culture model may be a suitable in vitro model for efficacy studies and mechanistic studies of enhancers with potential use in human buccal drug delivery.

Keywords: TR146 cell culture model; Buccal permeability; Enhancement; pH; Osmolality; Bile salt

1. Introduction

Buccal delivery offers a feasible non-invasive route of systemic delivery for drugs that are not administrable orally due to low bioavailability. However, for adequate systemic delivery of pep-

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0378-5173/99/$ - see front matter
PII: S0378-5173(99)00165-9

tide or protein drugs, a permeability enhancing drug delivery system is required (Merkle and Wolany, 1992). To evaluate the effect and the mechanism of, e.g. permeability enhancers, the availability of well characterized in vitro models of the human buccal epithelium is essential (Borchardt et al., 1996).

Due to morphological similarities and comparable water permeability (Lesch et al., 1989), porcine buccal mucosa has been considered a reasonable good model of human buccal mucosa for drug delivery studies (Zhang and Robinson, 1996). For rapid and efficient screening of drug permeability, cell culture techniques generally hold advantages over other in vitro models (Audus, 1996). The TR146 cell line originates from a neck node metastasis of a human buccal carcinoma (Rupniak et al., 1985), and the cell culture model has been proposed as an in vitro model of the human buccal epithelium (Jacobsen et al., 1995) and used for permeability studies with β-adrenoceptor antagonists (Jacobsen et al., 1995) and FITC-labelled dextrans (Nielsen et al., 1999). For further characterisation of the TR146 cell culture model as a model of the human buccal epithelium, it is important to evaluate the resilience of the permeability barrier towards non-physiological values of pH and osmolality, and towards permeability enhancers.

An increase in the amount of non-ionized drug is likely to increase the permeability of the drug across an epithelial barrier, and this may be achieved by a change in pH of the drug delivery system (Al-Sayed-Omar et al., 1987; Barsuhn et al., 1988; Nair et al., 1997). Further, the extracellular osmolality has been shown to affect the permeability across epithelial barriers (Dowty et al., 1992; Maitani et al., 1997). Bile salts have been shown to increase the permeability of hydrophilic drugs or model substances through the buccal mucosa of animals in vivo (Ishida et al., 1981; Aungst et al., 1988; Aungst and Rogers, 1989; Hoogstraate et al., 1996) as well as in vitro (Şenel et al., 1998). The mechanism by which bile salts affect mucosae and, thereby, enhance permeability across an epithelial barrier has been discussed extensively and may include: disruption of the plasma membrane causing leakage of phospholipids and proteins (Hosoya et al., 1994), loosening of the intercellular filaments resulting in decreased integrity of the epithelium, change in the composition of the intercellular matrix, micelle formation with the drug substance and/or inhibition of proteolytic enzymes (Ganem-Quintanar et al., 1997). The micellar forming properties of a bile salt are influenced by the presence of counter-ions (Carey and Small, 1972), and it has been suggested that the bile salt effect on a permeability barrier such as the gastric mucosa depends on the degree of bile salt ionisation (Eastwood, 1975). However, recent studies showed that bile salt induced enhancement of acyclovir permeability across porcine buccal mucosa was independent on the pH value between 4 and 9 (Shojaei et al., 1998).

The objective of this study was to evaluate the TR146 cell culture model with respect to the effect of pH, osmolality and selected bile salts as permeability enhancers. For evaluation of the enhancement effect, the increase in permeability of a hydrophilic marker, mannitol, was determined concurrent with studies on the sensitivity of the epithelial cells towards the bile salts. The results were compared to data obtained with porcine buccal mucosa in vitro.

2. Materials and methods

2.1. Materials

Sodium glycodeoxycholate (GDC), sodium glycocholate (GC), sodium taurocholate (TC) and folin–phenol reagent were obtained from Sigma Chemical Co. (St. Louis, MO, USA). D-[1-^{14}C]-mannitol was bought from New England Nuclear (Boston, MA, USA). The solutions of sodium chloride, citric acid buffer, phosphate buffer, carbonate buffer, glucose-Ringer (GR) as well as the solutions of copper sulphate, sodium/potassium tatrate, sodium carbonate and sodium hydroxide were prepared in Milli-Q water with chemicals from Merck (Darmstadt, Germany). Hanks Balanced Salt Solution (HBSS), Dulbecco's modified Eagles medium (DMEM), penicillin and streptomycin were purchased from Gibco BRL (Paisley,

UK). Foetal calf serum (FCS) was from Harlan Sera-Lab Ltd. (Belton, UK). Ultima Gold™ MV scintillation cocktail was 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). Culturing was performed as previously described (Jacobsen et al., 1995). Briefly, the culture was maintained at 37°C and 5% CO_2/95% air in 98% humidity, and the culture medium consisted of DMEM supplemented with 10% FCS, 100 IU/ml penicillin, and 100 μg/ml streptomycin. Subcultivation was performed using a solution of 0.5% (w/v) trypsin and 0.2% (w/v) EDTA. Prior use in the permeability studies, the cells were cultured on Falcon® 4.2 cm^2 permeable polyethylene terephthalate inserts with a pore size of 0.4 μm (Becton Dickinson Labware, NJ, USA) for 28–30 days. The seeding density was approximately 24 000 cells/cm^2. The passage numbers used in this study were from 17 to 29.

2.2.2. Porcine buccal mucosa

The porcine buccal mucosa was obtained from Roskilde Slagteriskole (Roskilde, Denmark). The buccal tissue was isolated immediately after sacrificing the pig and placed in ice cold GR until isolation of the mucosa (i.e. the epithelium, the basal lamina, and a minimum of submucosa) by use of a tissue slicer (Thomas Scientific, NJ, USA). The average thickness of the slices was 730 ± 120 μm ($n = 88$). The buccal mucosa was mounted in the Ussing chambers within 3 h after sacrificing the pig. Before the permeability experiments, the mucosa equilibrated for 1 h in oxygenated 37°C GR in the Ussing chambers.

2.2.3. MTS/PMS-assay

The dehydrogenase activity in the TR146 cells was used to estimate the sensitivity of the TR146 cells towards different test solutions, and measured according to the MTS/PMS assay optimized for the TR146 cell line (Jacobsen et al., 1996). A similar assay, the MTT assay (Mosmann, 1983), has proven useful in estimating the cellular sensitivity of buccal cells towards drugs (Zheng and Audus, 1994). The enzymatic activity in untreated TR146 cells was 100%, and the effects of the test solutions were measured as a decrease in relative sensitivity, i.e. high toxicity is reflected by a low relative sensitivity. The assay was performed using TR146 cells in the exponential growth phase testing concentrations of GDC, GC and TC ranging from 0.01 mM to 0.1 M, iso-osmolal buffers with pH values from 2 to 10 and sodium chloride solutions with osmolality values from 0 to 900 mOsm.

2.2.4. Critical micelle concentration

The critical micelle concentration (CMC) of each of the bile salts in HBSS was determined at 30°C by the Wilhelmy plate method using a K10T digital tensiometer (Krüss, Hamburg, Germany).

2.2.5. Permeability studies with the TR146 cell culture model

The permeability studies were carried out at 37°C for 4 h with horizontal mechanical stirring at a rate of 150 rpm. The diffusion area was 4.2 cm^2 and the donor and receptor volumes were 2.5 ml. The donor solutions consisted of an enhancer in HBSS, sodium chloride solutions of different osmolality in Milli-Q water, or iso-osmolal citric acid buffer, phosphate buffer, or carbonate buffer at different pH values in Milli-Q water. In all the studies, the donor solutions contained mannitol (120 nCi/ml) as a paracellular marker. The receptor solutions consisted of HBSS. After application of a donor solution to the apical side of the epithelium, samples of 100 μl were withdrawn from the basolateral side of the epithelium at fixed time intervals. Replacement of the withdrawn volume was done with HBSS. Samples of 100 μl were collected from the donor solutions at the beginning and at the end of an experiment. The samples were analysed by liquid scintigraphy. The transepithelial electrical resistance (TEER) of the TR146 cell layers was monitored before and after each experiment by the use of an Endohm™ culture cup connected to an EVOM voltohmmeter

(World Precision Instruments Ltd., Herts, UK). The initial TEER was $272 \pm 132\ \Omega \times cm^2$ ($n = 177$). Likewise, phase-contrast microscopic examinations, magnification × 150–300 (Nikon, Tokyo, Japan), were carried out before and after each experiment.

2.2.6. Permeability studies with the porcine in vitro model

In the Ussing chamber experiments with porcine buccal epithelium the diffusion area was 0.5 cm^2, the donor and the receptor volumes were 1.0 ml and stirring was done by a carbogen (95% O_2/5% CO_2) gas lift. The permeability experiments were carried out as described above except that GR was used instead of HBSS, and the concentration of mannitol in the donor solutions was 1200 nCi/ml. Throughout the experimental period the resistance (R) of the porcine buccal epithelium was calculated from measurements of the potential difference between the mucosal and the serosal surface and the corresponding short-circuit current. The initial R was $872 \pm 378\ \Omega \times cm^2$ ($n > 88$).

2.2.7. Protein assay

TR146 cell layers cultured for 30 days on permeable inserts were exposed apically to HBSS and to test solutions of 10 or 50 mM GC in HBSS under the same conditions as in the permeability experiments. Samples of 0.5 ml were withdrawn apically and basolaterally at 10, 120 and 240 min, and the amount of protein was determined by the method described by Lowry et al. (1951). The total amount of protein in cells cultured for 30 days on inserts was determined.

2.2.8. Data analysis

In the MTS/PMS assay, the sensitivity of the TR146 cells towards a test substance was determined relative to cells exposed to HBSS.

In the permeability studies, the change in TEER or R is expressed as the percentage of the initial value.

The apparent permeability coefficient (P_{app}) for the permeability of mannitol across the in vitro models was calculated according to Eq. (1):

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

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

The enhancement ratio (ER) was estimated according to Eq. (2)

$$ER = P_{app}\ (\text{enhancer})/P_{app}\ (\text{control}) \quad (2)$$

where P_{app} (enhancer) represents the permeability in the presence of an enhancer, at pH values or at osmolality values different from pH 7 and 300 mOsm, respectively. P_{app} (control) is the permeability in the corresponding control experiment.

The results of the protein assay are expressed as the percentage of protein released relative to the total amount of protein in homogenised cells.

Data are presented as mean ± SD (n), where n is the number of replicates. For significance testing, the Student's t-test is used at 95% level.

3. Results

3.1. MTS/PMS-assay

In Fig. 1, the sensitivity of cells exposed to bile salts in HBSS relative to cells exposed to HBSS is presented. It is noticeable that the TR146 cells were more sensitive towards the dihydroxy bile

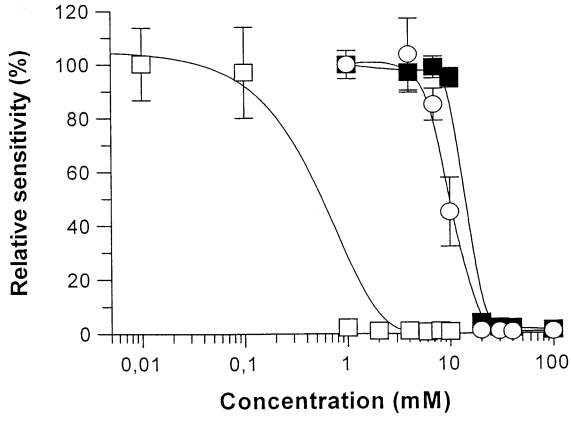

Fig. 1. The sensitivity of TR146 cells towards various concentrations of bile salts relative to unexposed TR146 cells determined by the MTS/PMS assay. Mean ± SD ($n = 4$). (■) taurocholate; (○) glycocholate and (□) glycodeoxycholate.

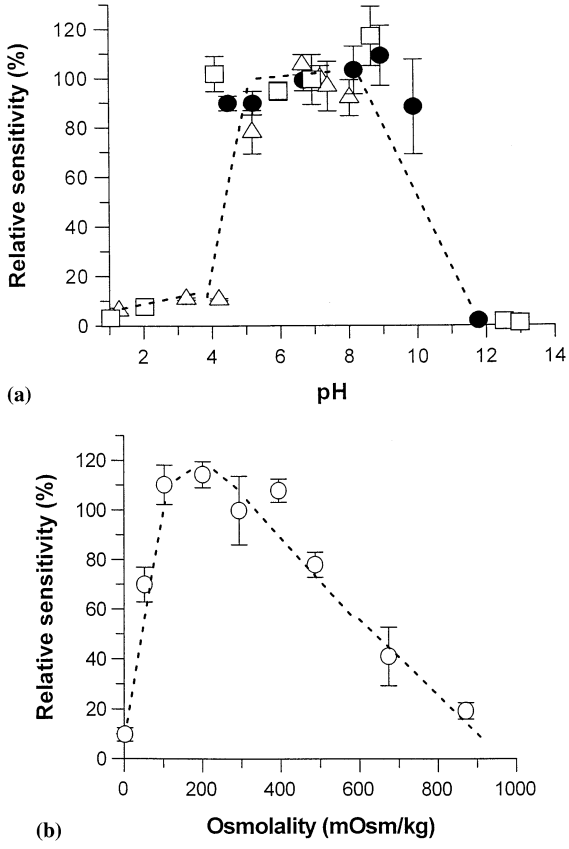

Fig. 2. The sensitivity of TR146 cells towards different media relative to iso-osmolal solutions of pH 6.9 determined by the MTS/PMS assay. Mean ± SD ($n = 4$). (a) pH values and (b) osmolality values. (□) phosphate buffer; (△) citrate buffer; (●) carbonate buffer and (○) sodium chloride in Milli-Q water.

salt, GDC, than towards the trihydroxy bile salts, GC and TC. Further, the cells were slightly more sensitive towards GC than towards TC at concentrations of 7 mM and above ($P < 0.01$). Fig. 2a depicts the sensitivity of TR146 cells exposed to various pH values relative to pH 6.9. It is shown that the TR146 cells were not sensitive towards pH changes in the approximate range 5.5–9.0. The sensitivity of the cells was independent on the type of buffer used. Fig. 2b shows that the relative sensitivity of the TR146 cells towards sodium chloride solutions was about 100% at osmolality values within the range of approximately 100 mOsm to approximately 400 mOsm.

3.2. Permeability studies with different pH and osmolality values

Table 1 presents P_{app}, relative TEER values and relative ER values obtained from permeability experiments with mannitol solutions of different pH values or osmolality values. Iso-osmolal buffers with a pH value of 5.2 and lower or a pH value of pH 8.1 and higher increased the permeability of mannitol across the TR146 epithelial barrier as compared to buffers with a pH of 6.9. The permeability of the TR146 cell culture model also increased significantly when the osmolality of solutions with a pH value of 6.9 was 52 mOsm and less or 488 mOsm and more as compared to an iso-osmolal solution.

In the porcine buccal in vitro model, permeability studies with a buffer of pH 2 and pH 12.5 showed neither significant increase in P_{app} of mannitol nor significant decrease in R of the porcine epithelium as compared to a buffer with pH 7.2. Furthermore, with the porcine in vitro model, no significant changes in P_{app} or R were promoted by solutions with osmolality from 0 mOsm to a saturated solution of sodium chloride (approximately 11 000 mOsm) as compared to an iso-osmolal solution (data not shown).

3.3. Permeability studies in the presence of bile salts

Fig. 3a, b, c depict the P_{app} values of mannitol permeability across the TR146 cell culture model and across the porcine in vitro model in the presence of various concentrations of TC, GC and GDC, respectively. Table 2 represents data of the relative TEER values for the TR146 cell culture model and the relative R values for the porcine in vitro model as well as ER values after enhancer exposure for 4 h. Further, microscopic observations of the effect on the TR146 cell culture model of 4 h exposure to the bile salt solutions are ranked between no effect (+) and pronounced damaging effect (+ + +), i.e. detachment of cells. It is observed that the P_{app} value of mannitol permeability across the TR146 cell culture model is approximately ten times the P_{app} obtained with the porcine in vitro model, irrespec-

tive of whether the barrier was exposed to an enhancer or not. This holds for both TC, GC and GDC.

The CMC values for TC, GC, and GDC were determined to 5, 4 and 1.5 mM, respectively. An increase in ER was obtained when the concentration of TC or GC in HBSS was 12.5 mM and higher, i.e. 2–3 fold higher than the CMC, and GC seemed to have a slightly stronger enhancing effect as compared to TC. The enhancing effect of GDC occurred at concentrations of the CMC value and higher. In both models the epithelium was more affected by GDC than by any of the trihydroxy bile salts, as enhancement was observed with lower molar concentrations of GDC than GC or TC. An increase in the ER value was consistent with a decrease in the integrity of the epithelium as reflected in TEER and R values. The visual inspection showed a decrease in the cell size ($++$), becoming gradually more pronounced ($+++$) with increased concentration of the bile salt. In studies with 10 mM GC, the ER did not increase further by increasing the osmolality of the donor solution up to 500 mOsm or decreasing the pH of the donor solution to 5.5 (data not shown). The porcine epithelium was more resilient towards bile salt exposures than the TR146 cell culture model. The standard deviation within data obtained with the porcine in vitro model was larger than with the TR146 cell culture model.

Fig. 4a depicts the relative amount of protein leakage from the apical side of the TR146 cell layers exposed apically to HBSS, 10 or 50 mM GC in HBSS for up to 240 min. Fig. 4b shows the relative amount of protein leakage from the basolateral side of the same TR146 filter-grown cells. It is evident that the amount of protein leakage increased with increasing concentrations of bile salt applied to the cell layers, and that the leakage of protein was only significant from the site of application. The protein leakage occurred within the first 120 min of exposure.

4. Discussion

4.1. Permeability studies with different pH and osmolality values

The results of the MTS/PMS sensitivity screening assay are indicative for the effect of pH and osmolality on the integrity of the barrier of filter-grown TR146 cells with respect to an increase in mannitol permeability correlating to a decrease in

Table 1
The apparent permeability (P_{app}) of mannitol across the TR146 cell culture model, the relative transepithelial electrical resistance (TEER) and the enhancement ratio (ER) for permeability studies with the TR146 cell culture model at different pH values or different osmolality values[a]

Exposure		$P_{app} \pm SD$ ($\times 10^7$) (cm/s)	TEER ± SD (% of initial TEER)	ER ± SD
pH	1.9	79.0 ± 0.9***	2.8 ± 2.0***	8.6 ± 0.7
	5.2	11.2 ± 1.6*	67.7 ± 10.1*	1.2 ± 0.2
	6.9	9.1 ± 0.8	100 ± 13.7	1.0 ± 0.1
	8.1	12.2 ± 1.2***	27.8 ± 5.1***	1.3 ± 0.2
	9.9	149.7 ± 37.1***	2.1 ± 1.8***	16.4 ± 4.3
Osmolality (mOsm)	0	3.5 ± 2.1***	5.7 ± 1.4***	6.5 ± 0.4
	52	65.9 ± 5.2***	20.1 ± 10.0***	4.6 ± 0.4
	103	15.1 ± 1.2	91.4 ± 16.6	1.0 ± 0.1
	294	14.5 ± 0.8	100.0 ± 11.0	1.0 ± 0.1
	488	26.2 ± 0.5***	60.5 ± 11.6***	1.8 ± 0.1
	871	40.8 ± 2.2***	37.1 ± 5.7***	2.8 ± 0.2

[a] Mean ± SD ($n = 3$).
* $P < 0.05$.
*** $P < 0.001$.

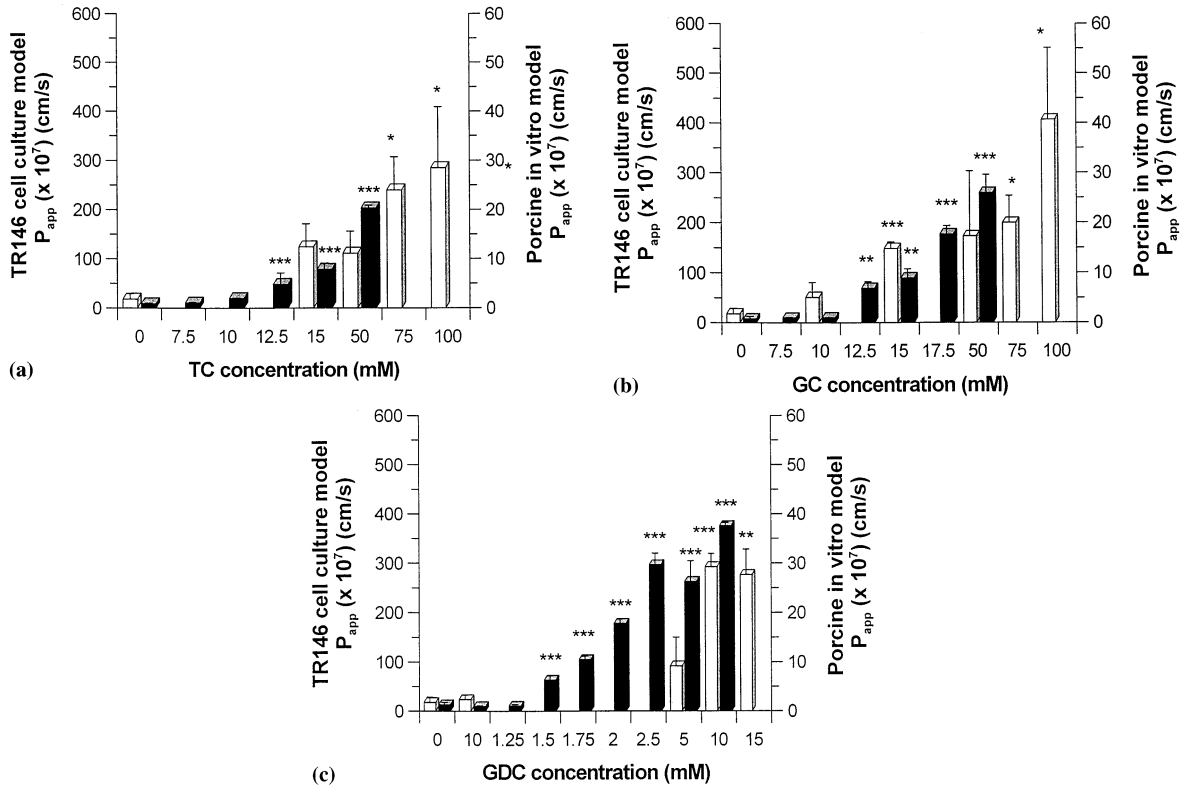

Fig. 3. The apparent permeability coefficients (P_{app}) for permeability of [^{14}C]-mannitol across the TR146 cell culture model (■) and the porcine in vitro model (□) exposed to (a) TC; (b) GC or (c) GDC. Mean ± SD ($n = 3$–6). (*) $P < 0.05$; (**) $P < 0.01$; (***) $P < 0.001$.

TEER. For example, osmolality values decreasing the relative sensitivity to approximately 50% or less significantly enhanced the P_{app} value. Differences in pH values and osmolality values of the donor solutions were shown not to affect the porcine buccal epithelial barrier properties with respect to mannitol permeability. This correlate to the results that irritation of human buccal mucosa was not provoked by exposure to solutions of pH 3–13 nor to a hyperosmolal mannitol solution (Place et al., 1988).

Differences in epithelial permeability of ionizable drugs due to changes in the pH or the osmolality of the solution may be assessed in the TR146 cell culture model as well as in the porcine in vitro model. However, the cell culture model is more sensitive towards non-physiological pH and osmolality than the porcine in vitro model.

4.2. Permeability studies in the presence of bile salts

The results of the present studies confirm the permeability enhancing effect of bile salts observed with porcine buccal mucosa (Hoogstraate et al., 1996; Şenel et al., 1998).

To achieve an effect on the permeability of filter-grown TR146 cells, bile salt concentrations with a relative sensitivity in the MTS/PMS assay of less than 20% were required. The sensitivity assay and the permeability study were carried out on TR146 cells in different growth phases, and it is recognized that the cell membrane of proliferating cells in the exponential growth phase, as used in the MTS/PMS assay, is more sensitive towards the bile salt exposure than the differentiated superficial cells in the 30-day-old cell culture model, which is supported by previous results (Nielsen et al., 1999).

Table 2
The relative transepithelial electrical resistance (TEER) for permeability studies with the TR146 cell culture model and the relative electrical resistance (R) for permeability studies with the porcine in vitro model as well as the enhancement ratios (ER) after 4 h exposure to different concentrations of TC, GC or GDC

Bile salt	Conc. (mM)	Cell culture model			Porcine in vitro model	
		TEER ± SD(%)	ER ± SD	Visual effect[a]	R ± SD(%)	ER ± SD
Absent	–	73.6 ± 23.7		+	118.2 ± 23.0	–
TC	7.5	98.5 ± 22.4	1.2 ± 0.3	+	n.d.	n.d.
	10.0	115.6 ± 12.0*	1.4 ± 0.2	+	n.d.	n.d.
	12.5	26.1 ± 7.6***	5.4 ± 0.9	++	n.d.	n.d.
	15.0	n.r.	n.r.	++	113.5 ± 9.8	6.9 ± 4.8
	50.0	n.r.	n.r.	+++	45.9 ± 24.2*	6.2 ± 4.4
	75.0	n.d.	n.d.	+++	40.7 ± 33.2*	10.2 ± 11.1
GC	7.5	83.0 ± 40.5	1.1 ± 0.9	+	n.d.	n.d.
	10.0	40.6 ± 13.4*	1.2 ± 0.9	+	122.3 ± 14.6	2.8 ± 2.4
	12.5	29.1 ± 10.0***	7.7 ± 6.2	++	n.d.	n.d.
	15.0	n.r.	n.r.	++	88.7 ± 37.9	8.2 > 4.9
	50.0	n.r.	n.r.	+++	54.8 ± 11.4*	11.7 ± 10.0
GDC	1.0	61.2 ± 13.6**	0.4 ± 0.03	+	129.9 ± 7.8*	1.3 ± 0.9
	1.25	79.7 ± 13.9	1.0 ± 0.3	+	n.d.	n.d.
	1.5	25.5 ± 2.4***	4.7 ± 0.3	++	n.d.	n.d.
	5.0	n.r.	n.r.	+++	49.3 ± 23.7*	5.1 ± 4.4

[a] Scoring of the visual effect of bile salt on the TR146 cell layers: (+) no effect; (++) slight effect, i.e. cell size decreased; (+++) severe effect, i.e. cell detachment. Mean ± SD ($n = 3$–6).

* $P < 0.05$.

** $P < 0.01$.

*** $P < 0.001$ (n.d.) not determined; (n.r.) not reported, as the electrical parameter TEER or $R \leq 25\%$.

In mechanistic studies, the enhancement effect of bile salts has been shown to correlate to accumulation in the epithelium (Şenel et al., 1998), leaching of proteins and phospholipids from the epithelial membrane (Hosoya et al., 1994), and decrease in the total amount of major polar lipids and cholesterol in the epithelium (Hoogstraate et al., 1997). The present results of protein leakage from the TR146 cell layers support this observation, and may explain the indicated change in permeability pathways for hydrophilic substances in the presence of GC (Nielsen et al., 1999). Interestingly, the effect of a GC solution seems only to occur on the superficial cell layers and does not proceed after about 120 min.

The more pronounced effect for dihydroxy than for trihydroxy bile salts (Nakane et al., 1996) corresponds with the results obtained in the studies with both the TR146 cell culture model and the porcine buccal mucosa. This is probably due to the fact that dihydroxy bile salts are more hydrophobic than trihydroxy bile salts and thereby less hydrated. This enables better penetration into the cell membrane and solubilisation of membrane components such as lecithin (Lindenbaum and Rajagopalan, 1984) by mixed micelle formation (Carey and Small, 1972). Further, the present studies indicate less enhancement of a taurine conjugated bile salt compared to a glycine conjugated bile salt, which have also been proposed by Hosoya et al. (1994). The permeability enhancing effect of the bile salts correlate to their micellar forming properties, but whereas the concentration of TC and GC had to be 2–3 fold higher than the CMC to increase the permeability GDC was effective at the CMC and above.

The degree of membrane perturbation by bile salts has been shown to be directly related to the

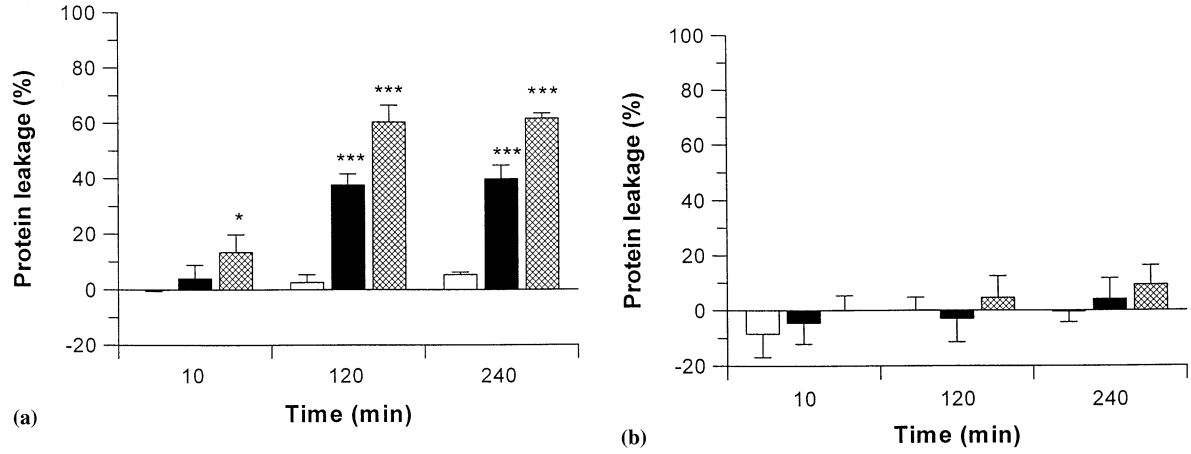

Fig. 4. Protein leakage from the (a) apical and (b) basolateral side of the TR146 cell culture model after apical exposure to HBSS (□); 10 mM GC (■) and 50 mM GC (▨) for 10, 120 and 240 min. The values are relative to the total amount of protein in filter-grown TR146 cells. $n = 3$, mean ± SD. (*) $P < 0.05$; (***) $P < 0.001$.

fraction of non-ionized bile salt (Eastwood, 1975), and it has also been shown that the rate of lecithin dissolution by bile salts is increased with increasing amounts of counter-ion in the solution (Lindenbaum and Rajagopalan, 1984). The enhancing effect of decreased pH or hyperosmolality in GC solutions could not be demonstrated by use of the TR146 cell culture model as greater changes in pH or osmolality from physiological solutions caused disruption of the cell layer integrity. In accordance with the results of the present study recent studies with porcine buccal mucosa in vitro have shown that the enhancing effect of glycocholate was independent on the pH value between pH 4 and 9 (Shojaei et al., 1998). Still, the lowest used pH value was approximately the pK_a of glycocholate (pK_a 3.8–4) (Carey and Small, 1972). To conclude whether the non-ionized form of glycocholate is more effective as an enhancer than the ionized form, further studies should be performed with the porcine in vitro model.

Most of the data obtained in the studies with the TR146 cell culture model are comparable to the results obtained in the porcine in vitro model, even though significance of enhancement with the latter is not as evident. The potential of buccal controlled drug delivery systems with bile salts as enhancers should be explored further, and this study has shown that the TR146 cell culture model has a potential in mechanistic studies of enhancement efficacy and enhancement mechanisms. Generally, the TR146 cell culture model holds the advantage of being well characterized and with a lower variability within the obtained data than the porcine in vitro model. Further, the TR146 cell culture model is more sensitive for variation regarding bile salt concentration, pH values and osmolality values.

5. Conclusion

Enhancement of the permeability across the TR146 cell culture model increased with increased concentrations of bile salt. The enhancement ratio decreased in the order glycodeoxycholate ≫ glycocholate > taurocholate along with decreasing epithelial integrity, as measured by the transepithelial electrical resistance. This ranking was consistent with results obtained with the porcine in vitro model. Mechanistic studies demonstrated epithelial protein leakage only from the superficial cells after application of GC to the apical surface of the TR146 cell culture model. The effect of the bile salt was not increased with prolonged exposure beyond 120 min. Deviations from the physiological values of pH and osmolality increased the permeability across the cell culture model. Yet, changes in pH or osmolality did

not increase the enhancing effect of glycocholate as compared to glycocholate in a neutral iso-osmolal solution.

Further, the results of the MTS/PMS assay correlated with a decrease in the permeability barrier of the TR146 cell culture model due to changes in pH or osmolality. Regarding the effect of the bile salt on the permeability of the cell culture model, this was less than expected from the results of the sensitivity assay.

In conclusion, the TR146 cell culture model is as suitable as the porcine in vitro model of the human buccal epithelium with regard to studies of bile salt enhancement. Along with the fact that the model possesses structural features similar to the human buccal epithelium, the present studies indicate that valid mechanistic evaluations of enhancement can be performed using the TR146 cell culture model derived from a human buccal carcinoma. In advance of such studies, the effect of enhancers on the intercellular matrix of the TR146 cell culture model should be performed and compared to the effect in human buccal epithelium. Better reproducibility of data and higher through-put of studies are advantages that the TR146 cell culture model holds over the porcine buccal in vitro model.

Acknowledgements

The authors would like to thank B. Dinitzen and E. Nielsen at the Royal Danish School of Pharmacy, and the staff at Roskilde Slagteriskole for technical assistance. PharmaBiotec III Research Center and Danish Animal Welfare Society are acknowledged for economic support.

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