Available online at www.sciencedirect.com
SCIENCE DIRECT
ELSEVIER
International Journal of Pharmaceutics 309 (2006) 123–128
INTERNATIONAL JOURNAL OF PHARMACEUTICS
www.elsevier.com/locate/ijpharm

Lack of effect of β-cyclodextrin and its water-soluble derivatives on in vitro drug transport across rat intestinal epithelium

Ying Zheng, Zhong Zuo, Albert H. L. Chow *

School of Pharmacy, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong, China

Received 28 July 2005; received in revised form 15 November 2005; accepted 15 November 2005
Available online 15 December 2005

Abstract

The present study aimed to investigate whether β-cyclodetxrin (β-CD) and its water-soluble derivatives, hydroxypropyl-β-cyclodextrin (HP-β-CD) and sulfobutyl ether β-cyclodextrin (SBE-β-CD), exert any effects on the permeation of two drug transport markers (propranolol and lucifer yellow) across rat intestinal epithelium. Rat ileum was stripped of its serosa and mounted inside an Ussing Chamber. Apparent permeability coefficients (P_{app}) of the markers from the mucosal to serosal side of the tissue were determined at 37 °C in the presence and absence of the β-cyclodextrins on the mucosal side. Potential difference (PD) was constantly monitored during each experiment to ensure maintenance of the viability and integrity of the tissue. Pre-incubation with 1% β-CD, 1% HP-β-CD or 1.48% SBE-β-CD on the mucosal side for 30 min did not significantly alter the PD and the propranolol permeability ($p > 0.05$). Co-incubation with 1% β-CD or 1% HP-β-CD exerted no significant effect on the P_{app} of both propranolol and lucifer yellow ($p > 0.05$), but co-incubation with 1.48% SBE-β-CD lowered the P_{app} of propranolol from $(1.71 \pm 0.44) \times 10^{-5}$ to $(0.19 \pm 0.04) \times 10^{-5}$ cm/s, which may be ascribed to the molecular complexation of propranolol with SBE-β-CD. All three β-cyclodextrins exert no apparent impact on both (passive) transcellar and paracellular drug transports.

Keywords: β-Cyclodextrins; Drug transport; Intestinal permeability; Ussing Chamber

1. Introduction

Cyclodextrins (CDs) are cyclic (α-1,4)-linked oligosaccharides composed of α-D-glucopyranose units which together yield a rigid cone-shaped structure. The structure is characterized by a relatively hydrophobic central cavity and a hydrophilic outer surface. The cavity provides a favorable binding site for suitably sized substrate (e.g. drug) molecules of similar polarity to form an inclusion complex via non-covalent bonding (Loftsson and Brewster, 1996). Only the 6-, 7, and 8-membered ring structures (α-, β- and γ-CD) occur naturally while other available CDs such as hydroxypropyl-β-cyclodextrin (HP-β-CD) and sulfobutyl ether β-cyclodextrin (SBE-β-CD) are chemically modified from their parent compound for improved aqueous solubility and parenteral safety (Stella and Rajewski, 1997).

In pharmaceutical formulation, β-CDs, particularly HP-β-CD and SBE-β-CD, have been widely used to enhance peroral drug absorption. The absorption-enhancing effects of β-CDs are believed to operate through two mechanisms. The first mechanism considers β-CDs to act as permeation enhancers, i.e. by direct action on the intestinal mucosal membrane permeability (Rajewski and Stella, 1996). The second mechanism views β-CDs to serve merely as drug carriers, and their role is simply to increase drug solubility and/or protect labile drugs against degradation in the gastrointestinal fluid, thereby providing more dissolved drug for absorption (Uekama et al., 1998; Shaker et al., 2003). It can be envisaged that if the former mechanism is indeed in operation, more safety studies on the β-CDs will be required before they can be utilized in drug formulation.

Conflicting reports have emerged in the literature over the past decade with regard to the potential disruptive effects of β-CDs on lipid cell membranes. The ability of parent CDs to solubilize cell membranes can be linked to the observed hemolytic activity of these CDs (β-CD > α-CD > γ-CD) on human erythrocytes in vitro (Miyazawa et al., 1995). However, using the Caco-2 cell monolayer model, it has been shown that β-CDs exhibited

* Corresponding author at: School of Pharmacy, The Chinese University of Hong Kong, 6/F, Rm 616, Basic Medical Sciences Building, Shatin, New Territories, Hong Kong SAR, China. Tel.: +852 26096829; fax: +852 26035295.
E-mail address: albert-chow@cuhk.edu.hk (A.H. L. Chow).

0378-5173/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2005.11.022

negligible influence on cell membrane permeability with the exception of dimethyl-β-cyclodextrin (DM-β-CD) (Hovgaard and Brønsted, 1995; Tötterman et al., 1997). In contrast, other reported work employing basically the same cell model demonstrated an increase in permeability of the cells to a peptide marker (by 2.94 or 2.69-fold) with either β-CD (1%) or HP-β-CD (1%) (Haeberlin et al., 1996). Using sulfanilic acid as a transport marker, similar permeability enhancement by β-CD (10 mM) has also been observed for rat intestinal tissue pretreated with a mucolytic agent (Nakanishi et al., 1992). It must be noted that results derived from cell culture studies are strictly not applicable to intact intestinal tissue as the cultured cells lack a mucus layer on their surfaces. Covering the entire surface of the digestive tract, the mucus layer acts as a barrier protecting the underlying delicate cells from damage by digestive enzymes, acid, abrasion and bacteria, and it is also a barrier to diffusion. The major structural components of the mucus layer are the mucins, which are huge glycoprotein molecules comprising a protein backbone embedded in oligosaccharide (sugar) chains. Cell models such as human Caco-2 cell monolayer that are devoid of a mucus layer are known to be more sensitive to the effects of absorption enhancers than the tissue models, which possess an intact mucus layer (Anderberg and Artursson, 1993). In addition, the tight junctions in the differentiated Caco-2 cell monolayer are more reflective of those in the colon than in the small intestine, thus affording higher transepithelial resistance than normally found across the small intestinal epithelium (Artursson, 1990).

To more closely reflect intestinal absorption that occurs predominantly in the small intestine, the present investigation has employed excised pieces of rat ileal epithelium, which exhibit normal electrophysiology and histology (Shen and Lin, 1994), for examining the influence of β-CD and its water-soluble derivatives, HP-β-CD and SBE-β-CD, on drug transport. The objective of the present work is to determine whether these β-CDs exert any direct permeability-enhancing effect on the rat intestinal epithelium. To this end, excised ileal epithelium was mounted inside an Ussing Chamber and drug transport across the epithelium was characterized using propranolol and lucifer yellow (delineating the transcellular and paracellular pathways) as the markers. Additionally, EDTA was used to ascertain the integrity of the tight junction between epithelial cells (Chung et al., 1998).

2. Materials and methods

2.1. Chemicals and reagents

Propranolol hydrochloride and lucifer yellow were purchased from Sigma (USA). β-CD was supplied by ICN Biomedicals Inc., Aurora, Ohio, USA. HP-β-CD [M.S. (average molar degree of substitution) = 0.8] was purchased from Sigma-Aldrich Inc., St. Louis, Mo, USA. SBE-β-CD [T.D.S (total degree of substitution) = 7; Captisol®] was donated by CyDex, Overland Park, Kansas, USA. All other chemicals and solvents were of analytical or HPLC grade, and all water used was deionized and double-distilled.

2.2. Osmolarity

Prior to all experiments, the osmolarities of incubation solutions (β-CDs dissolved in Krebs-Bicarbonate Ringer's solution) were measured by a vapor pressure osmometer (vapor pressure osmometer, WESCOR, Utah, USA).

2.3. Tissue preparation

Ileum from an anesthetized male Sprague–Dawley rat (250 g) was rapidly removed by surgery, washed with cold Krebs-Bicarbonate Ringer's (KBR) solution (114 mM NaCl, 25 mM $NaHCO_3$, 5 mM KCl, 1.1 mM $MgCl_2$, 1.25 mM $CaCl_2$, 1.65 mM Na_2HPO_4, 0.3 mM NaH_2PO_4, 25 mM $NaHCO_3$, 10 mM glucose, pH 7.4), and placed in a beaker with KBR solution aerated with a mixture of O_2/CO_2 (95:5) on ice (Dowty and Dietsch, 1997). The ileum was allowed to rest and cool for approximately 10 min before further treatment to minimize tissue damage during the preparation. Ileum was cut into 2 cm pieces along its mesenteric border and the serosa was removed using blunt dissection under an optical microscope. Care was taken to avoid taking segment of the Peyers patches (Polentarutti et al., 1999). The stripped tissue was mounted in a holder inside an Ussing Chamber over a surface area of 0.636 cm^2. Ten millimetre of KBR solution was added to each compartment (donor and receiver) of the chamber and the solutions were aerated as before. The chamber was kept at 37 °C by means of a thermostatic water circulator (Hoto-Holten A/S Gydevany, Allerød, Denmark).

2.4. Electrical measurement

The potential difference (PD), which reflects the voltage gradient generated by the tissue, was constantly monitored during each experiment using a four-channel Voltage Clamp (Ussing electrode kit, WPI, FL, USA). Potential difference of the mounted ileum was measured before each experiment. Any ileum with PD <3 mV was considered poorly viable and would be omitted (Polentarutti et al., 1999).

2.5. Transport studies

After the equilibration period, the marker compound (1 mM for propranolol or 0.1 mM lucifer yellow) was added to the donor compartment (apical side). At 15-min time intervals, samples (1 ml aliquots) were withdrawn from the receiver compartment (basolateral side). Following withdrawal of each aliquot, the compartment was replenished with the same volume of a blank buffer to maintain the volume constant. Concentrations of lucifer yellow or propranolol were measured respectively by fluorescence spectrophotometry or HPLC.

Apparent permeability coefficient was computed using the following equation:

$$P_{app} = \frac{(dC/dt) \times V}{A \times C},$$

where dC/dt is the change in concentration in the receiver compartment per unit time, V is the volume of the receiver compart-

ment, A is the area available for transport, and C is the initial concentration of the donor compartment.

The permeability of individual tissue preparation was calculated from the slope of the plot of marker concentration (in the receiver compartment) versus time within the range of 30–120 min. Excellent linearity ($r^2 = 0.95$–0.99; $n = 4$–6) was observed within this range in all cases.

2.6. Analytical methods

Samples of propranolol were analyzed isocratically by HPLC at ambient temperature using a Hypersil® BDS C18 reversed phase column (5 μm, 250 mm × 4.6 mm i.d., Thermo Hypersil Ltd., Chesire, UK), a Hypersil® BDS C18 guard column (5 μm, 10 mm × 4.6 mm i.d., Thermo Hypersil Ltd., Chesire, UK) and a Waters 2695 LC system equipped with a Waters 996 photodiode array detector and an autosampler (Waters, MA, USA). The mobile phase consisting of 30% acetonitrile and 70% 50 mM phosphate buffer (adjusted to pH 3 by concentrated phosphoric acid) was eluted at 1 ml/min. Twenty-microlitre samples were injected onto the column. UV detection was set at 230 nm. All calibration curves constructed with standard propranolol solutions within the appropriate concentration range displayed excellent linearity with r^2 values greater than 0.999. The intra-day and inter-day RSDs ($n = 4$) were within 6.6%.

Samples of lucifer yellow were measured by fluorescence spectrophotometry at excitation (E_x) wavelength of 424 nm and emission (E_m) wavelength of 525 nm.

2.7. Data analysis

All experiments were done in at least triplicate and the data were expressed as mean ± S.D. The permeability data for the control and various treatment groups were analyzed by ANOVA and the Post Hoc (Tukey and Dunnett) tests. A minimum p-value of 0.05 was used as the significance level for the tests.

3. Results and discussion

3.1. Effect of β-CDs on osmolarity and electrical parameters

Fig. 1 shows that the osmolarities of the β-CD solutions increased linearly with increasing concentration (0.5%–10%) of HP-β-CD/SBE-β-CD ($r^2 > 0.99$). The osmolarities of all test β-CD solutions (1% β-CD, 1% HP-β-CD or 1.48% SBE-β-CD in KBR solution) were within the range of 280–310 mOsm, which is isosmotic with intracellular fluids. Maintenance of isosmotic conditions during the transport study is considered critical, as it has been demonstrated with an in vivo rat perfusion model that an increase in luminal fluid osmolarity from 300 to 600 mOsm resulted in a decrease in net water flux and a concomitant decrease in the permeability of the paracellular marker, mannitol, across mucosal membrane in both jejunum and colon (Krugliak et al., 1994).

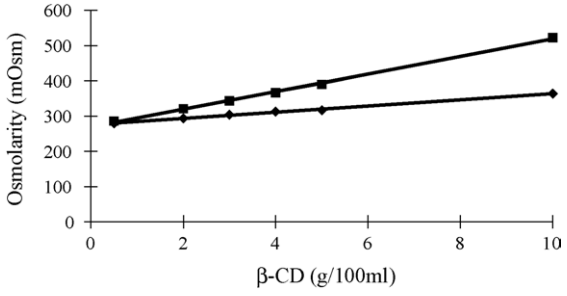

Fig. 1. Osmolarities of different concentrations of HP-β-CD or SBE-β-CD in KBR solution at room temperature. Key: (♦) HP-β-CD; (■) SBE-β-CD.

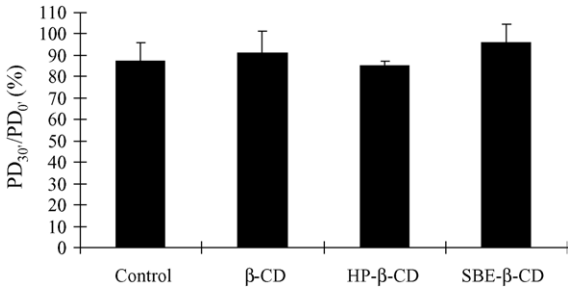

Fig. 2. Changes in potential difference (PD) after pre-incubation with 1% β-CD, 1% HP-β-CD or 1.48% SBE-β-CD for 30 min in Ussing Chamber ($n = 4$ or 12).

As shown in Fig. 2, the potential difference (PD) of the tissues was not significantly altered ($p > 0.05$) by pre-incubation with all three β-CDs for 30 min, indicating that these β-CDs at the concentrations employed exert no apparent disruptive effect on the tight junction. To verify the functionality of the tissue mounted in the Ussing Chamber, the tissue was incubated with EDTA, which serves to open the tight junction by chelating with extracellular Ca^{2+}, thereby increasing the permeation of compounds through the paracellular route (Chung et al., 1998). As depicted in Fig. 3, the PD of the tissue was reduced dramatically to nearly zero when being incubated with 5 mM EDTA for 30 min while the PD of the control tissue (i.e. without EDTA) remained constant.

The above results are in good agreement with those obtained from previous tissue morphological studies with β-CDs in rats. These studies did not reveal any significant tissue damage by histological examination when the rat intestinal mucosa was treated in situ with 10 mM α-CD/β-CD (Nakanishi et al., 1992) or 10% HP-β-CD/DM-β-CD (Shao et al., 1994) and when the rat nasal mucosa was exposed in vivo to 1.5% β-CD or up to

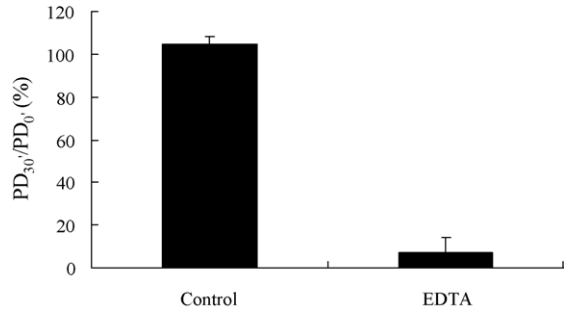

Fig. 3. Changes in potential difference (PD) after incubation with 5 mM EDTA for 30 min in Ussing Chamber ($n = 3$).

20% HP-β-CD (Asai et al., 2002). The lack of mucosal toxicity of cyclodextrins has been ascribed to their inability to actively penetrate the membrane bilayers (Shao et al., 1994).

3.2. Transport study

Apart from direct morphological observations and electrical measurements on tissues, the permeability of marker compounds is another important parameter for indirect assessment of the membrane integrity. Thus, the effects of the three β-CDs on the permeabilities of lucifer yellow (paracellular marker) and propranolol (transcellular marker) from the apical to basolateral side of rat intestinal epithelium were evaluated.

Figs. 4 and 5 show the effects of co- and pre-incubation with the β-CDs on the amount of propranolol transported across rat ileum over a period of 90 min. Apparent permeability coefficients (P_{app}) calculated from the slopes of the plots of propranolol concentration in the receiver compartment against time within the linear range from 30 min onward ($r^2 = 0.95$–0.99; $n = 4$–6) are presented in Table 1. The results indicate that co-incubation with 1% β-CD or 1% HP-β-CD caused no significant changes in the P_{app} of propranolol ($p > 0.05$) while the presence of 1.48% SBE-β-CD reduced the P_{app} of propranolol from $(1.71 \pm 0.44) \times 10^{-5}$ to $(0.19 \pm 0.04) \times 10^{-5}$ cm/s ($p < 0.05$).

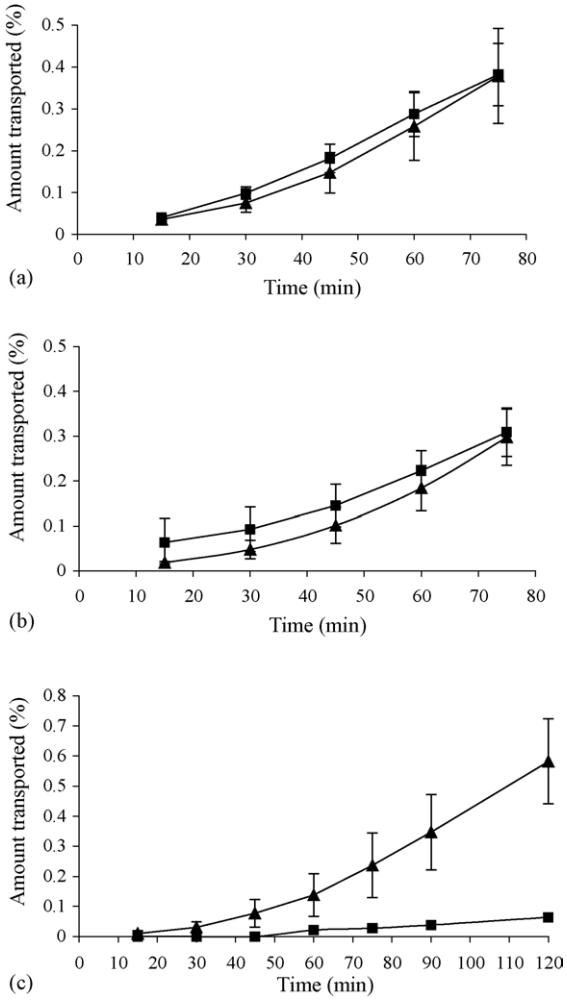

Fig. 4. Effect of co-incubation with 1% β-CD (a), 1% HP-β-CD (b) or 1.48% SBE-β-CD (c) on the amount of propranolol transported across rat ileum in Ussing Chamber ($n = 3$ or 4). Key: (■) with selected β-CD; (▲) control. Data show no statistically significant differences between the control and treatment groups in all cases except SBE-β-CD at the 5% level.

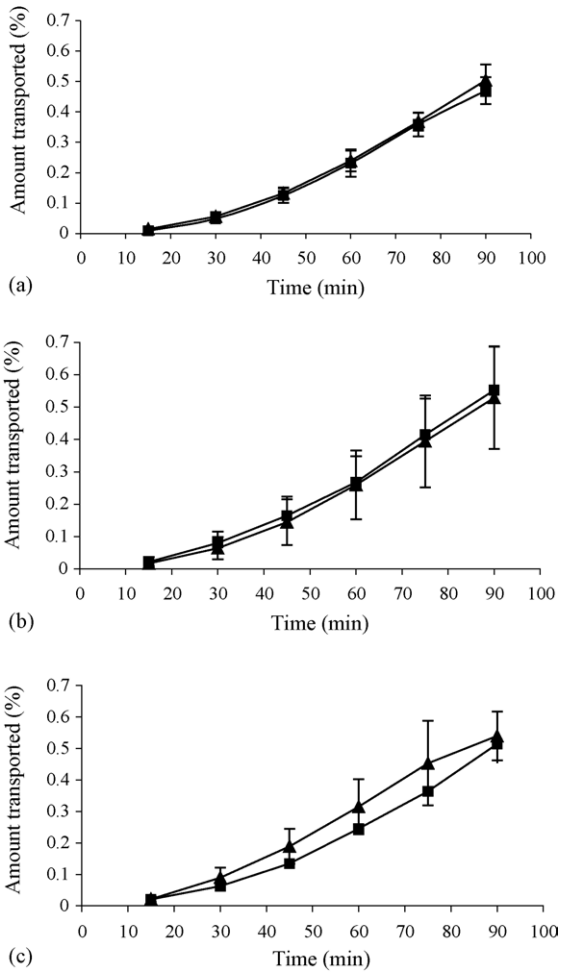

Fig. 5. Effect of pre-incubation with 1% β-CD (a), 1% HP-β-CD (b) or 1.48% SBE-β-CD (c) on the amount of propranolol transported across rat ileum in Ussing Chamber ($n = 3$ or 4). Key: (■) with selected β-CD; (▲) control. Data show no statistically significant differences between the control and treatment groups in all cases at the 5% level.

To clarify the roles of the various β-CDs in the transport process, the transport experiments were repeated by pre-incubating the tissues with the β-CDs at the same concentrations as before for 30 min prior to permeability determination in the absence of

Table 1
Permeability coefficients of propranolol across rat ileum in Ussing Chamber determined in KBR solution by pre- or co-incubation with β-CDs ($n = 3$ or 4)

Co-incubation	P_{app} ($\times 10^5$) (cm/s)	Pre-incubation	P_{app} ($\times 10^5$) (cm/s)
Control	1.71 ± 0.44	Control	2.10 ± 0.48
1% β-CD	1.67 ± 0.41	1% β-CD	1.88 ± 0.20
1% HP-β-CD	1.35 ± 0.07	1% HP-β-CD	2.08 ± 0.56
1.48% SBE-β-CD	0.19 ± 0.04[a]	1.48% SBE-β-CD	1.98 ± 0.35

[a] Significant compared with the control and other treatment groups ($p < 0.05$).

the β-CDs. No significant effect on the permeability of propranolol ($p > 0.05$) could be observed compared with the control group for all three β-CDs (Fig. 5; Table 1), suggesting that these β-CDs exert no direct effect on the membrane permeability.

It has been reported that β-CDs (including β-CD, HP-β-CD and SBE-β-CD) are capable of forming inclusion complexes with propranolol (Xie et al., 1997; Plätzer et al., 1999), and the complexation displays chiral selectivity which has been exploited in the separation of the drug enantiomers by capillary electrophoresis (Xie et al., 1997; Pak et al., 1998). In the present study, the marked decrease in propranolol permeability observed with SBE-β-CD may be explained by the ability of SBE-β-CD to form a relatively stable complex with propranolol under the stated experimental conditions.

It is generally known that only the free form but not the complex can transport across cell membrane (Loftsson and Brewster, 1996). If the concentration of free propranolol in the donor compartment was significantly reduced through complexation with SBE-β-CD, the use of total (instead of free) propranolol concentration in the calculation of P_{app} would lead to an underestimation of P_{app}. When the exposure of the tissue to β-CDs was limited only to the pre-incubation phase, complex formation involving β-CDs would not be possible, and hence there would be no apparent decrease in the P_{app} of propranolol.

It is important to note that while the complexation with SBE-β-CD caused an apparent reduction in permeability of propranolol across rat intestinal cells (due to non-absorbability of the complexed form) in the present in vitro study, its implication in the in vivo absorption of propranolol or other guest compounds via the same transport route in the gastrointestinal tract is expected to be very limited. This is because the complex is in dynamic equilibrium with the free form, and the rapid dissociation of the complex upon dilution with a large volume of fluids in the small intestine (Rajewski and Stella, 1996) will favor rapid absorption of the free form, which further drives the equilibrium in the direction of increasing free form concentration and thus allows more guest compound to be absorbed.

Also worth noting is that the concentrations of β-CDs (i.e. 1 g/100 ml β-CD, 1 g/100 ml HP-β-CD and 1.48 g/100 ml SBE-β-CD) selected for the present study are guided by osmolarity consideration (see Section 3.1) and are comparable to those used in other in vitro permeability studies of a similar theme employing the Caco-2 cell monolayer model (Hovgaard and Brønsted, 1995; Tötterman et al., 1997). These concentrations are much higher than what would normally be utilized in formulation for improving peroral drug absorption. Since no apparent permeation-enhancing effects have been observed with β-CDs at such high concentrations in vitro, it is unlikely that these β-CDs at their normal applied concentrations will have any significant bearing on the intestinal permeability in vivo.

To investigate whether the various β-CDs can alter drug transport via the paracellular route, the tissue was co-incubated with the β-CDs and lucifer yellow. No significant effect on the permeability of lucifer yellow ($p > 0.05$) was observed (Fig. 6; Table 2). However, co-incubation with EDTA (5 mM) on either side of the tissue enhanced the P_{app} of lucifer yellow from $(2.78 \pm 0.64) \times 10^{-6}$ to $(13.5 \pm 0.58) \times 10^{-6}$ cm/s, as shown in

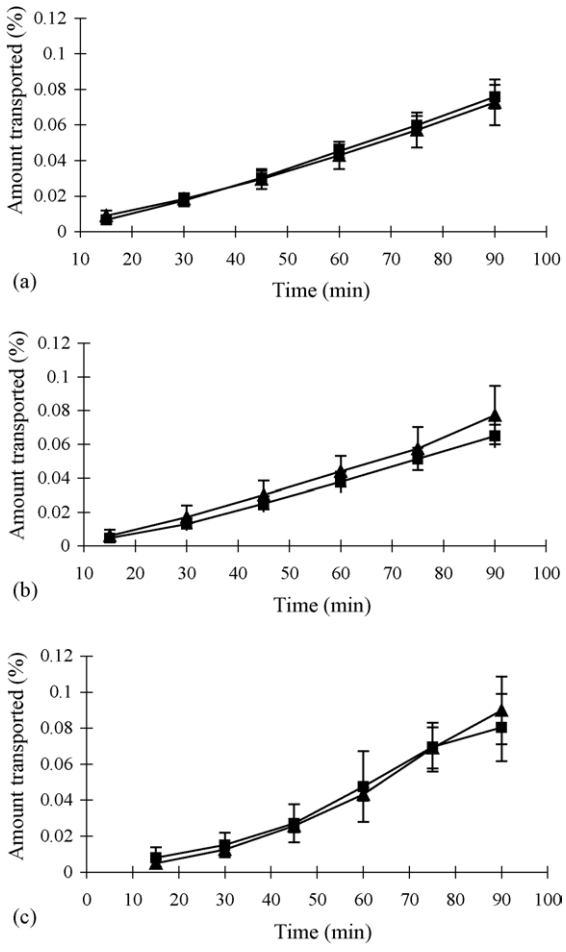

Fig. 6. Effect of co-incubation with 1% β-CD (a), 1% HP-β-CD (b) or 1.48% SBE-β-CD (c) on the amount of lucifer yellow transported across rat ileum in Ussing Chamber ($n = 3$ or 4). Key: (■) with selected β-CD; (▲) control. Data show no statistically significant differences between the control and treatment groups in all cases at the 5% level.

Fig. 7 and Table 2. In the control experiments (i.e. without EDTA and β-CDs), the P_{app} of lucifer yellow obtained was of the order of 10^{-6} cm/s (Table 2), which was ten times less than that of the transcellular marker, propranolol (Table 1). All these results suggest that the viability and tight junction of the ileum mounted inside the Ussing Chamber were well maintained.

The above observations are consistent with those reported in previous studies using the Caco-2 cell monolayer model to evaluate the effects of cyclodextrins on paracellular transport.

Table 2
Permeability coefficients of lucifer yellow across rat ileum in Ussing Chamber determined in KBR solution by co-incubation with β-CDs ($n = 4$) or 5 mM EDTA ($n = 3$)

Co-incubation	P_{app} ($\times 10^6$) (cm/s)
Control	2.78 ± 0.64
1% β-CD	2.56 ± 0.33
1% HP-β-CD	2.28 ± 0.21
1.48% SBE-β-CD	3.02 ± 0.69
5 mM EDTA	13.5 ± 0.58[a]

[a] Significant compared with the control and other treatment groups ($p < 0.05$).

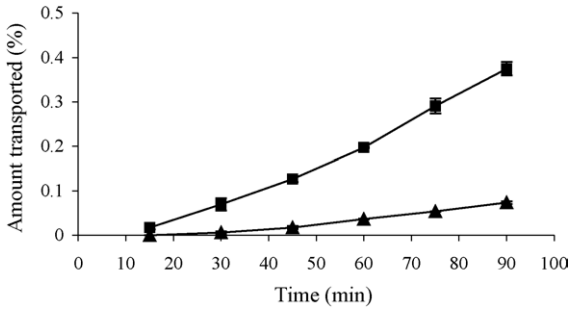

Fig. 7. Effect of co-incubation with EDTA (5 mM) on the amount of lucifer yellow transported across rat ileum in Ussing Chamber ($n = 3$). Key: (■) with EDTA; (▲) control. Data show statistically significant differences between the control and treatment groups at the 5% level.

Of all the cyclodextrins studied, only DM-β-CD (5%) caused a significant increase in cell permeability when being tested with the paracellular marker, polyethylene glycol 4000, whereas the other β-CDs (i.e. 1.8% β-CD and 5% HP-β-CD) had no noticeable effect (Hovgaard and Brønsted, 1995). Similarly, it has been shown employing mannitol as the paracellular marker that parent β-CD displayed no permeability enhancement by pre- or co-incubation with the Caco-2 cell monolayer or by varying transepithelial electrical resistance (Ono et al., 2001). Cytotoxcity against the Caco-2 cells has also been observed with DM-β-CD, but not with HP-β-CD and SBE-β-CD (Tötterman et al., 1997). All these previous findings based on the Caco-2 cell model together with the present results obtained with the rat tissue model strongly suggest that all three β-CDs (β-CD, HP-β-CD and SBE-β-CD) do not disrupt the epithelial integrity, and can be safely used in pharmaceutical formulation.

4. Conclusion

The present study using the Ussing Chamber in vitro model showed that all three β-CDs (β-CD, HP-β-CD, and SBE-β-CD) exert no significant impact on both transcellular and paracellular pathways of intestinal absorption, suggesting that the β-CDs serve as carriers rather than permeability enhancers for drugs administered via the peroral route.

Acknowledgment

Financial support from the Chinese University of Hong Kong (postgraduate studentship for YZ) is gratefully acknowledged.

References

Anderberg, E.K., Artursson, P., 1993. Cell cultures to assess drug absorption enhancement. In: A. (Bert) G de Boer (Ed.) Drug Absorption Enhancement: Concepts, Possibilities, Limitations and Trends, vol. 3, Harwood Academic Publishers, pp. 101–118.

Artursson, P., 1990. Epithelial transport of drugs in cell culture. I: a model for studying the passive diffusion of drugs over intestinal absorptive (Caco-2) cells. J. Pharm. Sci. 79, 476–482.

Asai, K., Morishita, M., Katsuta, H., Hosoda, S., Shinomiya, K., Noro, M., Nagai, T., Takayama, K., 2002. The effect of water-soluble cyclodextrins on the histological integrity of the rat nasal mucosa. Int. J. Pharm. 246, 25–35.

Chung, Y.B., Han, K., Nishiura, A., Lee, V.H., 1998. Ocular absorption of Pz-peptide and its effect on the ocular and systemic pharmacokinetics of topically applied drugs in the rabbit. Pharm. Res. 15, 1882–1887.

Dowty, M.E., Dietsch, C.R., 1997. Improved prediction of in vivo peroral absorption from in vitro intestinal permeability using an internal standard to control for intra- and inter-rat variability. Pharm. Res. 14, 1792–1797.

Haeberlin, B., Gengenbacher, T., Meinzer, A., Fricker, G., 1996. Cyclodextrins—useful excipients for oral peptide administration? Int. J. Pharm. 137, 103–110.

Hovgaard, L., Brønsted, H., 1995. Drug delivery studies in Caco-2 monolayers. I.V. Absorption enhancer effects of cyclodextrins. Pharm. Res. 12, 1328–1332.

Krugliak, P., Hollander, D., Schlaepfer, C.C., Nguyen, H., Ma, T.Y., 1994. Mechanisms and sites of mannitol permeability of small and large intestine in the rat. Dig. Dis. Sci. 39, 796–801.

Loftsson, T., Brewster, M.E., 1996. Pharmaceutical applications of cyclodextrins. 1. Drug solubilization and stabilization. J. Pharm. Sci. 85, 1017–1025.

Miyazawa, I., Ueda, H., Nagase, H., Endo, T., Kobayshi, S., Nagai, T., 1995. Physicochemical properties and inclusion complex formation of γ-cylcodextrin. Eur. J. Pharm. Sci. 3, 153–162.

Nakanishi, K., Nadai, T., Masada, M., Miyajima, K., 1992. Effect of cyclodextrins on biological membrane. II. Mechanism of enhancement on the intestinal absorption of non-absorbable drug by cyclodextrins. Chem. Pharm. Bull. 40, 1252–1256.

Ono, N., Arima, H., Hirayama, F., Uekama, K., 2001. A moderate interaction of maltosyl-α-cyclodextrin with Caco-2 cells in comparison with the parent cyclodextrin. Biol. Pharm. Bull. 24, 395–402.

Pak, C., Marriot, P.J., Carpenter, P.D., Amiet, R.G., 1998. Enantiomeric separation of propranolol and selected metabolites by using capillary electrophoresis with hydroxypropyl-beta-cyclodextrin as chiral selector. J. Chromatogr. A. 793, 357–364.

Plätzer, M., Schwarz, M.A., Neubert, R.H.H., 1999. Determination of formation constants of cyclodextrin inclusion complexes using affinity capillary electrophoresis. J. Microcolumn Separations 11, 215–222.

Polentarutti, B.I., Peterson, A.L., Sjöberg, K., Anderberg, E.K.I., Utter, L.M., Ungell, A.L.B., 1999. Evaluation of viability of excised rat intestinal segments in the Ussing Chamber: investigation of morphology, electrical parameters, and permeability characteristics. Pharm. Res. 16, 446–454.

Rajewski, R.A., Stella, V.J., 1996. Pharmaceutical applications of cyclodextrins. 2. In vivo drug delivery. J. Pharm. Sci. 85, 1142–1169.

Shaker, D.S., Ghanem, A.H., Li, S.K., Warner, K.S., Hashem, F.M., Higuchi, W.I., 2003. Mechanistic studies of the effect of hydroxypropyl-β-cyclodextrin on in vitro transdermal permeation of corticosterone through hairless mouse skin. Int. J. Pharm. 253, 1–11.

Shao, Z., Li, Y., Chermak, T., Mitra, A.K., 1994. Cyclodextrins as mucosal absorption promoters of insulin. II. Effects of β-cyclodextrin derivatives on α-chymotryptic degradation and enteral absorption on insulin in rats. Pharm. Res. 11, 1174–1179.

Shen, W.C., Lin, Y.J., 1994. Basic mechanisms in transepithelium transport enhancement. In: Hsieh, D.S. (Ed.), Drug Permeation Enhancement: Theory and Applications, vol. 3. Marcel Dekker, Inc., pp. 25–40.

Stella, V.J., Rajewski, R.A., 1997. Cyclodextrins: their future in drug formulation and delivery. Pharm. Res. 14, 556–567.

Tötterman, A.M., Schipper, N.G.M., Thompson, D.O., Mannermaa, J.P., 1997. Intestinal safety of water-soluble β-cyclodextrins in paediatric oral solutions of spironolactone: effects on human intestinal epithelial Caco-2 cells. J. Pharm. Pharmacol. 49, 43–48.

Uekama, K., Hirayama, F., Irie, T., 1998. Cyclodextrin drug carrier systems. Chem. Rev. 98, 2045–2076.

Xie, G., Skanchy, D.J., Stobaugh, J.F., 1997. Chiral separations of enantiomeric pharmaceuticals by capillary electrophoresis using sulphobutyl ether β-cyclodextrin as isomer selector. Biomed. Chromatogr. 11, 193–199.