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Effects of superporous hydrogels on paracellular drug permeability and cytotoxicity studies in Caco-2 cell monolayers

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Abstract

The aim of this study was to evaluate the effect of superporous hydrogel (SPH) and SPH composite (SPHC) as permeation enhancers for peptide drug delivery on Caco-2 cell monolayers. Moreover, the cytotoxic effects of these polymers were also studied using trypan blue test, MTT assay and propidium iodide staining. Transepithelial electrical resistance (TEER) studies revealed that both SPH and SPHC polymers were able to decrease TEER values to about 40% of initial values, indicating the ability of these polymers to open tight junctions. Recovery studies of TEER showed that the effects of polymers on Caco-2 cell monolayers were reversible, indicating viability of the cells after incubation with polymers. Both polymers were able to enhance the transport of the hydrophilic marker ^{14}C-mannitol up to 2.7 and 3.8-fold in comparison to the control group. The cumulative transport of fluorescein isothiocyanate labelled dextrans with a molecular weight of 4400 Da (FD4) and 19 600 Da (FD20) was enhanced by SPH and SPHC polymers by opening of tight junctions; however, this enhancement was inversely proportional to the molecular weight of marker compounds. Cytotoxicity studies confirmed that the transport enhancing properties of SPH and SPHC polymers were not caused by damage of the Caco-2 cell monolayers. The cells were able to exclude trypan blue as well as propidium iodide after incubation with SPH and SPHC polymers. MTT assay showed that the number of viable cells was higher than 95% after incubation with SPH and SPHC polymers. This indicates that the mitochondrial metabolic activities of the cells were preserved after application of the polymers. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Superporous hydrogels (SPH); SPH composite; Caco-2 cells; Cytotoxicity; Paracellular drug transport

1. Introduction

The intestinal absorption of peptide drugs is mainly limited by enzymatic degradation and low permeability of the intestinal epithelium. The in-

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testinal epithelium regulates the passage of natural compounds such as nutrients and foods, and serves as a barrier for paracellular passive transport of large hydrophilic molecules. This absorption barrier is composed of a single layer of columnar epithelial cells joined at the apical surface by tight junctional complex (Sakai et al., 1997; Artursson, 1990). The junctional complex forms a continuous seal, which segregates the apical and basolateral membrane compounds and conveys size and charge selectivity properties due to the fixed negative charges on the tight junction. Moreover, the stereochemical structure of hydrophilic compounds contributes to their selectivity for paracellular permeability (Pauletti et al., 1996; Daugherty and Mrsny, 1999). In order to improve the absorption of these poor permeability hydrophilic compounds, the junctional complex has to be altered in order to open the intestinal paracellular barrier. One strategy to overcome the permeability barrier is co-administration of hydrophilic drugs with permeation/absorption enhancers. There are several classes of absorption enhancers which have been investigated so far: surfactants (e.g. sodium lauryl sulphate), chelating agents (e.g. EDTA, salicylates), bile salts (e.g. sodium deoxycholate), fatty acids (e.g. oleic acid), non-surfactants (e.g. unsaturated cyclic urea), and polymers (e.g. polyacrylates, chitosans) (Meaney and O'Driscoll, 2000; Lindmark et al., 1998; Lee et al., 1991; Borchard et al., 1996). There are three major criteria for evaluating absorption enhancers: (1) effectiveness of the enhancers; (2) cytotoxicity effects; (3) mechanism(s) by which drug absorption is enhanced (Aungst et al., 1996). Co-administration of drugs with an absorption enhancer is believed to improve the transport of hydrophilic macromolecules via the paracellular pathway. However, in some cases application of enhancers causes cytotoxicity in vitro and in vivo, assuming that the improved permeability is mediated by mucosal membrane damage. For instance, sodium dodecyl sulphate (SDS), a well-known pharmaceutical wetting agent, could enhance the permeability of hydrophilic markers; however, SDS causes severe damage in Caco-2 cell monolayers (Anderberg and Artursson, 1993). Chitosan with a low degree of acetylation showed to be an effective drug absorption enhancer at low and high molecular weights and showed no toxic side effects (Schipper et al., 1997). Carbomer (0.5%) is also used as an absorption enhancer and opens the tight junctions by lowering the extracellular calcium concentration (Borchard et al., 1996). Consequently, the ideal absorption enhancer needs to improve the intestinal absorption of drugs without causing cell membrane damage (Quan et al., 1998).

Superporous hydrogels (SPH) and SPH composite (SPHC) are a new class of hydrogels which swell very quickly due to their highly porous structure. The differences between SPH and SPHC are found in their swelling ratios and mechanical stability. SPH has a higher swelling ratio, but is mechanically less stable than SPHC (Dorkoosh et al., 2000). Although mucoadhesive properties of SPH/SPHC polymers can not entirely be ruled out, the mechanism of increasing the retention of these systems depends on the mechanical fixation by swelling of the polymers (Dorkoosh et al., 2001). Mechanical pressure produced by swelling of SPH and SPHC polymers is suggested to cause the opening of intercellular tight junctions (Dorkoosh et al., 2002).

The present study was aimed at evaluating the potential of SPH and SPHC polymers to open the tight junctions by monitoring the transepithelial electrical resistance (TEER) and quantifying the permeability of hydrophilic model compounds (^{14}C-mannitol and fluorescently labelled dextrans) across Caco-2 cell monolayers. Carbomer is used in these studies as a positive control due to the fact that the chemical structure of carbomer is also made of acrylic acid derivatives which are the same as superporous hydrogel polymers (Borchard et al., 1996; Dorkoosh et al., 2000). Additionally, the cytotoxic effects of SPH and SPHC polymers were investigated using trypan blue, MTT assay and propidium iodide staining.

2. Materials and methods

2.1. Materials

^{14}C-mannitol (MW 182.2 Da; specific activity

0.2 μCi/ml) was obtained from Amersham Life Sciences (Little Chalfort, UK). FD4 (fluorescein isothiocyanate-dextran, MW 4400 Da), FD20 (MW 19 600 Da), thiazolyl blue (MTT) and trypan blue were purchased from Sigma-Aldrich Chemie (Steinheim, Germany). Propidium iodide was obtained from Molecular Probes (Leiden, The Netherlands). Carbomer (C934P) was donated by BF Goodrich (Cleveland, OH). SPH and SPHC were synthesized in our laboratory as described previously (Dorkoosh et al., 2000). All other chemicals were of analytical grade and were used as received.

2.2. Caco-2 cell cultures

The colonic adenocarcinoma cell line, Caco-2, was obtained from the American Type Culture Collection (Rockville, MD). The cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) (Life Technologies, Gibco BRL, UK) containing 10% v/v heat denaturated foetal calf serum (FCS), 1% v/v nonessential amino acids, 160 U/ml benzylpenicillin and 100 U/ml streptomycin (Sigma, St. Louis, MO). The cells were maintained at 37 °C in an atmosphere of 95% air and 5% CO_2 at 90% relative humidity. The cells were grown in 25 cm^2 culture flasks, the medium was changed every other day and the cells were trypsinized once per week. Caco-2 cells of passage numbers 84–94 were used in these experiments.

2.3. Transport studies

For transport studies the Caco-2 cells were cultured on porous polycarbonate filter membranes with a pore size of 0.4 μm and a surface area of 4.7 cm^2 in clusters of 6 wells (Costar Transwell®, Badhoevedorp, The Netherlands). When the cells were trypsinized, they were seeded at a density of 10^4 cells/cm^2 onto each filter. These cells were maintained at 37 °C in an atmosphere as described above. The medium was replaced every second day for 3 weeks. On the day of the transport experiments, the culture medium was replaced with an equal volume of Hank's balanced salt solution (HBSS) buffered with 30 mM HEPES at pH 7.2 (transport medium) and the cells were allowed to equilibrate for 1 h. The TEER was measured following equilibration using a Milicell® ERS meter connected to a pair of chopstick electrodes to ensure the integrity of the monolayers formed on the filters. TEER measurements were also performed during the experiment in order to check the effect of polymers on opening of the tight junctions at time intervals of 0 (this is 10 min after adding the polymers), 5, 15, 30, 60, 90, 120, 180, 240 min. In case of permeability studies using ^{14}C-mannitol across Caco-2 cell monolayers, firstly 10 mg SPH or 20 mg SPHC were applied apically (since the swelling ratio of SPH is higher than SPHC, it was necessary to use 20 mg SPHC polymer versus 10 mg of SPH polymer in order to cover the whole surface of the Caco-2 cell monolayers). After complete swelling of the polymers (10 min), 1.5 ml of the radioactive marker ^{14}C-mannitol solution in HBSS-HEPES (4 mmol/l, with a specific activity of 0.2 μCi/ml) was added on the apical side. For transport of FD4 and FD20 across the Caco-2 cell monolayers, 1.5 ml of FD4 or FD20 solution at concentrations of 1 mg/ml were added to the apical chamber. In all experiments 1 ml carbomer (C934P) solution (0.5% w/v) was used as a positive control, and in case of negative control no polymer was applied to the monolayers. Samples of 200 μl were withdrawn from the basolateral chamber at predetermined time intervals of 0, 5, 15, 30, 60, 90, 120, 180, 240 min and replaced with equal volumes of fresh HBSS-HEPES. After completion of the transport studies, the polymers were removed carefully and monolayers were rinsed with HBSS-HEPES and culture medium was applied on the monolayers. The monolayers were allowed to regenerate for 2 days at 37 °C in an atmosphere of 95% air and 5% CO_2 at 90% relative humidity. TEER was monitored at 5, 6, 24 and 48 h during the recovery period.

Apparent permeability (P_{app}) for each substance were calculated according to the following equation:

$$P_{app} = \frac{dQ}{dt}\frac{1}{AC_0 60} \quad (1)$$

where P_{app} is the apparent permeability (cm/s), dQ/dt is the permeability rate (amount permeated per minute during the whole period of 240 min), A is the diffusion area of the monolayers (cm^2), and C_0 is the initial concentration of the compounds studied. Statistical differences were calculated using one way ANOVA at a significant level of $P < 0.05$.

2.4. Analytical procedures

Samples of ^{14}C-mannitol were mixed with 3 ml scintillation cocktail (Ultima Gold, Groningen, The Netherlands) and the amount of radioactive marker transported at each time interval was determined by the liquid scintillation counter Tri-Carb 1500 from Packard Instrument (Meridan, USA).

FD4 and FD20 were analyzed using HPLC equipped with a fluorescence detector. The column (8 × 300 mm) was Suprema 30 (Polymer Standard Service, Mainz, Germany). Isocratic elution was performed with 0.05 M ammonium acetate buffer at pH 9.0 (adjusted with ammonia) containing 10% acetonitrile at a flow rate of 1 ml/min, and the injection volume was 50 μl. The excitation wavelength was 488 nm and the emission wavelength 520 nm. Both FD4 and FD20 were detected at a retention time of 6–7 min. The detection limit for FD4 and FD20 was 25 and 35 ng/ml, respectively.

2.5. Cytotoxicity studies

2.5.1. Trypan blue test

After completion of FD4 and FD20 transport experiments, the polymers were removed carefully. Both sides of Caco-2 cell monolayers were rinsed with HBSS buffered with 30 mM HEPES at pH 7.20. Subsequently, Caco-2 cell monolayers were incubated apically with 0.1% trypan blue solution in PBS (0.01 M) at 37 °C. The basolateral medium was replaced by PBS. After 30 min incubation, the dye was removed and monolayers were rinsed with PBS. Cells incubated for 5 min with 0.5% SDS solution in PBS and stained with trypan blue were used as positive control for cell membrane damage. Thereafter, monolayers were examined by light microscopy for dye exclusion. The intact monolayers showed no inclusion of dye, whereas the damaged cells showed dye inclusion.

2.5.2. MTT assay

MTT is a tetrazolium salt which is cleaved to a dark blue product by mitochondrial dehydrogenases in living but not in dead cells (Mossman, 1983). Caco-2 cells used for MTT assay were seeded onto 96 well culture plates at seeding density of 5×10^4 cells per well in DMEM culture medium. The cells were cultured in an atmosphere of 95% air and 5% CO_2 at 37 °C and 90% humidity for 48 h. Subsequently, culture medium was replaced with 150 μl HBSS-HEPES and 2 mg SPH or 5 mg SPHC was added to the wells. As negative control no polymer was added to the wells, and as an internal reference, 0.5% carbomer in HBSS-HEPES was applied to the cells. After adding the polymers, Caco-2 cells were further incubated at 37 °C for 4 h. Thereafter the polymers were removed; 100 μl MTT solution (5 mg/ml in PBS) was added to each well and the cells were incubated for another 4 h at 37 °C. The reaction product was then solubilized in 100 μl DMSO before quantifying the colour of reaction product using a plate reader (Bio-Rad, Herts, UK) at 590 nm.

2.5.3. Propidium iodide staining

Caco-2 cell monolayers were incubated with SPH and SPHC polymers and without any polymer (negative control) for 4 h in HBSS-HEPES. Thereafter, polymers were removed carefully. Both sides of the Caco-2 cell monolayers were rinsed with HBSS-HEPES and a solution of propidium iodide (50 μg/ml) in PBS was applied apically for 5 min. Then the dye was removed and monolayers were rinsed with PBS to eliminate the excess of the dye. The support filter with monolayers was cut carefully from the plastic insert and sandwiched between two coverslips and then mounted on a heating microscope stage (37 °C) of a confocal laser scanning microscope (CLSM).

A MRC-600 Lasersharp system (Bio-rad Laboratories, Richmond, CA) linked to a Zeiss IM 35 inverted microscope (Carl Zeiss, Oberkochen, Germany) with an objective of the magnification of 63 × was used to visualize the monolayers. Propidium iodide was detected at the excitation wavelength of 514 nm and emission of 617 nm. Cells excluding propidium iodide were considered to be viable (Anderberg and Artursson, 1993). Cells incubated with a 0.1% solution of SDS in PBS for 10 min were used as a positive control.

3. Results

3.1. Effect of polymers on the TEER of Caco-2 cell monolayers

The effect of SPH and SPHC polymers on opening of intestinal tight junctions was studied by measuring TEER values of the Caco-2 cell monolayers. The results are shown in Fig. 1A and are expressed as percentage of the initial values at $t = 0$ min. It is obvious that the effect of SPH and SPHC polymers on the epithelial integrity was

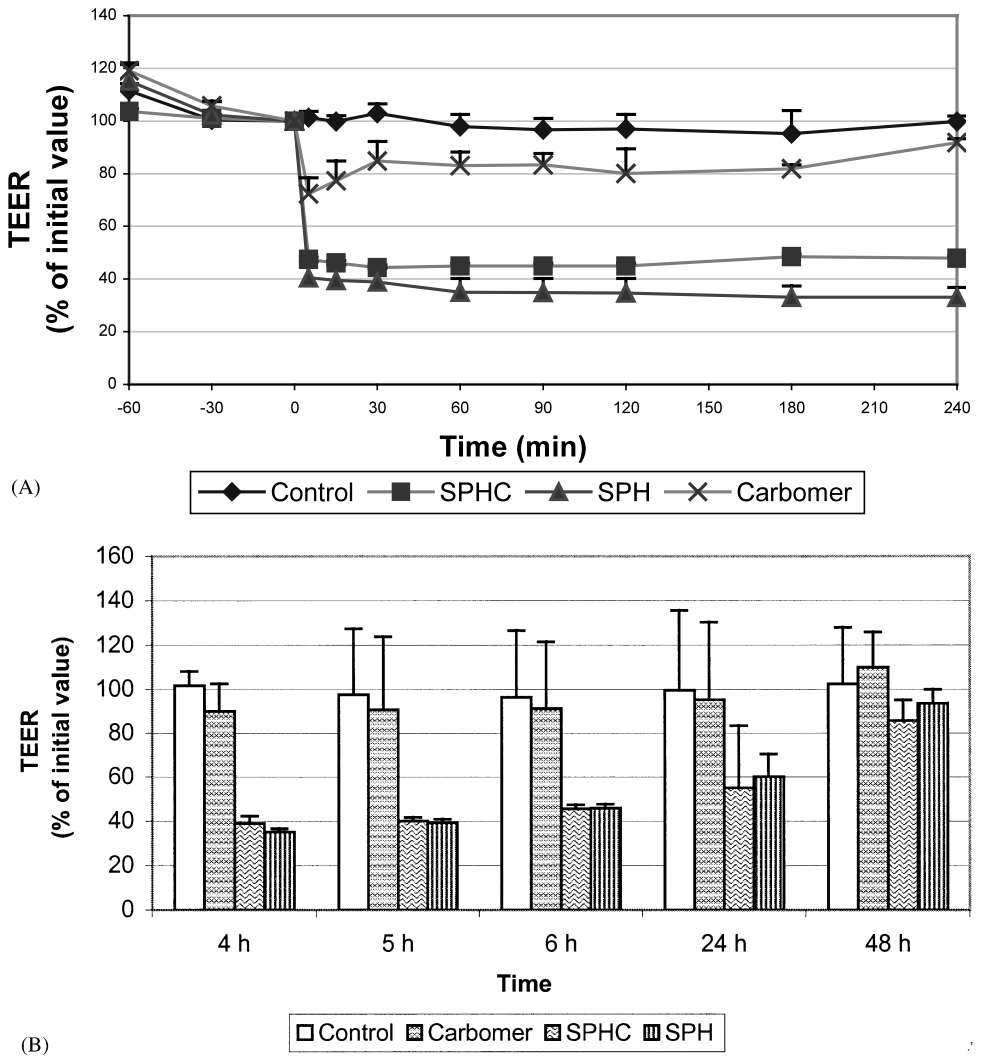

Fig. 1. (A) Effects of polymers on TEER of Caco-2 cell monolayers; (B) TEER recovery of Caco-2 cell monolayers after removing the polymers. Data are expressed as mean ± S.D. of three to five experiments.

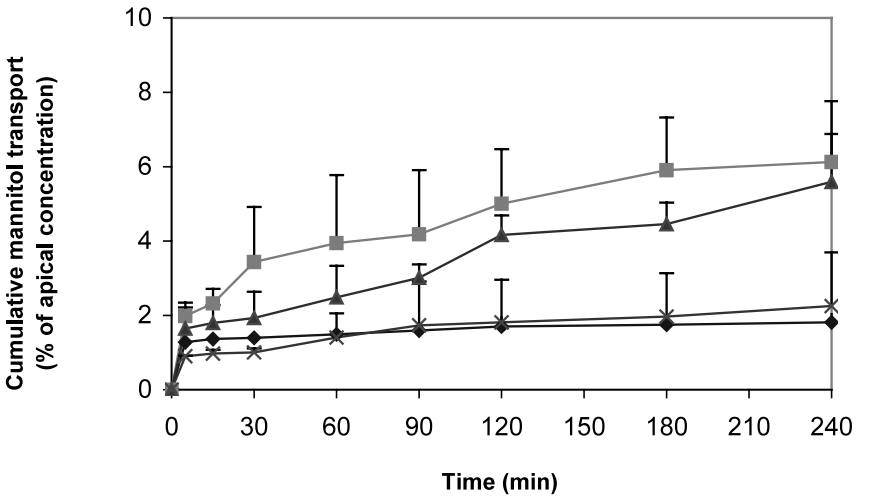

Fig. 2. Cumulative transport of ^{14}C-mannitol (MW 182.2 Da) across Caco-2 cell monolayers; (◆) control, (■) 20 mg SPHC, (▲) 10 mg SPH, (×) 0.5% carbomer. Data are expressed as mean ± S.D. of three to five experiments.

Table 1
Apparent permeability (P_{app}) for ^{14}C-mannitol, FD4 and FD20 transport across Caco-2 cell monolayers (mean ± S.D. of 3–5 experiments)

Polymer	^{14}C-mannitol (MW 182.2) $P_{app} \times 10^{-7}$ (cm/s), (ER)	FD4 (MW 4400) $P_{app} \times 10^{-8}$ (cm/s), (ER)	FD20 (MW 19 600) $P_{app} \times 10^{-8}$ (cm/s), (ER)
–	2.71 ± 0.52	0.24 ± 0.11	0.10 ± 0.02
Carbomer	3.52 ± 1.95 (1.3)	0.33 ± 0.10 (1.4)	0.10 ± 0.03 (1.1)
SPH*	7.34 ± 1.13 (2.7)	2.96 ± 0.63 (12.2)	0.85 ± 0.35 (8.6)
SPHC*	9.31 ± 2.82 (3.8)	3.53 ± 0.93 (14.6)	0.32 ± 0.04 (3.2)

* SPH and SPHC are significantly different from carbomer 0.5% and negative control ($P < 0.05$); transport enhancement ratios (ER) are shown in brackets.

immediate. After apical application of SPH (10 mg) and SPHC (20 mg) polymers, a significant decrease (about 40% of the initial value) was obtained within the first 5 min and remained stable during the time course of the experiments. These reductions were significantly different from carbomer and the negative control ($P < 0.05$). Carbomer (C934P) at concentrations of 0.5%, which was used as the positive control, also exhibited reduction in TEER values within 5 min; however, the TEER value then increased gradually during the time course of experiments. No reduction in TEER values was observed for the negative control (i.e. without any polymer).

Recovery of TEER values after removing of the polymers is depicted in Fig. 1B. Slight recovery was observed at 5 and 6 h for both SPH and SPHC treated monolayers. After 48 h incubation of the monolayers with culture medium, TEER was recovered almost completely to 93 and 85% of the initial value for SPH and SPHC, respectively, and not significantly different from the values measured for the control groups.

3.2. Transport studies

The low molecular weight and hydrophilic marker ^{14}C-mannitol was used to determine the increase of paracellular permeability of Caco-2 cell monolayers using SPH and SPHC polymers. As shown in Fig. 2, both SPH and SPHC caused a significant increase ($P < 0.05$) in transport of

^{14}C-mannitol compared to carbomer 0.5% and the negative control. The apparent permeability values (Table 1) showed an enhancement of 2.7-fold for SPH and 3.8-fold for SPHC in comparison to the negative control, whereas carbomer showed only a 1.3-fold increase in the transport of ^{14}C-mannitol across Caco-2 cell monolayers.

The transport profiles of the high molecular weight and hydrophilic marker compounds FD4 and FD20 are shown in Figs. 3 and 4, respectively. As evident from Fig. 3, both SPH and SPHC polymers improved markedly the cumulative transport of FD4 up to $2.01 \pm 0.43\%$ and $2.39 \pm 0.63\%$, respectively compared to carbomer ($0.22 \pm 0.07\%$) and negative control ($0.11 \pm 0.05\%$). As observed in Fig. 4, the cumulative transport of FD20 across the Caco-2 cell monolayers was lower than found with FD4. After 4 h, SPH and SPHC polymers increased FD20 transport to $0.57 \pm 0.23\%$ and $0.33 \pm 0.12\%$, respectively, compared to carbomer ($0.07 \pm 0.02\%$) and negative control ($0.07 \pm 0.01\%$). Comparing the P_{app} values of FD4 and FD20 (Table 1), it is

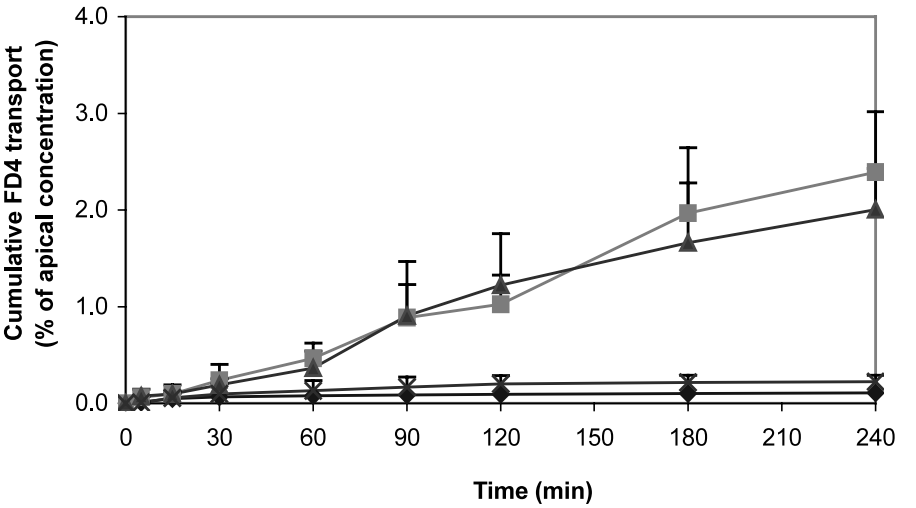

Fig. 3. Cumulative transport of FD4 (MW 4400 Da) across Caco-2 cell monolayers; (◆) control, (■) 20 mg SPHC, (▲) 10 mg SPH, (×) 0.5% carbomer. Data are expressed as mean ± S.D. of three to five experiments.

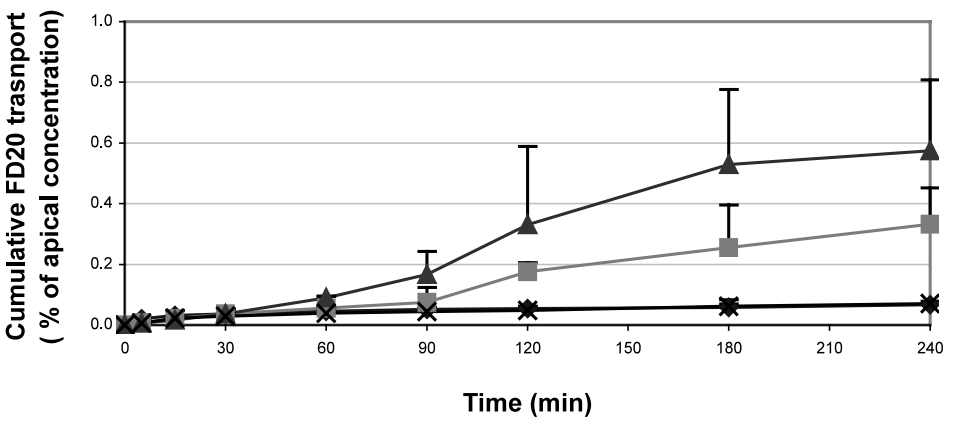

Fig. 4. Cumulative transport of FD20 (MW 19 600 Da) across Caco-2 cell monolayers; (◆) control, (■) 20 mg SPHC, (▲) 10 mg SPH, (×) 0.5% carbomer. Data are expressed as mean ± S.D. of three to five experiments.

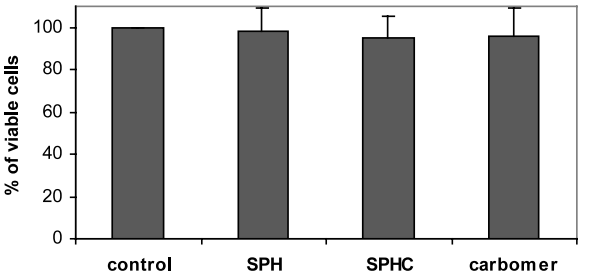

Fig. 5. Effect of polymers on mitochondrial dehydrogenase activity (MTT assay). Data are expressed as mean ± S.D. of three to five experiments.

obvious that the total transport of FD20 across the Caco-2 cell monolayers is less than FD4, but that the enhancement ratios for SPH and SPHC polymers compared to negative controls are quite similar.

3.3. Cytotoxicity studies

3.3.1. Trypan blue test

Trypan blue staining was used to study the permeability of the cytoplasmic membrane after application of polymers. Staining the Caco-2 cells with 0.1% trypan blue after completion of transport experiments revealed less than 1% of intracellular dye uptake (data not shown), demonstrating the viability of the cells during the transport experiments in the presence of SPH, SPHC or carbomer. Therefore, no cell damage was caused by application of the polymers to Caco-2 cell monolayers.

3.3.2. MTT assay

The effect of SPH and SPHC polymers on mitochondrial dehydrogenase activity is depicted in Fig. 5. The results showed no toxic effects caused by mucosal application of the polymers in comparison with the negative control; 98 ± 11, 95 ± 10 and 96 ± 14% of the cells were viable after application of SPH, SPHC and carbomer, respectively.

3.3.3. Propidium iodide staining

Caco-2 cell monolayers were examined for their viability after 4 h incubation with SPH and SPHC polymers by propidium iodide nucleic staining. In case of polymer applications (Fig. 6A and B), the monolayers appeared to exclude propidium iodide

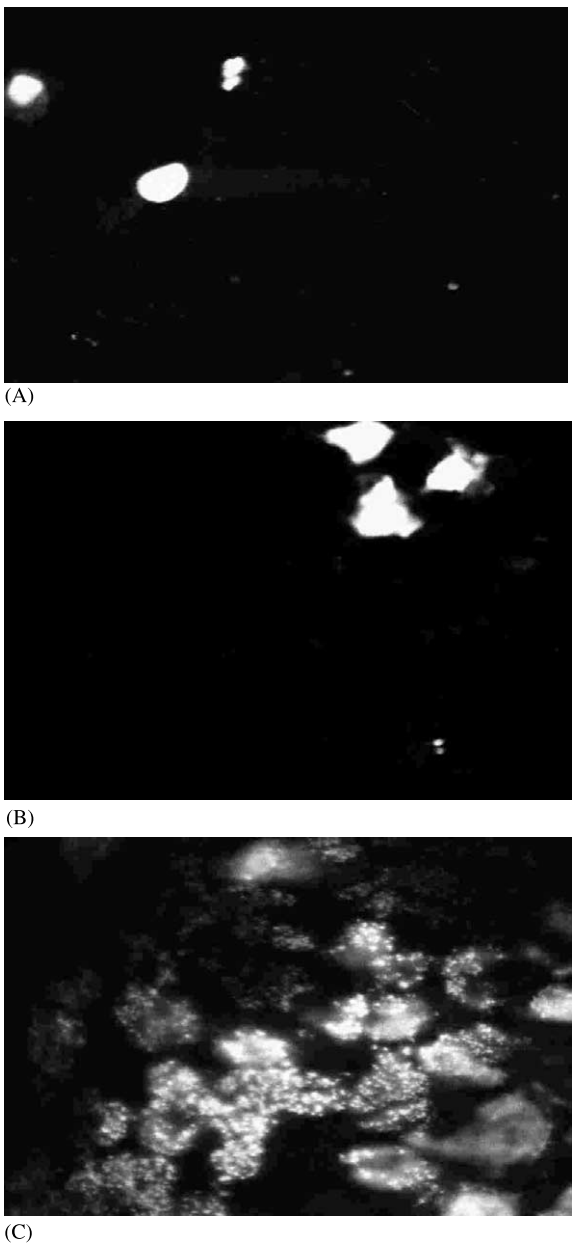

Fig. 6. CLSM image of Caco-2 cell monolayers using propidium iodide as impermeable dead cell staining probe; (A) cells incubated with SPH polymer, (B) cells incubated with SPHC polymer, (C) cells treated with 0.1% SDS for 10 min.

and showed no differences with the control group where the monolayers were incubated only with HBSS-HEPES. However, when 0.1% SDS was applied to the cells, propidium iodide was taken up by the cells, indicative for severe cell damage (Fig. 6C). Therefore, dye inclusion in polymer-treated cells was significantly less than in cells incubated with 0.1% SDS, demonstrating that the monolayers were intact after 4 h incubation with SPH and SPHC polymers.

4. Discussion

Integrity of Caco-2 cell monolayers and opening of tight junctions can be assessed by measuring the TEER and flux of radiolabeled molecules such as ^{14}C-mannitol across the monolayers (Artursson, 1990). As shown in Fig. 1, both SPH and SPHC polymers were able to decrease TEER significantly (compared to the control and 0.5% carbomer) by their swelling and applied mechanical pressure on monolayers. This TEER reduction was associated with an increase in flux of ^{14}C-mannitol across Caco-2 cell monolayers, indicating the capability of SPH and SPHC polymers for opening of tight junctions and modulating the paracellular permeability of the monolayers. This increased permeability might be caused by affecting the cell membrane integrity. Therefore, the reversibility of this effect is an important issue when screening these polymers as penetration enhancers. In the present study, it was observed that after removing the polymers from the monolayers, TEER values recovered to almost initial values within 2 days, indicating that the effects of SPH and SPHC polymers on tight junctions are reversible.

SPH and SPHC polymers were also evaluated for their effects on enhancing paracellular transport of the hydrophilic macromolecular model compounds fluorescein isothiocyanate-labelled dextrans with molecular weight of 4400 Da (FD4) and 19 900 Da (FD20). The reason for using FD4 and FD20 as model compounds for evaluation of SPH and SPHC polymers in Caco-2 cell monolayers was their hydrophilicity and high molecular weight; therefore, the transport route of these compounds is restricted to the paracellular pathway (Hosoya et al., 1993; Borchard et al., 1996). When SPH and SPHC polymers were apically added to the Caco-2 cell monolayers, they started to swell and to exert mechanical pressure on the cells which caused the opening of the tight junctions. The obtained P_{app} values for FD4 and FD20 in case of polymer-treated cells were much higher than for the control group. It has already been established that SPH and SPHC polymers are able to enhance the transport of FD4 across porcine intestine ex vivo by exerting mechanical pressure on the intestinal epithelium (Dorkoosh et al., 2002).

In the present study, P_{app} values of FD4 and FD20 obtained for the control experiments were similar to those previously published (Schipper et al., 1997), indicating complete integrity of the monolayers. Moreover, the permeability of FD20 in all transport experiments was less than FD4, which is due to higher molecular weight of FD20. This is also in agreement with previous observations (Hosoya et al., 1993), in which permeability coefficients of FDs in mucosae appeared to be inversely related to their molecular weights.

The cytotoxic effects of SPH and SPHC polymers on Caco-2 cell monolayers were examined with 3 different tests, including trypan blue exclusion assay, propidium iodide nucleic staining and mitochondrial dehydrogenase activity (MTT) assay. The trypan blue test is a vital staining method used to determine viability of cell cultures (Hovgaard et al., 1995). Trypan blue does not penetrate unperturbed cell membranes and it is therefore easy to observe dye exclusion using light microscopy. The present results showed the absence of any cell damage in controls and polymer-treated cells. Nevertheless, due to incapability of light microscopy to show a clear distinction between dead and living cells, further assessment was performed with propidium iodide staining using CLSM. After staining the cells with this compound, it is well possible to distinguish between dead and living cells (Anderberg and Artursson, 1993). Comparing SPH- and SPHC-treated cells with SDS-treated cells revealed that number of dead cells was substantially less in case of polymer application (Fig. 6), which

demonstrates the safety of SPH and SPHC polymers for mucosal application.

Using the MTT assay, SPH- and SPHC-treated cells were able to metabolize the mitochondrial substrate MTT by conversion into formazan crystals. This metabolic activity of cells is an appropriate approach for assessing the number of viable cells, since damaged or dead cells do not exhibit mitochondrial dehydrogenase activity (Liu et al., 1999). Thus the Caco-2 cell monolayers appeared to be viable after 4 h application of both SPH and SPHC polymers and even no damage was observed at the intracellular level.

In conclusion, SPH and SPHC polymers as novel excipients in peroral peptide drug delivery systems (Dorkoosh et al., 2001) are able to increase the paracellular transport of the hydrophilic low molecular weight marker ^{14}C-mannitol and the hydrophilic macromolecular compounds FD4 and FD20 across Caco-2 cell monolayers. Moreover, these polymers reversibly decrease the TEER values in Caco-2 cell monolayers, indicating the capability of SPH and SPHC for opening of tight junctions. Cytotoxicity tests revealed that both SPH and SPHC polymers are safe excipients and do not cause any damage to the Caco-2 cells after mucosal application.

Acknowledgements

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