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Charge-dependent interaction of self-emulsifying oil formulations with Caco-2 cells monolayers: binding, effects on barrier function and cytotoxicity

T. Gershanik [a], E. Haltner [b], C.-M. Lehr [b], S. Benita [a,*]

[a] *Department of Pharmaceutics, School of Pharmacy, P.O. Box 12065, The Hebrew University of Jerusalem, Jerusalem 91120, Israel*

[b] *Department of Biopharmaceutics and Pharmaceutical Technology, Saarland University, Saarbrucken, Germany*

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Abstract

A positively charged self-emulsifying oil formulation (SEOF), aimed to enhance oral bioavailability of drugs poorly soluble in water, was recently developed. In the present study the Caco-2 cell model was used for the investigation of the charge-dependent interactions of this SEOF with human intestinal epithelial cells. The positively charged emulsions affected the barrier properties of the cell monolayer at high concentrations and reduced the cell viability. However, at the dilution with aqueous phase used in the present study (1:2000), the positively charged SEOF did not induce any detectable cytotoxic effect. The binding of the fluorescent dye $DiIC_{18}(3)$ was much higher from the positively charged SEOF, compared to the negatively charged formulation, suggesting an increased closer adhesion of the droplets to the cell surface due to the electrostatic attraction. No transepithelial transport of this compound across Caco-2 cell monolayers was observed with any SEOF formulation.

Keywords: Self-emulsifying; Emulsion; Caco-2 cells; Barrier function; Binding study; Fluorescent dye

1. Introduction

Numerous potent drugs exhibit low oral bioavailability due to their poor aqueous solubility properties or presystemic metabolism. A popular approach to enhance the oral bioavailability of such drugs is the incorporation of the active component into various inert lipid or surfactant vehicles. Self-emulsifying oil formulations (SEOFs), isotropic mixtures of oil and surfactants, which can improve oral availability of drugs poorly soluble in water, are an example of such dosage forms. Taken in gelatin capsules these formulations disperse in the GI tract to form a fine emulsion, upon dilution with gastrointestinal fluids (Shah et al., 1994). A novel SEOF, which results in positively charged dispersed oil droplets upon dilution with an aqueous phase, elicited an

* Corresponding author. Address: David R. Bloom Center for Pharmacy, Hebrew University of Jerusalem, Israel. Tel.: +97-22-6758667; fax: +97-22-6757246.

E-mail address: benita@cc.huji.ac.il (S. Benita).

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increase in the oral bioavailability of progesterone in young female rats (Gershanik and Benita, 1996). More recently it was shown that the enhanced electrostatic interactions of positively charged droplets with the mucosal surface of the everted rat intestine are mainly responsible for the preferential uptake of model drug cyclosporine A (CsA) from the positively charged droplets (Gershanik et al., 1998).

Monolayers of differentiated Caco-2 cells show morphological and biochemical similarity to normal intestinal enterocytes, and they develop effective tight junctions (Artursson and Karlsson, 1991; Hidalgo et al., 1989). Therefore this cell line has become very popular as an in vitro model for the intestinal mucosa to determine various transport characteristics and toxic effects of drugs, and to design formulation strategies for membrane permeability enhancement (Meunier et al., 1995; Fagerholm et al., 1996).

It was shown recently that toxicity, binding and transport studies for lipid or surfactant vehicles or lipid conjugates can be carried out on Caco-2 cells, grown on 96-well tissue culture plates or polycarbonate membranes (Sattler et al., 1997; Lian and Ho, 1997). In the present study, the Caco-2 cell model was used to investigate the charge-dependent interactions of SEOFs with human intestinal cells, in particular with respect to absorption enhancement, cellular binding and uptake, as well as regarding possible changes of the epithelial barrier function and cytotoxicity.

2. Materials and methods

2.1. Materials

Ethyl oleate (EO) was purchased from Fluka AG (Buchs CG, Switzerland). Oleylamine (OA) was obtained from Akzo Nobel Chemicals (Amersfoort, NL). Polysorbate 80 and fluorescein was obtained from Sigma (St. Louis, MO, USA). The fluorescent dye, $DiIC_{18}(3)$ (long-chain dialkylcarbocyanine) was received from Molecular Probes (Leiden, NL). WST-1 reagent was purchased from Boehringer (Mannheim, Germany). Water was double distilled. Krebs-Ringer Buffer (KRB) was prepared using analytical grade salts.

2.2. Self-emulsifying oil formulation's preparation and characterization

The positively charged SEOF tested consisted of Tween 80 (25%), ethanol (30%), oleylamine (3%) and ethyl oleate (remaining difference to 100%), whereas negatively charged formulations were prepared by omission of OA from the SEOF. The preparation of the SEOFs consisted of weighing and mixing various components until a clear solution was obtained. $DiIC_{18}(3)$ was dissolved in SEOF at a concentration of 25 mg/ml.

Positively or negatively charged emulsions were prepared by the dilution of the appropriate SEOF from 1:500 to 1:4000 by KRB. ζ-potential was measured by Coulter DELSA 440 ζ-sizer. Emulsion droplet size was determined by photon correlation spectroscopy (PCS) method using Coulter Counter Supernanosizer N4SD™, Luton, UK.

2.3. Cell culture

Caco-2 cells were received from DSMZ (German collection of microorganisms and cell cultures, Braunschweig).

The cells were maintained at 37°C in Dulbecco's Modified Eagle's Medium (DMEM), supplemented with 10% fetal calf serum (FCS), 1% non-essential amino acids as described elsewhere (Sattler et al., 1997), and gentamycin (final concentration 50 μg/ml) in an atmosphere of 5% CO_2 and 90% relative humidity. All tissue culture media were obtained from Gibco through Life Technologies (Eggenstein, Germany). The cells were seeded on flat bottom 96-well tissue culture plates (Greiner Labortechnik, Frickenhausen, Germany) or on polycarbonate filter inserts (0.4 μm, 13 mm diameter) of Transwell® cell culture chambers (apical volume 0.5 ml, basolateral volume 1.5 ml; Transwell® Costar, Badhoevedorp, NL). Cells of passage number 94–99 were used throughout. The cells were allowed to grow and differentiate to confluent monolayers for 14–21 days.

2.4. Measurements of transepithelial electrical resistance

The filter-grown monolayers of 21–23 days old

were exposed to the SEOFs of different dilutions within a period of 3 h. The transepithelial electrical resistance (TEER) was measured using a volt/ohm-meter (EVOM, World Precision Instruments, Sarasota, FL), equipped with a pair of chopstick electrodes.

2.5. WST-1 reagent assay

The effect of SEOFs on the intracellular dehydrogenase activity was measured by WST-1 method. WST-1 is a tetrasolinium salt that is cleaved by mitochondrial dehydrogenase in living, but not dead cells to give a yellow product. (Ishiyama et al., 1996)

Caco-2 cells were seeded on flat bottom 96-well tissue culture plates and grown to confluence within 14 days prior to use. Cells were washed and incubated with 100 μl of diluted SEOFs or TritonX100 solutions (12 serial dilutions beginning from 10 mg/ml) for 1–3 h at 37°C. After the incubation the emulsions and blank buffer solutions were replaced with 200 μl of WST-1 reagent, diluted 1:20, while 100 μl of the 1:10 diluted WST-1 reagent was added to the wells containing TritonX100 solutions. The cells were incubated for 45 min, and UV absorption was measured at the wavelength of 450 nm. Cell viability was determined in triplicates and expressed as the percentage of the UV absorption determined on cells incubated with KRB as a reference.

2.6. Fluorescein transport across filter-grown cell monolayer

Transport experiments were initiated by washing the monolayers three times with KRB before addition of the test solutions: fluorescein solution in KRB (final concentration 100 μg/ml) or emulsions, were added to the apical side of the cells. At the beginning of the experiment the initial donor fluorescein concentration was determined from a 50 μl sample. No fluorescence was found in the basolateral chamber containing KRB. Subsequently, samples (50 μl) were taken from the receiver side at specified time intervals and replaced with an equal volume of KRB. Before a sample was taken the integrity of the monolayer was checked by measurement of the TEER. The transport experiments were carried out in triplicates over a time period of 3 h.

Fluorescein apparent permeability coefficients (P_{app}) in cm/min was calculated according to the following formula (Artursson, 1990; Hidalgo et al., 1989):

$$P_{app} = dc/dt \times V/AC_0,$$

where V is a volume of the receiver compartment (ml); A is a membrane surface area (cm^2); C_0 is the initial donor concentration of fluorescein (g/ml); dc/dt is the permeability rate (μg/min), which is the slope of a plot of a cumulative receiver concentration with time.

2.7. Binding of $DiIC_{18}(3)$ by cells grown on well plates

Caco-2 cells were seeded on flat bottom 96-well tissue culture plates and grown to confluence within 14 days prior to use. Binding studies were performed in air (5% CO_2) at 90% relative humidity and 37°C or 4°C (refrigerator), depending on the experimental design at SEOF dilutions of 1:2000 and higher. Calibration curves were prepared from the corresponding emulsions dissolved in the isopropanol. After cell incubation for 0.5, 1 or 3 h with the emulsions containing the dye, the cells were washed three times with KRB and then solubilized with isopropanol to determinate the uptake of $DiIC_{18}(3)$. Samples were immediately analyzed in a CytoFluor II microplate fluorescence reader (PerSeptive Biosystems, Wiesbaden, Germany) at excitation and emission wavelengths of 530 and 590 nm, respectively. Binding at each SEOF dilution was determined in triplicate and repeated with cells of different passages.

3. Results

3.1. Emulsion characterization

The preparation and characterization of the SEOFs used in this study is described elsewhere in details (Gershanik et al., 1998). Dilution of SEOF

with KRB resulted in the formation of positively or negatively charged emulsion droplets, the ζ-potential values of which were $+44.5 \pm 1.2$ or -34.5 ± 2.3 mV respectively. Most of the droplet population, 80–85%, were in the range 150–250 nm, and 15–20% were in the range 1–4 μm. Addition of the model lipophilic compound $DiIC_{18}(3)$ did not change the above droplet parameters.

3.2. WST-1 assay

Positively charged emulsions formed by 1:500 SEOF dilution were toxic to the Caco-2 cells and caused reduced mitochondrial dehydrogenase activity (the activity was reduced up to 58 ± 24% following 3 h incubation). The data were significantly different from control. The dilution of 1:1000 did not elicit any toxic effect prior to 3 h incubation. Negatively charged emulsions did not affect the cell enzymatic activity at the same dilutions.

3.3. Transepithelial electrical resistance measurements

Only positively charged emulsions at the higher dilution of 1:500 affected the tightness of the cell monolayer (Fig. 1). The transepithelial resistance in the wells with corresponding negatively charged SEOF dilutions, as well as with dilutions of 1:1000 and higher (data not shown) of both emulsion types were identical to those in blank wells, containing KRB.

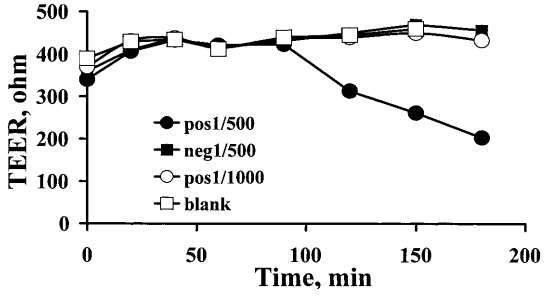

Fig. 1. Caco-2 cell transepithelial resistance as a function of SEOF charge and KRB dilution. (The effective surface area of filters was 1.13 cm^2.)

Table 1
Fluorescein apparent permeability ($cm/min \times 10^{-6}$) as a function of SEOF charge and dilution

Dilution	Negatively charged SEOF	Positively charged SEOF
1:500	53.1 ± 6.3	373.0 ± 83.6
1:1000	15.9 ± 4.6	62.3 ± 12.6
1:2000	6.6 ± 1.2	6.6 ± 6.7
1:4000	6.6 ± 0.6	6.6 ± 1.3
Blank buffer	8.0 ± 1.2	

3.4. Fluorescein transport experiments

The changes in the hydrophilic marker transport were observed in both, positively and negatively charged emulsions at the dilutions of 1:500 and 1:1000, but in the case of the positively charged droplets they were much more pronounced (Table 1).

3.5. Binding study

The binding of SEOFs labeled with $DiIC_{18}(3)$ to Caco-2 cells was measured at 3 h incubation time-points and at two temperatures: 4°C and 37°C. The data presented reflect only the results following 3 h incubation (Fig. 2). All the binding experiments were carried out at non-toxic SEOF dilutions of 1:2000 and higher (12.5 μg/ml and lower). The binding of the dye was much higher in case of the positively charged emulsions compared

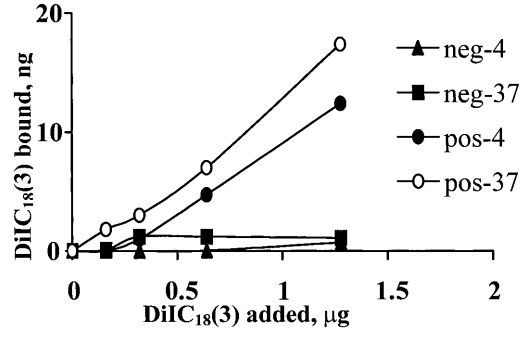

Fig. 2. Binding of $DiIC_{18}(3)$ from positively and negatively charged SEOFs to Caco-2 cells as a function of incubation temperature.

to the negatively charged emulsions, and increased with increasing concentration and incubation time, both at 4°C and 37°C. A saturation of binding was not observed at the concentrations studied. In contrast, binding of negatively charged emulsions remained very low, even at the highest concentration. Binding at 4°C was always just slightly decreased compared to the corresponding value at 37°C, suggesting a mechanism of passive diffusion/adsorption and the absence of any active transport process or endocytosis.

4. Discussion

The present study was performed in order to evaluate the potential of the positively charged SEOFs in enhancing the mucosal absorption of lipophilic drugs using Caco-2 cell model, with reference to mucosal cell cytotoxicity. Previously, it had been demonstrated that a positive charge increased the binding of SEOFs to rat intestinal mucosa in vitro (Gershanik et al., 1998). The importance of the electrostatic interactions of ionic and especially cationic compounds with the negatively charged apical membrane of Caco-2 cells monolayer has also been reported by others (Iseki et al., 1995; Choksakulnimitr et al., 1995; Schipper et al., 1996; Pauletti et al., 1997).

Increased drug absorption through the intestinal mucosa is often associated with reversible or irreversible damage caused to the intestinal cells and their barrier function. The practical use of absorption enhancing adjuvant is limited because they induce injury and irritation in the intestinal mucosal membrane. It was found that there is an almost linear relationship between the absorption enhancing effects of various absorption enhancers: surfactants, bile salts, chelating agents and fatty acids, and their cytotoxicity in Caco-2 cells (Quan et al., 1998). Both the positively and negatively charged SEOFs used in this study contain a rather high amount of non-ionic surfactants, necessary to provide the self-emulsifying ability of the formulation. These substances are potential absorption enhancers and may alter the epithelial barrier properties. However, in the SEOFs at least part of the added surfactants is located at the o/w interface of the emulsion droplet following emulsification. Therefore, the concentration of the free surfactant in the emulsion water phase is probably much lower than its nominal concentration in the entire emulsion. According to some other study performed on Caco-2 cells with two non-ionic surfactants, Solulan C24 and Solulan 16, their moderate toxic effect was caused mostly by the free surfactant present in the neosome suspension (Dimitrijevic et al., 1997). Generally, the non-ionic surfactants were found to be less toxic to Caco-2 cells than the ionic ones (Quan et al., 1998; Anderberg et al., 1991; Hurni et al., 1993). Investigations of a variety of macromolecule carriers in different cell cuture systems lead to the conclusion that especially polycationic macromolecules may induce cellular damage (Choksakulnimitr et al., 1995). Furthermore, the investigation of chitosans revealed that the binding and absorption enhancing effect of these polymers on epithelial cells are mediated through their positive charges (Kotze et al., 1998; Schipper et al., 1997). Therefore, the toxicity of the positively charged emulsion droplets represents a critical issue in the evaluation of their use as an oral carrier system for lipophilic drugs.

Measurements of TEER, the WST-1 test and monitoring of fluorescein transport have been used here to study the effect of the positively and negatively charged SEOFs at different dilutions on Caco-2 cells in terms of viability and epithelial barrier function. As could be expected, the positively charged emulsions were more toxic to the Caco-2 cells, and markedly affected the barrier properties of the cell monolayer. However, the dilution of 1:2000 was found to be non-toxic for both emulsion types, regardless of the method used. The primary luminal dilution of the SEOF in the stomach can be roughly estimated to be about 1:500 (0.5 ml capsule dissolved in the luminal volume of 250 ml) (Amidon et al., 1995). Taking into account the presence of the protecting mucus layer normally bound to the apical cell surface, and the peristaltic movements causing the quick spreading of the droplets along the GI tract, the above dilutions do not seem to be dangerous to the cells under the real physiological conditions.

A concentration-dependent decrease of the mitochondrial dehydrogenase activity was observed for positively charged emulsions in the dilution range used. The lack of such an effect for polysorbate (Tween 80), which is a component of both emulsion types, on the cells viability during the 3 h incubation corroborates data already published in the literature. Anderberg et al. (1991) showed no correlation between the CMC and damaging concentration of Tween 80, but the latter was strongly dependent on the cells exposure time. A concentration 20 fold higher than that used in the present study (0.3%) did not affect the intracellular dehydrogenase activity in Caco-2 cells exposed to this surfactant for a short time (10 min). However, noticeable damage was observed at the same concentrations after a 24-h exposure (Anderberg et al., 1991).

The results of the TEER measurements are consistent with those obtained by the WST-1 method: the rapid fall of the monolayer electrical resistance was observed with 1:500 dilution of the positively charged SEOF. The negatively charged SEOF at 1:500 and 1:1000 dilutions, as well as positively charged SEOF at the dilution 1:1000 did not affect the electrical properties of the monolayer, but they did altered its permeability to fluorescein at those dilutions. It was recently reported in the literature, that determination of the hydrophilic compounds permeability is generally more sensitive in the characterization of the surfactants effect on epithelial cell monolayers than the WST-1 method or TEER measurements (Anderberg et al., 1991).

The negatively charged SEOF caused only modest changes in the fluorescein permeability. Thus, the much more prominent effect of the positively charged droplets on the permeability of the hydrophilic marker could most likely be explained by the nature of the droplet charge which causes the electrostatic attraction of the droplets to the negatively charged cells surface. It was suggested that the mucosal charge density could be important for enhancement of mucosal absorption via the paracellular pathway (Schipper et al., 1997). Furthermore, it was found that the interaction of positively charged polymers, such as e.g. chitosans, with the cell membrane resulted in a structural reorganization of tight junction-associated proteins, followed by enhanced transport through the paracellular way (Schipper et al., 1997). The above findings conform with our results on the fluorescein transport experiments.

The $DiIC_{18}(3)$ binding experiments in this study were carried out with SEOF dilutions of 1/2000 and higher, where the barrier properties of the cell monolayer are not affected by the emulsions. Furthermore, as this fluorescent dye is lipophilic by nature it is likely to be absorbed by passive diffusion into the cell membrane and not using some paracellular pathway, which is the preferential route for hydrophilic molecules. It can be speculated that the higher binding of $DiIC_{18}(3)$ observed with the positively charged SEOFs is mediated by the intensified adhesion of the oil droplets to the cell surface due to electrostatic attraction. In this case the lipophilic dye partitions directly between the droplet and the cell membrane without the intermediate water diffusion step. In case of the negatively charged SEOFs, the dye must diffuse through the buffer solution towards the cell surface, and its membrane uptake from the emulsion droplets is limited by the poor water solubility. For both types of SEOFs cellular uptake of the dye was slightly temperature dependent, which can be explained by some increased diffusion coefficient of the dye at higher temperatures.

In additional experiments we tried to evaluate the influence of both SEOFs on the transport of $DiIC_{18}(3)$ across filter-grown Caco-2 monolayers, but there was no measurable transport of $DiIC_{18}(3)$ from the apical to the baso-lateral compartment (data not shown). This was also not observed even after adding 1% FCS to the basolateral compartment, which should facilitate the partitioning of the lipophilic dye from the cells to the aqueous buffer. However, relatively high amounts of the dye were associated with the cells at the end of these experiments. In control experiments with cell-free filters, the dye dissolved in the emulsion droplets freely penetrated

through the microporous polycarbonate membrane (P_{app} of $DiIC_{18}(3)$ was 0.22×10^{-6} and 0.41×10^{-6} cm/min for negatively and positively charged emulsions, respectively).

The reason for this phenomenon is probably the poor solubility of $DiIC_{18}(3)$ in the aqueous basolateral compartment, which is relatively small and of different composition compared to the plasma compartment relevant for drug absorption in vivo. As a result, lipophilic compounds tend to accumulate in the cell monolayer while the concentrations in the basolateral buffer remain very low. These results illustrate the difficulties in using cell-culture based in vitro test systems to study the transport of lipophilic, poorly water soluble drugs, and how their absorption can be influenced by pharmaceutical formulations. Still, however, such test-systems are very useful to study the interaction of colloidal drug carrier systems with the intestinal epithelium, especially with respect to binding, alteration of the barrier function and cytotoxicity.

5. Conclusion

Positively charged SEOF exhibited higher toxicity to Caco-2 cell monolayers than negatively charged SEOF. However, the concentrations, at which cytotoxic effects were observed in vitro, are not expected to occur in the real physiological conditions of the GI tract in vivo.

The positively charged emulsion droplets appeared to be more effective in enhancing the cellular binding and delivery of the model lipophilic compound $DiIC_{18}(3)$ to Caco-2 cell compared to negatively charged droplets. No transepithelial transport of this compound across Caco-2 cell monolayers was, however, observed with any SEOF formulation.

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References

Amidon, G.L., Lennernas, H., Shah, V.P., Crison, J.R., 1995. A theoretical basis for a pharmaceutical drug classification: the correlation of in vitro drug product dissolution and in vivo bioavailability. Pharm. Res. 12, 413–420.

Anderberg, E.K., Nystrom, C., Artursson, P., 1991. Epithelial transport of drugs in cell culture. VII: effects of pharmaceutical surfactant excipients and bile acids on transepithelial permeability in monolayers of human intestinal epithelial (Caco-2) cells. J. Pharm. Sci. 81, 879–887.

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.

Artursson, P., Karlsson, J., 1991. Correlation between oral drug absorption in humans and apparent drug permeability coefficients in human intestinal epithelial (Caco-2) cells. Biochem. Biophys. Res. Commun. 175, 880–885.

Choksakulnimitr, S., Masuda, S., Tokuda, H., Takakura, Y., Hashida, M., 1995. In vitro cytotoxicity of macromolecules in different cell culture systems. J. Contr. Rel. 34, 233–241.

Dimitrijevic, D., Lamandin, C., Uchegbu, I.F., Shaw, A.J., Florence, A.T., 1997. The effect of monomers and of micellar and vesicular forms of non-ionic surfactants (Solulan C24 and Solulan 16) on Caco-2 cells monolayers. J. Pharm. Pharmacol. 49, 611–616.

Fagerholm, U., Johansson, M., Lennernas, H., 1996. Comparison between permeability coefficients in rat and human jejunum. Pharm. Res. 13, 1336–1342.

Gershanik, T., Benita, S., 1996. Positively charged self-emulsifying oily formulation for improving oral bioavailability of progesterone. Pharm. Develop. Technol. 1, 147–157.

Gershanik, T., Benzeno, S., Benita, S., 1998. Interaction of the self-emulsifying lipid drug delivery system with mucosa of everted rat intestine as a function of surface charge and droplet size. Pharm. Res. 15, 863–869.

Hidalgo, I.J., Raub, T.J., Borchardt, R.T., 1989. Characterization of the human colon carcinoma cell line (Caco-2) as a model system for intestinal epithelial permeability. Gastroenterology 96, 736–749.

Hurni, M.A., Noach, A.B., Blom-Roosemalen, M.C., de Boer, A.G., Nagelkerke, J.F., Bremier, D.D., 1993. Permeability enhancement in Caco-2 cell monolayers by sodium salicilate and sodium taurodihydrofusidate: assessment of effect-reversibility and imaging of transepithelial transport routs by confocal laser scanning microscopy. J. Pharmacol. Exp. Ther. 267, 942–950.

Iseki, K., Kaido, K., Kobayashi, M., Sugawara, M., Myazaki, K., 1995. The effect of membrane surface potential on the permeability of anionic compounds across the apical membrane in human intestinal epithelial (Caco-2) cells. Biol. Pharm. Bull. 20, 794–799.

Ishiyama, M., Tominada, H., Shiga, M., Sasamoto, K., Ohkura, Y., Ueno, K., 1996. The combined assay of cell viability and in vitro toxicity with a high water-soluble tetrasolium salt, neutral red and crystal violet. Biol. Pharm. Bull. 19 (November), 1518–1520.

Kotze, A.F., Luessen, H.L., de Leeuw, B.J., de Boer, A.G., Verhoef, J.C., Junginger, H.E., 1998. Comparison of the effect of different chitosan salts and N-trimethyl chitosan chloride on the permeability of intestinal epithelial cells (Caco-2). J. Contr. Rel. 51, 35–46.

Lian, T., Ho, R.J., 1997. Cholera-toxin B-mediated targeting of lipid vesicles containing ganglioside GM1 to mucosal epithelial cells. Pharm. Res. 14, 1309–1315.

Meunier, V., Bourrie, M., Berger, Y., Fabre, G., 1995. The human intestinal epithelial cell line Caco-2, pharmacological and pharmacokinetic applications. Cell. Biol. Toxicol. 11, 187–194.

Pauletti, G.M., Okumu, F.W., Borchardt, R.T., 1997. Effect of size and charge on the passive diffusion of peptides across Caco-2 cell monolayer via the paracellular pathway. Pharm. Res. 14, 164–168.

Quan, Y.-S., Hattori, K., Lundborg, E., Fujita, T., Murakami, M., Muranishi, S., Yamamoto, A., 1998. Effectiveness and toxicity screening of various absorption enhancers using Caco-2 cell monolayers. Biol. Pharm. Bull. 21, 615–620.

Sattler, S., Schaefer, U., Schneider, W., Hoelzl, J., Lehr, C.M., 1997. Binding, uptake, and transport of Hypericin by Caco-2 cell monolayers. J. Pharm. Sci. 86, 1120–1126.

Schipper, N.G., Varum, K.M., Artursson, P., 1996. Chitosans as absorption enhancers for poorly absorbable drugs. 1: Influence of molecular weight and degree of acetylation on drug transport across human intestinal epithelial (Caco-2) cells. Pharm. Res. 13, 1686–1692.

Schipper, N.G., Olsson, S., Hoogstaate, J.A., de Boer, A.G., Varum, K.M., Artursson, P., 1997. Chitosans as absorption enhancers for poorly absorbable drugs. 2: M. of absorption enhancement. Pharm. Res. 14, 923–929.

Shah, N.H., Carvajal, M.T., Patel, C.I., Infeand, M.H., Malick, A.W., 1994. Self-emulsifying drug delivery systems (SEDDS) with polyglycolized glycerides for improving in vitro dissolution and oral absorption of lipophilic drugs. Int. J. Pharm. 106, 15–23.