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EFFECTS OF ABSORPTION ENHANCER SURFACTANTS ON CULTURE MODELS OF INTESTINAL AND VASCULAR BARRIERS

Summary of the Ph.D. thesis

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1. INTRODUCTION

Improving drug absorption is one of the main goals of pharmaceutical research. Pharmacokinetics and permeability across biological barriers are not satisfactory for a large number of active molecules. Bioavailability of drugs is influenced by several factors, such as physicochemical properties of the active agents, mainly low solubility and large molecular size, poor permeability across biological barriers, application sites, binding to plasma proteins, degradation by enzymes, or excretion.

Biological barriers like the skin, the intestinal, blood-fetal or blood-brain barriers separate the organism from the external environment and the body compartments from each other. They protect the organs, and they also hinder the cross of drugs. In case of oral drug delivery, which is the most common route of drug administration, the drug absorption is restricted by TJs connecting intestinal epithelial cells. In that way the permeability of cell membrane is a major determinant of drug penetration, but paracellular transport has also a huge impact on drug penetration.

Active agents with low permeability may need assistance for their penetration across barriers. Absorption enhancers are compounds that improve the absorption of drugs and enhance their bioavailability. Intestinal absorption enhancers can improve the pharmacological utilization of drugs by several mechanisms: (i) increasing drug solubility; (ii) opening tight junctions; (iii) temporarily disturbing the lipid bilayer packing or fluidizing membranes; (iv) complexation or ion pairing; (v) preventing degradation/metabolism.

Surface active agents, or surfactants, are amphiphilic molecules possessing both lipophilic and hydrophilic residues. They are widely used as solubilizing agents in oral, injectable and nasal formulations and many studies reviewed their absorption enhancing properties. Cremophors and Tweens are often used excipients in pharmaceutical research and industry for emulzifying or enhance the absorption of many drugs. They possess several advantageous properties, although some of them induced adverse reactions during their administration. Therefore there is a need for new, innovative absorption enhancers with more favorable properties and better pharmaceutical profile.

Sucrose esters, composed of sucrose and fatty acids, are widely researched for drug delivery as solubilizers and stabilizers or absorption enhancers. Sucrose esters were successfully tested in many different formulations and they are promising new candidates

for improving the bioavailability of drugs. Our research and my Ph.D. thesis focus on investigation and comparison of clinically applied and novel pharmaceutical excipients using *in vitro* culture models of the intestinal and vascular barriers.

2. AIMS

The main aim of the work was to reveal the complex mechanisms of the effect of non-ionic surface active agents, especially water soluble sucrose esters on drug penetration across the intestinal barrier using cell culture models. Three selected sucrose esters, potential novel pharmaceutical excipients, were compared to Tween 80, Cremophor RH40 and Cremophor EL, reference surfactants, used in various medicinal products. The major aims of the experimental study were the followings:

(I) investigation and comparison of the effects of surfactants on intestinal epithelial and vascular endothelial cell viability

(II) measurement of the effects of surfactants on drug permeability

(III) identification of the pathways for the drug penetration enhancement on intestinal barrier model

(IV) detailed analysis of intercellular tight junction morphology and membrane fluidity

(V) determination of the effects of surfactants on efflux pump activity

3. MATERIALS AND METHODS

3.1. Surfactants

Laurate sucrose ester (D-1216) was of pharmaceutical grade, palmitate (P-1695) and myristate (M-1695) sucrose esters were of analytical grade (Mitsubishi Kagaku Foods Co., Tokyo Japan). Tween 80, Cremophor RH40 and Cremophor EL (BASF, Ludwigshafen am Rhein, Germany) were of pharmaceutical grade and used as reference surfactants.

3.2. Cell culture

Human Caco-2 intestinal epithelial, human hCMEC/D3 brain endothelial, human MES-SA uterine sarcoma cell lines and its doxorubicin-selected derivative MES-SA/Dx5, and primary rat brain endothelial cells were used in the experiments. Caco-2 cells were grown in Eagle's minimal essential medium supplemented with 10 % fetal bovine serum, 1 % sodium-pyruvate and 50 μg/ml gentamicin in a humidified 37°C incubator with 5 % CO_2. D3 cells were grown in Endothelial Basal Medium-2 containing Endothelial Growth Medium-2 BulletKit (Lonza, Basel, Switzerland) supplemented with 2.5 % fetal bovine serum in a humidified 37°C incubator with 5 % CO_2. Primary cultures of brain endothelial cells were isolated from 3-weeks-old rats described by Veszelka *et al.* (Neurochem Int, 2007, 50:219-228). MES-SA, and its selected derivative expressing high levels of P-gp (MES-SA/Dx5) were used for testing P-gp functionality. The cells were grown in Dulbecco's Modified Eagle Medium supplemented with 10 % fetal bovine serum, 5 mM L-glutamine and 50 U/ml penicillin/streptomycin. For electric resistance measurements, permeability studies and electron microscopy Caco-2 cells were cultured on Transwell inserts (polycarbonate membrane, 0.4 μm pore size, Corning Life Sciences, Tewksbury, MA, USA). For staining of nuclei, junctions and F-actin cells were grown on glass coverslips (Menzel-Gläser, Braunschweig, Germany).

3.3. *In vitro* viability studies

Different methods were applied to determine the effects of non-ionic surfactants on cell viability: MTT dye conversion, lactate dehydrogenase release (LDH) measurement, double fluorescent staining of cell nuclei. Real-time cell impedance measurement also provided information on cell viability.

The treatment concentrations of sucrose esters P-1695, M-1695 and D-1216 varied between 3-3,000 μg/ml. Tween 80, Cremophor RH40 and Cremophor EL were used in 1-100,000 μg/ml concentrations. Untreated cells (control group) received only culture medium.

MTT dye conversion: The yellow 3-(4,5-dimethyltiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) dye is taken up by cells and converted by mitochondrial and cytoplasmic enzymes into blue formazan crystals. The dye conversion rate determines cell metabolic activity and viability. Caco-2 epithelial and hCMEC/D3 endothelial cells were treated and incubated with surfactants for 1 and 24 hours. Then cells were incubated with MTT solution and the amount of formazan converted by cells was determined by absorbance measurement with a microplate reader (Fluostar Optima, BMG Labtechnologies, Ortenberg, Germany).

LDH release measurement: The release of LDH enzyme, the indicator of cell membrane damage, was determined from culture supernants by Cytotoxicity Detection Kit/LDH (Roche, Basel, Switzerland). Caco-2 and hCMEC/D3 cells were treated for 1 or 24 hours with surfactants, then culture supernatants were incubated with Cytotoxicity Detection Kit and the resulted change in the absorbance of supernants was detected by microplate reader.

Double fluorescent staining of cell nuclei: Cell viability was also determined by a morphological test: *bis*-benzimide (Hoechst 33342) labels all cell nuclei blue, ethidium-homodimer-1 stains only dead cells red. Caco-2 and primary rat brain endothelial cells were treated with Cremophor RH40 and EL. *Bis*-benzimide and ethidium-homodimer-1 were added to the treated cells. After incubation cells were fixed and processed for junctional immunostaining.

3.4. Fluorescent actin-labeling and immunostainings for junctional proteins

Treatments induced changes in morphology and cell-cell connections of Caco-2 cells were confirmed by immunostaining for tight junction proteins claudin-1 and -4, cytoplasmic linker protein ZO-1 and adherens junction protein β-catenin. Filamentous actin (F-actin) was stained in intestinal culture model by fluorescently labeled phalloidin and cell nuclei by *bis*-benzimide dye. The morphology of TJ was also investigated in primary rat brain endothelial cells by immunostaining for claudin-5 protein. Cell layers were grown on glass coverslips and treated with surfactants. Cytochalasin D, an inhibitor of actin polymerization was applied as positive control. After treatments the cultures were fixed, permeabilized and blocked. The fixed cells were incubated with primary antibodies overnight. Incubation with secondary antibody Cy3-labeled anti-rabbit IgG, and Alexa Fluor 488 Phalloidin and *bis*-benzimide lasted for 1 hour. Stainings for claudin-1, claudin-4, ZO-1and β-catenin were examined by a Leica SP5 confocal microscope, while claudin-5 stainings were detected by a NikonEclipse TE2000 fluorescent microscope and photographed by a Spot RT digital camera.

3.5. Electron microscopy

Cells were treated with absorption enhancers then washed and fixed. After washing with the buffer several times, cells were postfixed in OsO_4 and dehydrated. Finally, the membranes of the culture inserts with the cells were removed from their support and embedded in Taab 812. Following polymerization, ultrathin sections were cut perpendicularly for the membrane using a Leica UCT ultramicrotome (Leica Microsystems, Milton Keynes, UK) and examined using a Hitachi 7100 transmission electron microscope (Hitachi Ltd., Tokyo, Japan).

3.6. Impedance and resistance measurements

Measurement of the impedance dynamically monitors living cells. The RTCA SP instrument (ACEA Biosciences, San Diego, USA) measures impedance at 10 kHz, which derives from interaction between cells and electrodes of 96-well E-plates (ACEA Biosciences). This interaction correlates with cell proliferation, viability or with transcellular ion flux in real-time.

Transepithelial electrical resistance was measured by an EVOM resistance meter using STX-2 electrodes (World Precision Instruments Inc., Sarasota, FL, USA) and expressed relative to the surface area ($\Omega \times cm^2$). TEER represents the paracellular permeability of cell layers for ions.

3.7. Permeability study

The flux of the drugs caffeine, antipyrine, atenolol, vinblastine and fluorescent dyes fluorescein and rhodamine 123 across Caco-2 epithelial cell layers was determined in apical to basal (AB) direction. The flux of vinblastine, fluorescein and rhodamine 123 was also measured in basal to apical (BA) direction. Caco-2 cells were seeded onto Transwell filters. After 21 days when the resistance of cell layer was high and stable, the culture medium at the apical compartment was replaced by 500 µl Ringer–Hepes containing drugs or marker molecules with or without absorption enhancers. The plates were kept in an incubator on a rocking platform for 1 hour in case of atenolol, caffeine, antipyrine and rhodamine 123. For vinblastine and fluorescein 2-hour permeability test was performed, and samples were taken from the acceptor phase at both 1- and 2-hour time points. After the incubation samples from the upper and lower compartments were collected and the concentrations of fluorescein and rhodamine 123 were determined by a fluorescent microplate reader (Fluostar Optima) while drug concentrations were measured by HPLC. The apparent permeability coefficients (P_{app}) were calculated in AB and BA directions, and the clearance of the molecules were also evaluated.

3.8. Measurement of plasma membrane fluidity in Caco-2 cells

Cells were labeled with TMA-DPH (1-(4-trimethylammoniumphenyl)-6-phenyl-1,3,5-hexatriene; Molecular Probes, Life Technologies) and fluorescence anisotropy was measured on a T-format fluorescence spectrometer (Quanta Master QM-1, Photon Technology International, Princeton, NJ, USA). After preincubation with buffer the treatment concentrations of surfactants were gradually increased during anisotropy measurements. A strong membrane fluidizer, benzyl alcohol was used as a positive control in the experiments.

3.9. Measurement of efflux pump activity

The activity of efflux pumps was determined by cellular accumulation of rhodamine 123 and calcein AM in Caco-2 epithelial cells. Treatments with surfactants or efflux pump inhibitors were performed in the presence of rhodamine 123. Following incubations cells were lysed with NaOH. Concentration of rhodamine 123 was determined by a fluorescent microplate reader. The calcein AM assay can be used for the measurement of the activity of efflux transporters P-gp, MRP-1 and MRP-2. The cellular transport of the non-fluorescent calcein AM is inhibited by efflux transporters, therefore only limited amount gets into the cells where intracellular esterases convert the dye to a fluorescent metabolite. Treatments with surfactants or efflux pump inhibitors were performed in the presence of calcein AM. Fluorescence was measured by a microplate reader.

To estimate the effect of sucrose esters on P-glycoprotein functionality, calcein AM accumulation was measured in the presence of the test substrates in MES-SA and MES-SA/Dx5 cells. Cells were preincubated with verapamil or surfactants. Thereafter calcein AM was added for further incubation. Cells were gated based on TO-PRO3 positivity. Samples were measured by a FACSCalibur™ flow cytometer (BD Biosciences, San Jose, CA, USA).

3.10. Statistical analysis

All data presented are means ± SD. Values were compared using analysis of variance (ANOVA) followed by Dunnett's test (GraphPad Prism 5.0, GraphPad Software Inc., San Diego, CA, USA). In case of fluorescein, vinblastine, and rhodamine 123 permeability and TMA-DPH anisotropy measurements two-way ANOVA followed by Bonferroni posttest was applied. Changes were considered statistically significant at $P < 0.05$. All experiments were repeated at least two times, the number of parallel samples varied between 4 and 12.

4. RESULTS AND DISCUSSION

4.1. Effects of non-ionic surfactants on viability of intestinal epithelial and vascular endothelial cells

Due to their amphiphilic properties surfactants can easily interact with cell membranes, perturb or disrupt the lipid bilayer. This effect depending on applied concentration and treatment time can be reversible, but higher concentrations may thin or rupture plasma membranes, damage the microvilli in the apical surface of cells, and lead to leakage of intracellular proteins or cell death.

The use of Cremophor EL in medical treatments with various dosage forms is accompanied by many adverse reactions, and understanding the side-effects of Cremophor EL is essential for safety consideration. The toxicity of Cremophor RH40 is less known and examined. In our study the cytotoxicity of Cremophors was tested in epithelial and endothelial cells using concentrations corresponding to clinical administration. Human intestinal epithelial cells were damaged by the Cremophors in a concentration-dependent manner (Fig. 1a-b).

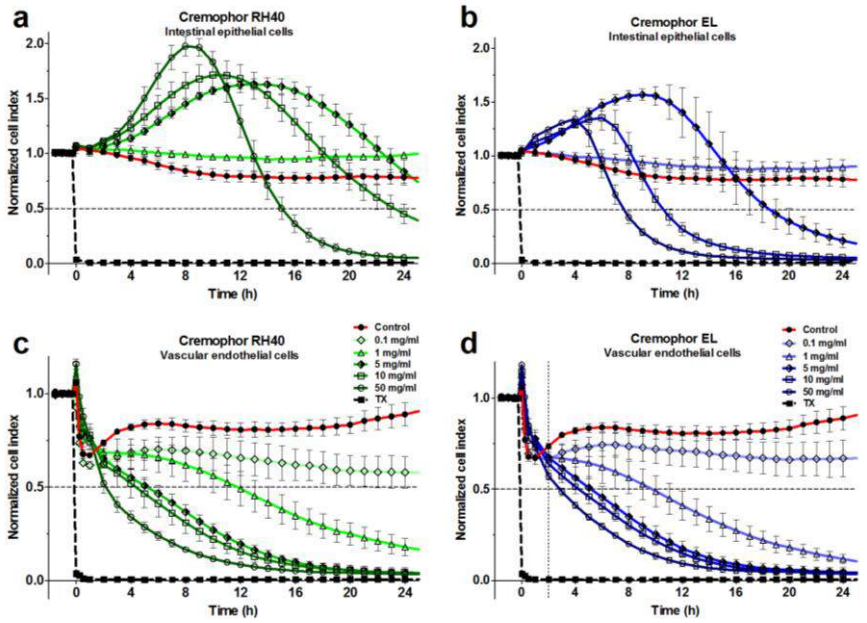

Figure 1. Effects of Cremophor RH40 and EL (0.1-50 mg/ml) on human Caco-2 intestinal epithelial (a, b) and hCMEC/D3 endothelial (c, d) cells measured by real-time cell microelectronic sensing method. Cell index is expressed as an arbitrary unit and calculated from impedance measurements between cells and sensors. Data are presented as mean ± S.D., n = 4. TX, Triton X-100 at 10 mg/ml concentration.

Kinetics of the toxicity of Cremophor RH40 and EL on endothelial cells was also demonstrated in the present work (Fig. 1c-d). The clinically observed side-effects might be related to changes in the viability and monolayer integrity of endothelial cells exerted by Cremophors.

An unexpected effect was seen in epithelial cells treated with surfactants using MTT assay and impedance measurement. The dye conversion and monolayer impedance were transiently increased in higher concentrations of both excipients. Such a change was also observed in endothelial cells, but it lasted only for one hour. MTT assay involves several cellular processes, which may be influenced by Cremophors. Activation of different G protein-coupled receptors in various mammalian cells lead to changes in cell shape and attachment resulting in a transient increase in impedance values. The effect of Cremophors on metabolic activity, endocytosis, or G protein-coupled receptors in epithelial or endothelial cells may cause this phenomenon and further experiments are needed to reveal this interaction.

Endothelial cells were more sensitive than epithelial cells for treatments with Cremophor RH40 and EL. Short term incubations with Cremophors resulted in changes only in endothelial but not in epithelial cells based on impedance measurement (Fig. 1). Cremophor RH40 was less toxic than EL in cultured epithelial and endothelial cells. The reason of the differences in the toxicity of the Cremophors is unknown. Distinct characteristics in the molecular structures may be related to the observed results.

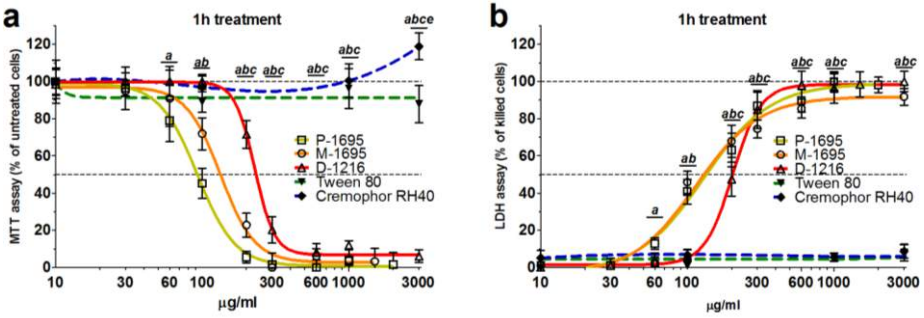

Figure 2. Toxicity of P-1695, M-1695, D-1216 sucrose fatty acid esters (10-3000 µg/ml) and Tween 80, Cremophor RH40 reference absorption enhancers (10-3000 µg/ml) on human Caco-2 intestinal epithelial cells measured by (a) MTT dye conversion and (b) LDH release methods. MTT values were compared to non-treated group (100 % viability). For LDH measurement values were compared to the Triton-X 100 treated group (100 % toxicity). Data are presented as mean ± S.D., n = 6; statistical analysis: ANOVA followed by Dunnett test; statistically significant differences ($P < 0.05$) were detected in the control group compared to the values measured in a: P-1695 group; b: M-1695 group; c: D-1216 group; e: Cremophor RH40 group.

Investigation of sucrose fatty acid esters on Caco-2 cells revealed that D-1216 laurate ester (carbon chain length: 12) was the least toxic, while myristate (carbon chain length: 14) and palmitate (carbon chain length: 16) esters had higher toxicity at both 1- and 24-hour treatments (Fig. 2). The toxic concentrations of the chosen reference surfactants Tween 80 and Cremophor RH40 are at least one order of magnitude higher than that of sucrose esters in our studies and in the literature indicating that reference molecules have a safer toxicity profile on cultured cells than sucrose esters.

4.2. Non-ionic surfactants and drug absorption

It is widely known that surface active agents can improve the absorption of drugs but the mechanisms are not fully explored. The present investigation aimed to reveal the pathways affected by the selected surfactants to induce absorption enhancement. Various methods were used to determine the effect of sucrose esters, Tween 80 and Cremophor RH40 on the para- and transcellular permeability of drugs and tracers.

Sucrose esters decreased the resistance and impedance of epithelial cell layers reflecting elevated ion penetration through the paracellular and transcellular pathways, respectively. However, reference surfactants did not change TEER of Caco-2 monolayers indicating no paracellular barrier opening. The resistance and impedance measurements suggest that sucrose esters enhance ion permeability through para- and trancellular pathways.

	Passive hydrophilic	Passive lipophilic	
	Atenolol	Caffeine	Antipyrine
P-1695 30µg/ml	1.46 ± 0.02 ***	1.01 ± 0.01	0.95 ± 0.06
M-1695 60µg/ml	2.37 ± 0.63 ***	0.99 ± 0.01	1.00 ± 0.02
D-1216 100µg/ml	1.58 ± 0.08 ***	0.98 ± 0.03	0.99 ± 0.03
Tween 80 1000µg/ml	2.11 ± 0.02 ***	1.06 ± 0.02	1.00 ± 0.02
Cremophor RH40 1000µg/ml	1.20 ± 0.15 ***	1.00 ± 0.03	0.98 ± 0.01

Table 1. Fold changes in the apparent permeability coefficients of atenolol, caffeine and antipyrine measured on confluent human Caco-2 intestinal epithelial cell layers after 1-hour treatment with P-1695, M-1695, D-1216 sucrose fatty acid esters and Tween 80, Cremophor RH40 reference absorption enhancers. Data are presented as mean ± S.D., n = 3; statistical analysis: ANOVA followed by Dunnett test; *** $P < 0.001$, all concentrations were compared to control.

Sucrose esters elevated the flux of the hydrophilic drug atenolol through Caco-2 cell layers, indicating the absorption enhancer properties of these surfactants (Table 1). Excipients

significantly increased the penetration of fluorescein in a concentration-dependent manner in the AB direction. Since treatments were applied apically, similarly to *per os* drug administration, moderate or no permeability enhancement was observed in the opposite direction. Tween 80 and Cremophor RH40 increased fluorescein permeability only in higher concentrations, therefore sucrose esters are more effective in small concentrations. Reference surfactants enhanced the permeability of all compounds, except the lipophilic model drugs caffeine and antipyrine.

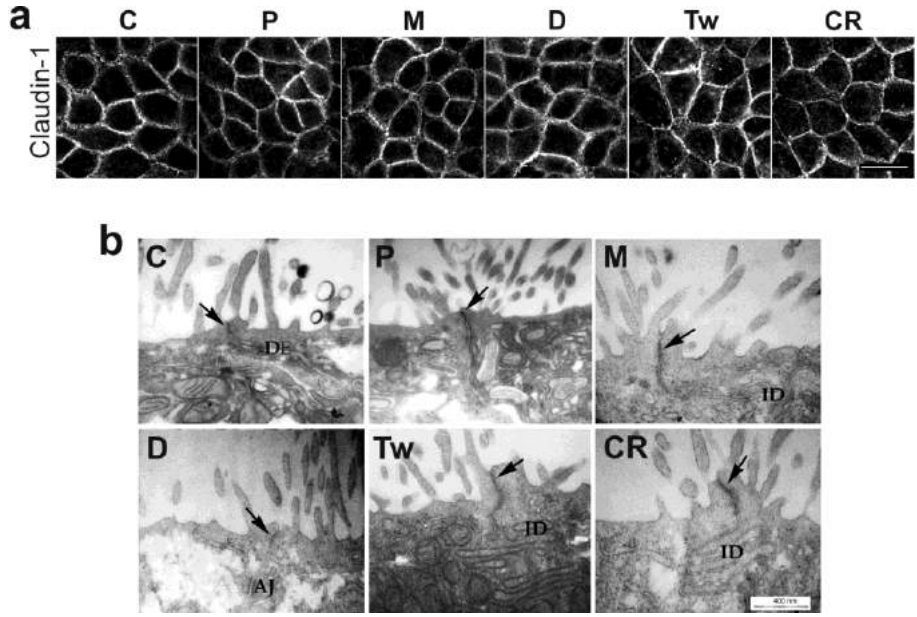

Figure 3. Effect of surfactants on cellular and junctional morphology, fluorescent immunostainings and electron microscopy. (a) Immunostaining for tight junction membrane protein claudin-1 in human Caco-2 intestinal epithelial cells after 1 hour treatment with sucrose esters and reference absorption enhancers. Bar = 20 µm. (b) Transmission electron microscopy at cell-cell connections after 1 hour treatment with sucrose esters and reference absorption enhancers; bar = 400 nm. Applied concentrations: P, 30 µg/ml; M, 60 µg/ml; D, 100 µg/ml; Tw, 1000 µg/ml; CR, 1000 µg/ml. Abbreviations: C, control; P, P-1695; M, M-1695; D, D-1216; Tw, Tween 80; CR, Cremophor RH40; arrow: tight junction; AJ: adherens junction; DE: desmosome; ID: interdigitation.

Immunostaining of junctional proteins, fluorescent labeling of F-actin and transmission electron microscopy were used to visualize intercellular connections and demonstrate the integrity of the paracellular barrier (Fig. 3). It was observed for the first time that sucrose esters and reference compounds did not cause any major change in the distribution of immunostaining for junctional proteins claudin-1 (Fig. 3a), ZO-1 and β-catenin on Caco-2 cells at the applied concentrations. Surfactants slightly changed the organization of F-actin at the junctional region and basal part of the cells, but did not cause visible opening of the junctions. This F-actin redistribution can be linked to

changes in the functional permeability of the junctions. Surfactants did not visibly open and change the morphology of TJs investigated by electron microscopy (Fig. 3b).

The effect of sucrose esters on cell membranes was previously suggested, but it was not investigated in living cells yet. The selected sucrose esters fluidized the plasma membrane of Caco-2 cells at lower concentrations than reference absorption enhancers, and caused stronger TMA-DPH fluorescence anisotropy reduction than Tween 80 or Cremophor RH40. Sucrose esters containing longer fatty acid chain increased better the membrane fluidity at lower concentrations, indicating a correlation between the length of fatty acid chain and effect on membrane fluidity. Other studies confirmed that surfactants, including our reference excipients, increase the fluidity of cellular plasma membranes, which is linked to enhanced membrane permeability and changes in the activity of membrane transporters and efflux pumps.

The resistance, impedance, permeability, morphology and membrane fluidity measurements indicate that several mechanisms are involved in the absorption enhancing effect of sucrose esters (Fig. 4). All surfactants elevated membrane fluidity, which can contribute to increased transcellular permeability. Sucrose esters decreased both resistance and impedance indicating an effect on the function of intercellular junctions and cellular membranes, thus enhancing drug permeability through both the trans- and paracellular routes. Reference molecules changed neither the morphology of cellular junctions, nor the resistance of cell layers, suggesting no effect on paracellular transport.

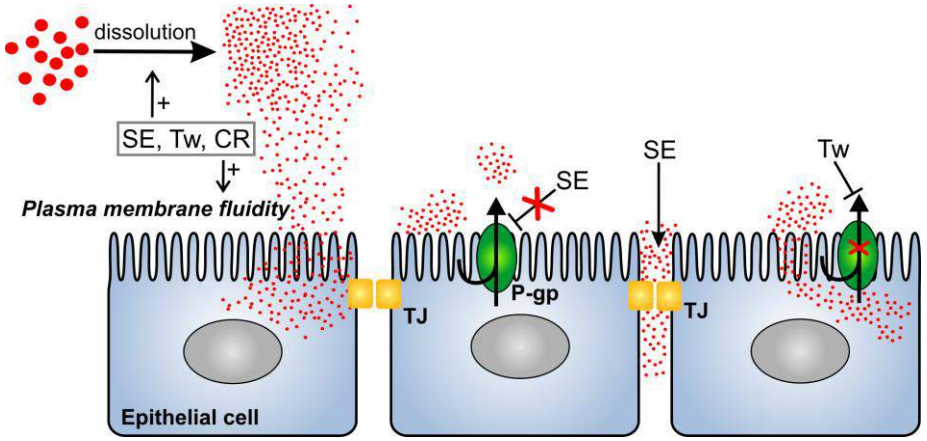

Figure 4. How sucrose esters, Tween 80 and Cremophor RH40 enhance permeability in epithelial cells? Sucrose esters and reference absorption enhancers may increase the penetration of molecules in several ways. (i) Surfactants enhance the dissolution of molecules and change plasma membrane fluidity which contribute to enhanced delivery of agents. (ii) Tween 80, but not sucrose esters, inhibits P-glycoprotein. (iii) Sucrose esters may alter the function but not the visible morphology of cellular junctions, while reference molecules have no effect. Abbreviations: CR, Cremophor RH40; P-gp, P-glycoprotein; SE, sucrose esters; TJ, tight junction; Tw, Tween 80.

4.3. Non-ionic surfactants and efflux pumps

Efflux transporters hinder drug delivery across biological barriers and blocking these pumps is considered as a way to increase drug penetration. In the present experiments sucrose esters, unlike inhibitors or reference surfactants, increased the permeability of vinblastine and rhodamine 123 in AB, but not in the other direction, suggesting no inhibitory effect on efflux pumps. Sucrose esters elevated rhodamine 123 and calcein AM accumulation in co-treatment, which may have suggested an inhibitory effect of efflux transporters. However, our experiments conducted on a model cell line expressing P-gp ruled out this possibility, as the sucrose esters did not influence the P-gp-mediated efflux in MES-SA/Dx5 cells (Fig. 5). Takaishi *et al.* suggested that elevated daunomycine flux in Caco-2 cells caused by sucrose esters is due to permeabilization of the cellular membrane (Takaishi *et al.*, Biosci Biotechnol Biochem, 2006, 70:2703-2711), but our work is the first to experimentally prove that enhanced permeability is unrelated to P-gp inhibition (Fig. 4 and 5).

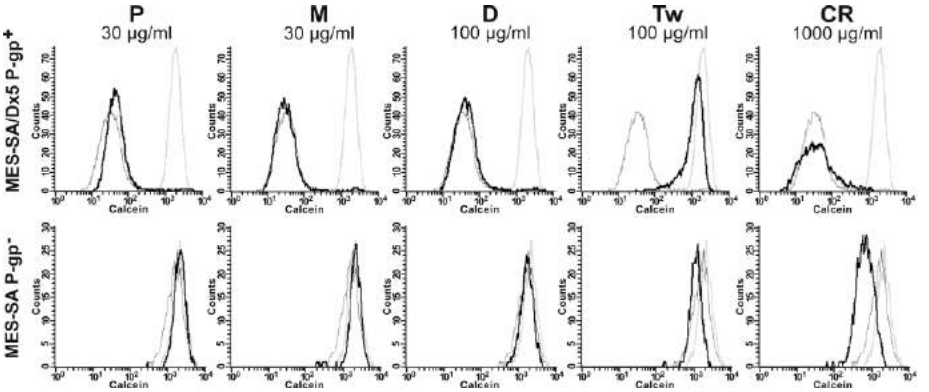

Figure 5. Effect of surfactants on calcein AM accumulation in P-gp positive MES-SA/Dx5 and P-gp negative MES-SA cell lines. Cellular calcein AM fluorescence was determined after the treatments with vehicle (dark gray), verapamil (light gray), and surfactants (black) and is shown in histogram. Applied concentrations: P-1695, 30 µg/ml; M-1695, 30 µg/ml; D-1216, 100 µg/ml; Tween 80, 100 µg/ml; Cremophor RH40, 1000 µg/ml; Verapamil, 100 µM. Abbreviations: C, control; M, M-1695; P, P-1695; D, D-1216; Tw, Tween 80; CR, Cremophor RH40; Ver, verapamil. The assay was repeated three times; the histograms show data from a representative experiment.

5. SUMMARY

Surfactants used in pharmaceutical products have several advantageous properties; they improve drug absorption and dissolution, and protect active molecules. However, adverse effects are also observed during the application of different surface active agents which necessitates the investigation of registered surfactants and novel absorption enhancer candidates.

Cremophor RH40 and EL are widely used excipients in oral and intravenous drug formulations. Studies indicate that Cremophors, especially EL, have toxic side effects, but few data are available on endothelial and epithelial cells which form biological barriers and are directly exposed to these molecules. In our investigation human intestinal epithelial and vascular endothelial cells were treated with Cremophor RH40 and EL in clinically relevant concentrations and their toxic effect was monitored by several methods. Cremophors caused concentration- and time-dependent damage in both epithelial and endothelial cells. Endothelial cells were more sensitive to surfactant treatment than epithelial cells, and Cremophor EL was more toxic than RH40 in both cell types. Our results support and complement the previously experimentally described toxic effects of Cremophor EL which may be related to the clinically recognized side-effects of medicines containing Cremophor EL. Sucrose fatty acid esters are increasingly investigated as novel excipients in pharmaceutical research and some of them are registered in Pharmacopoeias both in the European Union and in the United States. Several studies documented their advantageous properties but few data are available on their toxicity profile, mode of action and efficacy on intestinal epithelial models. Three water soluble sucrose esters, palmitate (P-1695), myristate (M-1695), laurate (D-1216), and two reference absorption enhancers, Tween 80 and Cremophor RH40 were tested on human Caco-2 intestinal epithelial cells. Sucrose esters in non-toxic concentrations significantly reduced resistance and impedance of cells, but not reference surfactants. All excipients increased permeability for drugs and fluidized plasma membrane, but did not visibly open tight junctions. Tween 80 inhibits P-glycoprotein but sucrose esters do not have the same effect. Our data indicate that in addition to their dissolution increasing properties sucrose esters can enhance drug permeability through both the transcellular and paracellular routes but this effect is unrelated to P-glycoprotein inhibition.

The presented results demonstrate the differences between the cellular actions of various non-ionic surfactant excipients, which can be important for the development of new pharmaceutical formulations and drug delivery systems.

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