Stimulation of Triglyceride-Rich Lipoprotein Secretion by Polysorbate 80: *In Vitro* and *in Vivo* Correlation Using Caco-2 Cells and a Cannulated Rat Intestinal Lymphatic Model

Fergal Seeballuck,[1] Emma Lawless,[1] Marianne B. Ashford,[2] and Caitriona M. O'Driscoll[1,3,4]

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Purpose. To assess the effects of polysorbates 80 and 60 on intestinal lipoprotein processing *in vitro*, using Caco-2 cells, and to compare the results with those obtained using an *in vivo* intestinal lymphatic cannulated rat model.

Methods. Caco-2 monolayers were used to monitor changes in lipoprotein secretion following exposure to excipients. *In vivo* data was obtained by monitoring intestinal lymphatic triglyceride levels following intraduodenal administration of the excipient to an anesthetised mesenteric lymph cannulated rat.

Results. Caco-2 cells digested the polysorbate 80 to liberate oleic acid, which was used by the cells to enhance basolateral secretion of triglyceride-rich lipoproteins including chylomicrons. This response was not seen with polysorbate 60. Polysorbate 80 elicited a similar response *in vivo* in the rat model, stimulating enhanced triglyceride secretion in mesenteric lymph. Inhibition of lipoprotein secretion by Cremophor EL in Caco-2 cells was reversed by co-administration with polysorbate 80.

Conclusions. Polysorbate 80 promoted chylomicron secretion in Caco-2 cells and counteracted the inhibitory effects of other surfactants. These properties, in tandem with its P-gp inhibitory activity, make polysorbate 80 an ideal excipient for lymphotrophic vehicles. The ability to predict the *in vivo* response to Polysorbate 80 implies that the Caco-2 model is useful for studying absorption mechanisms from oral lipid-based formulations.

KEY WORDS: bioactive excipients; Caco-2; lipoproteins; mesenteric lymph; polysorbate.

INTRODUCTION

As a result of their low oral toxicity, non-ionic surfactants are frequently used in the design of lipid-based oral drug delivery systems to aid vehicle dispersion and drug solubilization in the gastrointestinal tract (1). Recently, the bioactive nature of these excipients and their subsequent potential to alter physiologic and biochemical absorption barriers has stimulated much discussion. Various Pluronic block copolymers (2), Cremophor EL (3), TPGS (4) and polysorbate 80 (Tween 80) (5) have been shown to inhibit P-glycoprotein (P-gp) activity, resulting in enhanced permeability of drug substrates. In addition, Pluronic L81 has been reported to inhibit secretion of triglyceride-rich lipoproteins (TRL), most specifically, chylomicrons, in rats (6) and in Caco-2 cells (7). The effects of a wider range of surfactants on lipoprotein production and secretion were recently examined using the Caco-2 cell model (8). In this work, a number of Pluronic block copolymers (P85, L81 and F68) and Cremophor EL were all found to inhibit chylomicron secretion in a concentration dependent and reversible manner. Furthermore, a strong correlation was found between excipient-mediated inhibition of lipoprotein secretion and inhibition of P-glycoprotein (P-gp) efflux, implying a close association between the two biochemical processes (8). The ability of such bioactive excipients to simultaneously manipulate different cellular processes may have significant consequences for the absorption and bioavailability of co-administered lipophilic drugs, which are likely to be substrates both for P-gp (9) and for chylomicron-mediated transport via the lymphatic system (10). Although inhibition of P-gp may result in an increase in intestinal absorption, a simultaneous inhibition of chylomicron secretion may reduce the potential for lymphatic drug uptake.

It has widely been reported that certain lipid-based vehicles, particularly those containing long chain unsaturated fatty acids vs. saturated fatty acids, enhance intestinal lymphatic drug transport via stimulation of chylomicron production and subsequent association of the drug with the triglyceride core of the lipoprotein (10). The current work investigates the effects of polyoxyethylated sorbitan esters of fatty acids, namely polysorbate 60 and 80, on the production and secretion of chylomicrons using the Caco-2 cell model. Both these compounds combine the features of a non-ionic polyoxyethylated surfactant, with a fatty acid moiety. Polysorbate 60 (Tween 60) is an ester of the long chain saturated fatty acid, stearic acid (C18:0), and polysorbate 80 (Tween 80) is an ester of the long chain unsaturated fatty acid, oleic acid (C18:1).

Whole animal models, in particular the mesenteric lymph cannulated rat model, are most commonly used for studying intestinal lymphatic drug transport (11). The surgical technique involved in such models is difficult and time consuming. An alternative *in vitro* model capable of simulating *in vivo* conditions would avoid the use of animals and facilitate mechanistic studies at the cellular level not possible in the more complex animal model. A final aim of this work is to compare the enterocyte triglyceride secretory response to lipidic vehicles in Caco-2 cells with that achieved in the mesenteric lymph cannulated rat, and to investigate the existence of a correlation between the two models.

MATERIALS AND METHODS

Materials

Materials for cell culture were obtained from Gibco Life Science (Gibco Life Science, Paisley, UK). Essentially fatty acid free BSA, oleic acid (>99% pure), porcine pancreatin (8X USP), sodium taurocholate, polysorbate 80 and polysorbate 60 were obtained from Sigma Dublin, Ireland and used as supplied. Cremophor EL was a gift from BASF Corp. (Mount Olive, NJ, USA). [1-^{14}C] oleic acid (2.07 GBq/

[1] Department of Pharmaceutics and Pharmaceutical Technology, University of Dublin, Trinity College, Dublin 2, Ireland.
[2] Preformulation & Biopharmaceutics, AstraZeneca, Alderley Park, Macclesfield, Cheshire, England.
[3] Current address: School of Pharmacy, BioSciences Institute, University College Cork, Ireland.
[4] To whom correspondence should be addressed. (e-mail: caitriona.odriscoll@ucc.ie)

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mmol) was obtained from Amersham Pharmacia Biotech (Little Chalfont, UK). The monoclonal antibody, 1D1, was obtained from the University of Ottowa Heart Institute (Ottowa, Ontario, Canada); the anti-Apolipoprotein B, (human from sheep) was obtained from Boehringer Mannheim (Germany); and the alkaline phosphatase-conjugated rabbit affinity purified antibody to sheep IgG (whole molecule) from Cappel (Basingstroke, UK) . The triglyceride assay kit, PAP 150, was obtained from BioMérieux, (Marcy L'Etoile, France) and the non-esterified fatty acid (NEFA) test kit was obtained from Randox (Antrim, UK). All solvents used were of HPLC grade.

Methods

Cell Culture

Caco-2 cells were obtained from the European Cell and Animal Culture Collection (ECACC) and were cultured as previously described (8). The cells (passage 65-85) were seeded at a density of 6.3×10^4 cells/ cm^2 onto Transwell filter inserts with 0.4-μm pores (Costar, High Wycombe, UK). Inserts were placed into 6-well plates and cultured for 19–22 days to allow cell differentiation. Transepithelial electrical resistance (TEER) was measured using an EVOM voltohmmeter with chopstick electrodes (World Precision Instruments, Stevanage, UK).

Fatty Acid Vehicles

The "fed-state" simulated vehicle comprised 0.5 mM oleic acid bound to 0.125 mM bovine serum albumin (BSA) and was prepared as previously described (8). A "fasted-state" vehicle was prepared to monitor background (unstimulated) lipid turnover and comprised 0.125 mM BSA. The stearic acid vehicle was prepared in the same way as "fed-state," by substituting stearic acid for oleic acid. All vehicles were labeled with trace amounts of [^{14}C] oleic acid, which has been shown to be an effective general marker for lipid synthesis in cell work (8,12). Test excipients were added to "fed-" or "fasted-state" vehicles as required.

Excipient Toxicity

Toxicity of excipients to Caco-2 cells was determined by an MTT (3-(4,5 dimethylthiazolyl-2)–2,5-diphenyltetrazolium bromide) assay for intracellular dehydrogenase activity (13). MTT results are expressed as percent cell viability relative to that of cells incubated in culture medium alone over the same period. In addition, TEER values were used as a measure of monolayer integrity.

Vehicle Administration

Monolayers were rinsed thrice and then incubated in serum free culture medium for 4 h. Test vehicles were added to the apical side of monolayers (1.5 ml) and serum-free culture medium was added to the basolateral well (3 ml). Cells were incubated for 20 h at 37°C, 5% CO_2. This long incubation time was required to allow sufficient accumulation of secreted lipoproteins for accurate quantification and ultracentrifugation analysis and is standard in such studies (14).

Sample Preparation

For intracellular determination monolayers were rinsed and lysed as described (8). Samples of basolateral medium were centrifuged at $5000 \times g$ for 10 min to remove cell debris. ε-Amino-*n*-caproic acid (EACA), aprotinin, and phenyl methyl sulfonyl fluoride (PMSF) were added as protease inhibitors.

Lipid and Apoprotein B Analysis

Lipid analysis was conducted on cell lysate and basolateral samples. Lipids were extracted in chloroform: methanol (2:1) as described by Folch *et al.* (15). Lipid subclasses were separated from extracts via thin layer chromatography using petroleum ether:diethyl ethyl ether:acetic acid (80:20:1) as developing solvent. Lipid bands were visualized by exposure to iodine vapor, scraped into scintillation vials, and quantified via liquid scintillation counting following addition of Ultima Gold (Packard Bio, Baconsfield, UK) scintillation fluid.

The apoprotein B (apo B) content of basolateral samples and cell lysates was determined using an indirect sandwich ELISA as previously described (16).

Isolation of Lipoproteins

Lipoproteins were isolated from basolateral samples according to flotation density using density gradient ultracentrifugation as previously described (7).

Determination of Fatty Acid Content

The total free fatty acid content of polysorbate solutions was determined using a Randox (Antrim, UK) non-esterified fatty acid (NEFA) colorimetric test kit.

In Vitro Digestion of Polysorbate Surfactants

The sensitivity of polysorbate 80 to digestion was investigated in three separate experiments. Firstly, the ability of Caco-2 cells to digest the excipient was investigated by measuring the free fatty acid content of a solution following incubation with Caco-2 homogenate (equivalent to one monolayer in 1.5 ml media) over 20 h at 37°C. In a second experiment, apical medium was collected from Caco-2 cell monolayers after a 20 h incubation at 37°C and polysorbate 80 (0.25% w/v) was subsequently added and incubated for a further 20 h. Free fatty acid content was measured following this period. In a third experiment, sensitivity to intestinal luminal digestion was investigated by incubating polysorbate solutions with porcine pancreatin (8X USP, 0.04 mg/ml) in simulated intestinal fluid (5 mM sodium taurocholate with 1.25 mM lecithin in 10 mM Tris buffer, pH 6.8). Free fatty acid content was measured following a 2 h incubation. Controls were prepared in each case in the absence of polysorbate 80 and processed in the same manner as test systems to determine background absorbance. All determinations were made in triplicate.

Fatty Acid Composition of Polysorbates

The fatty acid composition of polysorbate 60 and 80 was determined using gas chromatography (GC). Fatty acids were initially transmethylated by boiling in boron-trifluoride methanol complex and extracted into hexane as described (17). Methylated fatty acid extracts were dried under nitrogen and stored at –70°C until analysis. Samples were reconstituted in hexane and analyzed via GC on a Shimadzu

(Milton Keynes, UK) GC-14A series gas chromatograph fitted with a Shimadzu C-R6A integrator and a BP 21 aluminum silica column (Scientific Glass Engineering Pty. Ltd, Milton Keynes, UK) using a carrier gas of oxygen free nitrogen as previously described (18). The fatty acid methyl esters were identified with a flame ionisation detector and their retention times compared against fatty acid methyl ester standards (Sigma).

Animal Model

All animal experiments were performed in accordance with EU directive 86/609 (as implemented in Ireland by Statutary Instrument No. 17/9) in association with the BioResources Unit of Trinity College Dublin, which is registered with the Department of Health. The facility is under the full-time direction of a veterinary surgeon who maintains the health and welfare program in compliance with appendix A of the directive.

Male Wistar rats (280–320 g) were fasted overnight and anesthetized for the duration of the experiment with sodium pentobarbital. The duodenum and the mesenteric lymph duct were cannulated as previously described (19). Animals were allowed to recover for 1 h following surgery. Test polysorbate 80 solutions (2%, 4%, 10% w/v in normal saline) or a saline control were subsequently administered intraduodenally at 1.5 ml/h over 2 h. Lymph was collected hourly for 8 h post-administration. To maintain body hydration and intestinal lymph flow, the duodenum was continuously perfused (except during administration of the test formulations) with normal saline at 1–1.5 ml/h via a constant infusion syringe pump. Lymphatic triglyceride levels were quantified directly using a Biomérieux colorimetric assay kit.

Data Analysis

Data analysis was conducted using Minitab 13.2 software. Where multiple samples were compared against a control, an analysis of variance (ANOVA) was conducted and significance relative to control calculated using Dunnett's multiple comparison test. Where multiple samples were compared against each other, an ANOVA was conducted and significance calculated using Tukey's multiple comparison test.

RESULTS

Effect of Polysorbate Surfactants on Lipoprotein Secretion in "Fed" Stimulated Caco-2 Monolayers

Control

Addition of the "fed-state" control vehicle to the apical side of Caco-2 monolayers stimulated secretion of triglyceride rich lipoproteins (TRL) of flotation density equivalent to chylomicrons and VLDL as determined by radiolabel incorporation. Under these conditions, approximately 5% of administered label was secreted as TRL over 20 h (Figs. 1a, 1b), as compared to <0.4% with the "fasted-state" control (Fig. 3b).

Addition of Polysorbate 80 to "Fed-State"

When polysorbate 80, the polyoxyethylene sorbitan ester of oleic acid, was added at increasing concentrations to the "fed-state" control, a further increase in the secretion of chylomicrons was observed. The response was concentration dependent, with significance relative to standard "fed-state" ($p < 0.05$) at 0.15% (w/v) and upward (Fig. 1a). Cell viability over the investigated concentration range was confirmed using an MTT assay, with 100% cell viability being maintained at 0.5% (w/v) polysorbate 80. Monolayer integrity, over the same concentration range, was confirmed by TEER values. VLDL secretion remained unchanged relative to "fed-state" levels over the investigated concentration range (Fig. 1a).

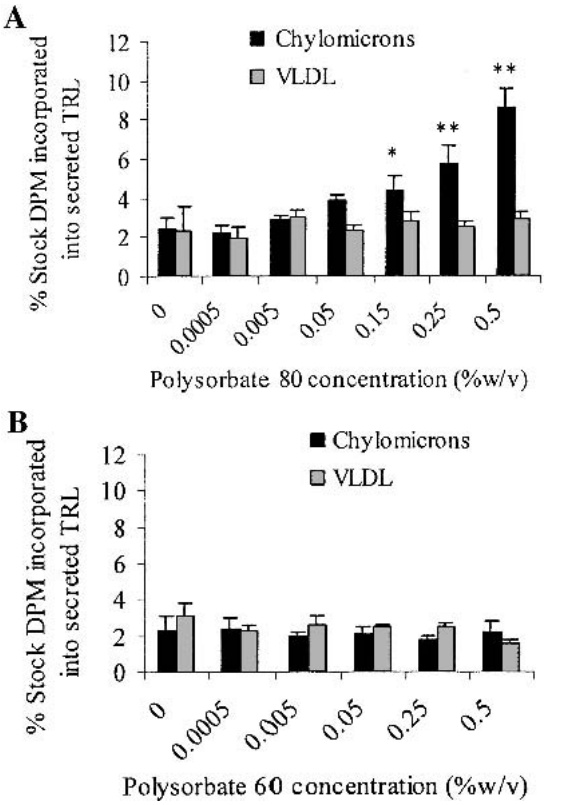

Fig. 1. Effect of increasing concentrations of polysorbate 80 (panel A) and polysorbate 60 (panel B) on TRL secretion in "fed" stimulated cells. Results are expressed as % of administered [^{14}C] oleic acid stock incorporated into the fractions. Values are the mean of 3-5 monolayers ± SD. Statistical significance of excipient effects relative to "fed-state" are shown: $^{**}p < 0.01$, $^{*}p < 0.05$.

Addition of Polysorbate 60 to Fed-State

In contrast addition of polysorbate 60, a polyoxyethylene sorbitan ester of stearic acid, over the same concentration range had no effect on either chylomicron or VLDL secretion levels when applied with "fed-state" (Fig. 1b). Cell viability remained at >90%, as measured via MTT and TEER values, at 0.25% w/v polysorbate 60, but was significantly reduced (50% dehydrogenase activity and 80% monolayer integrity, $p < 0.05$) at 0.5% w/v.

Effect of Polysorbate 80 on Cellular Accumulation and Secretion of Triglyceride and Apoprotein B in "Fed" Stimulated Caco-2 Monolayers

In the case of polysorbate 80, in addition to monitoring secretion of TRL (via ultracentrifugation), the total cellular accumulation and secreted basolateral content of triglyceride

and apo B were determined following 20 h incubation. Triglyceride and apo B, being key components of TRL, are useful indicators of TRL secretion levels (8). Apo B is incorporated into TRL in a 1:1 ratio, and hence presents a suitable means of quantifying the number of secreted lipoproteins and the triglyceride: apo B ratio can be used to indicate the triglyceride loading of secreted lipoproteins. The level of triglyceride secretion (Fig. 2a) in the presence of increasing concentrations of polysorbate 80 followed a similar trend to that of chylomicron secretion (Fig. 1a) with a concentration dependent increase. However, secreted apo B levels did not change significantly over the investigated concentration range indicating an increase in the size and lipid loading, but not the number, of secreted lipoproteins. This is consistent with incorporation of triglyceride into secreted chylomicrons as seen in the gradient data (Fig. 1a). Intracellular data showed no changes above "fed-state" levels in accumulation of triglyceride or apo B over the investigated concentration range (Fig. 2b). This suggests that the additional TRL stimulatory effects occur at the final assembly/ secretion steps of lipoprotein metabolism.

Effect of Polysorbate Surfactants on Lipoprotein Secretion in "Fasted-State" Caco-2 Monolayers

Addition of polysorbate 80 (0.25% w/v) to the fasted-state (i.e., in the absence of supplemental oleic acid) stimulated selective secretion of TRL (chylomicrons and VLDL) (Fig. 3a). This secreted lipoprotein profile was similar to that observed in the presence of free oleic acid (0.5 mM) (equivalent to "fed-state"), which is the constituent fatty acid of polysorbate 80 (Fig. 3b). In contrast, addition of polysorbate 60 to the fasted-state selectively stimulated the secretion of LDL (Fig. 3a). Again, this mirrored the secreted lipoprotein profile obtained following administration of the principal constituent fatty acid, stearic acid (0.5mM) (Fig. 3b).

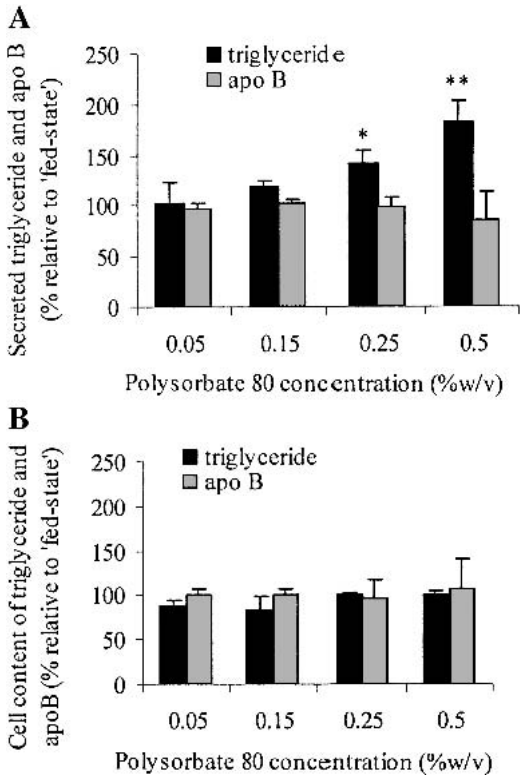

Fig. 2. Effect of increasing concentrations of polysorbate 80 in "fed" stimulated cells on the basolateral secretion (panel A) and cellular accumulation (panel B) of triglyceride and apo B over 20 h. Results are expressed relative to "fed-state" levels for triglyceride (estimated as % stock DPM incorporated) and apo B (measured via ELISA). Values are the mean of 3–5 monolayers ± SD. Statistical significance of excipient effects relative to "fed-state" is shown: $^{**}p < 0.01$, $^{*}p < 0.05$.

Mesenteric Lymph Cannulated Rat Model

Intraduodenal administration of increasing concentrations of polysorbate 80 (2–10% w/v) in saline to the rat resulted in a corresponding increase in triglyceride output in the mesenteric lymph (Fig. 4). At concentrations of 4% and 10% (w/v), polysorbate 80 significantly enhanced triglyceride output relative to the saline control ($p < 0.05$). These elevated lymphatic triglyceride levels are indicative of an increase in secretion of TRL including chylomicrons. Consequently, the response shown by the Caco-2 cells in the presence of polysorbate 80 is reflected *in vivo*, suggesting a correlation between the models.

Fatty Acid Composition and Digestion of Polysorbate Surfactants

Using gas chromatography, the fatty acid composition of polysorbate 80 was confirmed to be 75.6 ± 1.6% oleic acid (with approximately 7.5% as linoleic acid and the remainder as palmitic, palmitoleic and stearic acid) and that of polysor-

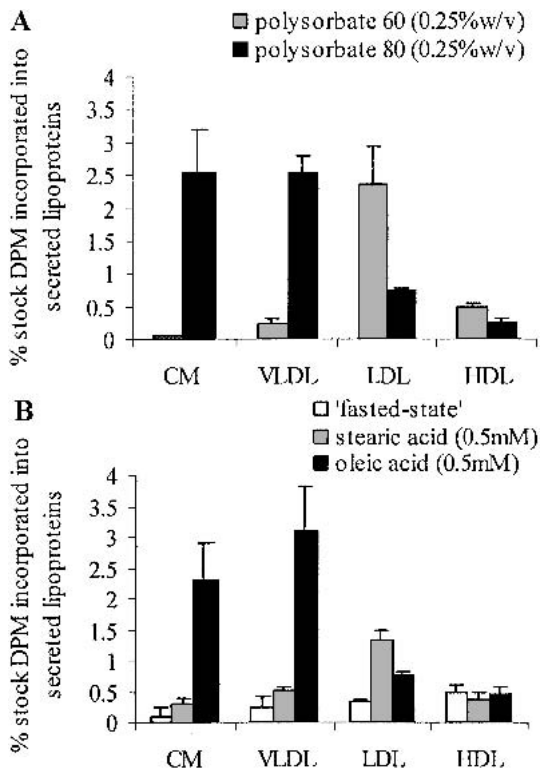

Fig. 3. Secreted lipoprotein profiles in "fasted" cells following exposure to polysorbate solutions (panel A) and fatty acid vehicles (panel B). Values are the mean of 4 monolayers ± SD.

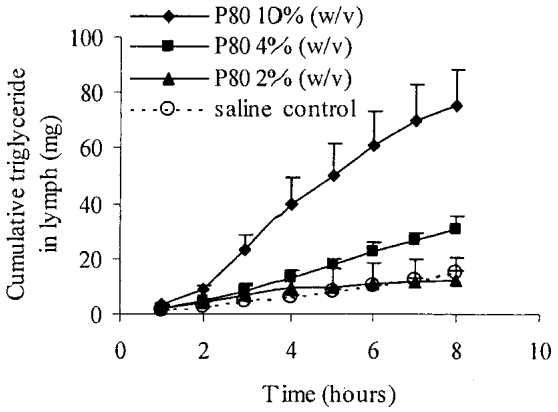

Fig. 4. Cumulative triglyceride content (mg triglyceride, mean ± SD) of rat mesenteric lymph following intraduodenal administration of increasing concentrations of polysorbate 80 (n = 6).

bate 60 to be 52.0 ± 0.6% stearic acid (with the remainder largely comprising palmitic acid). This is in accordance with product specifications as given by the supplier (Sigma-Aldrich, Dublin, Ireland). The key difference between the polysorbate surfactants is their fatty acid composition. Given the similarity between the secreted lipoprotein profiles in response to polysorbate 80 and oleic acid (Figs. 3a, 3b), it is clear that the oleic acid component of polysorbate 80 is responsible for the observed increase in TRL output (Fig. 1a). This may be due either to free fatty acid present in the excipient as supplied, or to free fatty acid liberated from the ester by hydrolysis.

The total free fatty acid content of 0.25% (w/v) polysorbate 80 solution was determined to be approximately 0.1mM (Table I). However, administration of polysorbate 80 (0.25% w/v) to Caco-2 cells in the fasted-state produced a significantly higher level ($p < 0.01$) of chylomicron secretion [2.54 ± 0.25% of administered stock as disintegration per minute (DPM)] than was obtained following administration of 0.1 mM oleic acid (0.17 ± 0.02% of administered stock DPM). The level of chylomicron secretion achieved with polysorbate 80 (0.25% w/v) was comparable to that produced by incubation with 0.5 mM oleic acid (2.26 ± 0.49% of administered stock DPM, Fig. 3b). This indicates that the free fatty acid content of polysorbate 80 as supplied is insufficient to promote the observed TRL response.

The sensitivity of polysorbate 80 to digestion and ester hydrolysis was therefore investigated. Relative to DMEM, incubation of polysorbate 80 (0.25% w/v) with Caco-2 homogenate resulted in a significant increase in the level of free fatty acid in the test solution ($p < 0.01$) (Table I). Incubation of polysorbate 80 (0.25% w/v) with apical medium harvested from Caco-2 monolayers also produced a significant increase in free fatty acid concentration ($p < 0.01$) (Table I), which was equivalent to 0.5 mM oleic acid, and hence could explain the level of chylomicron secretion observed with polysorbate 80. The presence of ester hydrolyzing activity in the apical medium may be due to secretion of enzyme or cell lysis. Finally, incubation of polysorbate 80 solution with porcine pancreatin under simulated intestinal conditions over 2 h also resulted in liberation of free fatty acid, thus confirming sensitivity to intestinal digestion.

Table I. Free Fatty Acid Content of Polysorbate 80 Solution (0.25% w/v) Prior to and Following *In Vitro* Digestion[a]

Incubation conditions for polysorbate 80 (0.25% w/v)	Free fatty acid concentration[b] (mM) (SD)
Solution in DMEM; preincubation	0.10 (0.02)
Incubation in blank DMEM (20 h)	0.12 (0.05)
Incubation with pancreatin (2 h)	1.34 (0.12)
Incubation (20 h) in apical medium (harvested from cell monolayers)	0.52 (0.09)
Incubation (20 h) with cell homogenate	1.45 (0.30)

[a] Values represent the mean (SD) (n = 3).
[b] Values were corrected for background readings of each test system incubated without polysorbate 80.

Effect of Polysorbate 80 on Cremophor EL–Mediated Inhibition of Chylomicron Secretion

Previously, we reported inhibition of chylomicron secretion by Cremophor EL (0.1% w/v) in the Caco-2 model (8). To investigate if this inhibitory response could be partly reversed or ameliorated by the promotional effects of polysorbate 80, blends of Cremophor EL:polysorbate 80 (1:1 and 1:2) were administered with the "fed-state". When polysorbate 80 was administered at a 1:1 ratio, a significant improvement in chylomicron secretion was observed relative to a Cremophor EL (0.1% w/v) control, resulting in a 60% reduction in inhibition ($p < 0.05$) (Fig. 5). Co-administration of polysorbate 80 at a 2:1 ratio with Cremophor EL resulted in complete reversal of the inhibitory effects of Cremophor EL. As expected, substitution of polysorbate 60 for polysorbate 80 in the blend failed to reverse the inhibition (Fig. 5).

DISCUSSION

To date, the mechanisms associated with enhanced oral drug absorption from lipid-based vehicles have been poorly elucidated. It is, however, accepted that intestinal lymphatic transport may play a significant role in absorption, particularly for lipophilic drugs (10). Lymphatic transport of lipophilic drugs is thought to occur in association with the lipid load of newly synthesized chylomicrons. Therefore, a lympho-

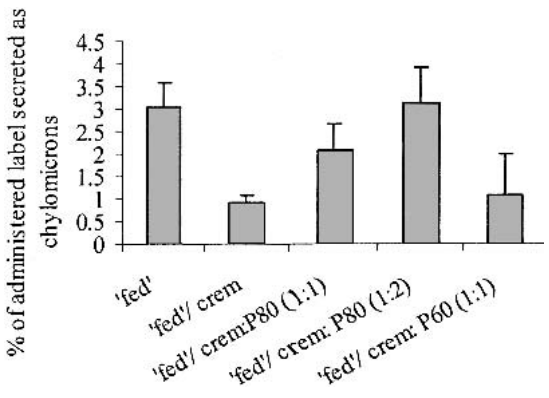

Fig. 5. Chylomicron secretion response in Caco-2 cells following administration of "fed-state" with Cremophor EL or "fed-state" with Cremophor EL/ polysorbate 80 or Cremophor EL/ polysorbate 60 combinations. Values are the mean of four monolayers ± SD.

tropic delivery system should be formulated to drive chylomicron formation and thus maximize transport via this route.

Polysorbate 80 (polyoxyethylene sorbitan mono-oleate) is a GRAS listed surfactant (20) frequently used in lipid-based vehicles, including microemulsions (1), to enhance solubilization of poorly water soluble drugs and to aid dispersion of the vehicle in the aqueous environment of the intestine (20,21). Polysorbate 80 has been shown to inhibit intestinal P-gp efflux (5), thus increasing the concentration and the residence time of a P-gp substrate within the enterocyte. The work described here has shown that polysorbate 80 has marked intestinal TRL stimulatory activity. The ability to simultaneously increase residence time, via P-gp inhibition, and to enhance the production of chylomicrons should provide greater opportunity for a co-administered lipophilic drug to become incorporated into the chylomicrons and hence be transported via the lymphatic system. These complementary bioactive properties make polysorbate 80 and ideal excipient for incorporation into lymphotropic vehicles.

The results of the current work demonstrate that polysorbate 80 is digested in the intestine, by pancreatic enzymes and also by Caco-2 cell monolayers in the absence of pancreatin, to liberate the long chain unsaturated fatty acid, oleic acid. Digestion was initiated by Caco-2 homogenate and also, to a lesser extent, by the apical medium harvested from cell monolayers. Susceptibility of polysorbate surfactants to enzyme hydrolysis by a number of mammalian and bacterial lipases and esterases has previously been reported (22), and sensitivity to pancreatin digestion has been shown here. Given that intestinal mucosal lipase and esterase activity has been identified in the rat (23–26), and intestinal mucosal esterase activity has been found in the human (27), it is possible that polysorbate 80 hydrolysis may be mediated by a combination of esterase and lipase activity in both the intestinal lumen and within the mucosa. The hydrolytic enzyme activity identified in the apical medium may be due to secretion of brush border enzymes into the medium, or may result from the release of intracellular enzymes via cell lysis.

Polysorbate 80 was able to compensate for the inhibitory effects of Cremophor EL on TRL secretion when presented to Caco-2 cells in a surfactant blend. This means that the solubilization power of both excipients can be exploited without significant negative effects on TRL turnover. This reinforces the need for an appreciation and understanding of the bioactive nature of individual excipients in order to facilitate a logical choice of excipients when designing multicomponent lipid-based formulations.

The ability of polysorbate 80 to enhance TRL secretion in the Caco-2 cells was qualitatively mirrored in the rat intestinal lymphatic model. However, responses occurred at quantitatively different concentrations in the two models, reflecting inherent differences in sensitivity. The Caco-2 model comprises a single, static layer of cells in constant contact with concentrated test formulations and consequently will be more sensitive to applied test compounds, such as surfactants (28). In contrast, in the rat model, solutions are perfused through intact intestine and are subsequently diluted by the gastrointestinal fluids and hydrating solution. Despite these conformational differences, the cell model can predict the *in vivo* response and is therefore a useful and complementary *in vitro* model for studying absorption mechanisms of lipid-based vehicles.

In summary, the properties of polysorbate 80 including; solubilization of lipophilic drugs, inhibition of P-gp efflux, stimulation of TRL, and the ability to counteract inhibition of TRL secretion by other co-administered surfactants, make it an ideal excipient for lymphotropic vehicles. Properties of the Caco-2 model including endogenous lipase activity and excipient-dependent TRL response make it a useful *in vitro* model for studying absorption mechanisms at the cellular level.

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