Water-in-Oil Microemulsions Containing Medium-chain Fatty Acids/Salts: Formulation and Intestinal Absorption Enhancement Evaluation

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Purpose. Water-in-oil (w/o) microemulsions have been developed which, in addition to non-ionic medium-chain glycerides, incorporate ionic lipids, primarily medium-chain fatty acids, such as caprylic (C_8) capric (C_{10}) and lauric (C_{12}) acids and their corresponding sodium salts. The absorption enhancing activity of w/o microemulsions incorporating these lipids was evaluated in the rat using Calcein (MW = 623) a water-soluble and poorly absorbed marker molecule.

Methods. Phase diagrams were constructed where C_8/C_{10} or C_{12} fatty acids were treated as lipophilic surfactants and their sodium salts as hydrophilic ones. The anesthetised rat model was employed to evaluate Calcein absorption upon a single intraduodenal administration from a solution and the various w/o microemulsions.

Results. A wide range of clear and transparent w/o microemulsions were obtained at ambient temperature either in liquid or solid form when a fixed blend of medium chain fatty acid/salt was titrated by a fixed ratio of the oil containing the oil-soluble mono- and diglycerides and deionized water or physiological saline. Upon intraduodenal administration in the anesthetised rat, the absorption of Calcein was improved from about 2% in aqueous solution up to about 37% in w/o microemulsions. Solid and liquid formulations were equally effective in improving bioavailability. The absorption enhancement activity of the fatty acids/salts followed the order $C_8 \approx C_{10} > C_{12}$. Absorption enhancement of Calcein was significantly reduced in the absence or presence of low levels of C_8/C_{10} mono-/diglycerides.

Conclusions. These results further support the use of medium-chain glycerides and fatty acids/salts in microemulsion formulations to improve intestinal absorption of water-soluble compounds.

KEY WORDS: w/o microemulsions; medium-chain glycerides; medium-chain fatty acids/salts; enhancer; intestinal absorption; calcein.

INTRODUCTION

Lipid microemulsions incorporating medium-chain glycerides have attracted much interest in recent years as oral dosage forms to improve drug dissolution and/or intestinal absorption (1). Early studies have shown that medium-chain glycerides and fatty acids improved intestinal absorption of water-soluble molecules, particularly in the lower gastrointestinal tract (2–4). Among these lipids, C_8/C_{10} mono-/diglycerides and sodium salts of C_8/C_{10} fatty acids were shown to be the most effective. In the case of medium-chain fatty acids, C_8/C_{10} fatty acid sodium salts have been demonstrated to enhance the rectal absorption of penicillins and cephalosporins in rats (2–4). Thus, enhanced rectal absorption of amphicillin (2), cefoxitin (3) and cefmetazole (4) has been observed in rats with sodium caprate or caprylate.

We have recently reported on the formulation and intestinal absorption enhancement of Calcein (5) and an RGD peptide (5,6) in rats from w/o microemulsions of different composition and particle size. The formulations used in those studies (5,6), which incorporated non-ionic lipids and surfactants, demonstrated that improved absorption was dependent on the lipid composition of the microemulsion, particularly on the presence of medium-chain glycerides (mono-/di- and triglycerides). In the present report, the evaluated w/o microemulsions, in addition to medium-chain glycerides, contained ionic lipids in the form of medium-chain fatty acids/salts. The results indicate: a) in pseudo-ternary phase diagrams the microemulsion existence field was significantly modified by the presence of ionic lipids; b) there was a significant contribution to the observed absorption enhancement of Calcein by medium-chain fatty acids/salts with the C_8/C_{10} fatty acids being the most effective; and c) absorption enhancement of Calcein was significantly reduced in the absence or presence of low levels of C_8/C_{10} mono-/diglycerides.

MATERIALS AND METHODS

Materials

Arlacel 186 (90:10% w/w of monoolein:propylene glycol, HLB = 2.8) was provided by ICI Americas, Inc. (Wilmington, DE). Capmul C_8 (C_8 mono-/diglycerides, 1/1 w/w, HLB = 5–6), Capmul MCM (C_8/C_{10} mono-/diglycerides, 1/1 w/w, HLB = 5–6), the oils Captex 355 (C_8/C_{10} triglycerides), Captex 200 (C_8/C_{10} diesters of propylene glycol) and Captex 8000 (C_8 triglycerides) were supplied by Karlshamns Lipid Specialties (Columbus, OH). Capmul C_8 is primarily estrerified with caprylic acid and according to the manufacturer its fatty acid distribution is 0.8% caproic (C_6), 97.8% caprylic (C_8) and 1.4% capric (C_{10}). Witepsol H-15 oil (90:10, % w/w of C_{12} glycerol triesters:diesters) with less than 2% C_{12} monoester and Imwitor 308 (80–90% wt. of C_8 monoglycerides, HLB = 6.0) were both provided by Hüls America, Inc. (Piscataway, NJ). Caprylic (C_8, HLB = 5.8), Capric (C_{10}, HLB = 4.8), and Lauric (C_{12}, HLB = 3.8) acid and sodium caprylate (HLB = 23.0), sodium caprate (HLB = 21.0) and sodium laurate (HLB = 13.9) as well as Tween 80 (polyoxyethylene sorbitan monooleate, HLB = 15.0) and propylene glycol were purchased from Sigma Chemical. Co. (St. Louis, MO). Super refined soybean oil was purchased from Croda Inc. (Mill Hall, PA). According to the manufacturer the fatty acid pattern of soybean oil is: 54% linoleic ($C_{18:2}$), 25% oleic ($C_{18:1}$), 6% linolenic ($C_{18:3}$), 4% stearic (C_{18}) and 11% palmitic (C_{16}). Myverol 18–92 (HLB = 3.7) which is distilled sunflower oil monoglycerides (90%

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Abbreviations: AUC, area under the plasma concentration-time curve; F, absolute bioavailability; GI, gastrointestinal; HLB, hydrophile-lipophile balance; i.d., intraduodenal; i.v., intravenous; MCM, medium-chain monoglycerides, ME, microemulsion; Na, sodium, W/O, water-in-oil.

glyceryl linoleate) was supplied by Eastman Chemicals (Kingsport, TN). High purity Calcein [5(6) carboxyfluorescein, MW = 623] was obtained from Molecular Probes, Inc. (Eugene, OR). Physiological saline (0.9% sodium chloride, USP), having a pH of 6.0 and osmolarity of 300 mOsm/liter was obtained from Baxter (Deerfield, IL).

Microemulsion Formulation/Phase Diagrams

Two different formulation strategies were employed to generate pseudo-ternary phase diagrams of oil, surfactant(s) and water or saline. In the first strategy, a given blend of the oil plus the low HLB surfactant (i.e, Captex oil and Capmul MCM) was titrated with a fixed blend of medium-chain fatty acid/fatty acid salt (i.e. caprylic acid/sodium caprylate) and water or saline looking for clear and transparent formulations at ambient temperature. In this strategy, the medium-chain mono-/diglycerides represent the primary low HLB surfactant whereas, the medium-chain fatty acid/salt represent a secondary low HLB/ high HLB surfactant, respectively. In a second approach, the oil (Captex 200) was titrated with the aqueous phase (1:1% w/w of water:propylene glycol) and a surfactant blend incorporating different surfactants at fixed weight ratio (Imwitor 308/ Tween 80/Capric Acid/Sodium Caprate, 2.2/4.4/2.4/1.0).

Microemulsion Preparation and Calcein Incorporation

Once the microemulsion existence field has been identified, w/o microemulsions were routinely prepared as previously described (5,6) by admixing appropriate quantities of the various components with gentle hand-mixing, vortexing or stirring if necessary to ensure thorough mixing. The solubility of Calcein at 25°C in 0.010 M Tris pH 7.4 exceeds 100 mg/ml and its oil/buffer partitioning at 37°C using Capmul MCM:Ringer's buffer (1:1, v/v) is 7/93 (5). This compound is negatively charged in the physiological pH range (5–9) and at pH 7.0 it carries a net negative charge of two. For the preparation of Calcein-incorporating microemulsions, the compound was first dissolved in the hydrophilic phase by dilution of a stock solution followed by the addition of other components in an order depending on the formulation approach used as described in the construction of phase diagrams. W/O microemulsions that are solid at room temperature (ME4, ME5 and ME7) were prepared by admixing the high melting oil (Witepsol H-15, m.p. 33–36°C) with the other components as described above. The solution of components was heated to a slightly elevated temperature (30–50°C) during mixing and then cooled to a solid at ambient temperature. Whilst higher temperatures (30–50°C) may be needed to solubilize all components during the preparation of microemulsion, the microemulsions which are liquid at ambient temperature can be formulated at this temperature. This is particularly advantageous for thermolabile compounds/ peptides. The compositions of the investigated w/o microemulsions are shown in Table 1.

Absorption Studies

The research with animals adhered to the "Principles of Laboratory Animal Care" (NIH publication # 85-23, revised 1985). The anesthetised rat model (7) was employed for the absorption studies using Spraque-Dawley male rats that have been fasted overnight. A group of five animals per formulation was used throughout the absorption studies. A single intraduodenal (i.d) administration of Calcein was performed in a group of 5 rats from a solution and the various w/o microemulsions at a dosing volume of 1.0 ml/kg. Prior to actual sampling and dosing, each rat was anesthetised with pentobarbitol (diluted with saline to a final volume of 1.0 ml) at 50 mg/kg i.p. The rats stayed anesthetised for the entire experiment. Dosing was achieved in the following way: a small incision 2–3 cm long was made on the abdominal midline, and then a purse-string suture was placed on the duodenal muscle. A small hole was made in the center of the purse-string suture in which a blunt 23 G needle attached to a tuberculin syringe was inserted to deliver the dose. Upon completion of dosing, the purse-string was tied to close the opening. The incision was closed with wound clips. A 0.2 ml blood sample was obtained via jugular catheter at various time intervals with the 0 min sample taken 15 min prior to administration of the dose. Plasma (0.1 ml) was removed from whole blood by centrifugation at 1600× g for 5 min, and then stored at −20°C in 4 × 0.025 ml aliquots.

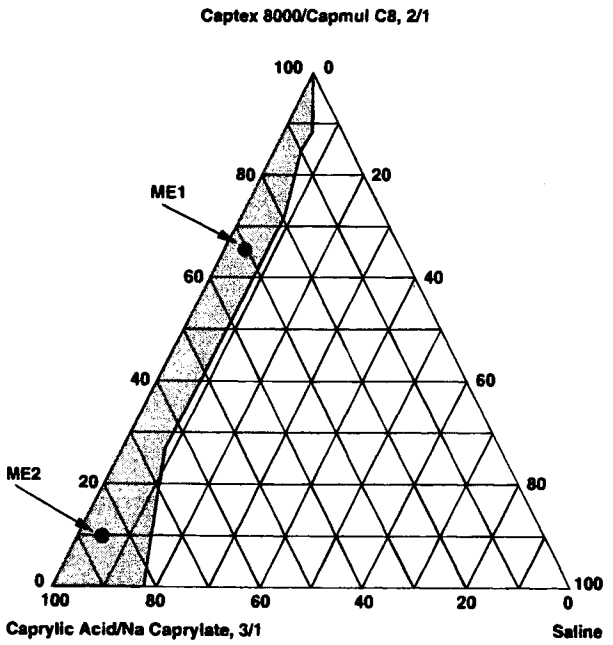

Fig. 1. Water-in-oil microemulsion existence field in the pseudo-ternary phase diagram of a system consisting of Captex 8000, Capmul C8, Caprylic Acid, Sodium Caprylate and saline. The w/o microemulsions ME1 and ME2 evaluated for absorption enhancement of Calcein are indicated. Other phases produced by this system are not shown.

Plasma levels of Calcein were determined by fluorescence spectroscopy using a Perkin Elmer LS 50 luminescence spectrometer (Perkin-Elmer Instruments, Exton, Pa). The excitation and emmision wavelenghts were set at 490 and 515 nm, respectively. Absolute bioavailability (% F) was calculated from the AUC (area under the plasma concentration-time curve) following i.d. or i.v dosing using the following equation (Eq. 1):

$$\%F = (AUC_{id}/AUC_{iv}) \times (Dose_{iv}/Dose_{id}) \times 100 \quad (1)$$

RESULTS AND DISCUSSION

Microemulsion Formulation

Fig. 1 presents a partial pseudo-ternary phase diagram of a system containing Captex 8000 (oil), Capmul C_8 (primary low

Table 1. Intraduodenal Bioavailabilities of Calcein from an Aqueous Solution and Different Water-in-oil Microemulsions in the Rat

ME	Composition (%, w/w)	% F[a] mean ± sem (n = 5)
Aqueous Solution	100 mM Calcein in isotonic 10 mM Tris, pH 7.4	1.3 ± 0.5
ME1	Captex 8000/Capmul C8/Caprylic Acid/ Na Caprylate/Aqueous (43.3/21.7/22.5/7.5/5.0)	36.3 ± 4.2
ME2	Captex 8000/Capmul C8/Caprylic Acid/ Na Caprylate/Aqueous (6.7/3.3/63.8/21.2/5.0)	29.9 ± 6.1
ME3	Captex 200/Imwitor 308/Tween 80/Capric Acid/ Na Caprate/Propylene Glycol/5N NaOH/Aqueous (44.5/9.8/19.6/10.7/4.4/4.9/1.2/4.9)	25.1 ± 4.6
ME4[b] (solid)	Witepsol H-15/Imwitor 308/Tween 80/Capric Acid/ Na Caprylate/Aqueous (42.6/23.8/12.4/11.4/4.8/5.0)	19.1 ± 2.7
ME5[b] (solid)	Witepsol H-15/Imwitor 308/Tween 80/Lauric Acid/ Na Laurate/Aqueous (42.7/23.8/12.3/11.4/4.8/5.0)	13.8 ± 2.6
ME6	Soybean Oil/Captex 355/Arlacel 186/Tween 80/Aqueous (30/30/20/15/5)	8.8 ± 2.4
ME7[b] (solid)	Witepsol H-15/Capmul MCM/Myverol 18-92/ Tween 80/Aqueous (38.3/7.9/4.3/38.6/10.9)	5.2 ± 1.6

[a] the administered Calcein dose (μmol/kg) was 2.5 from ME1 or ME2, 5.0 from ME3, ME4, ME5, ME6 and ME7 and 10.0 from the aqueous formulation. The administered volume of both the solution and microemulsions was kept at 1.0 ml/kg throughout.

[b] solid at room temperature and liquid at 37°C.

HLB surfactant), caprylic acid (secondary low HLB surfactant), sodium caprylate (high HLB surfactant) and saline. In order to formulate w/o microemulsions in this system where sodium salts of medium-chain fatty acids are solid, sodium caprylate was first solubilized in caprylic acid at a ratio of 1/3 (w/w) and then titrated with a fixed blend of Capmul C_8/Captex 8000 (1/2, w/w) and saline. The shaded area in the phase diagram represents clear and transparent w/o microemulsions (reverse micelles) that have been identified and characterized as previously described (5,6). Similar microemulsion existence fields were obtained at other caprylic/sodium caprylate ratios or when the caprylic acid/sodium caprylate blend was replaced by capric/sodium caprate or capric/sodium caprylate (8). Depending of the aqueous phase used, these w/o microemulsions can solubilized up to 15% saline (Fig. 1) or 35% deionized water (8). Due to the ionic nature of these microemulsions the difference between water and saline solubilization is most likely due to ionic strength effects. It is well known that ionic strength has greater effect on ionic compared to non-ionic systems. This is seen for example in the ionic strength dependence of the critical micelle concentration and aggregation number of micellar solutions or the phase behavior of oil/water/surfactant systems in general (9).

In another formulation strategy, the surfactant mixture containing Imwitor 308/Tween 80/capric acid/sodium caprate (2.2/4.4/2.4/1.0, w/w) was titrated with the oil (Captex 200) and aqueous phase that was a 1:1 mixture of propylene glycol and water. The pseudo-ternary phase diagram of this system is shown in Fig. 2. As can be seen, the w/o microemulsion existence field produced by this system is extended over a wide range of compositions (8). Interestingly, w/o microemulsions from this system, such as ME3 (Fig. 2, Table 1) can be converted to oil-in-water (o/w) microemulsions upon dilution with excess

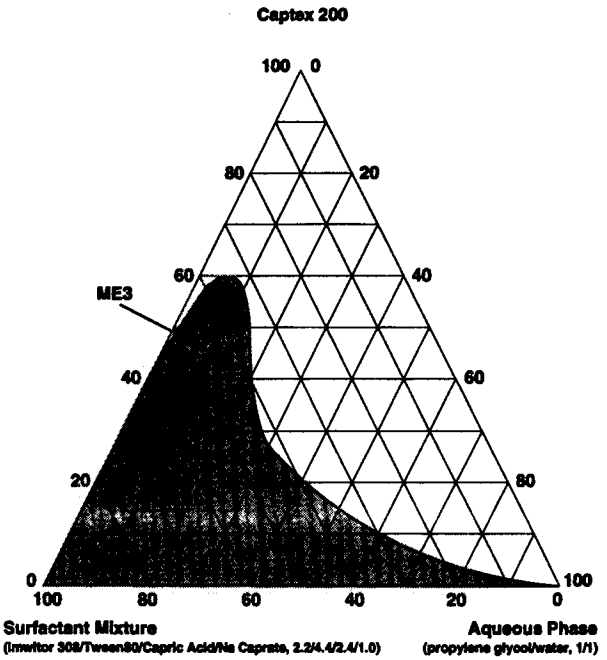

Fig. 2. Water-in-oil microemulsion existence field in the pseudo-ternary phase diagram of a system containing Captex 200, a Surfactant Blend and an Aqueous Phase. The surfactant blend was a mixture of Imwitor 308/Tween 80/Capric Acid/Na Caprate, 2.2/4.4/2.4/1.0, % w/w whereas the aqueous phase was a 1:1 mixture of propylene glycol and water. The w/o microemulsion ME3 evaluated for absorption enhancement of Calcein is shown.

of deionized water (≥20-fold), having an effective droplet diameter of 73 nm and polydispersity of 0.364. The other microemulsions of Table 1 upon dilution with excess water produced conventional o/w emulsions based on visual observation.

It is clear from the aforementioned phase diagrams that the w/o microemulsion existence field in systems containing medium-chain glycerides and fatty acids/salts can be modified, in terms of the amount of solubilized water, depending of the nature of the ionic surfactant used. In addition, while the formation of w/o microemulsions incorporating non-ionic lipids/surfactants is insensitive to the ionic strength of the aqueous phase (5), formulation of microemulsions incorporating ionic (anionic) surfactants can be affected by the ionic strength of the aqueous phase. The location and composition of the w/o microemulsion formulations, ME1, ME2 and ME3 that have subsequently been evaluated for absorption enhancement of Calcein are shown in the microemulsion existence fields of the corresponding phase diagrams.

Absorption Enhancement Evaluation

Calcein, a water-soluble and poorly absorbed marker molecule, was used as a model compound to evaluate absorption enhancement from the present w/o microemulsions since we have previously observed significant intestinal absorption enhancement of this molecule from non-ionic w/o microemulsions incorporating medium-chain glycerides and Tween 80 (5). The membrane permeability of Calcein in rabbitt ileum was found to be low (<0.010 cm/h, unpublished data). The absolute i.d. bioavailability of Calcein in the conscious rat from a Captex 355/Capmul MCM/Tween 80/isotonic 10 mM Tris pH 7.4, 65/22/10/3, (% w/w) w/o microemulsion (5) was found to be 44.9 ± 9.6 (mean ± sd, n = 6). While however, the conscious rat was employed in our earlier work (5), the present studies were focused on the more conveniently used anesthetised rat model. Intraduodenal bioavailability of Calcein in the conscious and anesthetised rat from an isotonic 10 mM Tris, pH 7.4 solution was found to be 2.4 ± 0.5%, n = 4 and 1.3 ± 0.5%, n = 5 (mean ± sem), respectively. Thus, in both rat models the i.d. bioavailability of Calcein is very similar and low to justify the evaluation of w/o microemulsions for bioavailability enhancement.

Plasma levels of Calcein as a function of time following i.d. administration to anesthetised rats from a solution and the w/o microemulsion formulations ME1 and ME2 are shown in Fig. 3. The composition of these two microemulsions is given in Fig. 1 and Table 1. The primary difference between ME1 and ME2 is in the content of medium-chain fatty acid/salt. While ME1 contains 30% (w/w) of a 3/1 w/w mixture of caprylic acid/sodium caprylate, ME2 contains 85% of the same mixture. Much higher plasma levels of Calcein were observed from the two microemulsion formulations than from the isotonic 10 mM Tris, pH 7.4 solution (Fig. 3). Absorption from microemulsions was fast with the C_{max} being reached within 30 min in agreement with our early report (5). The absolute i.d. bioavailabilities of Calcein from 10 mM Tris pH 7.4 isotonic solution, ME1 and ME2 were 1.3 ± 0.5, 36.3 ± 4.2 and 29.9 ± 6.1, respectively (Table 1). Thus, significant bioavailability enhancement (20 to 30-fold) was obtained from the microemulsion formulations. Since ME2 contains primarily (85%) the

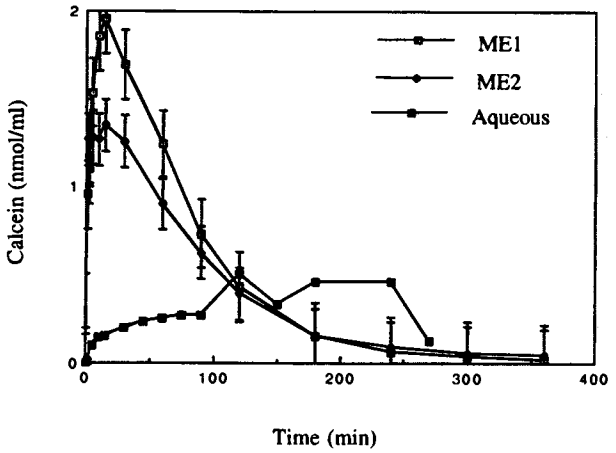

Fig. 3. Plasma concentration of Calcein as a function of time following intraduodenal administration to anesthetised rats from an aqueous and ME1 or ME2 microemulsion formulations. Results are means ± sem of five animals for both the aqueous and microemulsion formulations of Calcein. The composition of ME1 and ME2 microemulsions is given in Fig. 1 and Table 1. The aqueous formulation and aqueous phase of all evaluated microemulsions was an isotonic 10 mM Tris pH 7.4 incorporating 100 mM Calcein (Table 1).

3/1 mixture of caprylic acid/sodium caprylate, the improved absorption from this microemulsion is to a large extent due to the presence of these ionic lipids.

Fig. 4 presents the absorption profile of Calcein from ME3 and ME4 microemulsions the composition of which is given in Table 1. As can be seen these two microemulsions have several compositional differences: ME4 contains a high melting oil which makes the particle solid at room temperature but liquid at 37°C. ME3 is liquid at room temperature and in addition to medium-chain glycerides and Tween 80 it contains capric acid/sodium caprate and propylene glycol in the aqueous phase. Although fast absorption and significant plasma levels of Calcein were observed with either ME3 or ME4, the C_{max} in the

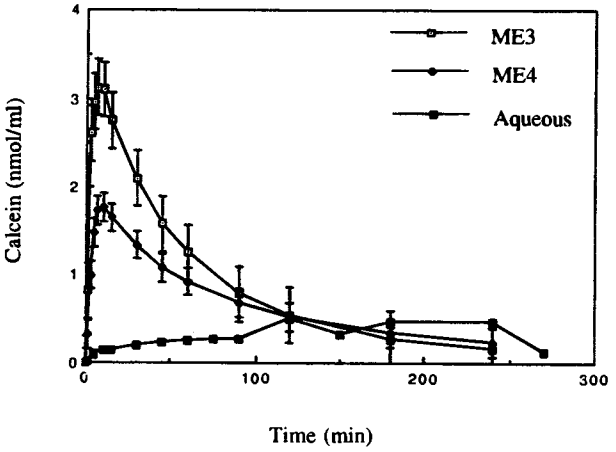

Fig. 4. Plasma concentration of Calcein as a function of time following intraduodenal administration to anesthetised rats from an aqueous and ME3 or ME4 microemulsion formulations. The composition of ME3 is indicated in both Fig. 2 and Table 1 whereas that of ME4 in Table 1 only. Results are means ± sem of five animals for both the aqueous and microemulsion formulations of Calcein.

case of ME4 was significantly less than that of ME3 (Fig. 4). The absolute i.d. bioavailabilities of Calcein from ME3 and ME4 were found to be 25.1 ± 4.6 and 19.1 ± 2.7, respectively (Table 1). That is, the physical form of the microemulsion at room temperature does not appear to play any role on the extent of the absorption enhancement. Both liquid and solid formulations were equally effective, provided, however, they contain optimum levels of the same lipid enhancer(s) in the form of either medium-chain mono-/diglycerides and/or fatty acid(s)/salt(s). Solid microemulsions may be more attractive as a commercial dosage form in a soft gelatin capsule from stability and processing considerations. Whether or not upon dilution with excess water the w/o microemulsion is inverted to an o/w microemulsion (ME3) or a conventional emulsion (ME4) doesn't seem to make any difference in regard to the extent of bioavailability enhancement. Although *in vitro* dilution may not be representative of the *in vivo* conditions, for water-soluble molecules the particle size of either the intact microemulsion particle or the inverted one does not play any major role on the extent of absorption (5,6).

ME5 microemulsion is similar in composition and physical form with ME4 (Table 1). Both microemulsions are solid at ambient temperature and have almost identical levels of the high melting oil (Witepsol H-15), C_8 monoglyceride (Imwitor 308) and aqueous phase (isotonic 10 mM Tris pH 7.4). The only difference in composition between these two microemulsions is that ME4 contains C_{10}/C_8 fatty acid/salt (capric/sodium caprylate) whereas, ME5 incorporates C_{12} fatty acid/salt (lauric/sodium laurate). The plasma levels of Calcein versus time from ME5 are shown in Fig. 5 and the absolute bioavailability of 13.8 ± 2.6 in Table 1. When this bioavailability is compared to that observed from ME4 (19.1 ± 2.7) it can be concluded that in terms of their absorption enhancement potential, fatty acids/salts can be ranked in the order of $C_8 \approx C_{10} > C_{12}$ (Table 1).

The bioavailability data from microemulsions ME1–ME5 in Table 1 and our earlier data (5,6) suggests that C_8/C_{10} glycerides in the form of mono-/diglycerides and/or free fatty acids/salts are primarily responsible for the observed absorption enhancement. Supporting this observation is the data where

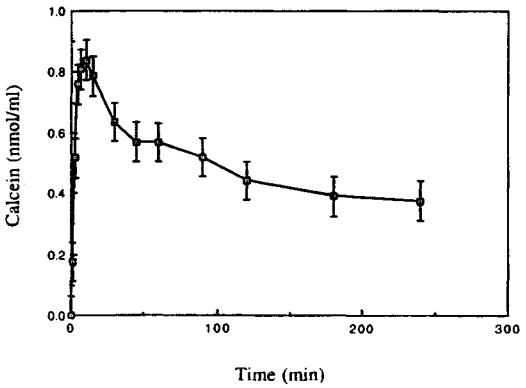

Fig. 5. Plasma concentration of Calcein as a function of time following intraduodenal administration to anesthetised rats from ME5 microemulsion. The composition of ME5 is indicated in Table 1. Results are means ± sem of five animals for both the aqueous and microemulsion formulations of Calcein. The plasma concentration of Calcein as a function of time from the aqueous formulation is shown in Figs. 3 and 4.

the i.d bioavailability of Calcein from a w/o microemulsion incorporating 30% C_8/C_{10} triglycerides (Captex 355), 30% long-chain triglycerides (Soybean Oil), 20% monolein (Arlacel 186), 15% Tween 80 and 5% aqueous phase was significantly reduced to a value of 8.8 ± 2.4 (ME6, Table 1). Optimum levels of C_8/C_{10} mono-/diglycerides are needed for significant absorption enhancement since, when the level of Capmul MCM, the only C_8/C_{10} glyceride present was reduced to about 8% in a w/o microemulsion (ME7, Table 1) incorporating Witepsol-H15 (38.3%), Myverol 18–92 (4.3%), Tween 80 (38.6%) and Calcein in isotonic 10 mM Tis pH 7.4 (5%), the i.d bioavailability of Calcein was only 5.2 ± 1.6 % (Table 1). Consistent with the absorption-enhancing action of Capmul MCM are also the results obtained from absorption studies of Calcein and PEG 4000 in both the conscious and anesthetised rat using w/o microemulsion/reverse micelle formulations of Capmul MCM (10).

In the small intestine, C_8/C_{10} triglycerides and diglycerides will be hydrolyzed by intestinal lipases to generate monoglycerides and free fatty acids that can be directly absorbed through the portal route and detected in the plasma (11). Thus, the *in vivo* species responsible for absorption enhancement are primarily monoglycerides and fatty acids. When these lipids are originally present in the formulation at optimum levels, the resulting absorption enhancement of hydrophilic molecules is greater than the one observed when only C_8/C_{10} triglycerides along with long-chain glycerides are present (ME6, Table 1). Further investigation should be focused on formulation optimization in regard to the levels of C_8/C_{10} monoglycerides and fatty acids/salts required for an optimum absorption enhancement.

The absorption-enhancing activity of C_8/C_{10} fatty acids and their sodium salts may be mediated by both transcellular and paracellular pathways (2–4,12–15). Results from colonic and rectal absorption studies indicate that caprylic and capric acids can increase transcellular permeability by disordering the membrane musoca (12). Using brush border membrane (BBM) vesicles from rat colon with their protein and lipid component labeled with fluorescent probes, the perturbing effects of caprate and caprylate on the membrane were examined by fluorescence polarization (12). Caprate interacted with both protein and lipids and caprylate mainly with proteins causing disordering of the BBM (12). Independent studies in the rat using fluorescently labeled BBM vesicles from the jejunum and colon (13) showed that jejunal cefmetazole absorption was increased significantly by capric acid but to a lesser extent than the colonic absorption of this water soluble antibiotic. In addition, impedance analysis and voltage clamp techniques demonstrated that capric acid increased paracellular permeability in the colon but not in the jejunum (13). The effects of capric acid on paracellular permeability were reversible and the junctional resistance, membrane capacitance and mannitol permeability returned to the control level following the removal of the fatty acid (14).

As far as the paracellular pathway is concerned, complexation of calcium by caprylic or capric acid would result in destabilization of tight junctions thus eliciting a paracellular absorption-enhancing effect (4, 13). Results from rectal absorption of hydrophilic compounds (cefmetazole, phenolsulfonphtalein, p-aminobenzoic acid, inulin, tryptan blue and FITC-Dextrans) in rats have shown that capric acid forms large aqueous openings through which compounds of MW < 10,000 daltons can diffuse freely (4, 13,15). It has been found that the

equivalent pore radius under normal conditions (~8 Å) can be increased up to 16 Å in the presence of various absorption enhancers with the pore radius being 11–12 and 13–15 Å in the presence of sodium caprylate or caprate, respectively (4, 15). It is worth mentioning that caprate is now clinically used in Japan as an effective enhancing adjuvant for amphicillin and ceftizoxim suppositories (15).

It is not clear whether the mechanism(s) by which medium-chain fatty acids promote absorption in the colorectum can also be applied to the small intestine. In addition, absorption-enhancing effects observed with C_8/C_{10} fatty acids alone may be modified in formulations with other lipids and surfactants. Clearly, a better understanding of these mechanistic issues, as well as other development issues with w/o microemulsions (1) is necessary in order to fully appreciate the potential of these lipids in oral drug delivery and absorption improvement.

CONCLUSIONS

A wide range of liquid or solid w/o microemulsions can be developed which in addition to non-ionic medium-chain glycerides, incorporate ionic lipids as absorption enhancers, such as medium-chain fatty acids/salts. Significant absorption enhancement of Calcein, a water-soluble marker molecule, has been observed from these microemulsions upon intraduodenal administration to rats. The physical state of the microemulsions does not play any major role on the extent of absorption enhancement which was found to be dependent on the chain-length of the fatty acid in the order of $C_8 \approx C_{10} > C_{12}$. Absorption enhancement of Calcein was significantly reduced in the absence or presence of low levels of C_8/C_{10} mono-/diglycerides. Additional studies are needed to further optimize the formulations and define the mechanisms by which medium-chain glycerides and fatty acids improve the oral absorption of water-soluble molecules from w/o microemulsion formulations.

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