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 (C8) capric (C10) and lauric (C12) 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 C8/C10 or C12 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 C8 > C10 > C12. Absorption enhancement of Calcein was significantly reduced in the absence or presence of low levels of C8/C10 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, C8/C10 mono-/diglycerides and sodium salts of C8/C10 fatty acids were shown to be the most effective. In the case of medium-chain fatty acids, C8/C10 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 ampicillin (2), cefoxitin (3) and ceftazidime (4) has been observed in rats with sodium caprate or cayprylate.

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 C8/C10 fatty acids being the most effective; and c) absorption enhancement of Calcein was significantly reduced in the absence or presence of low levels of C8/C10 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 C8 (C8 mono-/diglycerides, 1/1 w/w, HLB = 5–6), Capmul MCM (C8/C10 mono-/diglycerides, 1/1 w/w, HLB = 5–6), the oils Captex 355 (C8/C10 triglycerides), Captex 200 (C8/C10 diesters of propylene glycol) and Captex 8000 (C8 triglycerides) were supplied by Karshmanns Lipid Specialities (Columbus, OH). Capmul C8 is primarily esterified with caprylic acid and according to the manufacturer its fatty acid distribution is 0.8% capric (C10), 97.8% caprylic (C8) and 1.4% capric (C10). Witepsol H-15 oil (90:10, % w/w of C12 glycerol triesters:diesters) with less than 2% C10 monoester and Imwitor 308 (80–90% wt, of C8 monoglycerides, HLB = 6.0) were both provided by Hills America, Inc. (Piscataway, NJ). Caprylic (C4), HLB = 5.8, Capric (C10, HLB = 4.8), and Lauric (C12, 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 Croma Inc. (Mill Hall, PA). According to the manufacturer the fatty acid pattern of soybean oil is: 54% linoleic (C18:2), 25% oleic (C18:1), 6% linolenic (C18:3), 4% stearic (C18:0) and 11% palmitic (C16:0). Myverol 18–92 (HLB = 3.7) is which is distilled sunflower oil monoglycerides (90%
glyceryl linoleate) was supplied by Eastman Chemicals (Kingport, 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/Caprylic 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 Sprague-Dawley male rats that have been fasted overnight. A group of five animals per formulation was used throughout the absorption studies. A single intradu-

denial (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.

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 emission wavelengths 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):

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%F = \left( \frac{AUC_{id}}{AUC_{iv}} \right) \times \left( \frac{Dose_{iv}}{Dose_{id}} \right) \times 100
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**RESULTS AND DISCUSSION**

**Microemulsion Formulation**

Fig. 1 presents a partial pseudo-ternary phase diagram of a system containing Captex 8000 (oil), Capmul C8 (primary low