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Stability and in vitro antiproliferative activity of bioactive "Vitamin E" fortified parenteral lipid emulsions

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ABSTRACT

The objectives of this work were to engineer physically stable "Vitamin E" rich intravenous lipid emulsions and to evaluate their *in vitro* antiproliferative activity against MCF-7 (human mammary adenocarcinoma) and SW-620 (human colon adenocarcinoma) cell lines. Emulsions loaded with 70% vitamin E by total weight of the oil phase were stabilized with secondary emulsifiers and tested for their hemolytic effect and their plasma and electrolyte stability. Emulsions stabilized with sodium oleate and sodium deoxycholate were sensitive to electrolytes and exhibited significant hemolytic effect. On the other hand, addition of 2.5% poloxamer was found to stabilize the emulsions against electrolytes and physical stress, which was attributed to the steric effect of their polyoxyethylene (POE) head group. When tested for their antiproliferative effects, poloxamer-stabilized tocotrienol lipid emulsions were found to exhibit significantly higher anticancer activity than lipid emulsions enriched with tocopherol alone. The half maximal inhibitory concentrations (IC_{50}) of tocotrienols lipid emulsions against MCF-7 and SW-620 were 14 and 12 μM, respectively, whereas the IC_{50s} of tocopherol lipid emulsions were approximately 69 and 78 μM against MCF-7 and SW-620 cells, respectively.

1. Introduction

"Vitamin E" from palm oil refers to a family of eight molecules divided into two subfamilies known as tocopherols and tocotrienols (Fig. 1). Each subfamily consists of four isomers (α-, β, γ, and δ-) that differ in the number and position of methyl groups on the chromanol ring. Tocopherols and tocotrienols also differ in the degree of saturation in their phytyl side chain [1]. Vitamin E rich in tocotrienol isomers is often referred to as tocotrienol rich fraction or TRF. While tocopherols are widely used as antioxidants in food, cosmetic, and pharmaceutical products [2], tocotrienols were shown to display potent antiproliferative and apoptotic activity against breast cancer cells [3]. The long-term objective of this study was to develop stable and bioactive parenteral lipid emulsions fortified with high concentrations of "vitamin E". Unlike commercial fat emulsions such as Intralipid®, vitamin E lipid emulsions, however, require a complex blend of phospholipids with hydrophilic co-emulsifiers with high hydrophilic–lipophilic balance (HLB) values to overcome their poor intrinsic stability [4]. Due to its viscosity and polarity, vitamin E cannot be emulsified if phospholipids were the only emulsifiers used [1]. Presence of a hydroxyl group on the aromatic chromanol ring leads to a higher solubility of phospholipids in vitamin E making the emulsifier less available at the water interface [5]. Therefore, addition of hydrophilic co-emulsifiers to the phospholipids/vitamin E blends is more likely to create a stabilized interface [5]. In our preliminary studies [6] we confirmed that emulsions with high vitamin E loading cannot be processed using soybean phospholipids (Lipoid® E80S) alone. Rather, to facilitate the processing and manufacture of the emulsions by high-pressure homogenization, Lipoid® E80S was mixed with 0.5% polyoxyethylene sorbitan monooleate (Tween® 80), a hydrophilic emulsifier with a high HLB value. These emulsions, however, were unstable under stress conditions even at higher Lipoid® E80S and Tween® 80 concentrations, which pointed out to the need for secondary co-emulsifiers [6]. Therefore, the first objective of the present study was to compare the stabilizing effects of poloxamer 188, sodium oleate, and sodium deoxycholate as secondary co-emulsifiers by evaluating their hemolytic activity and by subjecting the emulsions to a series of stress conditions. The second objective of this study was to examine the biological activity of the stabilized formulations by evaluating the *in vitro* antiproliferative activity of parenteral lipid emulsions loaded with either TRF or α-tocopherol against MCF-7 (human mammary adenocarcinoma) and SW-620 (human colon adenocarcinoma) cells.

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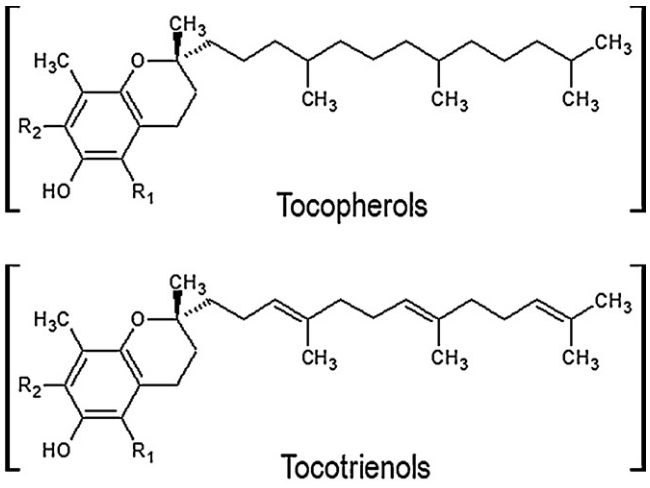

Isoform	R_1	R_2
Alpha (α)	CH_3	CH_3
Beta (β)	CH_3	H
Gamma (γ)	H	CH_3
Delta (δ)	H	H

Fig. 1. Generalized chemical structure of Vitamin E, which is a mixture of individual tocopherol and tocotrienols isoforms. Both tocopherols and tocotrienols have similar chemical structure. The difference between individual isoforms is in the degree of methylation of their chromane ring and the saturation of the phytyl chain.

2. Materials and methods

2.1. Materials

Vitamin E [(±)-α-Tocopherol] and insulin were purchased from Sigma (St. Louis, MO). Tocotrienol-rich-fraction of palm oil (TRF), which contains approximately 30% α-Tocopherol and 70% α, γ, and δ-tocotrienols was a gift from Beta Pharmaceutical Ltd (West Perth, Australia). Tween® 80 (polyoxyethylene sorbitan monooleate) was provided by Uniqema (New Castle, DE). Soybean Phospholipids (Lipoid® E80S) containing a minimum of 80% phosphatidylcholine and 7–9.5% phosphatidylethanolamine was provided by Lipoid GmbH (Ludwigshafen, Germany). Medium-chain triglyceride (MCT) was provided by Sasol (Miglyol® 812, Witten/Ruhr, Germany). Glycerol was purchased from Gallipot, Inc. (Saint Paul, MN). Lutrol® F 68 NF (Poloxamer® 188) was obtained from BASF (Florham Park, NJ). Sodium deoxycholate was obtained from Alfa Aesar (Ward Hill, MA). Sodium oleate was purchased from TCI AMERICA (Portland, OR). Hemoglobin reagent set was purchased from Teco Diagnostic (Anaheim, CA). Whole Rabbit Blood was obtained from Hemostat Laboratories (Dixon, CA). Sodium chloride solution (0.9%) was obtained from Hospria Inc. (Lake Forest, IL); 14.6% sodium chloride solution was obtained from LyphoMed, Inc. (Melrose Park, IL); and 10% calcium gluconate solution was obtained from American Regent, Inc. (Shirley, NY). Human Plasma was kindly donated by LifeShare blood center (Monroe, LA). Cell culture media RPMI + GlutaMax™–I, Trypsin and Phosphate buffer saline (PBS) were purchased from Invitrogen (Carlsbad, CA). MCF-7 and SW-620 cell lines were obtained from ATCC™ (Manassas, VA). Penicillin–streptomycin was obtained from cellgro® (Manassas, VA). Detergent reagent SDS was purchased from Trevigen Inc. (Gaithersburg, MD). Fetal bovine serum (HyCloneInc) was purchased through VWR (Westchester, PA). CellTiter-Glo® Luminescent Cell Viability Assay kit was purchased from Promega (Madison, WI). Double distilled (DI) water was used for all preparations. All other chemicals were of reagent grade or higher and were used without further modification.

2.2. Preparation of the emulsions

Emulsions (20 mL) were prepared by first mixing vitamin E with MCT (7:3, 20% w/w) to form the oil phase. The primary emulsifiers [1.2% Lipoid E80S and 0.5% Tween® 80] were dissolved in DI water to form the aqueous phase of the emulsion to which 2.25% glycerol was added to adjust tonicity. Co-emulsifiers (Poloxamer® 188, sodium oleate, and sodium deoxycholate) were then added to the aqueous phase at a concentration ranging from 0.5 to 3.5% by weight of the final emulsion. The oil and the aqueous phases were then mixed at 15,000 rpm for 2 min using an IKA® Ultra-Turrax T8 mixer (IKA® Works Inc., NC, USA) to form the crude pre-emulsion. A 20% (w/w) submicron emulsion was obtained by passing the coarse pre-emulsion through a high-pressure homogenizer (EmulsiFlex® C3, Avestin Inc., Ottawa, Canada) for 25 cycles and under homogenization pressure 25,000 psi (Pound per square inch).The pH was adjusted to 8 ± 0.05 using 0.1 N sodium hydroxide solutions. This was essential as lipid emulsions are most stable at pH values higher than 7.5 [7]. For sterilization studies, 2 mL of each emulsion was placed in a vial and sterilized by autoclaving at 121 °C for 15 min.

2.3. Characterization of the emulsions

Intensity-weighed mean particle size and population distribution (polydispersity index) of the emulsions were measured by photon correlation spectroscopy (PCS) at 23 °C and a fixed angle of 90° using Nicomp™ 380 ZLS submicron particle size analyzer (PSS Inc., Santa Barbara, CA). Emulsions were diluted with DI water in order to avoid multiple scattering and to achieve a scattering intensity of 300 kHz. The intensity-weighted mean diameter of the particles was calculated based on Stokes–Einstein law by curve fitting of the correlation function. Zeta-potential of the emulsions was measured using the same instrument (Nicomp™ 380 ZLS) under zeta mode. Samples were diluted with DI water and zeta-potential was measured using the Helmholz–Smoluchowsky equation. The percentage of vitamin E emulsified was determined by first removing any separated oil from the surface of the emulsion. A sample (0.1 mL) collected from the bulk of the emulsion was then diluted to 4 mL with methanol. From this stock, 0.1 mL was further diluted to 2 mL with methanol and analyzed for the amount of vitamin E remaining emulsified at 295 nm (Cary 50 probe-UV spectrophotometer, Varian Inc., Cary, NC). UV calibration curve was developed from the methanolic vitamin E solution. No spectral overlaps or interferences from lipids and other constituents of the emulsion were observed.

2.4. Scanning transmission electron microscope (STEM) studies

STEM was used to determine the morphology of the vitamin E lipid emulsions. Before analysis emulsions were diluted 500-fold with distilled water and negatively stained with 10% phosphotungstic acid for contrast enhancement. Staining was allowed to proceed for few minutes in air at room temperature. A small drop was then spread onto a Formvar® coated STEM copper grid (400 mesh), and the excess liquid was removed with kimwipe tissue paper. The grid containing the emulsion was observed at -5×10^{-5} Torr vacuum and 30 keV beam energy with a Type FP 2012/13 quanta 200 electron microscope (FEI, Hillsboro, OR). Particle sizing was accomplished *via* image processing with ImageJ Version 1.46m provided by the National Institutes of Health. Each