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Influence of vitamin E TPGS poly(ethylene glycol) chain length on apical efflux transporters in Caco-2 cell monolayers

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Abstract

D-alpha-tocopheryl poly(ethylene glycol) 1000 succinate (TPGS 1000) is a widely used form of vitamin E. TPGS 1000 is comprised of a hydrophilic polar (water-soluble) head and a lipophilic (water-insoluble) alkyl tail. TPGS 1000 has been used as a solubilizer, an emulsifier and as a vehicle for lipid-based drug delivery formulations. Most recently, TPGS 1000 has been recognized as an effective oral absorption enhancer. An enhancing effect is consistent with a surfactant-induced inhibition of P-glycoprotein (P-gp), and perhaps other drug transporter proteins; however, the exact inhibition mechanism(s) remain unclear. Therefore, in an attempt to generate additional knowledge, we have synthesized and tested various TPGS analogs containing different PEG chain length (TPGS 200/238/400/600/1000/2000/3400/3500/4000/6000). These results demonstrate a relationship between TPGS PEG chain length and influence on rhodamine 123 (RHO) transport in Caco-2 monolayers, a relationship which may be illustrated using a Weibull distribution.
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Keywords: P-glycoprotein; Vitamin E TPGS; Absorption enhancer; Poly(ethylene glycol); Caco-2

1. Introduction

Drug transporters significantly influence the pharmacokinetics (PK) and/or pharmacodynamics (PD) of drugs. In the case of intestinal drug absorption, drug transport can be influenced by active influx systems (e.g. human intestinal peptide transporter, hPepT-1; monocarboxylic acid transporter, MCT1; organic anion transporter, OATP) which greatly increase absorption of their substrates. On the other hand, absorption can be influenced negatively by apically located efflux pumps (e.g. multidrug resistance-associated protein, MRP2, or P-glycoprotein, P-gp) [1–4]. Efflux pumps pose a significant problem regarding substrate bioavailability, especially those classified as class II drugs according to the biopharmaceutical classification system, e.g. indinavir, talinolol, digoxin, or ketoconazole, drugs known for erratic absorption behaviour and significantly influenced by efflux pump expression levels and food effects [5,6].

Due to its high expression in almost all endothelial tissues, P-gp is probably the most important representative in the group of efflux pumps. P-gp, the product of the MDR1 gene, is an ATP-dependent multidrug efflux pump belonging to the ATP-binding cassette (ABC) superfamily of proteins [7]. P-gp is predominately located in the apical membranes of various epithelia (e.g. on the luminal surface of small intestine, colon, capillary endothelial cells of the brain and on kidney proximal tubules) [8–10]. P-gp protects cells from cytotoxic compounds by actively transporting against a concentration gradient and by reducing intracellular levels below their effective and/or toxic concentrations [11]. P-gp, and subsequent over-expression, plays a major role in the development of multiple drug resistance (MDR) in cancer cells [12]. As a result of its enterocyte localisation, and its broad substrate specificity, P-gp significantly limits the oral absorption of a large number of drugs [13]. Hence, inhibition of P-gp activity may be an effective way to enhance oral bioavailability.

Abbreviations: TPGS 1000, D-alpha-tocopheryl poly(ethylene glycol) 1000 succinate; P-gp, P-glycoprotein; RHO, rhodamine 123; PEG, poly(ethylene glycol); LDH, lactate dehydrogenase; CMC, critical micelle concentration.

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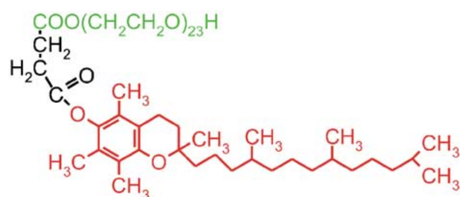

Fig. 1. General structure for TPGS analogs: red = hydrophobic vitamin E, green = hydrophilic poly(ethylene glycol) chain, black = succinate linker; average $n=4$ (TPGS 200) to 136 (TPGS 6000); R = H. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Various strategies to overcome MDR and influence intestinal absorption have been attempted. For example, highly selective P-gp inhibitors (e.g. cyclosporine A analog PSC-833, or Y335979, XR9576, and GF120918) have been developed [14–17]. Besides systematically synthesized inhibitors, other P-gp modulators have been discovered. For instance, several flavonoids, bile salts, phospholipids, and surfactants were found to improve absorption of P-gp substrates in vitro and in vivo [18–21]. Examples of non-ionic surfactants which may influence P-gp include Tween 80, Spans, several Pluronic block copolymers, Cremophor EL, and TPGS 1000 [22–27].

TPGS 1000 (D-alpha-tocopheryl poly(ethylene glycol 1000) succinate) was developed in the 1950s as a water-soluble form of vitamin E. TPGS 1000 is comprised of a hydrophilic polar (water-soluble) head and a lipophilic (water-insoluble) alkyl tail. Due to its surface active properties, it can be used as a solubilizer, an emulsifier and as a vehicle for lipid-based drug delivery formulations. Recently, TPGS 1000 has been described as an effective oral absorption enhancer for improving the bioavailability of poorly absorbed drugs: TPGS increased the sensitivity of P-gp expressing cells to several cytotoxic P-gp substrates in vitro and effectively blocked polarized transport of rhodamine 123 (RHO) and paclitaxel in transport assays [28]. In several studies, TPGS 1000 was found to be one of the most effective P-gp inhibitors amongst the surfactants [22,27,29,30]; however, other groups have reported no significant effect of TPGS 1000 [22,25].

In the present studies, we have synthesized and tested TPGS analogs for their ability to modify RHO efflux. RHO is widely used in literature as a model substrate to study P-gp activity [31–33], although other efflux pumps may influence RHO transport as well (e.g. BCRP) [34]. The analogs vary in their PEG chain length, residing between 200 and 6000 Da (Fig. 1). Besides the influence of these TPGS derivatives on RHO transport across Caco-2 cell monolayers, critical micelle concentration (CMC) and cytotoxicity indicators such as transepithelial electrical resistance (TEER) and lactate dehydrogenase (LDH) release were investigated.

2. Materials and methods

2.1. Materials

Commercial vitamin E TPGS 1000, vitamin E, vitamin E succinate and all TPGS derivatives were obtained from Eastman Chemical Company (TN, USA). Transwell permeable filter inserts (3460) were from Corning Incorp. Life Sciences (Acton, MA). Dulbecco's modified Eagle's medium (DMEM), non-essential aminoacids (NEAA) and fetal bovine serum (FBS) were purchased from GIBCO (Invitrogen Corp., Carlsbad, CA). The "cytotoxicity detection kit (LDH)" was from Roche Diagnostics Corp. (Indianapolis, IN). Rhodamine 123 (RHO), bovine serum albumin (BSA), and all other chemicals were purchased from Sigma-Aldrich (St. Louis, MO).

2.2. Synthesis

General synthetic procedure: vitamin E succinate (3.25 g, 6.12 mmol) was dissolved in dichloromethane (20 mL) and 1.1 equivalents of the corresponding polyethylene glycol added and stirred at room temperature. DMAP (4-dimethylaminopyridine; 0.1 equivalents) and DCC (*N*,*N'*-dicyclohexylcarbodiimide, 1.1 equivalents) were sequentially added. The reaction vessel was capped and stirred overnight. The reaction mixture was Büchner filtered, and the filtrate concentrated under reduced pressure to afford crude product(s). Products were then purified as their mono- and di-ester mixtures via preparative HPLC (Dynamax Microsorb C8, 250 × 41.4 mm I.d., 8 μ particles, 60 Å pore) using mobile phases (A, 25/75 methanol/acetonitrile (ACN); B, 25/75 *iso*-propyl alcohol (IPA)/ACN; C, IPA) with general gradient conditions of A for 24 min, B for 6 min and C for 12 min at a flow rate of ~80 mL/min.

2.3. Cell culture

Caco-2 cells, clone C2BBe1, were purchased at passage 60 from American Type Culture Collection (ATCC, Manassas, VA) and used at passages 70–92. Cells were grown to ~90% confluence in 75 cm^2 T-flasks with DMEM supplemented with 10% FBS and 1% NEAA. Culture medium was changed every second day and cells were grown at a temperature of ~37 °C in an atmosphere of ~85% relative humidity and ~5% CO_2. For the transport assay, cells were seeded on top of Transwell inserts (pore size 0.4 μm, 1.13 cm^2) at a density of ~60,000 cells/cm^2. Transepithelial electrical resistance (TEER) was measured and only monolayers with a TEER > 350 Ω* cm^2, with background subtracted, were used for transport studies.

2.4. Transport assay

Caco-2 monolayers were used 21–25 days after seeding. RHO transport was assessed in absorptive (apical to basolateral, Ap→Bl) and secretory (Bl→Ap) directions. Prior to RHO transport experiments, the monolayers were pre-incubated (1 h) with the corresponding TPGS analog (33 μM) on both sides. Subsequently, at $t=0$ min, a solution of RHO (13 μM in Krebs Ringer Buffer pH 7.4 (KRB)) was added to the donor compartment and pure KRB (pH 7.4) to the receiver compartment, both sides contained TPGS analog (33 μM). TPGS analog stock solutions (33 mM), except TPGS 200/238/ and 3400, were prepared immediately prior to transport experiments in

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