European Journal of Pharmaceutics and Biopharmaceutics 121 (2017) 97–103

Contents lists available at ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

journal homepage: www.elsevier.com/locate/ejpb

Research paper

Concomitant solubility-permeability increase: Vitamin E TPGS vs. amorphous solid dispersion as oral delivery systems for etoposide

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ARTICLE INFO

Keywords:
Drug absorption
Supersaturation
P-glycoprotein inhibition
Intestinal permeability
Oral drug delivery
Solubility

ABSTRACT

Vitamin E TPGS (TPGS) has both surfactant and P-glycoprotein (P-gp) inhibitory effects. While surfactants were previously found to cause solubility-permeability tradeoff, TPGS P-gp inhibitory effects may change this unfavorable interplay. The purpose of this research was to investigate the solubility-permeability interplay when using TPGS vs. amorphous solid dispersions (ASD) as oral drug delivery systems for the anticancer, P-gp substrate, lipophilic drug etoposide. The concentration-dependent effects of TPGS (0–100 mg/mL) vs. ASD on the solubility of etoposide, as well as the *in-vitro* (PAMPA) vs. *in-vivo* (intestinal rat perfusion) permeability of the drug were studied, and the resulting solubility-permeability interplay was analyzed. TPGS above CMC (0.3 mg/mL) increased etoposide solubility linearly, and ASD allowed significant supersaturation. Etoposide *in-vitro* PAMPA permeability decreased markedly with increasing TPGS levels, similarly to the solubility-permeability tradeoff previously defined for surfactants. In contrast, the presence of TPGS significantly increased etoposide *in-vivo* rat permeability, attributable to P-gp inhibition, similarly to the effect of the potent P-gp inhibitor GF120918 (10 μg/mL). High supersaturation achieved *via* ASD increased the drug's *in-vivo* permeability to the level obtained by TPGS or GF120918, supporting P-gp saturation. In conclusion, unique pattern of solubility-permeability interplay was found, involving concomitant increase of both the solubility and the permeability, as opposed to the previously reported tradeoff for solubilization methods and the unchanged permeability for supersaturation; P-gp inhibition/saturation by TPGS or by supersaturation allows simultaneous increase of both solubility and permeability, representing a significant advantage of such drug delivery approaches when suitable.

1. Introduction

As described in the biopharmaceutics classification system (BCS), the two key parameters that govern the overall process of oral drug absorption are the solubility of the drug dose in the aqueous gastrointestinal tract (GIT) milieu and the permeability of the drug through the intestinal membrane [1–5]. Nowadays, low aqueous solubility of new drug candidates is a common problem throughout many pharmaceutical companies. Oftentimes, this problem is tackled by using formulation techniques to allow improved aqueous solubility of the lipophilic agent. Despite the fact that significant solubility improvement may be obtained with these solubility-enabling formulations, we have recently shown that frequently some formulations should be used with caution; a tradeoff between solubility increase and permeability decrease was demonstrated when using cyclodextrins [6–10], surfactants [11,12], hydrotropy [13], and cosolvents [14–16]. On the other hand, no such tradeoff was evident when achieving supersaturation using amorphous solid dispersion [17–19]. This solubility-permeability interplay may explain why the effect of solubility-enabling formulations on the overall absorption is unpredictable, and may lead to increased, unchanged, or even decreased absorption.

Orally administered lipophilic drugs usually cross the intestinal membrane by passive transcellular absorption without the help of uptake transporters, however, once inside the cell these drugs may be substrates for apical efflux transporters [20,21]; efflux proteins placed at the apical membrane, e.g. P-glycoprotein (P-gp, MDR1), MRP2 and BCRP, can pump drugs from inside the enterocyte back into the intestinal milieu, limiting their absorption [22–24]. P-gp actively drives a

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http://dx.doi.org/10.1016/j.ejpb.2017.09.012
Received 4 July 2017; Received in revised form 15 September 2017; Accepted 24 September 2017
Available online 25 September 2017
0939-6411/

number of antineoplastic drugs, like etoposide, out of cancer cells and causes multidrug resistance. P-gp greatly affects the absorption of etoposide; for instance, Leu et al. have shown that P-gp contributes to the intestinal efflux of etoposide and P-gp-inhibiting compounds were able to increase the oral absorption of etoposide [25].

Vitamin E TPGS (D-α-Tocopherol polyethylene glycol 1000 succinate; TPGS) is a water soluble derivative of natural vitamin E. It is designed by esterification of vitamin E succinate with polyethylene glycol 1000 [26]. Vitamin E itself is classified as an oil-soluble vitamin but the solubility of vitamin E TPGS is comparatively very high in water: around 20% (w/w) at 25 °C. This physicochemical characteristic of TPGS is an important advantage of TPGS-based innovative formulations in the pharmaceutical field. In the 1960s TPGS was recommended as a solubilizer for oil-soluble vitamins, and in the 1980s it was applied for treatment of vitamin E deficiency and chronic cholestasis [27]. 20 years later, TPGS was established as a pharmaceutical solubilizing agent and absorption enhancer [28]. TPGS has long been engaged in oral dosage forms, and more recently new delivery applications are being investigated in parenteral, nasal, ophthalmic, and dermal drug delivery systems. It is an FDA approved pharmaceutically safe adjuvant. Formulations of TPGS have been developed for commercial anti HIV drugs: AGENERASE® (amprenavir) soft gel capsules and oral solution and APTIVUS® (tripanavir) oral solution and for anti HCV drug VIEKERA Pack™ (combination product of 3 APIs) tablets [29]. TPGS is a multirole excipient in pharmaceutical drug delivery systems; it is a non-ionic surfactant used as solubilizer for poorly soluble drugs, absorption enhancer, emulsifier, vehicle for lipid-based drug formulation, and antioxidant. Moreover, it is well known that TPGS has a strong P-gp inhibitory effect [30–33].

In previous studies, we have shown that surfactant-based formulations caused a permeability decrease that accompanied the solubility increase [12,34]. From this aspect, the use of TPGS as an excipient for etoposide may decrease its intestinal permeability. On the other hand, since etoposide is a P-gp substrate, the known P-gp inhibitory effects of TPGS may change the interplay between the solubility and the permeability. The purpose of this research was to investigate the solubility-permeability interplay when using TPGS vs. amorphous solid dispersions (ASD) as oral drug delivery systems for the P-gp substrate lipophilic anticancer agent etoposide. We have studied the concentration-dependent effects of TPGS vs. ASD on etoposides' solubility, the *in-vitro* vs. *in-vivo* permeability, and analyzed the resulting solubility-permeability interplay. Overall, this work allowed us to reveal a new pattern of solubility-permeability interplay; while we have previously shown cases in which the permeability either decreased or remained unchanged as the solubility increased, we now reveal an advantageous concomitant solubility-permeability increase.

2. Materials and methods

2.1. Materials

Etoposide, vitamin E TPGS, MES buffer and trifluoroacetic acid (TFA) were obtained from Sigma Chemical Co (St. Louis, MO). KCl and NaCl were purchased from Fisher Scientific Inc. (Pittsburgh, PA). Acetonitrile and water (Merck KGaA, Darmstadt, Germany) were UPLC grade. All other chemicals were of analytical reagent grade.

2.2. Formulations preparation

The TPGS-based formulations were prepared by dissolving different TPGS concentrations (0–100 mg/mL) in MES buffer (10 mM; pH 6.5). All formulations were freshly prepared 30 min prior to the different experiments.

The ASD powder of 20% etoposide in copovidon:eudragit L-100 (60:20) was prepared by rotovap as described previously [35]. Two hundred (200) mL of a 50:50% (v/v) methylene chloride:methanol mixture were added to 20% (w/w) API and 80% (w/w) copovidon:eudragit powder in a round-bottom flask attached to rotavapor under vacuum for solvent evaporation. Then, the flask was placed in a vacuum oven overnight at 25 °C, and the material was scraped and grinded with a mortar and pestle. Characteristics of the etoposide amorphous vs. crystalline etoposide were analyzed using polarized light microscopy, X-ray diffractometer, differential scanning calorimetry, and thermal gravimetric analysis.

2.3. Solubility and stability

Etoposide apparent solubility in 10 mM MES buffer (pH 6.5) containing increasing TPGS levels (0–100 mg/mL), was measured using the shake flask method, as previously reported [36]. Excess amounts of etoposide powder were placed in the different TPGS solutions and were shaken for 24 h. The samples were then centrifuged (14,000 rpm for 15 min), the supernatant was withdrawn, and immediately assayed for etoposide content by UPLC.

A stability study of supersaturated solutions prepared from the etoposide ASD was conducted, as previously reported [18,19]. Supersaturated etoposide solutions were prepared by dissolving suitable quantities of the 20% etoposide ASD powder in MES buffer, to achieve supersaturated solution of 2×, 4.5×, and 6.5× the equilibrium solubility of crystalline etoposide (196 μg/mL). The supersaturated solutions were sampled every 20 min and analyzed for etoposide concentration by UPLC. The solution stability was studied for 10 h which is sufficient timeframe to carry out the subsequent *in-vitro* and *in-vivo* permeability experiments.

2.4. In-vitro PAMPA studies

Parallel artificial membrane permeability assay (PAMPA) was used to study the *in-vitro* permeability of etoposide from the different formulations, on 96-well pre-coated filter plates (BD GentestTM, San Jose, CA), using a previously described method [37,38]. Etoposide solutions were prepared with different levels of TPGS in MES buffer pH 6.5, or different supersaturation level from the ASD powder. Etoposide concentration was calculated to achieve 75% saturation in all experimental groups. These solutions were added to the wells (300 μL/well) of the donor plate and blank MES buffer was added to the receiver plate wells (200 μL/well). The two plates were then coupled together and the assembled plate was incubated at room temperature without agitation for five hours. At the end of the incubation, the solution from the receiver plate wells was collected, and immediately assayed for etoposide content by UPLC. The PAMPA permeability coefficient of the compounds was calculated as per the manufacturer instructions, using the following formula:

$$P = \frac{\left\{-\ln\left[1-\frac{C_A(t)}{C_{eq}}\right]\right\}}{\left[A*\left(\frac{1}{V_D}+\frac{1}{V_A}\right)*t\right]}$$

where A is the filter area (0.3 cm^2), V_D is the donor well volume (0.3 mL), V_A is the acceptor well volume (0.2 mL), t is the incubation time (5 h), $C_A(t)$ is the compound concentration in the acceptor well at time t, and $C_{eq} = [C_D(t) * V_D + C_A(t) * V_A]/(V_D + V_A)$.

2.5. Single-pass intestinal perfusion studies in rats

All *in-vivo* study protocols were approved by Ben-Gurion University of the Negev Animal Use and Care Committee (Protocol IL-08-01-2015). Animals were housed and handled according to Ben-Gurion University of the Negev Unit for Laboratory Animal Medicine Guidelines. Male Wistar rats (Harlan, Israel) weighing 300–350 g were used for all studies.

SPIP (single-pass intestinal perfusion) permeability studies of