Head-To-Head Comparison of Different Solubility-Enabling Formulations of Etoposide and Their Consequent Solubility–Permeability Interplay

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ABSTRACT: The purpose of this study was to conduct a head-to-head comparison of different solubility-enabling formulations, and their consequent solubility–permeability interplay. The low-solubility anticancer drug etoposide was formulated in several strengths of four solubility-enabling formulations: hydroxypropyl-β-cyclodextrin, the cosolvent polyethylene glycol 400 (PEG-400), the surfactant sodium lauryl sulfate, and an amorphous solid dispersion formulation. The ability of these formulations to increase the solubility of etoposide was investigated, followed by permeability studies using the parallel artificial membrane permeability assay (PAMPA) and examination of the consequent solubility–permeability interplay. All formulations significantly increased etoposide's apparent solubility. The cyclodextrin-, surfactant-, and cosolvent-based formulations resulted in a concomitant decreased permeability that could be modeled directly from the proportional increase in the apparent solubility. On the contrary, etoposide permeability remained constant when using the ASD formulation, irrespective of the increased apparent solubility provided by the formulation. In conclusion, supersaturation resulting from the amorphous form overcomes the solubility–permeability tradeoff associated with other formulation techniques. Accounting for the solubility–permeability interplay may allow to develop better solubility-enabling formulations, thereby maximizing the overall absorption of lipophilic orally administered drugs. © 2015 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 104:2941–2947, 2015

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INTRODUCTION

Poor aqueous solubility is a major challenge in today's biopharmaceutics. Not only that ~50% of marketed drugs are classified as low-solubility compounds,[1–3] recent drug discovery statistics indicate that ~70% of new drug candidates are categorized as having poor water solubility.[4–7]

Many formulation techniques trying to tackle low aqueous solubility are in common use. These include surface-active agents,[8] lipid-based formulations,[9,10] cosolvents,[11] cocrystals,[12] nanonization techniques,[13] cyclodextrins,[14] amorphous solid dispersions (ASDs),[15] and more. Although significant solubility enhancement may be achieved with these formulations, we have recently discovered that in many cases the increased apparent solubility has its price, which is a parallel decrease in the apparent permeability of the drug.[16,17] Because the intestinal permeability of the drug and the solubility/dissolution of the drug dose in the gastrointestinal milieu are the two key parameters dictating the overall drug absorption following oral administration,[18–20] this tradeoff may explain why many times solubility-enabling formulations fail to improve the overall absorption. This solubility–permeability tradeoff was shown in several settings, including cases in which the increased solubility is accompanied by decreased free fraction that can explain the decreased permeability,[16,21–23] but also in cases that do not involve decreased free fraction of the drug.[16,24] It was recently discovered that this tradeoff can be overcome by using the amorphous form of the drug for solubility enhancement via supersaturation.[25–28]

Existing reports in the literature on the effect of formulation on the drugs' permeability focus on a single-formulation technique, and examine its effect on the apparent solubility/permeability. Although this information is accumulating in the literature, there is no head-to-head comparison of the impact of different solubility-enabling formulations on the solubility, the permeability, and the resulted solubility–permeability interplay. The purpose of this research is to provide this assessment.

We have chosen the low-solubility anticancer agent etoposide as a model drug, and formulated it in four different solubility-enabling formulations: surfactant, cyclodextrin, cosolvent, and ASD. For each formulation, we tested several strengths, to allow drawing the relationship between the excipient level and the enhancement of etoposide's solubility. We then investigated the *in-vitro* permeability of etoposide from each formulation, again using different excipient concentrations for each formulation. This enabled us to model the solubility–permeability interplay from the different vehicles, and to study it in one system that allows a head-to-head comparison. Overall, this research illustrates the underlying mechanisms dictating the solubility–permeability interplay, and allows a more intelligent and efficient use of solubility-enabling formulation strategies for enhanced oral drug absorption.

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MATERIALS AND METHODS

Materials

Etoposide, 2-hydroxypropyl-β-cyclodextrin (HPβCD), PEG-400, sodium lauryl sulfate (SLS), MES (4-morpholineethanesulfonic acid) buffer, trifluoroacetic acid (TFA), KCl, and NaCl were purchased from Sigma Chemicals Company (St. Louis, Missouri). Acetonitrile, water, and methanol (Merck KGaA, Darmstadt, Germany) were of ultra-performance liquid chromatography (UPLC) grade. All other chemicals were of analytical reagent grade.

Formulations Preparation

Four different etoposide solubility-enabling formulations were designed: surfactant, cyclodextrin, cosolvent, and ASD.

The preparation of the surfactant-, cyclodextrin-, and cosolvent-based formulations involved making MES buffer (10 mM; pH 6.5) solutions of the different excipients in the different strengths: (1) SLS solutions in three different levels, 2.5, 5, and 10 mM; (2) HPβCD solutions in three different levels, 5, 15, and 30 mM; and (3) PEG-400 solutions in three different levels, 10%, 20%, and 30% (v/v). After preparing the different solutions, the equilibrium solubility of etoposide in these solutions was studied, and etoposide powder was introduced to the formulations; for the permeability experiments, etoposide final concentration was made up at 60% the maximum solubility in each solution to keep thermodynamic activity constant across all formulations.

The ASD powder of 20% etoposide in copovidon was prepared by rotovap. Briefly, 20% (w/w) API and 80% (w/w) copovidon were loaded into a 500 mL round-bottom flask. Two hundred milliliters of a 50:50% (v/v) methylene chloride–methanol mixture was added to the flask. The flask was then mounted on a Buchi R215 rotovaper (Flawil, Switzerland) and rotated at 100 rpm at 40°C to dissolve the API and polymer. Once dissolved, the rotavaper was placed under vacuum to remove the solvent and dried for 30 min. The round-bottom flask was removed from the rotavaper and placed in a vacuum oven overnight at ambient temperature. The flask was removed, and the material was scraped and grinded with a mortar and pestle. Solid-state properties of the ASD versus crystalline etoposide were characterized using polarized light microscopy, X-ray diffractometer, differential scanning calorimetry, and thermal gravimetric analysis.

Solubility Experiments

Solubility studies were carried out in each formulation with different excipient strengths, to sketch the relationship between the excipient level and the enhancement of etoposide's solubility.

The equilibrium solubility of etoposide in the surfactant, cyclodextrin, and cosolvent formulations was determined at 25°C, in comparison with the drugs' solubility in MES buffer (10 mM, pH 6.5), using the traditional shake-flask method, as we have previously reported.[29] The solubility was measured at pH 6.5 to match the conditions of the permeability studies, and to mimic the small intestinal environment. Briefly, excess quantities of etoposide were added to glass vials containing the different formulations. The vials were tightly closed and placed in a shaking water bath (100 rpm) at 25°C. Equilibrium was confirmed by the comparison of 48 and 72 h samples. The vials were centrifuged (10,000 g, 15 min) and the supernatant was carefully withdrawn, filtered, and immediately assayed by UPLC.

For the surfactant-based formulations, MES buffer with different SLS levels (2.5, 5, and 10 mM) solutions were studied. For the cyclodextrin-based formulations, MES buffer with different HPβCD levels (5, 15, and 30 mM) solutions were studied. For the cosolvent-based formulations, MES buffer with different PEG-400 levels (10%, 20%, and 30% w/w) solutions were studied.

The binding constant, $K_{1:1}$, of the etoposide–HPβCD complexation was estimated from the phase–solubility diagram using the following equation[30,31]:

$$K_{1:1} = \frac{\text{Slope}}{\text{Intercept}\,(1 - \text{slope})}$$

where $K_{1:1}$ is calculated from the slope–intercept form of the linear equation of etoposide solubility as a function of increasing HPβCD concentration.

For the ASD formulation, a stability study of supersaturated solutions prepared from the etoposide ASD was carried out, as we have previously reported.[32] Briefly, supersaturated etoposide solutions were prepared by dissolving appropriate amounts of the 20% etoposide ASD powder in MES buffer, to obtain supersaturated solution of 2× (392 μg/mL), 4.5× (882 μg/mL), and 6.5× (1274 μg/mL) the equilibrium solubility of crystalline etoposide (196 μg/mL). The supersaturated solutions were allowed to stand at 25°C with no agitation, and were periodically sampled and assayed for etoposide concentration (UPLC). The solution stability was studied for 5 h, which would be sufficient to run the PAMPA experiments. To demonstrate true supersaturation, the equilibrium solubility of crystalline etoposide was measured in the presence of the ASD components, eudragit L-100, and copovidon and found to be unchanged by these polymers.

PAMPA Studies

Permeability studies through artificial membrane were carried out in the precoated PAMPA assay (BD Gentest™, San Jose, CA). The PAMPA plates were handled according to the manufacturer instructions, and the permeability of etoposide from the different formulations was calculated from the drug amount transported versus time, as we have previously reported.[33] Briefly, the donor wells were filled with 200 μL of the different etoposide formulations, the receiver wells were filled with 300 μL of blank buffers, and the PAMPA sandwich was incubated at 25°C. The receiver wells were collected every hour for 5 h.

The apparent permeability coefficient (P_{app}) values (cm/s) were calculated from the linear plot of etoposide amounts accumulated in the acceptor well versus time using the following equation:

$$P_{app} = \frac{dQ/dt}{AC_0}$$

where dQ/dt is the rate of etoposide appearance on the receiver well, C_0 is the initial concentration of the drug in the donor well, and A is the membrane surface area (0.048 cm^2). Linear regression was carried out to obtain the rate of etoposide appearance on the receiver well.

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