European Journal of Pharmaceutical Sciences 48 (2013) 775–780

Contents lists available at SciVerse ScienceDirect

European Journal of Pharmaceutical Sciences

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

Drug "supersaturation" states induced by polymeric micelles and liposomes: A mechanistic investigation into permeability enhancements

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ARTICLE INFO

Article history:
Received 11 October 2012
Received in revised form 11 December 2012
Accepted 11 January 2013
Available online 23 January 2013

Keywords:
Apparent solubility
Polymeric micelles
True supersaturation
Permeability enhancement
Lipophilic drug

ABSTRACT

The objective of this study was to investigate if the increase in apparent solubility induced by liposomalization or micellization of the poorly soluble drug hydrocortisone (HC) would lead to an enhancement of its permeability through biological membranes. For this purpose phosphatidylcholine liposome formulations as well as d-α-tocopheryl polyethylene glycol 1000 succinate (TPGS) micelle dispersions and polyvinylpyrrolidone (PVP) supersaturated solutions were prepared in order to increase the apparent solubility of HC. Both the apparent solubility of hydrocortisone (i.e. amount of drug entrapped plus non-entrapped in the carriers) as well as the concentration of molecularly dissolved drug (i.e. fraction non-entrapped into carriers, truly molecularly dissolved fraction) were characterized. Subsequently, the permeability of hydrocortisone was assessed for each type of formulation using the *in vitro* sheep nasal mucosa permeability assay. In all formulations where solubilizing agents are present, an enhanced flux of HC (compared to the pure drug powder suspension) is observed. The expected linear correlation between apparent solubilities and fluxes was not found, whereas, the concentrations of molecularly dissolved HC were found to be directly proportional to the respective fluxes. This is an experimental proof for the hypothesis that, of all the strategies to increase the apparent solubility of poorly soluble drugs, enhancement of the molecularly dissolved drug concentration (induction of true supersaturation) would lead to better permeation though membranes.

1. Introduction

In the last years low solubility of drug substances has become a major concern in pharmaceutical research and development. A number of techniques have been developed to improve solubility of poorly soluble active pharmaceutical ingredients (APIs). Thermodynamic solubility of drugs can be enhanced altering the drug at the molecular level, forming for example salts (in case of ionizable groups present in the drug molecules) or hydrophilic prodrugs (Muller, 2009; Ettmayer et al., 2004). Another strategy that can be employed to circumvent low solubility of drugs without chemical modification of the drug molecules is to induce apparent solubilization states. Apparent solubility can be defined as the concentration of a substance at apparent equilibrium. It is distinct from the true thermodynamic solubility, because in this case the equilibrium is time-limited (e.g. true supersaturated solutions) with the possibility of re-crystallization, or the drug molecules are not in direct contact with solvent molecules, but incorporated into or partially associated with (amphiphilic) carriers. True supersaturation is a temporary state of apparent solubilization in which the concentration of the freely dissolved drug is higher than the thermodynamic solubility under the same conditions (e.g. pH, temperature, etc.) (Brouwers, 2009). Metastable solid forms like polymorphs, amorphous material, solvates, and co-crystals have been proven to be efficient to induce supersaturation states (Pudipeddi and Serajuddin, 2005), but all of them may recrystallize in the form of the most stable crystal modification. Moreover, it is also well described in the literature that several hydrophilic polymers are capable of inducing and stabilizing supersaturated solutions without incorporating drug molecules into carrier systems (Brouwers et al., 2009; Mosharraf et al., 1999). Inclusion complexes with cyclodextrin derivatives, entrapment in liposomes and formation of micelles with amphiphilic molecules are also feasible alternatives for increasing apparent solubility of poorly soluble chemical entities (Loftsson et al., 2002; Fahr and Liu, 2007; Rangel-Yagui et al., 2005), but in this case small fractions of drug molecules are dissolved in the solvent (molecularly dissolved drug) and most of the drug is incorporated into the carriers. The resulting apparent solubility in this case will be the sum of carrier-incorporated drug and molecularly dissolved drug. Even if incorporation into amphiphilic carriers can enhance (apparent) solubility of poorly soluble entities in orders of magnitude, it is still unclear if there is a proportional improvement of drug permeability through

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http://dx.doi.org/10.1016/j.ejps.2013.01.006

biological membranes. Passive diffusion of drug molecules through membranes is described by Fick's firs law:

$$(J = DKS_0/h)$$

According to this equation, the flux of drug molecules (J) is directly proportional to the diffusion coefficient (D), the partition coefficient (K) and the solubility of the drug (S_0), but inversely proportional to the thickness of the membrane (h). Increased flux can be achieved altering the membrane structure (increasing D or K) or increasing the solubility of the drug (Walker and Smith, 1996; Sheu et al., 2003). However it is still unclear which parameters are the most effective to enhance the permeation process and which 'type' of solubility (truly dissolved drug concentration or apparent solubility) should be taken into account for a proper estimation of the apparent permeability coefficient (Papp) (Fischer et al., 2011). The suitability of both liposomes and d-α-tocopheryl polyethylene glycol 1000 succinate (TPGS) micelles as solubilizing agents have recently been studied for a new class of poorly soluble anticancer substances (di Cagno et al., 2011, 2012). For these chemical entities the nasal route of administration may be feasible for brain tumour delivery. However, not so much is reported in the literature about influence of the solubilizing agents on permeability through nasal mucosa tissue. The aim of the present study was to evaluate if solubilization of lipophilic substances with liposomes and TPGS micelles would increase drug permeability through nasal mucosa. Due to high cytotoxicity of quinolinone derivatives, hydrocortisone was chosen as a model drug for the permeability studies and sheep nasal mucosa was chosen as a model of *in vitro* biological membrane due to its similarity with human nasal mucosa (Illium, 1996). Moreover, a better understanding of the mechanistic aspects behind permeation of poorly soluble drugs under solubilizing conditions was aimed.

2. Materials and methods

2.1. Materials

Hydrocortisone (HC) (Fig. 1) and polyvinylpyrrolidone K15 (PVP-K15) were obtained from Fluka Chemie (Sigma-Aldrich GmbH, Steinheim, Germany). D-α-tocopheryl polyethylene glycol 1000 succinate (TPGS) was a kind gift from BASF SE (Ludwigshafen, Germany) and the phospholipids Phospholipon® used for the preparation of liposome were purchased from Phospholipid GmbH (Köln, Germany). Ethanol and acetonitrile as well as potassium di-hydrogen phosphate (KH_2PO_4) and sodium chloride (NaCl) were purchased from Carlo Erba Reagents SPA (Rodano, Italy) and di-sodium hydrogen phosphate (Na_2HPO_4) was provided by Merk KgaA (Darmstadt, Germany).

2.2. Preparation of the different formulations

2.2.1. Preparation of powder dispersion

A homogeneous crystal suspension of hydrocortisone was prepared by dispersing the micronized solid powder of HC in neutral (pH 7.4) phosphate buffer saline (PBS) and keeping the suspension under agitation for several days at 37 °C.

2.2.2. Preparation of liposomal and micellar dispersions

Both micelle and liposome dispersions were prepared using the classical "film" method. In brief, solutions of phospholipids (or TPGS, respectively) and HC were prepared in ethanol. The solvent was then removed by vacuum rotary evaporation (40 °C, 2 h; Laborota 4000 Efficient Heidolph Instruments GmbH, Schwabach, Germany). Liposome dispersions or respectively micelle dispersions (with a final concentration of the surfactant of approximately

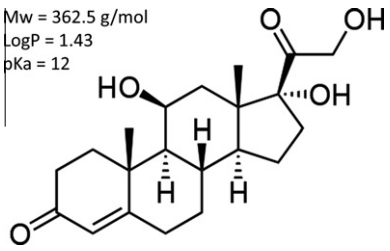

Fig. 1. Chemical structure of hydrocortisone.

50 mg/ml) spontaneously formed after reconstitution of the thin film in isotonic and isohydric phosphate buffer saline under manual agitation. Liposome dispersions were finally extruded through a 100 nm polycarbonate filter using a LiposoFast manual syringe extruder (Avestin Europe GmbH, Mannheim, Germany) in order to reduce and homogenize vesicle size. Ultracentrifugation (ALC Centrifuge, Milano, Italy) was used for removing any possible remaining crystals of hydrocortisone from both micelle and liposome dispersions respectively (18,000 g, 35 min, 5 °C).

2.2.3. Preparation of supersaturated HC solution

Supersaturated solution of HC was prepared by dispersing solid powder of the drug in PBS containing PVP (5%, w/v). The obtained suspension was stirred for 5 days at 37 °C till equilibrium was insured. Undissolved HC was removed by ultracentrifugation (conditions as above) and subsequent filtration of the dispersion through a 0.2 μm pore-size polycarbonate filter.

2.3. Characterization of HC formulations

2.3.1. Determination of HC apparent solubility

The analytical method previously described by di Cagno et al. (2011) was used to quantify apparent solubility of HC in each type of formulation. In brief, an exact volume (1 ml) of each homogeneous dispersion was rationally diluted with ethanol and placed in ultrasound bath for 20 min to insure complete destruction of the drug carriers (micelles, liposomes or greater dispersion of solid crystals of HC). Subsequently, the solution was filtered through a 0.2 μm pore-size filter and the concentration of the drug detected by high-performance liquid chromatography (HPLC) using a Shimadzu LC-10ATvp apparatus (Shimadzu Corporation, Kyoto, Japan) equipped with an ultraviolet detector (LC-10Avp) and a reverse phase Phenomenex column (Torrance, USA) model Synergy 4-U Hydro-RP 80A (C18). Each analysis was repeated in three replicates ($n = 3$).

2.3.2. Quantification of molecularly dissolved HC

To quantify the amount of HC freely molecularly dissolved in the aqueous medium (i.e. the fraction which is not incorporated into the carrier systems), a modification of the dialysis method previously described (Frank et al., 2012) was employed. Small volumes of neutral PBS (pH 7.4) were placed inside commercially available small glass tubes sealed at one end with a Visking Tubo Dialysis membrane (Medicell International Ltd., London, UK) (cut-off size 14000 Dalton) used as acceptor compartment. The tube was immersed into the respective formulation under investigation (donor compartment) and the system was kept at constant temperature (37 °C) for several days till equilibrium was insured. The HC concentration in the respective acceptor chamber was then detected via HPLC or directly by UV–*vis* spectroscopy using a UV-1610 spectrophotometer (Shimadzu Co.). All analyses were replicated three times.