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Combined use of bile acids and aminoacids to improve permeation properties of acyclovir

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ABSTRACT

The aim of this work was to develop a topical formulation with improved permeation properties of acyclovir. Ursodeoxycholic (UDC) and dehydrocholic (DHC) acids were tested as potential enhancers, alone or in combination with different aminoacids. Equimolar binary and ternary systems of acyclovir with cholic acids and basic, hydrophilic or hydrophobic aminoacids were prepared by co-grinding in a high vibrational micromill. Differential scanning calorimetry (DSC) was used to characterize the solid state of these systems, while their permeation properties were evaluated in vitro through a lipophilic artificial membrane. UDC was more than 2 times more effective than DHC in improving drug AUC and permeation rate. As for the ternary systems drug-UDC-aminoacid, only the combined use of L-lysine with UDC acid produced an evident synergistic effect in enhancing drug permeation properties, enabling an almost 3 and 8 times AUC increase compared to the binary UDC system or the pure drug, respectively. The best systems were selected for the development of topical cream formulations, adequately characterized and tested for in vitro drug permeation properties and stability on storage. The better performance revealed by acyclovir-UDC-L-lysine was mainly attributed to the formation of a more permeable activated system induced by the multicomponent co-grinding process.

1. Introduction

Acyclovir, an analogue of the natural nucleoside 2′-deoxyguanosine is considered a first-line drug in the anti-herpetic therapy, due to its inhibitory activity against herpes simplex virus types 1 (HSV-1), and 2 (HSV-2), varicella-zoster virus (VZV), and Epstein Barr virus (DeClerc et al., 1980; Whitley and Gnann, 1992; Balfour, 1999). Topical formulations for the therapy of herpetic cutaneous infections have several potential advantages over systemic therapy, including targeting of drug to the specific site of infection, higher local drug concentrations, reduced circulating drug levels, with consequent reduction of side effects, better patient compliance. However, currently available topical acyclovir formulations, though widely evaluated, exhibited only limited efficacy, and this has been attributed mainly to the poor ability of the drug to penetrate through the skin barrier layer (Freeman et al., 1986; Spruance et al., 2002). Moreover, this problem also gives rises to bioinequivalence problems among commercial topical formulations (Trottet et al., 2005).

The stratum corneum is known as the principal obstacle to the absorption of drugs through the skin (Ritschel and Hussain, 1988) and different approaches have been developed in the attempt to overcome its barrier functions and improve drug skin permeation. Among these, an interesting strategy is represented by the use of effective permeation enhancers, i.e., pharmaceutically-inert excipients able to promote the absorption of active components. Penetration enhancers can act by reversibly reducing the skin resistance to drug transport, or by changing the partitioning behavior of the drug at the stratum corneum level, or by affecting the drug thermodynamic activity (Williams and Barry, 2004; Pathan and Setty, 2008; Afouna et al., 2003; Tolle-Sander et al., 2003; Dey et al., 2009). Among the various classes of substances proposed and tested as potential penetration enhancers, bile salts and acids are particularly interesting, due to their endogenous nature and their surfactant, solubilizing and wetting activities, which make them suitable to enhance drug permeability through different biological membranes, including the skin (Carelli and Di Colo, 1993; Sawicki, 2001; Johansson et al., 2002; Naveen et al., 2011). Moreover, it has been shown that combinations of enhancers with appropriate substances can give rise to a possible synergistic effect in their ability to enhance skin permeability when compared to individual components (Karande et al., 2004, 2007; Maestrelli et al., 2011; Jug et al., 2014).

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Mechanochemical activation carried out by co-grinding drugs with suitable carriers, as such or in mixtures with auxiliary substances has been successfully employed to improve unfavorable characteristics of drugs (Maestrelli et al., 2011, 2004; Jug et al., 2014; Zerrouk et al., 2004). The mechanical energy supplied to a sample during a high-energy grinding process gives rise to a metastable structure that very rapidly relaxes to a more stable condition by releasing heat, breaking (particle size reduction), losing crystallinity until to complete amorphization, thus forming an activated material (Colombo et al., 2009). In order to improve the stability of the activated material, the addition to the grinding sample of at least one more component is necessary; this acts as a stabilizer, by interacting through van der Waals or hydrogen bonds with the drug molecule, yielding to stable products conserving their activation (Bodyrev, 2004). The role of the stabilizer is of critical importance, since its properties and its ability of interacting with the drug molecules can significantly affect the physicochemical properties of the final product, and its performance.

Based on all these considerations, the present work was aimed at improving the permeation properties of acyclovir by co-grinding with ursodeoxycholic (UDC) and dehydrocholic (DHC) acids, selected as potential permeation enhancers. Moreover, the possible synergistic effect provided by the addition of different kinds of aminoacids was also investigated, considering the positive effect shown by these components in improving the physicochemical properties and bioavailability of different drugs (Piel et al., 1997; Mura et al., 2003, 2005; Corvi Mora et al., 2003; Arakawa et al., 2008). In particular, in order to evaluate the possible role of the nature of the aminoacid molecule, different aminoacids were selected, with different basic (L-arginine and L-lysine), hydrophilic (L-serine) and hydrophobic (L-leucine) properties.

Equimolar coground binary (drug-cholic acid) and ternary (drug-cholic acid-aminoacid) systems were characterized for solid state interactions by DSC analysis, whereas their permeation properties were investigated in vitro using a lipophilic artificial membrane simulating the skin (Mura et al., 1993). Such an artificial membrane proved to give good correlation with the results of permeation experiments performed by both the rabbit ear model (Corti et al., 1998; Mura, 2014, 2007; Maestrelli et al., 2005), indicating that it can be considered a reliable simplified model for a rapid and well reproducible screening of topical formulations. The best products were then selected for the development of a topical cream formulation, which was adequately characterized for technological properties, stability under storage and drug permeation rate through the lipophilic artificial membrane simulating the skin.

2. Experimental

2.1. Materials

Acyclovir (9-((2-hydroxyethoxy)-methyl)-guanine), ursodeoxycholic acid (UDC) and its sodium salt (UDCNa), dehydrocholic acid (DHC) and its sodium salt (DHCNa), L-lysine (L-lys), L-arginine (L-arg), L-serine (L-ser) and L-leucine (L-leu) and Na dodecylsulphate were supplied from Sigma (USA). Poloxamer 407 (Pluronic F127) was a kind gift from BASF (Germany). The excipients of the creams (vaseline, cetostearilic alcohol, liquid paraffin and propylene glycol) were supplied from Galeno (Italy). Water obtained from a Millipore water purification system was used throughout the study.

2.2. Methods

2.2.1. Preparation of binary and ternary solid systems

Ground (GR) equimolar binary and ternary systems at 1:1 and 1:1:1 molar ratios, respectively were prepared by co-grinding the drug with ursodeoxycholic or dehydrocholic acid in the absence or presence of each tested aminoacid in a high vibrational micromill (Retsch GmbH, Germany) at a frequency of 24 Hz for 30 min. Grinding jars (volume 12 cm^3) and stainless steel balls (9 and 12 mm diameter) were used.

2.2.2. Permeation studies of acyclovir from binary and ternary systems

Permeation studies were carried out using a Sartorius Model SM 16750 absorption simulator (Sartorius Membranfilter GmbH, Germany) termostated at a temperature of $32 \pm 0.5\,^{\circ}C$. A cellulose nitrate membrane impregnated with lauryl alcohol (membrane weight increase 90–110%) was used as artificial lipophilic membrane simulating the epidermal barrier (Mura et al., 1993, 2014, 2007; Corti et al., 1998; Maestrelli et al., 2005). The membrane was placed in the diffusion cell connected to the donor and receptor compartments, the first constituted by 100 mL of aqueous suspensions of 50 mg acyclovir, as such or as binary or ternary coground product with cholic acids and aminoacids, and the second consisting of 100 mL of pH 7.4 phosphate buffer solution. At fixed times, samples were withdrawn from the receptor compartment, spectrometrically assayed at 254 nm (UV/vis 1601 Shimadzu), and replaced with fresh medium. The correction for the cumulative dilution was calculated. Each experiment was performed at least three times and the results were averaged (C.V. $< 1.5\%$).

It was verified that no interferences occurred at the selected wavelength (254 nm), by performing permeation experiments with the different components but in the absence of acyclovir.

The stability of the impregnated-membrane was checked at the end of each test, by exploiting the following method: the lauryl alcohol was extracted (with 1 mL isopropanol for 5 times) and gaschromatographically assayed (PerkinElmer Mod. 900 connected to an Hitachi PerkinElmer CC12 recorder) by injection of 0.5 μl sample using an inox anakrom column (ABS 100-110 mesh). The results were compared with those obtained from membranes impregnated but not used for release tests. The residual amount of lauryl alcohol on the membrane at the end of experiments ranged between 92 and 95% independently of the content of the donor compartment.

The AUC (area under the drug permeation curve) was calculated using the trapezoidal rule.

2.2.3. Differential scanning calorimetry

DSC analyses were carried out with a Mettler TA4000 Star^e software apparatus (Mettler Toledo, Switzerland) equipped with a DSC 25 cell. The instrument was calibrated for temperature and heat flow with standard Indium samples. Samples of about 5–10 mg were exactly weighed (Mettler M3 microbalance) in pierced Al pans and scanned under static air at a heating rate of $10\,^{\circ}C$/min over a 30–300 °C temperature range.

2.2.4. Fourier transform infrared spectrometry

FT-IR spectra were performed by a Shimadzu IRPrestige-21 apparatus (Shimadzu Corporation, Japan) on KBr tablets.

Spectra were recorded in the 4000–400 cm^{-1} range (10 scans, resolution 4 cm^{-1}).

2.2.5. Preparation of creams

Topical creams containing 5% (w/w) acyclovir or its equivalent as selected binary or ternary systems with UDC acid (or its sodium salt) and L-lys were prepared using a traditional preparation method of O/A emulsions according to the composition of commercial cream formulations (Cycloviran, Zovirax™, Acyvir™). The oily phase, consisting of Vaseline (15% w/w), cetostearilic alcohol (8% w/w) and liquid paraffin (6% w/w) was melted at $65\,^{\circ}C$. The aqueous phase was prepared by adding Na dodecylsulphate