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Development of liposomal and microemulsion formulations for transdermal delivery of clonazepam: Effect of randomly methylated β-cyclodextrin

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ABSTRACT

Transdermal administration of clonazepam, a poorly water-soluble benzodiazepine, is an interesting strategy for overcoming the drawbacks of its oral administration. With this aim, two nano-carrier formulations, based on ultra-deformable liposomes and microemulsions, have been developed to favour clonazepam transdermal delivery. Considering the solubilizing power of methyl-βcyclodextrin (Me-βCD) toward clonazepam and its potential positive influence on transdermal drug delivery, the effect of its addition to these formulations was investigated. Artificial lipophilic membranes simulating the skin allowed a rapid evaluation of the drug permeation properties from the systems, compared with those from an aqueous drug suspension, with or without Me-βCD. The best formulations were further characterized by permeation through excised rabbit ear skin. All the formulations increased drug permeability, ranging from 2-fold (liposomes without Me-βCD), up to over 4-fold (microemulsions containing Me-βCD). The different formulations allowed for pointing out different possible permeation enhancing mechanisms of Me-βCD: increase in drug solubility and thermodynamic activity in the vehicle, when added to the drug aqueous suspension; interactions with the vesicle bilayer, in case of liposomal formulations; interactions with the skin membrane lipids, as evidenced in experiments with excised rabbit ear for microemulsions containing Me-βCD, that were then selected for further *in vivo* studies.

1. Introduction

Transdermal drug delivery has been attracting increasing attention over the last years, due to the number of advantages offered over oral or intravenous administration, such as reduced systemic toxicity, absence of hepatic first-pass metabolism and better control of blood levels. Unfortunately, only a limited number of active compounds appear to be able to penetrate the skin at a rate sufficiently high to exert systemic effects and have therapeutic efficacy (Hadgraft, 2001; Notman and Anwar, 2013). Therefore, several strategies have been proposed and investigated in the attempt of overcoming this drawback and improving drug permeability through the skin.

Liposomal formulations constitute a promising approach for the development of effective dermal and transdermal drug delivery systems (Neubert, 2011). In particular, the potential of liposomes as carrier systems for transdermal drug delivery can be improved by adding suitable pharmaceutically-acceptable "edge-activators" in the vesicle bi-layer, able to increase its elasticity, thus obtaining ultra-deformable liposomes, also named *transfersomes* (Cevc, 2004; El Zaafarany et al., 2010).

Another interesting formulative strategy, which can be exploited for promoting transdermal drug delivery, is represented by microemulsions, thermodynamically stable, isotropic liquid mixtures of oil and water stabilized by an interfacial film of a suitable surfactant-cosurfactant mixture (Moulik and Paul, 1998). Microemulsions present ease of preparation, high solubilization capacity both for hydrophilic and lipophilic drugs and good penetration enhancing effects when administered on the skin (Peltola et al., 2000; Rhee et al., 2001; Kogan and Garti, 2006; Chen et al., 2006; Heuschkel et al., 2008). The effectiveness of both such kinds of formulations in promoting transdermal drug delivery can be further improved by the addition of a suitable "skin penetration enhancer" (Pahri et al., 2012). Depending on the type of enhancer and on the nature of the drug, different mechanisms could be involved, such as increase of the effective concentration of the drug in the vehicle, improvement of drug partitioning from the formulation to the skin, increase of the drug diffusion coefficient,

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decrease of the skin barrier properties (Moser et al., 2001). Cyclodextrins are reported among the different possible skin penetration enhancers, even though their mechanism of action is still under debate (Loftsson and Másson, 2001; Loftsson et al., 2007). The combination in a same formulation of different penetration enhancers, including cyclodextrins, can give rise sometimes to a synergistic effect (Loftsson and Màsson, 2001; Loftsson et al., 2007; Karande and Mitragotri, 2009; Pahri et al., 2010).

Different authors evidenced the potential advantages over conventional dosage forms of the transdermal administration of different kinds of benzodiazepines (Nokhodchi et al., 2003; Kravchenko et al., 2003; Balaguer-Fernandez et al., 2010; Soler et al., 2012). Among these, clonazepam (CLZ), a potent benzodiazepine derivative mainly employed for its anxiolytic, hypnotic and antiepileptic properties, is considered a very interesting candidate for transdermal administration, due to its pharmacological characteristics, such as high first pass metabolism, wide blood levels oscillations, low dose size, need for long-term treatment (Ogiso et al., 1989; Mura et al., 1996, 2000; Corti et al., 1998; Puglia et al., 2001). On the other hand, the very low water solubility of CLZ gives rise to a dissolution rate-limited absorption, generally recognized as directly related to poor and/or erratic absorption and bioavailability (Amidon et al., 1995). For such a reason, in a recent work we investigated and compared the complexing and solubilizing efficacy of different native and chemically-modified cyclodextrins toward CLZ, as a first step for the future development of innovative transdermal delivery systems of the drug (Mennini et al., 2014). Among the examined carriers, the randomly methylated-beta-cyclodextrin (Me-βCD) provided the best results in terms of increase in drug solubility and dissolution rate (Mennini et al., 2014).

On the basis of all the above considerations, and in continuation of our previous studies on transdermal delivery of CLZ (Mura et al., 1990, 1992, 1996, 2000; Corti et al., 1998), the present work was aimed at the development of a new innovative and effective transdermal delivery system of CLZ. With this purpose, we developed different ultra-deformable liposomal and microemulsion formulations containing potential skin penetration enhancers and investigated their performance in improving the drug permeation properties. Moreover, considering the potential co-enhancer effect of cyclodextrins in transdermal drug delivery (Loftsson et al., 1998, 2007), and also based on the high solubilizing efficacy of Me-βCD toward CLZ (Mennini et al., 2014), the effect of the addition of such CD to both liposomal and microemulsion formulations was also evaluated. The permeation properties of CLZ from these delivery systems through both skin-simulating lipophilic artificial membranes and rabbit ear excised skin, used as a percutaneous absorption model, were evaluated and compared with those of a simple drug aqueous suspension (Mura et al., 1996, 2007).

2. Materials and methods

2.1. Materials

Cholesterol (CHL), Clonazepam (5-(2-chlorophenyl)-7-nitro-3H-1,4-benzodiazepin-2(1H)-one) (CLZ), lauryl alcohol, L-α-phosphatidylcholine from egg yolk (PC), octadecylamine (OCT), oleic acid, polyoxyethylene sorbitan monolaurate (Tween 20), sodium cholate hydrate (SC), sorbitan monoleate (Span 80), sorbitan trioleate (Span 85), triethanolamine (TEA) were purchased from Sigma–Aldrich Co. (St. Louis, MO, USA). Randomly methylated-beta-cyclodextrin (Me-βCD) was kindly provided by Wacker Chemie Gmbh (Munich, Germany). Caprylic/capric triglycerides (Labrafac CC), caprylocaproyl macrogol-8 glycerides (Labrasol), highly purified diethylene glycol monoethyl ether (Transcutol HP), linoleoyl macrogol-6 glycerides (Labrafil M2125CS), medium-chain triglycerides (Labrafac Lipophile WL1349), octyldodecylmyristate, polyglyceryl-3 dioleate (Plurol Oleique CC 497), polyglycolized glycerides (Labrafac Hydro WL 1219), propylene glycol dicaprylocaprate (Labrafac PG), and propylene glycol monolaurate (Lauroglycol 90) were kindly donated by Gattefossé Italia s.r.l. (Milan, Italy). Isopropyl myristate and polyethylene glycol 400 (PEG 400) were purchased from Merck Schuchardt OHG (Hohenbrunn, Germany). Carbopol 940 was obtained from Lubrizol (Cleveland, OH, USA). Water was obtained from a Milli-Q water purification system (Millipore, Billerica, MA, USA). All other chemicals were of analytical grade.

2.2. Screening of oils for microemulsions

The solubility of CLZ in the different oil phases (Labrafil M2125CS, Labrafac CC, Lauroglycol 90, Labrafac PG, Labrafac Hydro WL 1219, Labrafac lipophile WL1349, oleic acid, isopropyl myristate) was determined, in order to select the oil with the highest solubilizing power to use as the oil phase in the microemulsions. An excess amount of drug was added to 5 mL of each oil; each sample was sealed, initially shaken using a vortex mixer and then kept under magnetic stirring at 25 ± 1.0 °C for 48 h to reach equilibrium. The suspensions were then centrifuged at 3000 rpm for 15 min mantaining constant the temperature. The supernatant was filtered through a 0.45 μm cellulose acetate membrane filter and the concentration of CLZ in the filtrate was determined by HPLC, as described below. The experiments were performed in triplicate.

2.3. High-performance liquid chromatography (HPLC) assay of clonazepam

Quantitative assay of CLZ was carried out by HPLC (Merck Hitachi, Darmstadt, Germany) equipped with an Elite Lachrom UV–vis detector (Merck Hitachi). A Hypersil RP C18 column (Thermo Electron Co., Waltham, MA, USA), 2.4 μm particle size, 100 mm × 4.6 mm, was used as stationary phase. The mobile phase was a 30:70 v/v mixture of acetonitrile:water; the flow rate was 0.9 mL/min. UV detection was carried out at 310 nm. The injection volume was 20 μL. The temperature was maintained at 40 ± 1.0 °C. The retention time of CLZ under these experimental conditions was about 8 min. A calibration curve in the 5–20 mg/L concentration range was prepared. The method was validated performing repeated analyses of decreasing analyte concentrations (Ermer, 2001). The lower limit of quantification and the limit of detection were 0.6 mg/L and 0.25 mg/L, respectively.

2.4. Construction of phase diagrams and preparation of microemulsions

Pseudo-ternary phase diagrams were constructed by progressive titration with water of the component mixtures. Each surfactant (Labrasol, Span 80, Span 85, Plurol Oleique CC 497, Tween 20) was mixed in a 1:1 v/v ratio with Transcutol, selected as co-surfactant. Each surfactant/co-surfactant (S/CoS) mixture was then mixed with Labrafac Hydro WL 1219, the oil phase selected on the base of previous solubility studies, in different oil:S/CoS v/v ratios. Each mixture was then titrated by adding water drop by drop up to clouding. The surfactant which allowed the obtainment of the largest existence area of the microemulsion (Tween 20) was selected for the subsequent step where the experiments were repeated at different S/CoS v/v ratios.

Analysis of the obtained pseudo-ternary phase diagrams allowed the choice of the best components and selection, within