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Full Length Article

The effect of diethylene glycol monoethyl ether on skin penetration ability of diclofenac acid nanosuspensions

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ABSTRACT

The poor ability of many drugs to cross skin layers is the main limiting factor for the exploitation of the transdermal route for drug delivery. As a consequence, several approaches have been proposed to overcome the skin barrier, such as the inclusion of penetration enhancers in the topically applied drug solutions and emulsions. In this work, the penetration enhancer diethylene glycol monoethyl ether was included in novel diclofenac acid nanocrystal formulations, developed using the wet media milling technique and Poloxamer 188 as stabilizer.

The nanosuspensions were characterized by different techniques such as scanning electron microscopy, differential scanning calorimetry, X-ray powder diffractometry, Fourier-transform infrared spectroscopy and photon correlation spectroscopy. The influence of diethylene glycol monoethyl ether on (trans)dermal delivery of diclofenac nanosuspensions was evaluated by *in vitro* studies using Franz diffusion cells and pig skin.

Results: demonstrated that the presence of diethylene glycol monoethyl ether influences the Poloxamer 188 ability to stabilize the nanocrystals during the milling process, leading to larger particles as compared to penetration enhancer-free nanosuspensions. As previously reported, the *in vitro* permeation studies indicate the nanosizing as a key factor in the dermal penetration of topically applied diclofenac. Surprisingly enough, the inclusion of increasing amounts of the penetration enhancer in the formulation decreased the diclofenac accumulation in the stratum corneum, while showing no significant effect on the drug delivered to the epidermis. Overall, the present results exclude a synergistic effect of the nanosizing approach and the addition of diethylene glycol monoethyl ether in regard to the skin penetration of diclofenac applied as a nanosuspension.

1. Introduction

There is great interest in the skin as route for both local and systemic drug administration. Indeed, topical drug delivery has several advantages over the other routes of administration such as reduction of drug systemic toxicity, improved bioavailability for drugs that suffer the gastrointestinal environment and/or hepatic first effects and also enhancement of drug local activity and patient compliance. However, the skin, in particular the stratum corneum (SC), forms a formidable barrier to drug penetration thereby limiting topical and transdermal bioavailability. In order to overcome the impermeability of intact human skin and to enhance drug transport across the skin, several strategies have been developed, which include passive and active penetration enhancement or technologies properly used to bypass the stratum corneum. The different strategies involve passive methods such as the use of nanoparticles [1,2] and molecules which act as penetration enhancers [3–6] as well as active methods which involve the use of external energy (ionophoresis, electroporation and low-frequency ultra-sound) [7].

Diethylene glycol monoethyl ether (Transcutol® P, TRC) seems to be very attractive as skin penetration enhancer due to its non-toxicity, biocompatibility, miscibility with polar and non-polar solvents [8]. It has been suggested that TRC can lead to the formation of a cutaneous depot of topically applied drugs due to its ability to increase skin permeability. Indeed, it acts causing the swelling of stratum corneum intercellular lipids without altering their multiple bilayer structure [3,9,10]. However, TRC has also been reported to increase the skin penetration of topically applied compounds without a concomitant increase in transdermal permeation [11].

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Another novel approach to increase skin permeability of poorly water soluble drugs is the production of nanocrystals (pure drug crystals with an average diameter below 1 μm stabilized with a small amount of stabilizer). The use of the nanocrystals technology has shown to be very effective for improving dermal bioavailability of lipophilic substances with good skin permeability [12]. Indeed, conversion of the microsized drug particles into nanocrystals not only increases the dissolution velocity [13–15], but also the drug saturation solubility. The improvement of these parameters determines an increased concentration gradient between the topically applied formulation and the skin, which significantly improves the bioavailability of the drug [16–18].

The objective of this investigation was the combination of two technologies such as the use of a chemical penetration enhancer and the nanosizing approach.

Indeed, in this study, we developed diclofenac acid (DCF) nanosuspensions containing different concentrations of diethylene glycol monoethyl ether (NS-TRC), and we studied the relative contributions of the chemical penetration enhancer and of the size reduction of crystals to the transdermal permeation and/or skin accumulation of diclofenac [19].

Diclofenac, 2-[(2,6-dichlorophenyl)amino] phenyl acetic acid, is a potent nonsteroidal anti-inflammatory drug (NSAID) with very low aqueous solubility and gastrolesive actions. It is used in inflammatory and painful conditions of rheumatic and non-rheumatic origin [20]. Three polymorphic forms of diclofenac acid are reported: two are monoclinic and are referred as HD1 (space group $P2_1/c$) and HD2 (space group $C2/c$). In both forms molecules are linked to each other through the carboxyl groups giving rise to centrosymmetric dimers [21]. Third polymorph is an orthorombic form (HD3, space group *Pcan*) where no intermolecular hydrogen bond is present [22]. In this study the polymorphic form HD2 has been used.

Characterization of the nanosuspensions, prepared using the wet media milling technique, was carried out by different techniques: scanning electron microscopy (SEM), differential scanning calorimetry (DSC), X-ray powder diffractometry (XRPD), Fourier transform infrared spectroscopy (FTIR) and photon correlation spectroscopy (PCS).

With the aim to investigate the potential of the combination of nanosizing and chemical penetration enhancer on the (trans)dermal DCF delivery, the NS-TRC were subjected to *in vitro* skin penetration studies using Franz diffusion vertical cells and newborn pig skin, and results were compared with those obtained by applying DCF nanosuspension without TRC, DCF coarse suspension and a commercial topical formulation containing DCF sodium salt.

2. Experimental

2.1. Materials

Diclofenac sodium salt was purchased from Galeno (Comeana, Italy). Diethylene glycol monoethyl ether (Transcutol® P, TRC) was kindly provided by Gattefossé (Saint Priest, France). Diclofenac sodium salt commercial formulation, Diclofenac Sandoz® gel 1% is produced by Sandoz S.p.a. (Origgio, Varese, Italy). Kolliphor® P188 (Poloxamer 188, P188) and all other reagents were purchased from Sigma–Aldrich (Milan, Italy).

2.2. Preparation of DCF acid

Diclofenac acid crystal form was obtained following the procedure reported in a previous work [20]. Briefly, a saturated aqueous solution of diclofenac sodium salt was acidified with diluted HCl until a white precipitate of DCF acid was observed. The precipitate was filtered out, washed with bidistilled water to remove residual HCl and dried at 40 °C overnight.

Table 1
Composition of DCF nanosuspensions.

Components (% w/w)	Formulations			
	NS-TRC 0%	NS-TRC 0.5%	NS-TRC 1%	NS-TRC 5%
DCF	0.92	0.92	0.92	0.92
Poloxamer 188	0.46	0.46	0.46	0.46
Transcutol	–	0.5	1	5
Water	98.6	98.1	97.6	93.6

2.3. Preparation of DCF coarse suspension

The DCF coarse suspensions were prepared by dispersing DCF bulk powder in an aqueous solution of P188 (0.46% w/w) and TRC at different concentrations (0, 0.5, 1 or 5% (w/w) using an Ultra Turrax T25 basic (IKA, Werke, Germany) for 5 min at 6500 rpm. The drug concentration was the same of the commercial product, used as reference (3.1 mmol/100 g).

2.4. Preparation and characterization of DCF nanosuspensions

Diclofenac nanosuspensions were prepared using a wet media milling technique. Drug bulk powder was dispersed in an aqueous solution of P188 (0.46% w/w) and TRC at different concentrations (0, 0.5, 1 or 5% (w/w) using an Ultra Turrax T25 basic for 5 min at 6500 rpm (Table 1). This coarse suspension was divided in 1.5 ml conical microtubes containing about 0.4 g of 0.1–0.2 mm yttrium-stabilized zirconia-silica beads (Silibeads® Typ ZY Sigmund Lindner, Germany). The microtubes were oscillated at 3000 rpm for 60 min using a beads-milling cell disruptor equipment (Disruptor Genie®, Scientific Industries, USA). The obtained nanosuspensions of each microtubes were gathered and then separated from the milling beads by sieving. The drug concentration was the same of the commercial product, used as reference (3.1 mmol/100 g).

The average diameter and polydispersity index (PI; a measure of the size distribution width) of the nanosuspensions were determined by photon correlation spectroscopy (PCS) using a Zetasizer nano (Malvern Instruments, Worcestershire, United Kingdom). Samples were backscattered by a helium–neon laser (633 nm) at an angle of 173° and a constant temperature of 25 °C. Zeta potential was estimated using the Zetasizer nano by means of the M3-PALS (Phase Analysis Light Scattering) technique. Just before the analysis, the DCF nanosuspensions were diluted with bidistilled water. The morphology of nanosuspensions was evaluated using scanning electron microscopy (SEM) (S-4100, HITACHI). Samples were fixed on a brass stub using carbon double-sided, coated with gold blazers SCD 004 sputter coater for 2 min and observed under an excitation voltage of 5 kV.

2.5. Solubility studies

The water solubility of DCF acid was measured for the DCF bulk powder, coarse suspensions and nanosuspensions. The formulations (n = 3) were kept under constant stirring for 48 h in a thermostated bath at 37 °C. At preselected time intervals, samples were withdrawn and centrifuged. 0.2 ml of the clear supernatant was filtrated, diluted with methanol and analyzed by HPLC. DCF content was quantified at 280.4 nm using a chromatograph Alliance 2690 (Waters, Milan, Italy), equipped with a photodiode array detector and a computer integrating apparatus (Empower 3). The column was a SunFire C18 (3.5 μm, 4.6 mm × 100 mm, Waters), and