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Novel nanosized formulations of two diclofenac acid polymorphs to improve topical bioavailability

Rosa Pireddu [a], Chiara Sinico [a], Guido Ennas [b], Francesca Marongiu [a], Rita Muzzalupo [c], Francesco Lai [a,*], Anna Maria Fadda [a]

[a] Dept. Scienze della Vita e dell'Ambiente, Sezione di Scienze del Farmaco, CNBS, University of Cagliari, via Ospedale 72, 09124 Cagliari, Italy
[b] Dipartimento di Scienze Chimiche e Geologiche, University of Cagliari and Unità di Ricerca del Consorzio Nazionale di Scienze e Tecnologie dei Materiali (INSTM), Cittadella Universitaria di Monserrato, SS 554 bivio Sestu, 09042 Monserrato, CA, Italy
[c] Dipartimento di Farmacia e Scienze della Salute e della Nutrizione, Università della Calabria, Edificio PolifunzionaleArcavacata di Rende, Cosenza, Italy

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ABSTRACT

In this work, nanocrystal formulations, containing two different diclofenac acid crystal forms, were developed with the aim to improve dermal drug bioavailability. Nanosuspensions were obtaining using wet media milling technique and were characterized in terms of size distribution, morphology, zeta potential, differential scanning calorimetry and X-ray powder diffractometry. The ability of the nanocrystals to improve dermal drug bioavailability was investigated in vitro using Franz diffusion vertical cells and newborn pig skin, in comparison with diclofenac acid coarse suspensions and a commercial topical formulation containing diclofenac sodium. Nanocrystals exhibited a mean diameter ranging between 279 and 315 nm and a PI lower than 0.25, as shown by PCS measurements. The XRDP and DSC analysis clearly indicated that the preparation process did not modify the diclofenac polymorphic forms. In vitro transdermal delivery experiments showed an improved skin deposition and permeation of the nanocrystals compared to coarse suspensions and diclofenac sodium commercial topical formulation. These results highlight the fundamental role of the crystal size on drug solubility and, thus, on the ability of a poorly soluble drug to cross the skin and accumulate in the deeper skin layers.

1. Introduction

Diclofenac (DCF) is one of the most commercially successful non-steroidal anti-inflammatory drugs (NSAIDs), which was launched by Ciba–Geigy as a sodium salt oral formulation in 1973. As all NSAIDs, DCF exerts anti-inflammatory, analgesic and anti-pyretic actions via inhibition of the cyclooxygenase I (COX 1) and cyclooxygenase II (COX II) enzymes (Warner et al., 1999). However, recent research indicates that the pharmacologic activity of DCF goes beyond COX inhibition and includes multimodal and, in some instances, novel mechanisms of action, such as inhibition of the thromboxane-prostanoid receptors or inhibition of lipoxygenase enzymes and activation of the nitric oxide-cyclic guanosine monophosphate pathway (Gan, 2010). DCF is widely prescribed for the treatment of a variety of localized pain and inflammatory conditions including arthritis and soft tissue injuries. Although it is one of the best-tolerated NSAIDs, several systemic drawbacks often appear following chronic oral administration (Odom et al., 2014). Therefore, a topical DCF formulation with improved skin permeation may be useful in the treatment of not only local skin inflammation but also inflammation and pain involving deeper structures such as bones, joints and muscles.

Since 1973, several pharmaceutical preparations have been developed for DCF application to the skin, and a variety of DCF salt products are currently available on the market. By contrast, because of its low water solubility, no DCF acid based commercial product exists. Indeed, even though this molecule possesses a good permeation rate due to its high lipophilicity, poor solubility causes a slow release rate from the formulation which in turn limits the penetration and permeation of an effective drug concentration into and through the skin. The final result is low dermal drug bioavailability. In order to overcome the slow dissolution rate of such drugs, various approaches have been studied such as the use of surfactants, co-solvents, inclusion and complexation compounds. In particular, cosolvents have been widely used as vehicles as well as penetration enhancers in the topical formulations of drugs. In addition to affecting the drug solubility in the vehicle, cosolvents may alter the structure of the skin and modify the penetration rate. Thus, cosolvents can affect both drug release and percutaneous

* Corresponding author.
E-mail address: frlai@unica.it (F. Lai).

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absorption. It was demonstrated that the use of propylene glycol a cosolvent in combination with isopropyl myristate as penetration enhancer produced synergistic enhancement of diclofenac sodium skin permeation (Arellano et al., 1998).

However, these approaches have been related to different side effects including skin irritation and can be only used for drugs having specific characteristics (sufficient solubility in excipients, appropriate shape and molecular size for incorporation in cyclodextrin etc.) (Williams et al., 2013).

An alternative to such methods is the nanonization of pure drug crystals with the preparation of the so-called nanocrystals (100–1000 nm), one of the most interesting drug delivery technologies of the last twenty years.

Nanocrystals are prepared as a suspension (nanosuspension) in an outer liquid phase, usually water but also water miscible liquids or non-aqueous media. Nanosuspensions are stabilized using ionic stabilizers (sodium dodecyl sulfate, lecithin, etc.) or non-ionic surfactants or polymers (Pluronic® surfactants, polyethylene glycol, Tween 80, polyvinylpyrrolidone, cellulose polymers, etc.).

Fundamentally, nanosuspensions can be prepared by bottom up or top down technologies or by a combination of both (Salazar et al., 2012). In the bottom up technology, the low water soluble drugs are (molecularly) dissolved in a solvent and then precipitated in different ways as nanocrystals in a surfactant solution (Dolenc et al., 2009). The top down technology is based on particle disintegration techniques including beads wet milling (Ghosh et al., 2012; Merisko-Liversidge et al., 2003) and high pressure homogenization (Keck and Müller, 2006; Müller et al., 2001).

Nanosuspensions have been studied for different routes of administration; however, the oral one has been the most studied because of the improved clinical efficacy, the main advantages being enhanced bioavailability, reduced fed/fasted state and subject variability. Therefore, the currently approved and marketed nanocrystal based products are mainly for oral administration (Möschwitzer, 2013).

Until now, the use of nanocrystals for dermal applications has not been intensively studied and only a few articles regarding this topic have been published (Ghosh and Michniak-Kohn, 2013; Lai et al., 2013; Romero et al., 2014; Zhai et al., 2014a). However, this technology could be very effective for improving dermal bioavailability of lipophilic substances with good skin permeability, such as DCF acid. Indeed, in 2007 the first anti-aging and skin-protective cosmetic products based on nanosuspensions of poorly soluble antioxidants rutin (Juvena) and hesperidin (La prairie) have been introduced on the market (Zhai et al., 2014b).

The aim of the present investigation was to study the ability of nanocrystal technology to improve the dermal bioavailability of the poorly water-soluble drug DCF acid. In order to investigate the role of the nanocrystal polymorphic structure in cutaneous bioavailability, two different DCF acid polymorphic forms (HD1 and HD2) were used to prepare nanosuspensions using a wet media milling technique with poloxamer 188 as a stabilizer.

Three polymorphic forms of diclofenac acid are reported in literature: two forms referred as HD1 (space group P21/c) and HD2 (space group C2/c) are monoclinic. In both forms molecules are linked to each other through the carboxyl groups giving rise to centrosymmetric dimers (Castellari and Ottani, 1997). Third polymorph is an orthorhombic form (HD3, space group *Pcan*) where no intermolecular hydrogen bond is present (Jaiboon et al., 2001). In all the forms, a bifurcated intramolecular hydrogen bond involves N–H group.

Characterization of DCF nanosuspensions was carried out by different techniques: scanning electron microscopy (SEM), differential scanning calorimetry (DSC), X-ray powder diffractometry and photon correlation spectroscopy (PCS). The efficacy of the nanocrystals in improving dermal drug bioavailability was evaluated by in vitro skin penetration and permeation studies using Franz diffusion vertical cells and newborn pig skin, in comparison with DCF coarse suspension and a commercial topical formulation containing diclofenac sodium.

2. Materials and methods

2.1. Materials

Diclofenac sodium salt (DCF_{Na}) was purchased from Galeno (Comeana, Italy). Lutrol®F68 (Poloxamer 188) was a gift from BASF AG (Ludwigshafen, Germany). DCF_{Na} commercial formulation, Diclofenac Sandoz® gel 1% is produced by Sandoz S.p.a. (Origgio, Varese, Italy). All the other compounds were of analytical grade and used as received from Sigma–Aldrich (Milan, Italy).

2.2. Preparation of DCF acid crystal forms

HD2 DCF acid crystal form was obtained following the procedure reported in a previous work (Lai et al., 2009). Briefly, a saturated aqueous solution of diclofenac sodium salt was acidified with diluited HCl until a white precipitate of DCF acid was observed. The precipitate was filtered, washed with bidistilled water to remove residual HCl and dried at 40 °C overnight. Starting from HD2 DCF satured solution in dimethyl sulfoxide (DMSO), HD1 DCF acid crystal form was obtained by precipitation with bidistillated water. The precipitate was washed with the minimum amount of bidistilled water and after several cycles of centrifugation was dried at 40 °C overnight.

2.3. Preparation of coarse suspensions

Coarse drug suspensions were prepared dispersing HD1 or HD2 DCF acid bulk powder in Poloxamer 188 bidistilled water solution using an Ultra Turrax T25 basic (IKA, Werke) for 2 min at 6500 rpm. DCF acid coarse suspensions were prepared using both the same drug amount of the commercial gel formulation (0.0031 mol/100 g, CS HD1 I and CS HD2 I) and the double amount (0.0062 mol/100 g, CS HD1 II and CS HD2 II). Drug/surfactant ratio (w/w) was 2:1 (Table 1).

2.4. Preparation of nanosuspensions

Nanosuspensions were prepared using a wet media milling technique. Drug bulk powder (HD1 or HD2) was dispersed in aqueous Poloxamer 188 solution 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 different times 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.

Table 1
Composition of HD1 and HD2 coarse suspension (CS HD2 II, CS HD2 I, CS HD1 II, CS HD1 I) and nanosuspension formulations (NS HD2 II, NS HD2 I, NS HD1 II, NS HD1 I.

Components (% w/w)	Formulations			
	NS HD1 I (CS HD1 I)	NS HD1 II (CS HD1 II)	NS HD2 I (CS HD2 I)	NS HD2 II (CS HD2 II)
HD1	0.92	1.84	–	–
HD2	–	–	0.92	1.84
Poloxamer 188	0.46	0.92	0.46	0.92
Water	98.62	97.24	98.62	97.24