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Influence of modified cyclodextrins on solubility and percutaneous absorption of celecoxib through human skin

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Abstract

We evaluated the ability of two modified cyclodextrins, hydroxypropyl-β-cyclodextrin (HP-β-Cyd) and 2,6-di-*O*-methyl-β-cyclodextrin (DM-β-Cyd), to influence the percutaneous absorption through isolated human stratum corneum and epidermis (SCE) of celecoxib (CCB). Previous studies demonstrated that DM-β-Cyd includes the drug, producing a significant increase of water solubility (0.5 mg/ml at 25 °C) and dissolution rate of CCB. In this work chemical-physical characterization studies were performed to evaluate the ability of HP-β-Cyd to include CCB. We showed that only an external interaction could exist between CCB and HP-β-Cyd that positively influences the water solubility of the drug (0.12 mg/ml at 25 °C for CCB-HP-β-CyD system and 4.12×10^{-3} mg/ml at 25 °C for free CCB). In vitro percutaneous experiments were performed using samples in solution and in suspension containing different Cyd concentrations. Both HP-β-Cyd and DM-β-Cyd enhanced drug flux through SCE by means of an increase of dissolution rate of the drug as well as a direct action on the stratum corneum (SC). Histological analysis of treated SCE showed a protective effect of the two Cyds towards an invasive action shown by CCB on SC.

Keywords: Celecoxib; Modified cyclodextrins; In vitro percutaneous studies; Isolated human skin; Histological analysis

1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are the most active agents used in inflammatory diseases. They act by inhibiting the enzymatic activity of cyclooxygenase (COX), showing not only therapeutic effects but also side effects.

Celecoxib (CCB) is the first synthesized NSAID able to selectively inhibit COX-2 activity. For this reason, CCB shows high efficacy in the treatment of osteoarthritis and rheumatoid arthritis (Simon et al., 1998; Annoni and Strumia, 2000 and references therein), but no gastrolesivity or interference with platelet function was observed at therapeutical concentrations. However, different authors (Marques et al., 2003; Goeschke and Braathen, 2004; Yang et al., 2004) reported the appearance of benign skin damage with generalized pustular exanthema in patients treated systemically with CCB, due to the presence of sulphonamide group in the molecule. Topical application of CCB could increase the presence of the drug locally, reducing, in the mean time, the risk of systemic skin toxicity as a result of a reduced dose.

CCB is a 4-[5-(4-methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl]benzenesulfonamide and shows high apolar characteristics (Fig. 1). The drug is insoluble in water and could show per se poor percutaneous absorption properties. Moreover, the protective role played by the stratum corneum could make the topical application of CCB ineffective. Cyclodextrins (Cyds) could influence the percutaneous absorption of CCB by both a solubilizing action on the drug (Felton et al., 2002; Rode et al., 2003; Babu and Pandit, 2004), thus increasing its availability at the absorption site, and by an interaction with the free lipids present in the stratum corneum (Swartzendruber et al., 1987), improving transdermal penetration of CCB (Vitória et al., 1997; Loftsson and Masson, 2001; Ventura et al., 2001).

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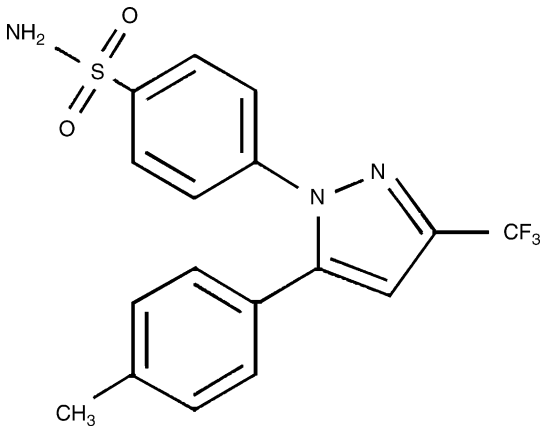

Fig. 1. Chemical structure of CCB.

We recently prepared and characterized the inclusion complex of CCB with 2,6-dimethyl-β-CyD (DM-β-Cyd) (Ventura et al., 2005). DM-β-Cyd is able to include CCB, increasing the water solubility and dissolution rate of the drug. Permeation of CCB through the CaCo-2 cell monolayer was significantly increased in the presence of the macrocycle.

In this work we evaluated the ability of DM-β-Cyd and hydroxypropyl-β-Cyd (HP-β-Cyd) to influence the percutaneous absorption of CCB through isolated human stratum corneum and epidermis (SCE). In vitro permeation experiments were performed using Franz cells. Characterization studies in the solid state and in aqueous solution were performed, compared to CCB-DM-β-Cyd inclusion complex, to evaluate the ability of HP-β-Cyd as a complexing carrier for CCB. Histological analysis of isolated human SCE treated with the two complexes, free Cyds and CCB alone, were performed to evaluate the different mechanism by which Cyds act.

2. Materials and methods

2.1. Materials

CCB was obtained by repeated extractions with methanol from a marketed capsule formulation (Solexa®; Pfizer) and the purity (99%) was assayed by ^{1}H NMR, HPLC and elemental analysis (Calcd. for $C_{17}H_{14}F_3N_3O_2S$: C, 53.54; H, 3.7; N, 11.01; S, 8.39; found: C, 52.99; H, 3.81; N, 10.84; S, 8.91). HP-β-CyD, with 0.6 degree average substitution, was kindly provided by Roquette Italia (Cassano Spinola, Italy). DM-β-Cyd was purchased from Cyclolab R&D Laboratory (Budapest, Hungary) and used without further purification.

All other chemicals and solvents were of analytical reagent grade and obtained from Sigma–Aldrich (Milano, Italy). Deionized double-distilled water was used throughout the study.

2.2. Preparation of the CCB-Cyds solid samples

CCB-DM-β-Cyd and CCB-HP-β-Cyd solid samples were prepared by the freeze-drying method as described in our previous paper (Ventura et al., 2005). Briefly, a water/methanol solution (50:50, v/v) containing DM-β-Cyd or HP-β-Cyd was added to an excess amount of solid CCB. After stirring at room temperature for 2 days, the suspensions were filtered through a 0.45 μm Nylon filter (Millipore, Bedford, U.S.A.) and the filtrates were freeze-dried using the Modulyo 4 K system (Edwards, Crawley, U.K.). The obtained CCB-DM-β-Cyd solid sample (10 mg) was solubilized in methanol (5 ml) and analyzed by HPLC to determine the drug-Cyd molar ratio. CCB-HP-β-Cyd solid sample (10 mg) was suspended in methanol (5 ml) and stirred for 2 h. The suspension was filtered and the solution was analyzed for molar ratio.

CCB-Cyds physical mixtures were prepared in 1:2 molar ratio by simple mixing in a mortar for 15 min.

2.3. Differential scanning calorimetry (DSC)

DSC scans were recorded on a Mettler DSC 12E (Mettler Toledo Italia, Milano, Italy); equipped with a Haake thermocryostate mod. D8-G (Haake, Karlsruhe, Germany). A Mettler TA89E and FP89 system software was used for data acquisition. Indium was used to calibrate the instrument. Each sample was scanned at a speed of 10 °C/min in the 30–300 °C temperature range.

2.4. Circular dichroism (CD) spectra

CD spectra were performed on a Jasco J-600D recording spectropolarimeter (Jasco, Inc., Easton, MD, USA). CCB alone or in the presence of different concentrations of both Cyds (1:10 or 1:100 molar ratio) were solubilized in a water/methanol solution (70/30, v/v) and stirred before the analysis for 12 h.

2.5. ^{1}H NMR studies

^{1}H NMR spectra were recorded, at a probe temperature of 303 °K, on a VARIAN Unity Inova Instrument at 200 MHz (Varian, Palo Alto, CA, USA), For the analysis, CCB (0.4 mg) and the Cyds (1:1 and 1:2 molar ratios for CCB-DM-β-Cyd; 1:1, 1:2 and 1:3 molar ratios for CCB-HP-β-Cyd) were poured into vials and added to 1 ml of D_2O/CD_3OD solution (50/50, v/v). After stirring for 24 h, 0.7 ml of these solutions were submitted to analysis. Free CCB, DM-β-Cyd and HP-β-Cyd were solubilized in the same solvent mixture. No internal standards were added to the samples due to their interaction with Cyd cavity, the residual sign of CD_3OD at 3.3 ppm was used as reference.

2.6. Water solubility and dissolution rate determination

Water solubility of CCB-HP-β-Cyd solid samples, compared to free CCB and CCB-DM-β-Cyd, was determined by suspending excess amounts of each sample in 2 ml of water and stirring at room temperature for 2 days. The suspensions were then filtered (0.45 μm Nylon Millipore filter) and analyzed by HPLC.

Dissolution rates of the same samples were carried out according to the USP 25th paddle method. An amount of 50 mg of free CCB or a corresponding amount in complexes and physical mixtures were suspended in 900 ml of water and stirred at 100 rpm at 37 ± 0.5 °C. At fixed time intervals the concentration