International Journal of Pharmaceutics 358 (2008) 151–158

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Effect of HPβCD on solubility and transdermal delivery of capsaicin through rat skin

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ARTICLE INFO

Article history:
Received 26 September 2007
Received in revised form 20 February 2008
Accepted 3 March 2008
Available online 18 March 2008

Keywords:
Capsaicin
Hydroxypropyl-β-cyclodextrin
Hydrogel
In vitro percutaneous studies
Histological analysis

ABSTRACT

We evaluated the ability of hydroxypropyl-β-cyclodextrin (HPβCD) to influence the percutaneous absorption of capsaicin (CP) through isolated rat skin. Phase solubility analysis and phase distribution studies suggested the potential of HPβCD as a solubilizer and permeation enhancer for CP. In vitro permeation studies showed the trend that, the penetration flux (J_s) of CP increased with the increasing concentration of HPβCD from 0 to 2.20% (w/v), and then decreased dramatically when the concentration of HPβCD kept on increasing up to 15% (w/v). 2.20% (w/v) of HPβCD provided both just adequate solubilization and preferred J_s for the permeation of CP (0.075%, w/v). Similar change patterns of the permeation parameters were also observed in the hydrogels, but the J_s of CP was reduced significantly along with the increasing concentration of Carbopol U21. Histological analysis showed an invasive action of HPβCD on the stratum corneum (SC) of rat skin, which could only reduce the lag time (T_L) but could not increase the J_s of CP. On the other hand, the complexation of HPβCD with CP could attenuate this invasive action. It is inferred that excess of HPβCD could not only disturb the percutaneous absorption of CP but also disrupt the structure of SC.

1. Introduction

Capsaicin (CP, Fig. 1), the active ingredient of hot peppers of the genus capsicum, exhibits broad bioactivity, including antinociception, antihypertension and lipid-lowering activities (Hayes et al., 1981; Wang et al., 1984; Monsereenusorn et al., 1982) and has been employed topically to treat various diseases such as rheumatoid arthritis, osteoarthritis, diabetic neuropathy and post-therapy neuralgia (Fusco and Giacovazzo, 1997). The high degree of first-pass metabolism of capsaicinoids in rats and mice was observed (Sietsema et al., 1988; Donnerer et al., 1990) and the half-life of CP by intravenous administration from rats was also detected very short (7.06 min) (Kawada et al., 1985). Therefore, topical delivery should be applied for this drug to circumvent the hepatic metabolism and achieve better bioavailability. It is well known that vehicles of external preparations may greatly influence the flux and extent of drug permeating through the skin. Some investigators prepared the topically applied formulations of CP, including hydrogel, solution, ointment and cream and in vitro percutaneous absorption experiments were also performed with these formulations (Wang et al., 2001; Magnusson and Koskinen, 2000; Fang et al., 1996a,b). However, most of these formulations employed organic solvents or surfactants, such as ethanol, propylene glycol and sodium laurylsulfate as the solubilizer of CP, which often disturbed or interacted with the intercellular lipids or keratin of human skin (Williams and Barry, 2004). Furthermore, it was observed that the percutaneous effect of CP was reduced with increasing concentrations of ethanol and isopropanol (Fang et al., 1996b). Consequently, further studies are still necessary to exploit a new solubilizer for CP with permeation enhancement.

Hydroxypropyl-β-cyclodextrin (HPβCD) is able to form hydrophilic inclusion complexes with many lipophilic compounds in aqueous solution, which can enhance the aqueous solubility of the lipophilic drugs without changing their intrinsic ability to permeate lipophilic membranes (Loftsson and Masson, 2001). HPβCD has exhibited the potential as a solubilizer and penetrating enhancer for topically applied delivery by increasing the availability of dissolved drug molecules immediate to the biological membrane surface or by direct action on the stratum corneum (Loftsson et al., 2006; Ventura et al., 2006). However, some other studies considered that HPβCD had no effect on drug transport through human skin or through mouse skin with any manners (Simeoni et al., 2004; Shaker et al., 2003). To our knowledge, there is not any report to date concerning the influence of HPβCD on the percutaneous absorption of CP.

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doi:10.1016/j.ijpharm.2008.03.001

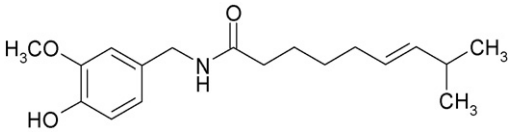

Fig. 1. Chemical structure of capsaicin.

This study aimed at exploring the effect of HPβCD on the permeation behavior of CP in solution and hydrogel. We characterized the interaction of CP with HPβCD in solution by phase solubility studies and in solid state by X-ray diffractometry (XRD) and differential scanning calorimetry (DSC) analyses. Phase distribution studies were performed to evaluate the effect of HPβCD on the observed distribution coefficients (D_{obs}) of CP at either pH 7.0 and 5.5 which was close to the acidity of human skin. The change patterns of in vitro permeation parameters of CP through excised rat skin were investigated in the presence of HPβCD at different concentrations. The permeation parameters of solutions were further compared with that of Carbopol U21 hydrogels. The rat skin samples pretreated with free CP, HPβCD alone and their complex were examined with transmission electron microscope (TEM) to explore the enhancement mechanism of HPβCD on the percutaneous absorption of CP.

2. Materials and methods

2.1. Materials

Capsaicin was purchased from Wuhan Yuancheng Technology Development Co., Ltd. (Hubei, PR China) and the purity was 99.15%. Hydroxypropyl-β-cyclodextrin (HPβCD, MW 1380, MS 5.9) was a gift from Xinxin Excipients Inc. (Jiangsu, PR China). *N*-Octanol (99%) was purchased from Shanghai Lingfeng Chemical Co., Ltd. (Shanghai, PR China). Carbopol U21 was a gift from Goodrich (Charlotte, USA). All other chemicals and solvents were of at analytical reagent grade or HPLC reagent grade. Double-distilled water was used throughout the study.

2.2. Preparation of the CP/HPβCD solid samples

The freeze-dried product of CP and HPβCD was prepared. 0.5 g of CP and 2.26 g of HPβCD (1.64×10^{-3} mol each of CP and HPβCD) were dissolved in 8 ml of ethanol/water solution (6/2, v/v) to obtain a solution containing CP and HPβCD in 1:1 molar ratio. After stirring for 48 h at room temperature, the solution was freeze-dried using the Modulyo 4 K system (Edwards, Crawley, U.K.). The obtained white powder was stored in sealed glass container at 25 °C for further investigations. The CP content of the complex was determined by dissolving an accurately weighed quantity in ethanol/water solution (50/50, v/v) followed by HPLC assay.

The physical mixture was prepared by simple mixing CP/HPβCD in 0.5:2.26 (w/w) ratio in a mortar for 15 min.

2.3. X-ray diffractometry

The X-ray diffraction pattern of the solid sample was recorded using Philips X-ray diffractometer (PW-1710) equipped with graphite monochromator, under the following operating conditions: Ni filtered Cu Kα radiation, 30 kV voltage, 20 mA current and scan speed 1° 2θ min^{-1}.

2.4. Differential scanning calorimetry

DSC scans of the solid sample were recorded on a PerkinElmer instrument equipped with a low temperature cell. The sample weight was 3.5 mg (approximately) and the heating rate was 10 °C/min.

2.5. Phase solubility studies

Phase solubility diagram of CP/HPβCD system was obtained according to Higuchi and Connors' method (1965). CP in excess of its solubility was weighed into a series of screw-capped vials containing aqueous solutions of HPβCD at concentrations ranging from 0 to 0.10 M. The sealed vials were agitated on a rotary shaker for 48 h under 27 °C and equilibrated for further 24 h. The clear supernatant was passed through 0.45 μm Millipore filter. The filtrates were immediately diluted with methanol followed by HPLC assay.

2.6. Phase distribution studies

The procedure for determining the observed distribution coefficients (D_{obs}) of drug distributing between *n*-octanol and aqueous solutions containing HPβCD at different concentrations was described previously (Másson et al., 2005). Solutions of CP were prepared at a concentration of 1 mg/ml in *n*-octanol that had been saturated with aqueous solutions containing 0, 1.0, 2.20, 3.0, 10.0, or 15.0% (w/v) of HPβCD at pH 7.0 (new distilled water) and 5.5 (phosphate buffer solution (PBS)). 3 ml aliquot of the CP *n*-octanol solution were transferred to 10 ml of vials, respectively, then 3 ml aqueous HPβCD solutions saturated with octanol were also added into the corresponding vials. The vials were shaken with a mechanical shaker for 24 h at room temperature. The phases were then separated with separating funnels and samples from each phase were analyzed by HPLC. The partition coefficient was calculated as the ratio between the concentrations in the octanol phase and the aqueous phase.

2.7. Preparation of solutions/suspensions

HPβCD was added to water at 0, 1, 2.20, 3, 10 and 15% (w/v) concentrations, respectively to obtain clear aqueous solutions. CP was added at 0.075% (w/v) concentration to these solutions equivalent to the strength of commercial topical preparations of CP, and the mixtures were stirred at room temperature for 12 h before the permeation experiments. Then the uniform suspensions (free CP, the sample prepared in the presence of 1% (w/v) of HPβCD) and solutions (the samples prepared in the presence of HPβCD at 2.20, 3, 10 and 15% (w/v) concentrations) were obtained.

2.8. Preparation of hydrogels

Various hydrogels with CP at 0.075% (w/v) concentration were prepared equivalent to the strength of commercial topical preparations of CP.

To prepare CP/HPβCD hydrogels containing 0.3–0.8% (w/v) of Carbopol U21 in the presence of HPβCD at 2.20% (w/v) concentration, 0.3, 0.5 and 0.8 g of Carbopol U21 was dissolved in 50 ml of water, respectively with continuous stirring for 2 h, and then these solutions were adjusted to pH 5.5 with 10 M of NaOH solution stirred for 1 h at room temperature. 2.20 g of HPβCD and 0.075 g of CP were dissolved in 20 ml of water with continuous stirring for 2 h; three parallel solutions were prepared with this method. Then the Carbopol U21 solutions and CP/HPβCD solutions were mixed together, respectively. At last, sufficient quantity of water was added to these three solutions to obtain 100 g of hydrogels. These hydrogels were stirred at room temperature for 2 h before the permeation experiments.

CP/HPβCD hydrogels containing 3, 10 and 15% (w/v) of HPβCD in the presence of 0.3% (w/v) of Carbopol U21 were also prepared with the method mentioned above.