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Preparation and solid-state characterization of bupivacaine hydrochloride cyclodextrin complexes aimed for buccal delivery

Mario Jug[a,*], Francesca Maestrelli[b], Marco Bragagni[b], Paola Mura[b]

[a] Department of Pharmaceutics, Faculty of Pharmacy and Biochemistry, University of Zagreb, A. Kovačića 1, HR-10000 Zagreb, Croatia
[b] Department of Pharmaceutical Sciences, University of Florence, via U. Schiff 6, I-50019 Sesto Fiorentino, Florence, Italy

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ABSTRACT

Binary products of bupivacaine hydrochloride (BVP HCl), an amide type local anesthetic, with parent β-cyclodextrin (β-CD) and its soluble β-cyclodextrin-epichlorohydrin polymer (EPI-β-CD) were prepared and evaluated as a first phase in the development of a novel mucoadhesive formulation aimed for buccal delivery of this drug. The solid products were obtained by physical mixing, ball milling in high-energy mills, co-evaporation and lyophilisation, in order to rationally select the most effective preparation technique. The solid products obtained were carefully characterised by differential scanning calorimetry (DSC), X-ray powder diffractometry (XRPD), Fourier transform infrared spectroscopy (FTIR) and environmental scanning electron microscopy (ESEM). The impact of the preparation techniques on the physicochemical properties of plain drug was also studied. Results of solid-state analysis revealed more intense interactions of BVP HCl with EPI-β-CD than with native β-CD, accompanied by stronger reduction of drug crystallinity in the samples, probably favoured by the amorphous nature of the polymeric carrier. While summarising the results of DSC and XRPD analyses, it seems that ball milling of drug/cyclodextrin binary mixtures was particularly efficient in inducing solid-state interaction between the components and it can be considered as the method of choice for preparation of complexes of BVP HCl with β-CD and EPI-β-CD. *In vitro* dissolution properties in artificial saliva of ball-milled BVP HCl and corresponding CD complexes were investigated by simulating the conditions present at the surface of the buccal mucosa. The obtained results confirmed that complexation of BVP HCl with β-CD and EPI-β-CD is a suitable tool for properly tailoring the dissolution properties of the drug and it can be favourably exploited for the development of an effective buccal drug delivery system.

1. Introduction

Bupivacaine hydrochloride is an amide type local anaesthetic (LA) that is widely used in management of the pain during and after dental and oral surgery procedures. The drug is commonly administered as injectable solution containing a vasoconstrictor, such as epinephrine [1,2]. The onset of action following dental injections is usually 2–10 min and anesthesia may last two or three times longer than lidocaine and mepivacaine [3]. However, there is a need to further improve the effectiveness of regional administration of LA in the oral cavity and to find suitable alternative formulations to the current mode of drug administration. This would highly increase the patient comfort, especially in needle sensitive persons and children. Moreover, such novel formulations may be very useful in treatment of radiation-induced oral mucositis that occurs during radiation therapy of the carcinoma in the oral cavity or as side-effect of cytostatics administration. This condition is very painful and has a significant impact on the quality of the patients' life [4].

A possible approach to achieve the above stated goals may be the development of a buccal mucoadhesive drug formulation that will ensure the regional delivery of LA to the oral cavity [5,6]. The drug release from such a formulation has to be properly modulated in order to obtain fast onset and prolonged duration of pharmacological action of the drug, which requires the use of suitable carriers [7]. Cyclodextrins (CDs), cyclic oligosaccharides consisting of 6, 7 or 8 α-1,4 linked glucopiranose units are suitable candidates for such role, because they are capable to favourably modify undesired biopharmaceutical properties of the drug, such as low chemical stability and limited aqueous solubility by inclusion complex formation [8]. In particular, β-CD complexation of a typical LA such as benzocaine allowed a marked improvement of its solubility and a significant reduction of its toxicity [9]. Moreover, CDs are able to control drug release from polymeric matrices [10], overcoming obstacles such as the limited amount of dissolution medium in the mouth and the barrier properties of the oral mucosa [11,12]. Furthermore, they

* Corresponding author. Tel.: +385 1 4612 608; fax: +385 1 4612 608.
E-mail address: mjug@pharma.hr (M. Jug).

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are biocompatible molecules and in low concentration they do not cause buccal tissue irritation [13].

Therefore, we prepared a series of solid binary systems of BVP HCl with selected CDs, as a first phase in the development of a novel mucoadhesive formulation aimed for buccal delivery of this drug. Preliminary studies indicated that water soluble β-cyclodextrin-epichlorohydrin polymer (EPI-β-CD) can be a suitable carrier for BVP HCl, since it showed particularly good complexing and solubilizing properties towards the drug [14]. The favourable effect of this β-cyclodextrin polymer on solubility and bioavailability of several drugs has been demonstrated [15], and confirmed also in human volunteers [16]. Moreover, although there are not specific toxicological investigations about the toxicity of this β-cyclodextrin polymer on the buccal mucosa, it is plausible that soluble polymeric cyclodextrins, due to their high molecular mass and high hydrophilicity, are not adsorbed and merely serve as temporary carriers [15,16]. Native β-cyclodextrin (β-CD) was used as a reference.

A careful analytical characterization of drug-CD solid systems is an essential step in the development of an effective pharmaceutical formulation [17,18]. In particular, since the effectiveness of CDs can be strongly affected by the technique used for the complex preparation [19,20], drug-CD binary systems were prepared by various methods (i.e. ball milling in a high-energy mill, co-evaporation and lyophilisation), and characterised by differential scanning calorimetry (DSC), X-ray powder diffractometry (XRPD), and infrared spectroscopy (FTIR), in order to detect the most suitable preparation technique. The influence of the different techniques used for the binary systems preparation on the physicochemical properties of the plain drug was also evaluated. Furthermore, *in vitro* dissolution studies of the best systems were performed using dissolution tests that would allow the simulation of the conditions present in the buccal mucosa, according to guidelines published by Azarmi et al. [21].

2. Materials and methods

2.1. Materials

Bupivacaine hydrochloride (BVP HCl) was kindly donated by S.I.M.S. (Italy). The cyclodextrins included in this study comprised β-cyclodextrin (β-CD; Kleptose 4PC, Roquette, France) and soluble β-cyclodextrin-epichlorohydrin polymer (EPI-β-CD; Cyclolab R&D Ltd, Hungary). All others chemicals and solvents used in this study were of analytical reagent grade.

2.2. Methods

2.2.1. Preparation of solid binary products

Solid binary products of BVP HCl with β-CD or EPI-β-CD were prepared in equimolar drug:cyclodextrin ratio, according to the results of previous phase solubility studies [14] and results published by Dollo et al. [22], using different techniques. Physical mixtures (PM), of BVP HCl with selected cyclodextrins were prepared by gentle mixing of the accurately weighed components using pestle and mortar. Co-evaporated products (COE) were obtained by separately dissolving the drug and the corresponding amount of cyclodextrin in ethanol and water, respectively. The obtained solutions were mixed together and the solvent was removed using a rotary evaporator (Laborota 4000, Heidolph Instruments GmbH, Germany). The ethanol was added with the aim to reduce the amount of the solvent necessary to dissolve the drug and to decrease the boiling point of the solvent, thus facilitating its removal. Ball-milled products (BM) were prepared by co-grinding equimolar drug/cyclodextrin mixtures in a high-energy vibration mill (Retsch, GmbH, Germany) at 24 Hz for different times (30, 45 and 60 min). Each time, the degree of drug residual crystallinity was checked by DSC analysis as described in the following section. Lyophilised products (LYO) were prepared by adding the equimolar amount of the drug into the aqueous solution of each cyclodextrin tested. The sample was stirred until complete dissolution of the drug. The obtained solution was frozen and the solvent was removed using a Lyovac GT2 freeze-dryer (SRK System Technik GmbH, Germany).

To evaluate the effect of the applied techniques for the binary systems preparation on the physicochemical characteristics of the drug, samples of BVP HCl have been treated according to the same procedures, omitting the cyclodextrin from the preparation. All samples were kept in desiccator until further analysis.

2.2.2. Differential scanning calorimetry (DSC)

DSC thermal curves of the solid products were recorded using a Mettler TA 4000 Star^e apparatus equipped with a DSC 25 cell (Mettler Toledo, Switzerland). The instrument was calibrated with indium and zinc prior to analysis of samples under static air atmosphere unless other stated. Accurately weighed samples (2–5 mg, Mettler M3 Microbalance) were placed in sealed aluminium pans with pierced lid and scanned at a heating rate of 10 °C min^{-1} over the temperature range of 30–300 °C. The relative degree of the drug crystallinity (*RDC*) in the samples was calculated according to Eq. (1):

$$RDC = \frac{\Delta H_{sample}}{\Delta H_{drug}} \times 100\% \quad (1)$$

where ΔH_{sample} and ΔH_{drug} are the measured heat of the fusion of the sample and of the crystalline drug, respectively, normalised to the drug content in the sample.

2.2.3. X-ray powder diffractometry (XRPD)

The XRPD spectra of the samples were obtained at ambient temperature with a Bruker D8 apparatus (Θ/Θ geometry) using a Cu Kα radiation and a graphite monochromator. The samples were analysed in the 2.5–30 2Θ range, with a scan rate of 0.03 s^{-1}.

2.2.4. Fourier transformed infrared spectroscopy (FTIR)

The FTIR spectra of all solid products were recorded by PerkinElmer Model 1600 spectrometer (Wellesley, USA). The samples were prepared by the potassium bromide disc method (3 mg sample in 297 mg KBr) and scanned in the range of 4000–400 cm^{-1} at 2 cm^{-1} resolution.

2.2.5. Environmental scanning electron microscopy (ESEM)

The samples were fixed on a brass stub using a double-sided adhesive tape and observed using an environmental scanning electron microscope XL 30 ESEM FEG (Philips, Netherlands).

2.2.6. In vitro dissolution test

To determine the *in vitro* dissolution properties of the solid products in simulated saliva, two different techniques were used. The aim was to mimic the conditions that are present on the surface of the buccal mucosa, according to the guidelines described by Azarmi et al. [21]. Simulated saliva, consisting of 2.38 g Na_2HPO_4, 0.19 g KH_2PO_4, 8.00 g NaCl in 1000 mL of distilled water with pH value adjusted to 6.75 by the use of orthophosphoric acid, was used as dissolution medium [23].

The first method used was the dispersed amount technique modified according to Mohamed and Khedr [24] and it was referred in the text as "MDA technique". The dissolution properties of BVP HCl and its binary systems with β-CD and EPI-β-CD were determined by introducing the solid product equivalent to 30 mg of