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Poly(ethylene oxide)/hydroxypropyl-β-cyclodextrin films for oromucosal delivery of hydrophilic drugs

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ABSTRACT

In this study, we highlight the potential of the mucoadhesive film made from a poly(ethylene oxide)/hydroxypropyl-β-cyclodextrin (PEO/CD) mixture in the oromucosal delivery of hydrophilic drugs, with a specific focus on dexamethasone phosphate disodium salt (Dexa). CD formed a complex with Dexa in solution and did not interact with mucin as highlighted from the spectrophotometric and spectrofluorimetric analysis. Similarly, CD and PEO did not affect mucin conformation, suggesting no direct interaction between the unstirred water layer and film components. Remarkably, PEO/CD/Dexa films dissolved more slowly than those made of PEO alone also in phosphate-buffered saline (PBS) pH 6.8 and gave a time-control on Dexa delivered dose. These combined effects resulted in a higher amount of Dexa accumulated in the mucosa, which can be highly beneficial in case of local diseases. Furthermore, Dexa amount able to diffuse through porcine buccal mucosa was lower when film contained CD, highlighting how CD can act as a modulator of drug transport also in the case of water-soluble drugs. In summary, our results demonstrate the versatility of PEO/CD films in mucosal delivery of hydrophilic corticosteroids paving the way to a novel approach in the treatment of mouth diseases.

1. Introduction

The local treatment of oromucosal diseases, from the most serious *pemphigus* to the common *aphthous stomatitis*, currently relies on the use of mouthwashes, gels, and spray. As main drawback, poor drug availability in the mucosa is attained due to the short residence time of the formulation *in situ* and its fast dilution in saliva followed by swallowing. To overcome these issues, different mucoadhesive drug delivery systems, including tablets, patches and films, have been developed (Hearnden et al., 2012; Paderni et al., 2012). Amid them, mucoadhesive thin films are handy and retentive dosage forms that can be directly attached to the damaged site (Morales and McConville, 2011; Senel et al., 2012). This makes them very attractive for delivery of drugs to localized and mild diffused oral diseases, providing a more accurate dosage compared to other oromucosal formulations, such as gels and sprays (Preis et al., 2013). The efficient and fast absorption of the entrapped molecule in the injured tissue offers the advantage to limit systemic side effects, allowing the rapid resolution of the local pathology at low drug doses. If adequately engineered in term of flexibility and retention time, oral thin films may also protect the underlying disease tissue, thus reducing pain and increasing treatment effectiveness (Dixit and Puthli, 2009; Morales and McConville, 2011). Therefore, high level of patient compliance and comfort are expected.

While intensive research has been devoted to optimize oral thin film technologies to allow drug systemic absorption (Hassan et al., 2010), as in the case of antipain drugs (e.g., Onsolis®, Suboxone®, Breakyl®) or nicotine replacement therapy in tobacco dependence (e.g., NiQuitin®), much less is known on the design rules of bioadhesive films to attain a local effect. To withstand oromucosal delivery of drugs expected to act in the mucosa, the film should exhibit i) prolonged mucoadhesion to the injured site; ii) slow release of the loaded active; iii) drug diffusion in the mucosa; iv) low systemic absorption; v) polymeric platform dissolution upon drug release. Furthermore, discomfort for the patient due to film application should be minimal.

We have recently developed a novel oral thin film technology for oromucosal release of bioactive agents (Quaglia et al., 2012). The technology consists of mucoadhesive, flexible and comfortable

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films based on a mixture of high molecular weight poly(ethylene oxide)s (PEOs) and hydroxypropyl-β-cyclodextrin (CD), the latter acting as a multi-purpose excipient. In fact, CD incorporation in the film is crucial to optimize mechanical properties and drug distribution in the matrix, as well as to slow down drug release and film dissolution rates in simulated buccal fluids. We previously evaluated the usefulness of PEO/CD films as a platform for the oromucosal delivery of sparingly soluble drugs, such as triamcinolone acetonide (Miro et al., 2013). Nevertheless, limited studies can be found in the literature on buccal thin film technology applied to local delivery of hydrophilic drugs. With this idea in mind, here we demonstrate the versatility and potential of PEO/CD films as a polymeric platform for the local treatment of oromucosal diseases with water-soluble drugs. To this purpose, PEO/CD films were loaded with a model water-soluble corticosteroid, that is dexamethasone phosphate disodium salt (Dexa). After a thorough investigation of interactions between Dexa, CD, PEOs and mucin, Dexa/PEO/CD films were produced and fully characterised, paying particular attention to drug content uniformity, time of adhesion to the mucosa and release rate in biologically relevant conditions. Finally, diffusion experiments through the porcine buccal mucosa were performed to evaluate mucosal accumulation of the delivered drug.

2. Materials and methods

2.1. Materials

Dexamethasone 21-phosphate disodium salt (Dexa), type II mucin from porcine stomach, phenolphthalein and rhodamine B (Rhod) were purchased from Sigma Aldrich (Italy). NF grade poly (ethylene oxide)s (Polyox WSR 205, approximate MW 600 kDa; and Polyox WSR 301, approximate MW 4000 kDa) were from Dow Chemical Company (Midland, MI, USA). Polyox WSR 205 and 301 were always employed as a blend at 1:1 w/w ratio and referred in the following as PEOs, while (2-hydroxypropyl)-β-cyclodextrin (CD, ΔS 0.99) was a gift from Roquette Frère (France). All the other chemicals were of analytical reagent grade.

2.2. CD/Dexa interactions

The variation of Dexa UV–vis spectrum in the presence of CD was measured to determine the stability constant of the Dexa/CD complex. To this end, aqueous solutions of Dexa at the concentration of 0.08 mM (corresponding to 0.04 mg/mL) were obtained by diluting aliquots of a Dexa stock solution with water (control) or the appropriate CD aqueous solution to get final concentrations of CD within the range 0.36-14.5 mM (corresponding to 0.5–20 mg/mL). UV–vis absorption spectra of triplicate samples were recorded immediately by a spectrophotometer (model 1800, Shimadzu) fitted out with 1-cm quartz cell (Hellma®). Data were processed by the double reciprocal method according to the Benesi–Hildebrand's equation (Benesi and Hildebrand, 1948):

$$\frac{1}{\Delta ABS} = \frac{1}{K_f \cdot [Dexa] \cdot \Delta\varepsilon \cdot [CD]} + \frac{1}{[Dexa] \cdot \Delta\varepsilon} \quad (1)$$

where ΔABS is the difference in absorbance at 240 nm between the CD-complexed and free drug, Δε is their difference in the molar absorptivities, [Dexa] is Dexa molar concentration and [CD] is the molar concentration of uncomplexed CD (assumed as the total amount of CD added). Data were fitted by linear regression according to Eq. (1) by an Excel software package. Considering the formation of a Dexa/CD complex with a 1:1 stoichiometry, the apparent stability constant K_f was calculated from the intercept/slope ratio.

2.3. Interactions of Dexa, PEO and CD with mucin

The interactions between mucin and thin film components (i.e., Dexa, CD and PEO) was studied by spectrophotometry and spectrofluorimetry. Briefly, a stock aqueous dispersion of mucin (0.2 mg/mL) was obtained under stirring overnight. The dispersion was added with either water (control) or appropriate volumes of aqueous stock solutions of Dexa (100 μg/mL), CD (5 mg/mL) and PEOs (2 mg/mL) in order to achieve samples at a fixed mucin concentration (0.1 mg/mL) containing different amounts of: i) Dexa (5, 10, 25, 50 μg/mL); ii) CD (2.5, 2, 1, 0.5 mg/mL); iii) PEO (1, 0.5 mg/mL); iv) a Dexa/CD/PEO mixture (10 μg/mL, 0.5 mg/mL and 0.5 mg/mL, respectively according to component ratio in the film). UV–vis spectra were recorded in a wavelength range of 200–600 nm to determine the maximum absorption wavelength of mucin (i.e., λ260 nm). Then emission fluorescence spectra were recorded at a fixed excitation wavelength (λex 260 nm) in the range 270–600 nm by a spectrofluorophotometer (model RF-6000, Shimadzu). The fluorescence quenching of mucin in presence of Dexa was evaluated by Stern–Volmer Eq. (2):

$$\frac{F_0}{F} = 1 + K_{sv}[Dexa] \quad (2)$$

where F_0 is the fluorescence intensity of mucin alone and F is the fluorescence intensity of mucin in the presence of increasing concentration of Dexa [Dexa]. K_{sv} is the Stern-Volmer quenching constant, which describes a collisional quenching of fluorescence.

2.4. Dexa quantitative analysis

Dexa was quantified by reverse-phase High-Performance Liquid Chromatography (RP-HPLC). The HPLC system consisted of a LC-10ADvp liquid chromatograph, a SIL-10ADvp auto-injector, a SPD-10Avp UV–vis detector and a C-R6 integrator (Shimadzu, Italy). The HPLC analysis was performed on a Luna 5-μm C18 column (250 × 4.6 mm, 300 Å) (Phenomenex) in isocratic elution mode. The mobile phase was a mixture of acetonitrile and water with TFA 0.1% (v/v) (30:70 v/v). The flow rate was 1 mL/min and the detection wavelength 240 nm. In these conditions, the retention time of Dexa was 8.5 min (total run time 11 min). The linearity of the response was verified over the concentration range 0.25–25 μg/mL ($r^2 \geq 0.999$).

2.5. CD quantitative analysis

CD was quantified by UV analysis of the fading of a phenolphthalein alkaline solution (De Rosa et al., 2005). The phenomenon was due to the formation of the colourless stable phenolphthalein/CD inclusion complex (molar ratio 1:1) and was directly related to the amount of CD added to the solution (Zarzycki and Lamparczyk, 1998). Briefly, a stock phenolphthalein solution in methanol (3 mM) was diluted 1:100 in 0.05 M carbonate buffer at pH 10.5 just prior to use. Into a test tube, 2.6 mL of the phenolphthalein working solution were added to 400 μL of a CD sample and the absorbance of the resulting solution was immediately measured at 553 nm (phenolphthalein λ_{max}) by means of UV–vis spectrophotometry. All measurements were performed in triplicate at room temperature. The linearity of the response was verified over the CD concentration range 0.01–1.00 mg/mL ($r^2 > 0.99$).

2.6. Film preparation and characterization

Films were prepared by a solvent casting procedure as previously described (Miro, d'Angelo et al., 2013). Briefly, 36 mL of PEOs (126 mg) and Dexa (3 mg) aqueous solutions containing or