European Journal of Pharmaceutics and Biopharmaceutics 108 (2016) 83–90

Contents lists available at ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

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

Research paper

Laponite as carrier for controlled *in vitro* delivery of dexamethasone in vitreous humor models

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ARTICLE INFO

Article history:
Received 13 April 2016
Revised 18 August 2016
Accepted in revised form 29 August 2016
Available online 2 September 2016

Keywords:
Dexamethasone
Controlled delivery
Laponite
Corticosteroids
Vitreous humor

ABSTRACT

Laponite clay is able to retain dexamethasone by simple physisorption, presumably accomplished by hydrogen bonding formation and/or complexation with sodium counterions, as shown by solid state NMR. The physisorption can be somehow modulated by changing the solvent in the adsorption process. This simple system is able to deliver dexamethasone in a controlled manner to solutions used as models for vitreous humor. The proven biocompatibility of laponite as well as its transparency in the gel state, together with the simplicity of the preparation method, makes this system suitable for future *in vivo* tests of ophthalmic treatment.

1. Introduction

Among the treatments for posterior segment ocular diseases, intravitreal injection is considered an effective approach, as it delivers the therapeutic agent directly into the eye [1,2]. In spite of some advantages with respect to systemic administration, such as lower side effects, intravitreal injection presents some potential risks associated with the need for repeated injections [3]. In the last years, alternative drug delivery systems are being developed to overcome this kind of limitations by reducing the frequency of injections, and hence the potential complications [4–6]. For example, dexamethasone (DEX) has been released from biodegradable subconjunctival implants of a poly(ortho ester) [7], intravitreal implants of poly(ε-caprolactone) [8], or poly(lactic-co-glycolic acid) [9]. The degradation of the polymeric matrix produces the slow release of DEX, but this methodology requires a surgical intervention to implant the material [8,10].

Very recently an intravitreal injectable implant has been described using DEX encapsulated into a thermogel matrix of poly(lactic acid-co-glycolic acid)–poly(ethylene glycol)–poly(lactic acid-co-glycolic acid) triblock copolymer, able to lengthen the ocular retention time of DEX from several hours to more than one week [11]. In this case the matrix was prepared by copolymerization of lactic acid and glycolic acid, using poly(ethylene glycol) as initiator. Other hydrogels based on poly(ethylene glycol) and poly(valerolactone) [12] or hyaluronic acid [13,14] have been also used for DEX release, but not in ophthalmic applications.

Clay minerals play different roles in health care formulations [15,16], mainly for topical and gastrointestinal diseases, but only recently they have been envisaged as drug delivery modulators [17–19]. Natural clays show a certain degree of variability in their properties, such as chemical composition, particle size, and color. Hence, it is very difficult to envisage their use in intravitreal applications, and only in one case they have been used as components of polyurethane composites for implants releasing dexamethasone acetate upon degradation of the polymer [20]. However, synthetic clays are prepared with reproducible composition and textural properties. As an example, laponite (LAP) is a white synthetic smectite clay, a layered hydrous magnesium silicate with an empirical formula of $Na_{0.7}[(Si_8Mg_{5.5}Li_{0.3})O_{20}(OH)_4]$ that forms transparent colloidal dispersions in water [21]. As sodium cations are exchangeable, this property has been used to immobilize cationic drugs, usually molecules with protonated amino groups, that

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http://dx.doi.org/10.1016/j.ejpb.2016.08.015
0939-6411/

can be released in aqueous saline solutions. This is the case of itraconazole [22] (anti-fungal drug), donepezil [23] (Alzheimer's disease), tetracycline [24] (antimicrobial agent), and doxorubicin [25] (antitumoral). *In vivo* studies with the doxorubicin-laponite system have shown a high antitumor efficacy of the system with very low toxicity [26], whereas the laponite disks can be modified with folic acid for targeted drug delivery [27].

Here, we describe the first report of immobilization of a non-ionic drug, dexamethasone, on laponite clay, the nature of the drug-clay interaction and the *in vitro* behavior of the material in controlled delivery.

2. Materials and methods

2.1. Materials

Dexamethasone (DEX) was from Aldrich. Laponite (LAP) was from Rockwood Additives. Ethanol, acetone and acetonitrile were HPLC grade from Scharlab. CD_2Cl_2, CD_3OD, acetone-d_6 and dmso-d_6 were from Aldrich. Sodium hyaluronate 3% solution (Healon® EndoCoat OVD) was from Abbott Medical Optics. Saline solution (9 mg/mL NaCl) was from Fresenius Kabi España.

2.2. Preparation and characterization of DEX/LAP

The required amount of DEX (20, 10 or 5 mg) was dissolved in ethanol or acetone (10 mL) and LAP (100 mg) was added to this solution. The slurry was stirred at room temperature for 1 h and then the solvent was removed in a rotary evaporator. In that way, the whole amount of DEX was deposited on the laponite support. The solid was dried at room temperature overnight under vacuum over P_2O_5.

DEX contents were assessed (less than 0.5% error) by carbon analysis of the dried powders in a Perkin-Elmer EA-2400 Series II elemental analyzer. Extraction of the samples with acetonitrile for 1 h led to the complete release of DEX to solution, as determined by HPLC (see below). FT-IR spectra (250 scans) of KBr pellets were registered using a Thermo-Nicolet Nexus 5700 spectrometer in the range from 4000 to 600 cm^{-1} at a resolution of 4 cm^{-1}. ^{13}C CP-MAS NMR (cross-polarization magic angle spinning nuclear magnetic resonance) spectra were recorded in a Bruker Avance III WB400 spectrometer with 4 mm zirconia rotors spun at magic angle in N_2 at 10 kHz. Spectra (1000–25,000 scans depending on the sample) were measured using a ^1H $\pi/2$ pulse length of 3 μs, with a contact time (ramp) of 3 ms, spinal 64 proton decoupling sequence of 5.3 μs pulse length and recycle delay of 7 s. Dipolar dephasing (DD) spectra were recorded under the same conditions with a dephasing delay of 50 μs and a refocusing ^{13}C π pulse of 8 μs. Solution NMR spectra were recorded in a Bruker Avance 400 spectrometer. Step scanned (1 s/step) X-ray diffraction patterns were collected in the Servicio de Difracción de Rayos X y Análisis por Fluorescencia del Servicio General de Apoyo a la Investigación de la Universidad de Zaragoza at room temperature from 3° in 2θ up to 35°, using a D-Max 2500 Rigaku system with a rotating anode. CuKα radiation with a wavelength of 1.54 Å at 40 kV and 80 mA was used.

2.3. In vitro DEX release studies

Release was studied in two extraction media, saline solution and a model of vitreous humor made up of 0.5% sodium hyaluronate in saline solution. The release tests were performed by dispersing DEX/LAP (20 mg) in the extraction medium (2 mL of saline or 2 g of the vitreous model) under stirring at 120 rpm at room temperature. After 24 h the dispersion was centrifuged at 14,000 rpm for 20 min. The liquid phase was removed and analyzed, while the solid was re-dispersed in fresh extraction medium for a new cycle.

The analysis of DEX was carried out by HPLC in a Waters 2695 system equipped with a Phenomenex Kinetex C18 column (75 mm × 4.6 mm × 2.6 μm) coupled to a Waters 2995 PDA detector. The mobile phase was acetonitrile/water (35:65 v/v) pumped at a flow rate of 0.2 mL/min at 35 °C. Detection at 250 nm was used. The amount of DEX was calculated by a seven point calibration curve against 6-α-methylprednisolone (internal standard). Aliquots of the extraction medium (200 μL) were mixed with acetonitrile (2 mL) and 100 μL of a solution of standard (20 ppm in acetonitrile). The solution was then vortexed for 1 min and sonicated for 5 min to ensure the complete mixing, and finally centrifuged at 3000 rpm for 5 min. The supernatant was collected and evaporated under vacuum, then redissolved in 200 μL of acetonitrile, filtered through a 0.22 μm PTFE syringe filter and analyzed by HPLC. The presence of laponite in the supernatant was not detected in any case.

3. Results and discussion

3.1. Characterization of adsorbed DEX

In contrast with other drugs immobilized on LAP [22–25], DEX is not cationic and it cannot interact with the solid surface through electrostatic forces. Thus, it was deposited on LAP by suspending the clay in an ethanol solution of the drug, with three different DEX/LAP weight ratios, 1:20, 1:10 and 1:5, labeled as low, medium and high, as the optimal situation would be the use of the minimum amount of support for intravitreal injections. The solids were obtained by simply evaporating the solvent under reduced pressure.

As the immobilization of DEX on LAP cannot be due to electrostatic interactions through the exchange of sodium cations, the distribution of DEX on the surface is not easily predictable. X-ray diffraction was used to determine whether the dexamethasone molecules were able to enter the interlayer space of laponite, leading to an increase in the basal spacing of the clay. As can be seen in Fig. 1A, the powder patterns are poorly oriented, and the 001 line is not clearly defined and it appears as a shoulder. In DEX(high)/LAP, the sharp diffraction lines corresponding to pure orthorhombic DEX (JCPDS database) are observable, indicating the presence of DEX crystallites. This pattern is different from the one registered with the as-purchased DEX (Fig. 1). This difference might be explained by the process of solution and crystallization on the laponite surface used in the preparation of the DEX/LAP samples. Oriented samples of LAP can be obtained by addition of some drops of a solvent, for example acetone. In such case (Fig. 1B), the basal spacing of laponite (14.3 Å) is in good agreement with the layer thickness (9.3 Å) [21] and the interlayer space (5.0 Å) is compatible with the presence of partially hydrated sodium cations (diameter of the fully hydrated cation = 7.2 Å [28]) in the interlayer space. However, the treatment of DEX/LAP with acetone produces the redistribution of DEX, given its solubility in acetone, as shown by the presence of DEX particles even at low content. In that case, a basal spacing of 21.5 Å is observable, which can be attributed to the presence of DEX crystallites in the interlayer space. Although the presence of intercalated DEX molecules between the clay layers is not directly detected, it can be inferred from this result.

The IR spectrum of DEX (Fig. 2A) is mostly overlapped by the bands corresponding to the laponite structure, mainly the broad and intense band due to Si—O—Si bonds (1300–1000 cm^{-1}). The presence of the organic molecule in DEX(high)/LAP is detectable in the 3000–2800 cm^{-1} zone, corresponding to the stretching of