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Research paper

In vivo eye surface residence determination by high-resolution scintigraphy of a novel ion-sensitive hydrogel based on gellan gum and kappa-carrageenan

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ABSTRACT

In last years, sensitive hydrogels have become a breakthrough in ophthalmic pharmaceutical technology aimed at developing new strategies to increase the residence time of active substances. In a previous paper, we qualitatively demonstrated the capacity of a new ion sensitive hydrogel to increase the residence time. Nevertheless, the clearance of the gel from the ocular surface was not quantifiable with the used methodology. The aim of the present work was to use a well-established approach based on scintigraphy to quantitatively estimate the residence time of the previously proposed hydrogel. The rat corneal residence time of a topic ophthalmic formulation containing gellan gum and kappa carragenan (0.82% w/v) labeled with ^{99m}Tc-DTPA radiotracer was evaluated and compared with the residence of an aqueous solution. Ophthalmic safety studies such as eye irritation or passage through the cornea were also carried out. After 1.5 h of contact, 77% of the hydrogel remained in the ocular surface, presenting kinetics of disappearance one-phase decay and a half time of 262 min. We conclude that the novel

Abbreviations: GG, Deacylated Gellan gum; KC, Kappa-Carrageenan; RTCA, xCELLigence Real-Time Cell Analyzer System; CI, cell index; HET-CAM, Hen's Egg Test Chorionallantoic Membrane; SLF, simulated eye tears.

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ophthalmic hydrogel developed with kappa carrageenan and gellan gum remains for long periods of time on the corneal surface, presenting a drop that fits an exponential decay.

1. Introduction

The development of new strategies to increase the residence time of active substances is currently an active field of research in ophthalmic pharmaceutical technology [1,2]. Stimuli-sensitive hydrogels are very different from passive hydrogels because they can detect changes in environmental properties, such as ionic environment [3,4], response through changes in texture, mechanical properties, shape or/and size [5]. As a consequence of the external stimuli, in situ gelling polymers respond undergoing a sol–gel transition that produces the hydrogel formation [6]. Ion sensitive hydrogels were a breakthrough in this field, and different active substances are already being incorporated for improving the treatment of ophthalmic diseases [7,8]. Regarding to this, we have previously shown the feasibility of a prolonged fluconazole release over time [2].

A detailed study of the residence time on the corneal surface of the ophthalmic formulations is valuable and essential for the development of such vehicles [9], with different approaches described in the past few years, such as ex vivo techniques to provide systems that simulate the ocular perfusion clearance [10], *in vitro* methods based on 3D printing [11] and even mathematical models that estimate the effect of the viscosity of formulations on the nasolacrimal drainage [12]. These approaches constitute indirect methods aimed at emulating the effect of such formulations on the ocular surface. Nevertheless, there are also direct in vivo methods that allow estimations of the residence time of ophthalmic formulations. Such methods are based on in vivo imaging techniques such as nuclear magnetic resonance [6], positron emission tomography [13] and scintigraphy. The latter is by far, the most commonly used technique for determining the residence time of different artificial tears [14,15], thermosensitive hydrogels [16] or ion sensitive hydrogels [17].

In a previous paper we used MRI (Magnetic Resonance Imaging) and direct visualization techniques in order to demonstrate the capacity of the ion sensitive hydrogels to increase their ocular residence. However, these techniques did not allow to quantify the clearance of the gel from the ocular surface [6]. Now, we used the well-established approach based on scintigraphy in order to estimate the residence time of the previously described new hydrogel [18]. This approach consists in a scintigraphy study using a planar gamma camera after instillation of a drop of ^{99m}Tc-DTPA on the interior of the eye. Dynamic images are then acquired every few minutes in order to evaluate the drainage [19]. The obtained scintigraphy images offer the possibility of estimating the residence time [20], becoming thus a valuable technique to complement our previous study.

Furthermore, multiple studies of ophthalmic safety, eye irritation, or passage through the cornea were also carried out, which are indispensable when radiopharmaceuticals are used in such a sensitive organ as the eye. In this regard, it should be mentioned that none of the previous studies with scintigraphy analysed such parameters, even though such approach has been widely used for some time [16,21].

2. Material and methods

2.1. In vitro and ex vivo assays

2.1.1. Ion-sensitive hydrogel with radiotracers

The ion-sensitive hydrogel used in this work is based on gellan gum and kappa-carrageenan. It was prepared with an overall polymer concentration of 0.82% (w/v), of which 80% is gellan gum and 20% is kappa-carrageenan. Deacylated Gellan gum (GG) (Kelcogel® CG-LA) and kappa-Carrageenan (CK) (GENUGEL® carrageenan GC-130) were provided by CPKelco®. The concentrations and ratios of the polymer were derived from a previous work [6]. The solutions were prepared by dispersing polysaccharides in warm distilled water (55 °C), stirring for 24 h and subsequently filtered for sterilization in a horizontal laminar flow hood.

In order to perform the radioactive labeling, two radiotracers were incorporated into the hydrogel: Technetium-99m (^{99m}Tc) and ^{99m}Tc pentatate [diethylene triamine pentaacetic acid] (^{99m}Tc-DTPA). Firstly, ^{99m}Tc was prepared in the form of ^{99m}Tc-sodium pertechnetate by elution in sterile saline from a ^{99}Mo/^{99m}Tc generator. An aliquot of the eluate (0.3 ml, 3 GBq) was extracted into an equal volume of n-butanone. The supernatant was transferred to a clean vial and dried under a stream of warm dry air in a laminar flow cabinet. The residue was redissolved in 100 µl DTPA solution, reconstituted with sterile water without electrolites. These radiotracers were then used for radiolabelling the hydrogel. Thereby, 100 µL of each radiotracer were incorporated in 1 mL of each hydrogel. The homogenization was performed magnetic stirrer at room temperature for 30 min.

The uniformity of the radiotracer concentration in the hydrogel was tested by collecting 10 samples of 10 µL from each formulation, and then measuring the radioactive activity in each sample by using a high-precision dose calibrator (Atomlab™ 500, Biodex Medical Systems Inc., New York, US).

The labeling stability of the hydrogel and the loss of radiotracer in presence of simulated eye tears (SLF) were estimated by using Franz diffusion cells with GVS 0.20 µm cellulose acetate membranes (membrane surface area of 0.785 cm^2). SLF were prepared as previously detailed by Ceulemans et al. [22]. It is an electrolyte solution containing 1.7893 g/l KCl, 6.3118 g/l NaCl, 2.1842 g/l $NaHCO_3$, 44.4 mg/l $CaCl_2$ and 47.6 mg/l $MgCl_2$. Physiological pH (7.4 ± 0.1) was adjusted by adding the required amount of 0.1 M HCl. Sink conditions were achieved in the receptor compartment with SLF, the volume of the receptor fluid being 6 mL. During the experiment, the receptor compartment was continuously homogenized using an incubating orbital shaker VWR® at 200 rpm and 33 °C. Serial sampling was performed at 10, 20, 30, 60, 90, 120 and 320 min. Each experiment was repeated three times in order to evaluate the dispersion of the results. The diffusion coefficient of the radiotracers in the hydrogel (D) were estimated by fitting the experimental data to the Fick equation. Next equation, derived from Fick's second law [23], was employed to estimate the radiotracers diffusivity in the hydrogel (D): $\frac{Q_t}{Q_\infty} = 4 \cdot \sqrt{\left(\frac{D \cdot t}{\pi \cdot h^2}\right)}$ where Qt