Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biomaterials 26 (2005) 1293–1298
Biomaterials
www.elsevier.com/locate/biomaterials

Ocular release of timolol from molecularly imprinted soft contact lenses ☆

Haruyuki Hiratani[a,*], Akihito Fujiwara[a], Yuka Tamiya[a], Yuri Mizutani[a], Carmen Alvarez-Lorenzo[b]

[a] *Menicon Co., Ltd. 5-1-10 Takamoridai, Kasugai, Aichi 487-0032, Japan*
[b] *Departamento de Farmacia y Tecnologia Farmaceutica, Facultad de Farmacia, Universidad de Santiago de Compostela, 15782 Santiago de Compostela, Spain*

Received 28 January 2004; accepted 8 April 2004

Available online 28 May 2004

Abstract

The aim of this study was to evaluate "in vivo" the usefulness of molecular imprinting technology to obtain therapeutic soft contact lenses capable of prolonging the permanence of timolol in the precorneal area, compared to conventional contact lenses and eyedrops. Soft contact lenses (diameter 14 mm, center thickness 0.08 mm) consisted of *N*,*N*-diethylacrylamide (DEAA; main component of the matrix), methacrylic acid (MAA; functional monomer) and ethylene glycol dimethacrylate (EGDMA; cross-linker) were prepared by the conventional methodology (non-imprinted) or by applying a molecular imprinting technique using timolol as the template (imprinted ones). After washing and reloading, timolol release studies carried out in rabbits showed that the soft contact lenses made by the molecular imprinting method (34 μg dose) provided measurable timolol concentrations in the tear fluid for 2.0- and 3.0-fold longer than the non-imprinted contact lenses (21 μg dose) and eyedrops (doses of 34 and 125 μg), respectively. Furthermore, the area under the timolol concentration–time curve (AUC) was 3.3- and 8.7-fold greater for imprinted contact lenses than non-imprinted contact lenses and eyedrops, respectively. The timolol concentration of the eyedrops did not affect the precorneal residence time of drug significantly. On the other hand, timolol loading capacity of the contact lenses was improved by the molecular imprinting method; the sustaining of the drug levels in the tear fluid being proportional to the loading capacity of the contact lenses. These results indicate that imprinted soft contact lenses are promising drug devices able to provide greater and more sustained drug concentrations in tear fluid with lower doses than conventional eyedrops.

Keywords: Hydrogel; Molecular imprinting; Timolol; Contact lens; Controlled drug release

1. Introduction

The design and synthesis of biocompatible and comfortable materials useful as basis of sustained drug dosage forms for ocular delivery is still under development, despite the numerous efforts carried out by researchers and clinicians to enhance ocular bioavailability [1,2]. Upon instillation of eyedrops, most of the instilled amount is quickly removed from ocular surface owing to the precorneal elimination factors, such as drainage and tear turnover. In consequence, both poor drug ocular bioavailability and untoward side effects can be observed. To achieve a proper duration of drug contact with the cornea, various types of ophthalmic dosage forms such as viscous solutions, ointments or therapeutic soft contact lenses have been proposed [3–8]. Although soft contact lenses have the excellent comfortable feeling and biocompatibility, the drug-loading capacity of a conventional lens is quite low and, in consequence, it is difficult to achieve a therapeutic concentration in the eye [9,10].

In previous studies, we have demonstrated that soft contact lenses fabricated by the so-called molecular imprinting method have a drug loading capacity 2- to 3-fold greater than that of the contact lenses made by a conventional method [11–13]. Furthermore, the

☆ The work described in this paper is the subject of patent applications filed by Menicon Co.

*Corresponding author. Tel.: +81-568-95-3311; fax: +81-568-95-3317.
E-mail address: h-hiratani@menicon-net.co.jp (H. Hiratani).

0142-9612/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biomaterials.2004.04.030

adsorption affinities for the drug used as template were 9- to 20-fold higher in the case of the imprinted contact lenses, depending on the matrix composition of the contact lenses [13]. These in vitro results indicate that adsorption sites capable of capturing the target drug can be effectively encoded into the polymer network by the molecular imprinting and, in consequence, improve the drug loading capacity of the contact lenses. Additionally, imprinted soft contact lenses were able to sustain timolol release in vitro for several hours [11–13]. The general strategies and experimental details of the molecular imprinting and its potential in the pharmaceutical field have been the subject of recent reviews [14–19].

The aim of this study is to test "in vivo" the efficacy of imprinted systems to sustain drug levels in the tear film. A prolonged delivery in the precorneal area can significantly increase the ocular bioavailability of the drug and therefore reduce the dosage frequency. Furthermore, it can potentially increase the safety of topical therapy by minimizing drainage loss of drug. To carry out the study, timolol was used as model drug and its release profile to rabbits tear film, from presoaked soft contact lenses prepared by molecular imprinting, was compared with that observed with the corresponding non-imprinted contact lenses and with those of two eyedrop solutions; one administered at the same dose as the imprinted contact lens (34 μg) and the other was the clinically used 0.25% timolol eyedrop (125 μg).

2. Materials and methods

2.1. Materials

N,*N*-Diethylacrylamide (DEAA) was purchased from Kojin (Tokyo, Japan). Methacrylic acid (MAA) and ethylene glycol dimethacrylate (EGDMA) were from Tomey (Nagoya, Japan). 2-Hydroxy-2-methyl-1-phenyl-propane-1-one (Darocure) was obtained from Merck (Darmstadt, Germany). These monomers were all distilled prior to use. Timolol maleate salt was purchased from Sigma (St. Louis, MO, USA). Commercially available timolol eyedrop, Brunne® ophthalmic solution 0.25%, was supplied by Menicon Co. Ltd (Nagoya, Japan). The diluted timolol ophthalmic solution, 0.068%, was prepared by dissolving 0.68 mg of the timolol maleate salt in 1 ml of distilled water. All chemicals were of reagent grades.

2.2. Preparation of the timolol imprinted soft contact lenses

The soft contact lenses were prepared by the molecular imprinting method as described previously [12]. Briefly, MAA (50 mM), EGDMA (140 mM), timolol maleate (12.5 mM), and Darocure (0.4 vol%, photoinitiator) were dissolved in DEAA. The monomer solution was injected in polypropylene molds (contact lens shape; 11.7 mm diameter, 0.07 mm center thickness) and free-radical polymerization was initiated with UV irradiation (10 mW/cm^2 at 365 nm) for 20 min at room temperature. The dimension of the molds were decided in order to obtain a soft contact lens having 14 mm diameter and 0.08 mm center thickness in the swollen state. Control (non-imprinted) contact lenses were synthesized simultaneously under exactly the same conditions, though in the absence of timolol. After polymerization, all contact lenses obtained were washed with deionized distilled water and then with 0.9% NaCl solutions to remove the timolol and unreacted monomers. The complete removal of timolol was ensured by analyzing the washing solutions spectrophotometrically.

2.3. Water content of contact lenses

The weight of each type of contact lenses at the dry state (W_d) and after reaching equilibrium in distilled water at $37 \pm 0.5^\circ$C (W_s) was measured in triplicate. The water content, Q, was calculated as follows:

$$Q = (W_s - W_d)/W_s. \quad (1)$$

2.4. Timolol loading capacity and in vitro release behavior

Each contact lens was loaded by immersion in 10 ml of 1 mM timolol solution for 3 days, then rinsed with water, and placed in 10 ml of 0.9% NaCl at 37°C for 13 days, as reported previously [12]. The amount of timolol released at different time intervals was determined spectrophotometrically (UV-3150, Shimazu, Kyoto, Japan). Timolol loading capacities of the imprinted and non-imprinted contact lenses were estimated as the total amount of timolol released from the gels at the end of the experiment. The contact lenses were sterilized by autoclaving (20 min, 121°C, 0.2 MPa) before use in in vivo experiments.

2.5. In vivo release behavior

2.5.1. Animals

Male Nippon albino rabbits weighing 3.3–3.7 kg were housed individually in standard cages in a light-controlled room at $20 \pm 1^\circ$C and $50 \pm 5\%$ relative humidity, with no restriction of food or water. During the experiments the rabbits were placed in restraining boxes: they were allowed to move their heads freely, and their eye movements were not restricted. All experiments conformed to the "Principles of Laboratory Animal Care" (NIH publication #85–23, revised 1985).