International Journal of Pharmaceutics 410 (2011) 23–30

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

The stabilization mechanism of latanoprost

Akiko Ochiai[a,*], Kazumi Danjo[b]

[a] Drug Formulation Department, Central Research Laboratories, Kaken Pharmaceutical Co., Ltd., 301 Gensuke, Fujieda, Shizuoka 426-8646, Japan
[b] Faculty of Pharmacy, Meijo University, 150 Yagotoyama, Tempaku-ku, Nagoya 468-8503, Japan

ARTICLE INFO

Article history:
Received 25 January 2011
Accepted 7 March 2011
Available online 11 March 2011

Keywords:
Latanoprost
Adsorption
Hydrolysis
Stabilization mechanism
Micelle

ABSTRACT

The content of latanoprost is likely to decrease in solution because of the adsorption to eye drop containers and hydrolysis. We reduced these problems and established a formulation of latanoprost eye drops which is stable at room temperature. We assume that the additive surfactants form micelles and stabilize latanoprost in this formulation. In this study, we elucidated the latanoprost stabilization mechanism.

It was revealed by Arrhenius analysis that the adsorption to eye drop containers and hydrolysis of latanoprost were temperature-dependent. In addition, polyethylene glycol monostearates inhibited the adsorption and hydrolysis of latanoprost at 1 mg/mL, which exceeded the critical micelle concentration. By the fluorescent probe method, it was suggested that the surfactants were associated with benzalkonium chloride and formed complex micelles consisting of about 10 molecules, and latanoprost interacted with the micelles at 1:1. By ^{1}H NMR, it was revealed that adsorption was inhibited by arranging the hydrophobic group toward the center of complex micelles and that hydrolysis was inhibited by interaction between the ester group and the complex micelles.

It was shown that the latanoprost is stabilized by the interaction with complex micelles. It was effective for the inhibition of both adsorption and degradation.

1. Introduction

Prostaglandin derivatives are likely to degrade in aqueous solutions and adsorb to containers (Morishima et al., 2002; Sakai and Ohtori, 2005). Temperature-dependent reduction of the latanoprost content, a therapeutic drug for glaucoma, has also been reported (Morgan et al., 2001), but, to our knowledge, there has been no report on its mechanism. In our previous report, we confirmed that the latanoprost content reduction was due to adsorption to the eye drop container and hydrolysis accompanying the production of latanoprost acid (Ochiai et al., 2010). To overcome these problems, we established a latanoprost eye drop formulation which can be stored at room temperature by adding the surfactants polyethylene glycol monostearate 25 (MYS-25) and 40 (polyoxyl 40 stearate, MYS-40). In this formulation, the surfactants used as additives may have formed micelles and stabilized latanoprost. In the present study, we elucidated the mechanism of latanoprost stabilization in solution by these additives. The adsorption and degradation of latanoprost were investigated employing Arrhenius analysis (Yoshioka, 1995), and temperature dependence was confirmed. In addition, we investigated the influences of the concentration and molecular weight (number of added moles) of the 2 surfactants as well as the cause of latanoprost degradation observed when the additives were added at a high concentration in the previous report (Ochiai et al., 2010). Furthermore, the latanoprost stabilization mechanism was elucidated by analyzing the micelle structure and state of interaction between the micelles and the latanoprost employing the fluorescent probe method (Ueno et al., 1988; Wolszczak and Miller, 2002) and nuclear magnetic resonance (NMR) spectroscopy (Ueno et al., 1992; Bernardez, 2008).

2. Materials and methods

2.1. Materials

Latanoprost (Everlight Chemical Industrial Corporation, Taiwan), benzalkonium chloride (BZC, Maruishi Pharmaceutical Co., Ltd., Osaka), MYS-25, MYS-40 (Nihon Surfactant Kogyo K.K., Tokyo), sodium chloride, disodium hydrogen phosphate hydrate, sodium dihydrogen phosphate dihydrate, polyethylene glycol 1540 (PEG1540), pyrene, 1-dodecylpyridinium chloride (DPCI), sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS), and D_2O (Wako Pure Chemical Industries Ltd., Osaka) were used. For the container, an eye drop container made of low-density polyethylene (LDPE) (Taisei Kako Co., Ltd.) and a glass ampule were used.

* Corresponding author. Tel.: +81 054 635 7182; fax: +81 054 635 7264.
E-mail address: ochiai_akiko@kaken.co.jp (A. Ochiai).

0378-5173/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2011.03.006

2.2. Model formulations

Model formulations shown in Table 1 were prepared.

2.3. Latanoprost content

The model formulations were used as sample solutions. The latanoprost contents of the sample solutions were measured by liquid chromatography under the following conditions (number of measurements: 3): The liquid chromatograph used was Alliance (Nihon Waters K.K., Tokyo). For the column, mobile phase, and detector, a reverse-phase silica column (octadecyl silanized silica gel, particle size: 5 μm, inner diameter: 4.6 mm, length: 25 cm), mixture of sodium 1-hexanesulfonate aqueous solution and acetonitrile, and ultraviolet absorption spectrophotometer were used, respectively.

2.4. Latanoprost acid content

Hydrochloric acid was added to the model preparations to prepare sample solutions. Under the analytical conditions of latanoprost content measurement described above, the main degradation product of latanoprost, latanoprost acid, in the sample solutions was measured (number of measurements: 3).

2.5. Adsorption to the eye drop container

Latanoprost adsorbed to the eye drop container was measured referring to the reported method (Wong et al., 2006). The eye drop container (bottle and nozzle) was washed with water and dried, cut into about 2 mm × 2 mm pieces, and extracted with acetonitrile. Using the extract as a sample solution, measurement was performed under the analytical conditions described above (number of measurements: 3).

2.6. Water loss

The mass of the eye drops was measured at the initial and measurement points of the stability test using an electronic balance (XP205, Mettler-Toledo Inc., Tokyo), and the water loss was determined from the change in the mass (number of measurements: 3).

2.7. Free polyethylene glycol content

The model preparations were used as sample solutions. The free polyethylene glycol (PEG) content was measured in the sample solutions using liquid chromatography/mass spectrometry (HP1100, Agilent Technologies, Tokyo/LCQ DECA XP, Thermo Fisher Scientific K.K., Kanagawa) (number of measurements: 3). For the column, mobile phase, ionization method, and detection, a reverse-phase silica column (octadecyl silanized silica gel, particle size: 5 μm, inner diameter: 4.6 mm, length: 25 cm), mixture of water and acetonitrile, electrospray ionization, and positive ion mode were employed, respectively.

2.8. Fluorescence spectrum measurement

Fluorescence spectra of the sample solutions were measured using a fluorometer (F-4500, Hitachi Ltd., Tokyo). The excitation wavelength was 341 nm, and fluorescence spectrum was measured within a range of 350–500 nm (number of measurements: 3).

2.9. ^{1}H NMR spectrum measurement

Using an NMR spectrum measurement device (JNM-A500, JEOL Ltd., Tokyo), ^{1}H NMR spectra of the sample solutions were measured. The conditions were: resonance frequency, 500 MHz; measurement temperature, 23 °C; and number of scans, 10,000. For the standard substance, DSS was used (number of measurements: 1).

2.10. Stability test of model preparations

Formulation No. 1 was stored at 30, 40, 50, and 60 ± 2 °C in a dark place and the stability was evaluated. Each preparation was sampled at 0, 1, 2, and 4 weeks, and the latanoprost and latanoprost acid contents, adsorption to the eye drop container, and water loss were measured. The latanoprost content was presented as the mean rate of remained drug (%) regarding the initial content as 100%. Formulation No. 2–16 and 22–24 were stored at 60 ± 2 °C for 4 weeks in a dark, and the influences of the concentrations and molecular weights of the additives on stability were investigated. In addition, the free PEG content was measured in Formulation No. 2 stored for 4 weeks. For the container, an eye drop container made of LDPE was used, and the fill volume was 2.5 mL.

2.11. Stability test of PEG-added model preparations

Formulation No. 1 and 17–21 were stored at 60 ± 2 °C for 8 weeks in a dark place and the influence of free PEG on stability was investigated. Each preparation was sampled at 0, 2, 4, and 8 weeks, and the latanoprost and latanoprost acid contents were measured. For the container, glass ampules were used, and the fill volume was 2.5 mL.

2.12. Structural analysis of micelles

Sample solutions were prepared by adding 0.1 μg/mL of pyrene to the control formulation and Formulation No. 1 and 2, and the fluorescence spectrum was measured. As an index of micelle formation, the fluorescence intensity ratio of the first to third peak (I_1/I_3) of the fluorescence spectrum of pyrene was determined (Ueno et al., 1988; Wolszczak and Miller, 2002). In addition, 0.1 μg/mL of pyrene was added to Formulation No. 25–33, followed by the addition of 10–50 μmol/L of DPCI as a quencher to prepare sample solutions, and the fluorescence spectrum was measured. The fluorescence intensity ratio of the first peak of pyrene (I) in the fluorescence spectrum to that in the absence of DPCI (I_0) was determined, and the association number was calculated using Eq. (1) (Ueno et al., 1988; Suzuki, 1993):

$$\ln\left(\frac{I_0}{I}\right) = \frac{[Q]\cdot n}{[Ct] - [\text{momoner}]} \quad (1)$$

where n is the association number, [Q] is the quencher (DPCI) concentration (mol/L), [Ct] is the surfactant concentration (mol/L) and [momoner] is the monomer concentration (mol/L).

2.13. Micelle–latanoprost interaction (Ueno et al., 1992; Bernardez, 2008)

Using D_2O as a solvent, sample solutions with the same compositions as those of the control formulation and Formulation No. 1 and 2 were prepared, and the ^{1}H NMR spectrum was measured. Sample solutions with the same compositions as those of Formulation No. 25–33 were also prepared using D_2O and the ^{1}H NMR spectrum was measured (number of scans: 16).