

ALL-TRANS-RETINOIC ACID LOADED OCULAR MICROSPHERES: PREPARATION AND IN-VITRO CHARACTERIZATION

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ABSTRACT

Retinoic acid microspheres were formulated using PLGA (50:50) polymer with solvent evaporation technique. In-vitro characterization of microparticles were determined. Retinoic acid microspheres were prepared by a yield of 42,79%. The average particle size was measured to be 3,291µm. Total drug content in microspheres were determined to be 1,65% and retinoic acid release from PLGA microspheres were continued for 11 days.

INTRODUCTION

Dry eye and keratoconjunctivitis sicca (KCS) are most common disorders of the ocular surfaces connected with reduction or instability of the precorneal tear film. The conjunctival goblet cell regression is one of the important consequence of eye dryness. In the conjunctiva, some epithelial differentiation including goblet cell loss, increased cellular stratification and keratinization occurred as a result of KCS and this is called squamous metaplasia (1). Conventional non-surgical treatments are not directed specifically to reversing the squamous metaplasia. All trans retinoic acid (tretinoin) has been reported to be effective in reversing the epithelial changes in dry eye disorders (2). Different dosage forms of retinoic acid including ophthalmic ointments, emulsions and microparticles have been attempted (3,4). The objective of the present study was to design PLGA microparticles of retinoic acid. The rationale of retinoic acid microparticulate system is suggested some advantages including the formulation of poorly soluble and chemically labil drug (retinoic acid), and improvement

of corneal contact time of the drug.

METHODS

Preparation of PLGA (50:50) Microspheres

Microspheres containing retinoic acid were prepared by solvent evaporation technique. For this purpose, 10 mg retinoic acid and 300 mg PLGA (50:50) were dissolved in 5 ml dichloromethane. The mixture was added dropwise into the aqueous continuous phase containing polyvinylalcohol and sodium oleat (4:1). The medium was stirred firstly by Ultraturrax (T25 basic, IKA, Labortechnik, Germany) at 22.000 rpm for 5 min and then stirred continuously (1500 rpm) at room temperature for 2 hour until the evaporation of dichloromethane was completed. Finally, the resulting microspheres were collected by centrifugation at 15.000 rpm for 15 min; washed with water and dried at room temperature. All procedure was carried out in the dark room.

Drug Loading

10 mg of microspheres were weighed properly and added into 10 ml of ethanol : phosphate buffer solution (pH 7,4) (7:3). They were kept in an ultrasonic bath for 3 min. After the removal of supernatant by centrifugation, the remaining microparticles were dissolved in 5 ml of dichloromethane and this solution was extracted with 10 ml of ethanol : phosphate buffer (pH 7,4) (7:3) for 1 hr in order to transfer retinoic acid from dichloromethane. When the extraction was completed, dichloromethane was evaporated and the resulting solution was filtered through a membrane filter with a

pore size of 0,45 μm and retinoic acid concentration was determined by high pressure liquid chromatography (HPLC). The chromatographic system (HP Agilent 1100–USA) equipped with a C18 column (5 μm , 250x4,6 mm, Phenomenex-USA) was used. The mobile phase was a mixture of methanol : acetonitrile : water : acetic acid (80:10:10:0,5). The flow rate was set to 1 mL/min, the detection was performed at 356 nm. Total drug content was calculated as the sum of retinoic acid amounts both on the surface and inside of the microspheres.

Particle Size Distribution

The particle size distribution of PLGA microspheres containing retinoic acid was measured using laser diffraction particle sizer (Malvern Mastersizer 2000, UK). For measurement procedure the microspheres were suspended in distilled water containing 0,1% Tween 80.

Surface Morphology

A Scanning Electron Microscope (Jeol-SEM ASID-10. Device in 80 KV, Japan) was used to evaluate surface characteristics of microparticles. Microspheres were mounted on the metal stubs with conductive silver paint and then sputted with a 150 Å thick layer of gold in a Bio-Rad apparatus.

In Vitro Release

The in vitro release profiles of PLGA (50:50) microspheres were investigated in ethanol : phosphate buffer solution (pH 7,4) (7:3). Given amount of microspheres were suspended in the release medium at $37 \pm 0,5$ °C in horizontally shaken flasks corresponding to each time point of the experiment. The released amount of retinoic acid was assayed by HPLC.

RESULTS AND DISCUSSION

PLGA microspheres containing retinoic acid were prepared by a yield of 42,79 %. The average particle size was measured to be 3,291 μm . Total drug content in microspheres were determined to be 1,65

%. SEM photographs revealed that microspheres were homogenous and had a spherical surface (Fig.1). PLGA microspheres released retinoic acid during 11 days (Fig.2).

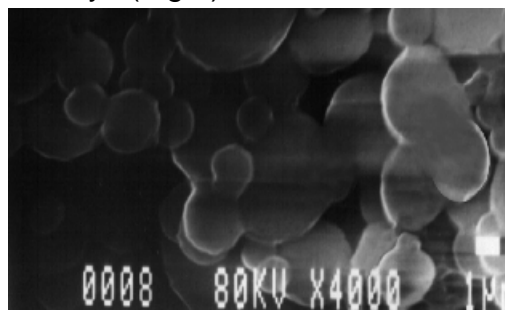


Figure 1. SEM photographs of retinoic acid microspheres

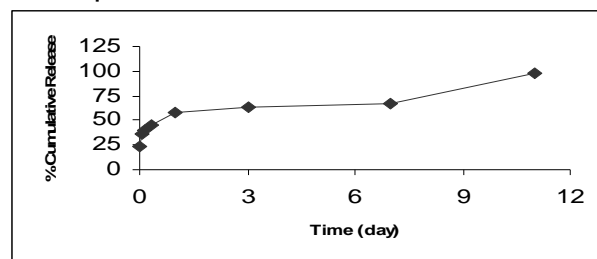


Figure2. Dissolution profiles of retinoic acid microspheres

CONCLUSION

In this preliminary part of the study, in vitro release of retinoic acid from PLGA microparticles appeared to be extended. Furthermore, stability assessment and in vivo evaluation of the formulation will be performed.

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