

Preparation And In-Vitro Characterization of PLGA (50:50) Microparticles Containing All-Trans-Retinoic Acid

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All-trans retinoic acid (atRA) is a vitamin A acid, regulates differentiation and proliferation of epithelial tissues. Antiproliferative effect of atRA for retinal pigment epithelium has been reported (1). It has been proved that atRA is effective to reverse the squamous metaplasia in conjunctiva, caused by dry eye syndrome (2,3). On the other hand, atRA possess clinical potential in the treatment of epithelial and hematological malignancies (4-6). In spite of the effectiveness of atRA in several therapeutic areas, clinical use of the drug is limited because of its side effects. Microparticulate systems may offer several advantages for the formulation of poorly soluble and chemically label drugs such as atRA, and also for reducing the possible toxicity and side effects of the drug. The objective of the present study is to prepare polylactid-co-glycolide (PLGA) microparticles of atRA and to evaluate their in vitro characteristics.

Retinoic acid microparticles were prepared by solvent evaporation technique. In a duplicate procedure, 10 mg retinoic acid and 300 mg PLGA (50:50) were dissolved in 5 ml dichloromethane. Each was added dropwise into the portion aqueous continuous phase containing 1% and 0,5% of polyvinyl alcohol and sodium oleat mixture (4:1). The media were 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 microparticles 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. For drug loading study 10 mg microparticles of each sample 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 2 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, and 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 microparticles. The particle size distribution of PLGA microparticles containing retinoic acid was measured using laser diffraction particle sizer (Malvern Mastersizer 2000, UK). For measurement procedure the microparticles were suspended in distilled water containing 0,1% (w/v) Tween 80. A Scanning Electron Microscope (Jeol-SEM ASID-10. Device in 80 KV, Japan) was used to evaluate surface characteristics of microparticles. The in vitro release profiles of PLGA (50:50) microparticles prepared in two strenghts of PVA and SO were investigated in ethanol : phosphate buffer solution (pH 7,4) (7:3). Given amount of microparticles 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.

PLGA microparticles of retinoic acid were prepared by the yields of 72,58%, and 74,19% for 0,5% and 1% concentration of PVA and SO in the samples, respectively. The average particle sizes were measured to be 1,62 μm and 1,52 μm for 0,5% and 1% PVA+SO containing microparticles, respectively. Total drug content was found to be 51,22% in the microparticles containing 0,5% PVA+SO. In the case of 1% PVA+SO content, total drug amount was 45,02%. SEM photographs revealed that microparticles were homogenous and had a spherical shape. PLGA microparticles released 99,8% of retinoic acid in 12 days.

In vitro release of retinoic acid from PLGA microparticles appeared to be extended when it was compared to conventional eye drops. Further stability assessment and in vivo evaluation of the formulation will be performed in future.

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