Characterization and Evaluation of Triamcinolone, Raloxifene, and Their Dual-Loaded Microspheres as Prospective Local Treatment System in Rheumatic Rat Joints

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ABSTRACT: In this study, injectable microspheres were developed for the local treatment of joint degeneration in rheumatoid arthritis (RA). Microspheres loaded with triamcinolone (TA), a corticosteroid drug, and/or raloxifene (Ral), a cartilage regenerative drug, were prepared with a biodegradable and biocompatible polymer, polycaprolactone (PCL). Microspheres were optimized for particle size, structural properties, drug release, and loading properties. *In vitro* release of Ral was very slow because of the low solubility of the drug and hydrophobic nature of PCL. However, when coloaded with TA, both drugs were released at higher amounts compared with their single forms. Smallest particle sizes were obtained in dual drug-loaded microspheres. *In vitro* cytotoxicity tests showed biocompatibility of microspheres. *In vivo* bioefficacy of these microspheres was also examined in adjuvant-induced arthritis model in rats. *In vivo* histological studies of control groups showed development of RA with high median lesion score (5.0). Compared with control and intra-articular free drug injections, microsphere treatment groups showed lower lesion scores and better healing outcomes in histological evaluations. Results suggest that a controlled delivery system of TA and RAL by a single injection in inflamed joints holds promise for healing and suppressing inflammation. © 2014 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 103:2396–2405, 2014

Keywords: drug delivery; triamcinolone; raloxifene; polycaprolactone; intra-articular applications; microspheres; site specific delivery; controlled release; polymeric biomaterials

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic disease that mainly attacks and causes inflammatory joint damage. As a consequence, RA can severely affect movement and thereby the quality of life of the patients.[1,2] Traditional treatment of the disease requires long-term systemic drug administration, which mostly results with unwanted side effects. Another drawback of systemic treatment is lower allocation of drugs into synovial fluid. Hence, local intra-articular injection of drugs is also carried out.[3] Nevertheless, local injection requires either high single-dose administration to provide the agent for long periods or frequent injections. Currently, triamcinolone (TA) injections are preferred, though their effect can sometimes be short lived, and can also cause rare complications of steroid arthropathy, after injection flare and cutaneous atrophy.[4] At this point, drug delivery systems such as microspheres might be a solution by controlling the amount and period of drug release at the target site.

Here, polycaprolactone (PCL),[5–8] a biocompatible and biodegradable polymer, was used for the preparation of microspheres. TA acetonide was chosen as it is the corticosteroid commonly used in RA treatment owing to its wide spectrum anti-inflammatory and immunosuppressive activity.[2,3,9–13] Raloxifene (Ral) is a selective estrogen receptor modulator drug commonly used in the treatment of osteoporosis. Ral was chosen as it has also been reported to be effective against RA in clinical cases and shown to be efficient in joint regeneration.[14–18] Therefore, delivery systems of these bioactive agents were developed for improved response. Microspheres were optimized for: (1) a suitable size and shape for injection into the joint space, (2) sustained delivery of the drug(s), and (3) provide the required dose of drugs with feasible amount of microsphere. In recent years, there has been an increasing focus on intra-articular drug delivery systems.[3,19–21] All these studies highlight the importance for intra-articular long-term delivery systems in the treatment of joint diseases as given in detail in the review of Butoescu et al.[3] To maintain drug at therapeutic concentrations for extended periods, it is either required to be repeatedly injected into the intra-articular space or administered with an injectable depot formulation.[22]

Abbreviations used: PCL, polycaprolactone; RA, rheumatoid arthritis; Ral, raloxifene; TA, triamcinolone.

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Table 1. Data on Particle Size, Encapsulation Efficiency (EE), Loading, and Diffusion Exponent of Release Results of Microspheres

Microspheres	Cumulative Undersized 10% (μm)	Average Diameter (μm)	Cumulative Undersized 90% (μm)	EE (%)	Loading (%)	*n*
PCL–TA (10:1)	29.97	80.89	164.77	33.87 ± 0.04	3.09 ± 0.03	0.436
PCL–TA (10:2)	26.49	86.72	178.34	107.67 ± 0.75	17.95 ± 0.13	0.470
PCL–TA (10:4)	32.44	100.44	198.71	103.88 ± 0.82	29.68 ± 0.24	0.646
PCL–Ral (10:1)	32.51	88.17	238.96	95.60 ± 1.35	8.69 ± 0.12	0.634
PCL–Ral (10:2)	28.89	95.31	200.54	78.06 ± 6.36	13.01 ± 1.06	0.323
PCL–TA–Ral (10:4:2)	15.91	68.77	151.37	49.76 ± 5.75; TA 47.45 ± 0.92; Ral	14.22 ± 1.64; TA 7.91 ± 0.15; Ral	0.670 0.590

Diffusion exponent (*n*) obtained using Peppas' equation for release outcomes of PCL microspheres.

This study also contributes to the relevant literature for the use of TA and Ral in PCL microspheres either alone or in coloaded form for treatment of RA for the first time. Besides that, this study aims to develop a new treatment approach by dual delivery of an anti-inflammatory (TA) and cartilage regenerative agent (Ral) at the same time.

MATERIALS AND METHODS

Poly(vinyl alcohol) (PVA), gelatin (from porcine skin, type A), PCL (MW = 14 000 Da), TA, Ral, methanol and chloroform, and Alamar Blue were purchased from Sigma–Aldrich Chemical Company (Saint Louis, Missouri). Dimethyl sulfoxide (DMSO) was purchased from Applichem GmbH (Darmstadt, Hesse, Germany). Dulbecco's modified Eagle's medium (DMEM) low glucose with L-glutamine, DMEM high glucose with L-glutamine, and fetal bovine serum (FBS) were purchased from Biochrom AG (Berlin, Germany). Trypsin–Ethylenediaminetetraacetic acid and penicillin/streptomycin were from PAA Laboratories (Pasching, Upper Austria, Austria).

Preparation of Microspheres

Polycaprolactone microspheres were prepared by using modified solid-in-oil-in-water (s/o/w) solvent evaporation technique.[23] Different polymer–drug ratio groups were used (Table 1). PVA (2%, w/v) and gelatin (1%, w/v) were used as the aqueous phase (in a total volume of 200 mL). For the oil phase (o), the polymer (PCL) solution in chloroform (200 mg in 5 mL) was prepared. Then, drug(s) (TA, Ral, or both) were added in solid form into the polymer solution at different amounts (80 or 40 mg drug was added for PCL–drug ratios of 10:4 and 10:2, respectively) and mixed thoroughly. This mixture was added drop-wise to the aqueous phase while stirring continuously at 1000 rpm. Stirring was continued for about 12 h for the complete evaporation of the organic solvent. Microspheres were collected by centrifugation at 3461 g (Hettich, Tuttlingen, Germany) and then washed with distilled water three times.

Determination of Drug Encapsulation Efficiency and Loading

The amount of TA in the microspheres was determined by dissolving them in chloroform and obtaining optical densities at 255 nm with a UV spectrophotometer (Hitachi U-2800A, Chiyoda, Tokyo, Japan). For the analysis of Ral, microspheres were dissolved in chloroform, and DMSO was then added. Ral concentrations were quantified by optical density measurements at 300 nm. In the dual drug-loaded microspheres, drug amount detections were performed in two stages because of differences in the solubility of the drugs. In the first stage, microspheres were dissolved in chloroform and optical density values were measured at 255 nm for TA quantification. In the second stage, DMSO was added to measure the second drug, Ral, at 300 nm. Drug measurement methods were validated for noninterference of the polymer and the other drug by using known concentrations of polymer/other drug in extraction studies.

Encapsulation efficiency (EE) was calculated as the percentage of the ratio of drug amount in microspheres to the input drug used Eq. (1).

$$\mathrm{EE}(\%) = (\text{Experimental drug loading}/\text{Theoretical drug loading}) \times 100 \quad (1)$$

Loading was calculated as the percentage of the ratio of drug amount of microspheres to the amount of microspheres used Eq. (2).

$$\text{Loading}(\%) = (\text{Amount of drug in microspheres}/\text{Amount of microspheres}) \times 100 \quad (2)$$

Release Studies

Microspheres were placed in tubes (5 mg microsphere/tube) containing phosphate-buffered saline (PBS; 10 mM, pH 7.4, 5 mL). Tubes were placed in a shaking water bath set at 37°C and 50 rpm. The tubes were centrifuged at 3461 g for 12 min to settle the microspheres after the first 4 h of release, and 1 mL samples from release media were taken. Fresh PBS (1 mL) was added to release media and tubes were placed into water bath again. In the following release periods, samples were taken from release media daily (up to 28 days) and the total media were refreshed. Release studies were carried out in quadruplets. Drug quantifications were carried out with high-performance liquid chromatography (HPLC) system (UV/Vis Detector SPD 20-A, Degasser DGU-20A_3, Liquid chromatography LC-20AT, Auto-injector SIL-10AD VP, Column oven CTO-10AS VP; Shimadzu, Kyoto, Kansai, Japan). Briefly, a C18 column (Luna-Phenomenex, Torrance, California) together with Phenomenex HPLC guard cartridge system was used with the mobile phase MeOH–dH_2O (85:15, v/v) at a flow rate of