International Journal of Pharmaceutics 441 (2013) 285–290

Contents lists available at SciVerse ScienceDirect

International Journal of Pharmaceutics

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

Intra-articular delivery of liposomal celecoxib–hyaluronate combination for the treatment of osteoarthritis in rabbit model

Ji Dong[a,1], Dinghua Jiang[b,1], Zhen Wang[b], Guizhong Wu[b], Liyan Miao[a,*], Lixin Huang[b,**]

[a] Department of Clinical Pharmacology Research Lab, the First Affiliated Hospital of Soochow University, 188 Shi Zhi Street, Suzhou 215006, China
[b] Department of Orthopaedic, the First Affiliated Hospital of Soochow University, 188 Shi Zhi Street, Suzhou 215006, China

ARTICLE INFO

Article history:
Received 20 July 2012
Received in revised form 26 October 2012
Accepted 18 November 2012
Available online 27 November 2012

Keywords:
Intra-articular injection
Liposome
Celecoxib
Hyaluronate
Combination
Osteoarthritis

ABSTRACT

Oral administration of celecoxib (Clx), which is the traditional treatment for osteoarthritis (OA), is accompanied by a high risk for cardiovascular events, while intra-articular injection of hyaluronate (HA) is a well-documented treatment for knee OA. To improve OA therapy while reducing the adverse effects, we formulate Clx-loaded liposomes embedded in HA gel, then administer the liposomal Clx–HA combination via intra-articular injection. Clx-loaded liposomes showed high efficiency encapsulation (>99%). In vitro release studies demonstrated that the release of Clx from lipsosomes was delayed by the combination of HA with liposomes. We examined the effect of intra-articular injection of liposomal Clx–HA combination on cartilage degeneration in rabbit knee OA model. The rabbits were treated with a single intra-articular injection of a single drug, either Clx liposome or HA, or liposomal Clx–HA combination. Using an incapacitance tester and the histopathological study, it was verified that the liposomal Clx–HA combination was more effective than a single drug in pain control and cartilage protection.

1. Introduction

Osteoarthritis (OA) is a most common disease in old people, and is characterized by destruction of articular cartilage, osteophyte formation, subchondral bone sclerosis, and secondary synovitis (Buckland-Wright, 2004; Haywood et al., 2003). Current available therapy is symptomatic treatment, directed to relieve the pain and regain function. For example: oral administration of celecoxib (Clx) relieves pain and inflammation (Clemett and Goa, 2000); intra-articular injection of hyaluronate (HA) lessen mechanical damage (Moreland, 2003; Sun et al., 2006).

Clx is used as a first line drug for the treatment of OA. Clx is a COX-2 inhibitor that exhibits analgesic and anti-inflammatory activities. The mechanism of its action is the inhibition of prostaglandin synthesis by COX-2 without inhibiting COX-1 (Clemett and Goa, 2000). HA, a high molecular weight polymer of glucosamine and glucuronic acid residues, is a principal constituent of the normal synovial fluid and contributes significantly to its rheological properties and joint homeostasis (Moreland, 2003). Intra-articular injection of high molecular weight HA is now widely used in the treatment of OA. The main mechanism by which HA prevents cartilage destruction and slows OA disease progression is considered to be its action in maintaining the viscoelastic property of synovial fluid, because this is reduced by inflammation in OA patients (Moreland, 2003; Sun et al., 2006). Due to different mechanisms without mutual interference, the concomitant use of Clx and HA is frequently prescribed for OA patient.

Although Clx is proved to induce fewer gastrointestinal toxicities compared to traditional NSAIDs, it has been reported to raise a high risk for cardiovascular events that are associated with chronic use and higher doses (Solomon et al., 2005; Wong et al., 2005). The approach we propose, to improve OA therapy while reducing the adverse effects is: (i) to formulate Clx-loaded liposomes embedded in HA gel (ii) to administer the liposomal Clx–HA combination locally, via intra-articular injection to the inflamed joint. This local route maintains the conventional treatment by HA and switches the conventional route of Clx from oral to local injection.

Derived from naturally occurring, biodegradable, and nontoxic lipids, liposomes are good candidates for local targeting of therapeutic agents to the site of interest, while reducing the systemic exposure. Liposomes have been shown to be very efficient in the local treatment of joint diseases in laboratory animals and also in humans. For example, when administered in human patients, hydrocortisone-entrapping liposomes remarkably improved subjective and objective indices of inflammation at 48 h, with a slow return to the pre-injected state after 2 weeks (de Silva et al., 1979).

In the present study, we have taken a new direction that was to pursue not only each drug alone, but the potential of the combination of drugs. Clx and HA are both efficient in the treatment of

* Corresponding author.
** Corresponding author. Tel.: +86 512 67780040; fax: +86 512 67780040.
E-mail addresses: miaolysuzhou@163.com (L. Miao), szhuanglx@yeah.net (L. Huang).
[1] These authors contributed equally to this work.

0378-5173/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.ijpharm.2012.11.031

OA, but in two different mechanisms, expected to have synergistic effect. The present investigation was aimed to evaluate whether treatment with the drug combination was better than a single drug using the rabbit OA model.

2. Materials and methods

2.1. Materials

Celecoxib (Clx) (99.65%) was purchased from Excel Pharmaceutical Co. (Zhejiang China). S100 Soybean phosphatidylcholine (SPC) was purchased from Lipoid (Ludwigshafen, Germany). Cholesterol (CHOL) was purchased from Huixing Biochemistry Reagent Co. (Shanghai China). Chloroform was obtained from Damao chemical reagent factory (Tianjin China). Hyaluronate (HA) 2.5 MDa was obtained from Mengjie biochemistry Co. (Shanghai China). Penicillin injection was produced by North China Pharmaceutical Company (Hebei China). Chloral hydrate was obtained from Kunshan Niansha Auxiliary Agent Co. (Jiangsu China). Tween 80 was purchased from JingKe HongDa Biotechnology Co. (Beijing China).

2.2. Preparation of liposomes and liposomal Clx–HA combination

Liposomes containing Clx were prepared by film technique (Bangham et al., 1965). Clx (5 mg), SPC (100 mg), CHOL (20 mg) were dissolved in 5 ml chloroform in a round-bottom flask. The chloroform was removed using a R201 rotary evaporator (Shensheng, China) under reduced pressure to form a thin film over the wall of the flask. The dried film was then hydrated with sterile normal saline followed by vigorous vortex until the entire film was suspended.

As a vehicle for liposomal Clx–HA combination, HA at the concentration of 20 mg/ml in distilled water was made. HA (0.2 g) was dispersed in 10 ml sterile distilled water by heating at 37 °C for 24 h. For preparation of liposome gel, HA gel and liposome suspension were mixed 1:1 ratio on volume basis by a vortex mixer.

All the containers and materials were sterile and no pyrogen and the preparation procedures were completed in an bioclean environment.

2.3. Characterization of liposomes

2.3.1. Particle size measurement

The average diameter of the liposomes was determined by photo correlation spectroscopy (PCS), using a Zetamaster (Malvern Instruments, Malvern, UK) at a temperature of 25 ± 0.1 °C. The intensity of the laser light scattered by the samples was detected at an angle of 90° with a photomultiplier. Liposome suspensions were suitably diluted with distilled water and vortexed before the size was measured. At least three independent samples were taken, each of which was measured three times. From this analysis, the *z*-average value was obtained, which was an approximation of the diameter of the liposomes. Samples were analyzed 24 h after their preparation.

2.3.2. Scanning electron micrographic studies

Images were recorded on a Hitachi S-4700 scanning electron micrograph (magnification: 15,000; accelerating voltage: 15.0 kV). The liposomes dispersion was diluted appropriately. Few drops of the dispersion was placed on the grid and allowed to dry. After the samples dried thoroughly, the image was captured.

2.3.3. Determination of drug encapsulation efficiency

Entrapment efficiency was determined using ultrafiltration separation as per reported method (Patlolla et al., 2010). In brief, Clx liposome (0.5 ml) formulation was transferred to the upper chamber of Nanosep® centrifuge tubes fitted with an ultrafilter (molecular weight cut-off 10 kDa, Pall Life Sciences) and centrifuged at 7000 rpm for 30 min. The aqueous phase collected at the bottom of ultrafilter membrane was subjected to high-performance liquid chromatography (HPLC) analysis to determine the Clx content.

The entrapment efficiency was calculated by the following equation:

$$\text{Percentage entrapment efficiency} = \frac{W_{\text{total}} - W_{\text{free}}}{W_{\text{total}}} \times 100$$

where "W_{total}" is the mass of the total amount of drug present in the system and the "W_{free}" is the mass of the free drug detected in the filtrate of lower chamber of Nanosep® post-centrifugation of the aqueous dispersion.

In order to verify the total amount of drug present in the system, Clx liposome formulation was diluted suitably with methanol to destroy the liposome structure, releasing the drug into the solution and then final clear solutions were analyzed for drug content by HPLC.

2.4. HPLC analysis of Clx

The HPLC system comprised of 717 plus auto sampler, 1525 binary pump, and 2487 Waters dual λ absorbance detector (Waters, USA). The mobile phase consisting of acetonitrile, water, and acetic acid (65:35:0.1% v/v) was pumped through the Kromasil C18 column (5 μm, 4.6 mm × 250 mm) at a flow rate of 1.0 ml/min and the eluent was monitored at 250 nm.

2.5. In vitro drug release studies

In vitro drug release studies were conducted with dialysis membrane bags (Molecular weight cut off: 10,000, Hercules, USA) using a RC2-8B dissolution apparatus (Tianjing, China) for 72 h with the help of 500 ml of phosphate buffer saline (PBS) (pH 7.4) containing 1% (v/v) Tween-80 as the dissolution medium. Cellulose membranes were soaked overnight in the dissolution medium. To the pre-swollen cellulose membrane bags, 1 ml of the liposome formulation or liposomal Clx–HA combination was placed and both the ends of bags were tied to prevent any leakage. Later, dialysis bags were gently inserted at the bottom of the dissolution vessel and the paddles were rotated at 100 rpm for 72 h at 37.0 ± 0.1 °C. At regular time intervals 1 ml of the sample was collected and replaced with an equal volume of the dissolution medium. The amount of Clx released in to the medium was determined with the help of HPLC analysis. The experiments were carried out in triplicate.

2.6. Animal experiments

2.6.1. Induction of OA

All protocols involving the use of animals were approved by the University of Soochow Animal Care and Use Committee. New Zealand rabbits weighing 2.5–3 kg were provided by the Experimental Animal Center of Soochow University. The experimental OA model was prepared according to the Hulth modeling way (Hulth et al., 1970). Under anesthesia with 10% chloral hydrate (4 ml/kg body weight), the rabbit's right knee joint was incised aseptically about 2 cm down the medial aspect of the patella to expose and cut anterior and posterior cruciate ligament and medial collateral ligament, and medial meniscus was resected. Postoperatively, all the animals were permitted free cage activity and 800 000 U penicillin was injected intramuscularly into the thigh, once per day, for one week after surgery.