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Ibuprofen-loaded porous microspheres suppressed the progression of monosodium iodoacetate-induced osteoarthritis in a rat model

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ABSTRACT

The objectives of this study were (1) to fabricate ibuprofen-loaded porous microspheres (IBU/PMSs), (2) to evaluate the *in vitro* anti-inflammatory effects of the microspheres using LPS-induced inflammation in cultured synoviocytes, and (3) to evaluate the *in vivo* effect of the IBU/PMSs on the progression of monosodium iodoacetate (MIA)-induced osteoarthritis (OA) in a rat model. A dose-dependent *in vitro* anti-inflammatory effect on pro-inflammatory cytokine markers (matrix metallopeptidase-3 (MMP-3), matrix metallopeptidase-13 (MMP-13), cyclooxygenase-2 (COX-2), a disintegrin and metalloproteinase with thrombospondin motifs-5 (ADAMTS-5)), interleukin-6 (IL-6), and tumor necrosis factor (TNF-α) was observed by confirming with real-time PCR analyses. *In vivo*, treatment with IBU/PMSs reduced MIA-stimulated mRNA expression of MMP-3, MMP-13, COX-2, ADAMTS-5, IL-6, and TNF-α in rat synoviocytes. In addition, we demonstrated that intra-articular IBU/PMSs suppressed the progression of MIA-induced OA in the rat model *via* anti-inflammatory mechanisms. In conclusion, IBU/PMSs are a promising therapeutic material to control the pain and progression of OA.

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1. Introduction

Osteoarthritis (OA) is a well-known degenerative joint disease involving articular cartilage and subchondral bone and commonly occurs in middle-aged and elderly adults [1]. OA causes pathophysiologic changes in tissues that induce pain and stiffness of joints, preclude daily living and social activities, and ultimately cause long-term disability [1]. Common treatments for OA include anti-inflammatory agents such as non-steroidal anti-inflammatory drugs (NSAIDs), analgesics, hyaluronic acid (HA) injections, or corticosteroids injections. Either administered orally or intra-articularly, these drugs are known to alleviate the symptoms and prevent the progression of OA [2–7]. At present, oral administration of NSAIDs or analgesics is the most common OA treatment. Despite their popular use, these treatments may cause serious gastrointestinal side effects including bleeding, ulceration, and perforation of the stomach, small intestine, or large intestine [8,9]. Intra-articular administration of NSAIDs or analgesics is useful in the treatment of joint inflammation and pain and can minimize systemic adverse effects of the drug. Indeed, intra-articular administration is directed to the site of action and can circumvent the assault on the gastrointestinal tract. However, the biggest disadvantage of intra-articular administration of the drug is its short duration of action in the joint cavity [3,10]. Currently, HA and corticosteroids are the most commonly used intra-articular drugs for treatment of OA. When used in the knee, intra-articular HA injection serves diverse roles including shock absorption, joint lubrication, anti-inflammatory effects, chondroprotection, proteoglycan synthesis, and cartilage matrix alterations [11]. However, the effects of intra-articular HA injections are short-lived and multiple injections are required, potentially causing patients pain and discomfort [12]. Intra-articular corticosteroid injections may reduce patients' symptoms up to three weeks after the injection

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[13], but these can suppress of regeneration of articular cartilage [10,14].

Oral ibuprofen (IBU), an NSAID, is broadly used for treating OA and rheumatoid arthritis (RA) due to relatively low risk of adverse gastrointestinal effects and renal effects at anti-inflammatory doses [15,16]. Although oral IBU is effective at ameliorating symptoms of OA and RA, it has a short half-life (1–5 h) in the joint cavity [17]. The development of intra-articular drug delivery system (DDS) with IBU may enable maintenance of an effective concentration of the drug in the joint cavity for a prolonged period while not causing adverse events. Thus, the developed system would have potential to alleviate symptoms and restore patient activity levels.

Recently, Baruch et al. demonstrated that IBU-loaded poly (lactic-co-glycolic acid, PLGA) microspheres co-entrapped with cells led to prolonged release of IBU [16]. Moreover, Bédouet et al. reported that PEG-hydrogel microspheres (MS) conjugated to ibuprofen inhibited the synthesis of prostaglandin E2 in articular cartilage [18]. However, the anti-inflammatory effect of an intra-articular IBU injection has not yet been demonstrated in an animal model of OA.

Thus, to evaluate the potential of this treatment in an OA-induced rat model, we designed a system of IBU-loaded porous PLGA microspheres (IBU/PMSs) that is administered *via* intra-articular injection. First, we fabricated IBU/PMSs using a fluidic device method. We then evaluated the anti-inflammatory effects of the IBU/PMSs *in vitro* in cultured synoviocytes by assessing their effects on pro-inflammatory cytokine marker gene expression including matrix metallopeptidase-3 (MMP-3), matrix metallopeptidase-13 (MMP-13), cyclooxygenase-2 (COX-2), and a disintegrin and metalloproteinase with thrombospondin motifs-5 (ADAMTS-5). Last, we assessed the *in vivo* efficacy of IBU/PMSs in a rat model with monosodium iodoacetate (MIA)-induced OA.

2. Materials and methods

2.1. Materials

Poly(D,L-lactide-co-glycolide) (PLGA, 50:50, molecular weight: 30,000–60,000), poly(vinyl alcohol) (PVA, molecular weight: 13,000–23,000, 98% hydrolyzed), dichloromethane, gelatin from porcine skin, and monosodium iodoacetate (MIA) were supplied from Sigma-Aldrich (St. Louis, MO, USA). Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), phosphate-buffered saline (PBS), and penicillin-streptomycin were purchased from Gibco BRL (Rockville, MD, USA). Ibuprofen was kindly obtained from Samjin Pharmaceutical Corporation (Seoul, Korea).

2.2. Fabrication of ibuprofen (IBU)-loaded porous microspheres

A fluidic device with discontinuous and continuous phases was used to fabricate IBU-loaded porous microspheres [19]. In brief, PLGA (2 wt%) was dissolved in dichloromethane (DCM) and then ibuprofen (1, 3, or 5 wt%) was added at the amount of PLGA (2 wt%). Subsequently, gelatin (3 wt%) and PVA (0.5 wt%) were added. The resulting solution was emulsified with a homogenizer (Ultra-Turrax T-25 Basic, IKA) at 13,500 rpm for 1 min. The homogenized solution was used as the discontinuous phase, and the PVA solution was used as continuous phase. The flow rate of both the discontinuous and the continuous phase was 0.5 mL/min. Microspheres were collected and immersed in warm water at 45 °C under gentle stirring for 4 h to remove residual gelatin and then were rinsed three times with distilled water. The microspheres were lyophilized for 3 days to generate the IBU-loaded porous PLGA microspheres (IBU/PMSs). In order to generate a vehicle control, porous microspheres without IBU were also fabricated by the same procedure.

2.3. Morphologies and pore sizes of porous microspheres with and without IBU

The morphologies of PMSs, IBU (1%)/PMSs, IBU (3%)/PMSs, and IBU (5%)/PMSs were determined using scanning electron microscopy (SEM, S-2300, Hitachi, Tokyo, Japan). The specimens were coated with gold using a sputter-coater (Eiko IB, Japan) and the SEM was set at 3 kV. The pore sizes of the microspheres were determined using the ImageJ program (ver 1.2). To determine the optimal loading IBU, 30 mg of IBU (1%)/PMSs, IBU (3%)/PMSs, or IBU (5%)/PMSs was dissolved in DCM. Phosphate buffered saline (PBS) was added to the aforementioned solutions and the resulting mixtures were gently vortexed for 3 h. The supernatant was used to calculate the optimal loading amount of IBU. The loading amount of IBU was determined by a Flash Multimode Reader (Varioskan™, Thermo Scientific, USA) at 359 nm. Drug loading and loading efficiency were determined using Eqs. (2) and (3), respectively.

$$\text{Drug loading} = \frac{\text{weight of ibuprofen in microspheres}}{\text{microspheres sample weight}} \times 100 \quad (2)$$

$$\text{Loading efficiency} = \frac{(\text{actual weight of ibuprofen in sample})}{(\text{theoretical weight of ibuprofen})} \times 100 \quad (3)$$

2.4. In vitro *release of ibuprofen*

Approximately 30 mg of IBU (1%)/PMSs, IBU (3%)/PMSs, or IBU (5%)/PMSs was suspended in 1 mL of PBS (pH 7.4) in a 2-mL e-tube at 37 °C under continuous agitation (100 rpm). At predetermined times, PBS was drawn off completely and same volume of fresh PBS was added. The release of IBU was assessed by quantifying the concentration of IBU in the collected PBS using a UV/Vis spectrophotometer at 359 nm.

2.5. Isolation and cultivation of synoviocytes

Synoviocytes were primarily isolated from synovial membranes in rat. In brief, rat synovial membranes were isolated and rinsed with PBS. The membranes were minced with scissors and a scalpel into pieces <3 mm and suspended in an equal volume of 0.06% (wt/v) collagenase type I (Sigma, St. Louis, MO, USA) overnight at 37 °C with gentle shaking. The tissues were mixed with an equal volume of Dulbecco's Modified Eagle's Medium (DMEM, Gibco, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (FBS) and 1% antibiotics (100 U/mL penicillin and 0.1 mg/mL streptomycin), followed by centrifugation at 700 rpm for 3 min at room temperature (RT). The cells were seeded in 100-mm tissue culture dishes and allowed to grow in a humidified incubator with 5% CO_2 at 37 °C.

2.6. Cytotoxicity test

The cytotoxicity test of PMSs, IBU (1%)/PMSs, IBU (3%)/PMSs, and IBU (5%)/PMSs was evaluated in rat synoviocytes. The synoviocytes were seeded at 1×10^5 cells/mL with 30 mg each of PMSs, IBU (1%)/PMSs, IBU (3%)/PMSs, or IBU (5%)/PMSs and maintained with DMEM supplemented with 10% FBS and 1% antibiotics (penicillin 100 U/mM, streptomycin 0.1 mg/mL). The cells were then incubated for 3 days. At predetermined time intervals of 1 and 3 days, the medium was aspirated and CCK-8 proliferation kit (Dojindo Molecular Technologies Inc, Tokyo, Japan) reagents were added. Accordingly to the manufacturer's instructions, the synoviocyte cultures were further incubated for 1 h at 37 °C. The optical density of the live cells was measured using a microplate reader (Bio-Rad, Hercules, CA, USA) at 450 nm.

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