SCIO-469 loaded Microparticles for Osteoarthritis treatment: fluorescence imaging and drug delivery

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ABSTRACT SUMMARY

SCIO-469 loaded microparticles offer a new imaging capability of controlled release technology for the treatment of osteoarthritis (OA). The intrinsic fluorescence of the drug substance allows localization by imaging and it shows and showing a high potential to treat OA.

INTRODUCTION

OA is the most common type of arthritis or degenerative joint disease. It is a leading cause of chronic disability which affects progressively cartilage, synovial membrane, subchondral bone and periarticular tissues. Pain appears during or after articular activity and fades with rest. The various hyaluronic acid (HA) and glucocorticoid formulations, which are currently commercially available for IA treatment, often do not provide adequate and prolonged pain relief. Because of the localized nature of the disease, generally affecting one or two joints, intra-articular (IA) drug injection is an attractive treatment approach.

Figure 1. Chemical structure of SCIO 469

In the present study we report a formulation strategy to develop IA injection of SCIO-469 (Fig. 1), a selective ATP-competitive p38 mitogen-activated protein kinase (MAPK) inhibitor (IC50 = 9 nM for p38a in vitro) [1]. Signaling through p38α MAPK reduces inflammatory effects which play an important role in OA and in rheumatoid arthritis (RA). The drug has reached Phase II trials using oral administration of SCIO-469 in patients using active RA ([2]-NCT00043732). For effective OA treatment, SCIO-469 requires a microparticle delivery system with week-to-month controlled release.

EXPERIMENTAL METHODS

Microparticles were prepared by a double emulsion-solvent extraction method. Briefly, 1 mL of organic phase containing 300 mg mL⁻¹ Poly(D,L-lactide-co-glycolide) (PLGA 75:25, RG-752S, inherent viscosity 0.16 - 0.24 dL·g⁻¹) and 10 mg mL⁻¹ SCIO-469 in ethyl acetate added with 10 % ethanol (w/v) as a co-solvent was emulsified with 0.15 mL of purified water by means of a sonicator for 10 s using VibraCell™ VC 50T. This primary emulsion was emulsified with 2 mL of 2 % PVAL solution using a T25 Ultra-Turrax for 1 min at 5448 RCF. This final emulsion was then poured into 20 mL of water and mechanically stirred for 4 h at room temperature. The resulting microparticles were separated at 276 RCF using a Jouan BR 3.11 centrifuge (IG), washed 3 times with MilliQ water, freeze-dried and finally stored at 4 °C.

Size measurements were performed using laser-light diffraction (Mastersizer) after dispersing the particles in water and the size was expressed as D[4;3]. SCIO-469 content of the microparticles was assessed by HPLC, using a 100-5 C18 Nucleosil column (125/4.6, Macherey Nagel), an acetonitrile, 0.1 % (v/v) trifluoroacetic acid (TFA):water,0.1 % (v/v) TFA 1:1 mobile phase at flow rate of 1 mL min⁻¹ (detection at 254 nm) after solubilization of the particles in acetonitrile. The suspensions were centrifuged at 276 RCF in order to separate the PLGA from the drug substance-containing solution.

Scanning electron microscopy (SEM) was performed on gold-coated freeze-dried samples.
with a JEOL JSM-6400 microscope at an accelerating voltage of 3 kV in order to determine the surface characteristics of the particles.

The distribution of the SCIO-469 fluorescent within the microparticles was investigated using a confocal scanning light microscopy (CLSM) Zeiss LSM700 confocal microscope. An excitation wavelength of 405 nm, close to the maximum absorption of the drug substance SCIO-469, was selected and confocal images were taken with a 63 × Oil immersion objective. The green fluorescence channel was used as an emission filter. CLSM data acquisition was analyzed by Image J software to obtain 2D and 3D micrographs.

RESULTS AND DISCUSSION

Due to the higher viscosity of the 19 kDa RG-752S polymer organic solution, particles obtained were micron-sized. The SCIO-469 microparticles were homogeneous with narrow and monomodal distributions (Table 1). SCIO-469 is water-soluble and part of the active substance was eliminated throughout the solvent evaporation.

<table>
<thead>
<tr>
<th>SCIO-469 microparticle</th>
<th>D[4; 3] (µm)</th>
<th>Drug encapsulation efficacy (%)</th>
<th>Drug loading (mg %)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>7.97</td>
<td>39.4</td>
<td>1.3</td>
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Table 1. SCIO-469 Microparticle characterization

The scanning electron micrographs (Fig. 2a) revealed that the microparticles are spherical and exhibit a smooth surface without pores or channels. SCIO-469 contains a fluorescent indole group (Fig. 1). Its excitation and emission wavelength values, respectively 395 and 496 nm, were determined using BioTeck microplate reader at 21 °C. CLSM image (Fig. 2b) showed a uniform distribution (coefficient of fluorescence variation ±0.17) of the drug inside the matrix particle. These different types of structures result from the fact that during the solvent evaporation and polymer precipitation the emulsion microdroplets coalesce, creating internal aqueous phase droplets (white arrow). The hardening of emulsion droplets with some inner droplet of internal aqueous phase results in the formation of capsule-like structures. Also 3D micrograph (Fig. 2c) allows visualizing surface structure of samples as well as the 3D distribution of drug SCIO-469 which emitted fluorescence inside PLGA microparticle system.

Figure 2. Scanning electron microscopy images (a) CLSM micrographs 2D (b) and 3D (c) of SCIO-469 microparticles. Scale bars = 10 µm.

CONCLUSION

Intrinsic drug fluorescence properties allowed a direct characterization of drug distribution using CLSM. This report sheds some light on the potential of fluorescent SCIO-469 biodegradable microparticles to design tailored solutions addressing intra-articular drug delivery challenges.

REFERENCES