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Original Research Article

Preparation and pre-clinical characterization of sustained-release ketoprofen implants for the management of pain and inflammation in osteoarthritis

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Abstract

Purpose: *To prepare and evaluate sustained-release ketoprofen implants for prolonged drug release and activity.*
Methods: *Ketoprofen implants were prepared with poly (lactic-co-glycolic acid) (PLGA) and chitosan in the form of tablets. The implants were analyzed for drug loading, thickness, hardness, swelling, in vitro drug release, as well as in vivo analgesic and anti-inflammatory activities.*
Results: *The implants were round, smooth in appearance, uniform in thickness and showed no cracks or physical defects on the surface. Their friability was < 1 % while drug content ranged from 89.98 ± 2.06 to 92.95 ± 1.65 %. In vitro drug release ranged from 70.23 to 92.04 % at the end of 5 days. Implants containing higher amounts of PLGA produced the highest swelling (40.24 ± 1.08 %). Implant IKT3 showed maximum analgesic activity (7.75 ± 1.00 s) and shortest time of maximum analgesia (2.5 h) in hot plate method. Inhibition of rat paw edema for IKT1, IKT2 and IKT3 was 79.95, 69.98 and 82.24 %, respectively, after 24 h.*
Conclusion: *Ketoprofen-loaded implant IKT3 (4:4:2 ratio of PLGA, chitosan and ketoprofen) provides relatively quick onset and prolonged duration of analgesic effect. Thus, ketoprofen implants have a potential for development into therapeutic products for prolonged management of pain and inflammation in osteoarthritis.*

Keywords: *Osteoarthritis, Ketoprofen implant, Prolonged analgesia, Poly(lactic-co-glycolic acid), Chitosan*

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INTRODUCTION

Osteoarthritis is a disorder involving degeneration of articulate cartilage in the joints. It causes moderate-to-severe joint pain and reduced motion due to breakdown and the loss of cartilage in one or more joints [1,2]. Put more precisely, osteoarthritis is not a disease, but rather it is a common complex disorder with multiple risk factors (like knee/hip osteoarthritis, obesity, high bone density, aging, and reduced muscle strength). Osteoarthritis has been reported to affect more than 20 million individuals in the USA alone; it is the foremost cause of

mortality in the aged population (more than 70 years of age) and costs the USA over US$100 billion per annum [3-6].

Osteoarthritis may be of the primary or secondary type. Increased stress on major weight-bearing joints or on weakened joints causes primary osteoarthritis. In general it affects the knees, finger joints, cervical and lumbar spine and the big toe. On the other hand, secondary osteoarthritis generally results from either chronic or any sudden injury (due to trauma, septic arthritis, metabolic disorder or developmental disorders) to a joint. The most common symptom of osteoarthritis is pain in the affected joints [7,8].

To manage pain and inflammation in osteoarthritis, non-steroidal anti-inflammatory drugs (NSAIDs) are frequently prescribed. However, prolonged use of NASIDs is associated with many side effects which may range from gastric discomfort (ulcers, irritation, and bleeding), to kidney failure and cardiac side effects (cardiac arrest and stroke) [9-11].

An implant may be defined as a polymeric system which is prepared to fill/ replace/support or enhance a biological structure and/or to provide prolonged drug delivery. A subcutaneous drug implant may be one of the most practical approaches for delivering drugs in the microenvironment of the affected joint/bones so as to achieve continuous and prolonged drug administration. Biodegradable implants prepared with poly glycolic acid (PGA), poly L-lactic acid (PLLA), and polylactic co-polymer have been investigated for use in small fingers and toes in treating osteoarthritis and rheumatoid arthritis [12-14]. The shape and size of the implant may vary depending on the site of administration. The implant may be pin, rod, cube, film, sponge or tablet-shaped.

Ketoprofen (chemically known as 2(3-benzoyl phenyl) propionic acid is a very widely prescribed NSAID for the treatment of RA and OA. Ketoprofen inhibits cyclooxygenase -1 (COX-1) and COX-2, which are enzymes that inhibit the production of prostaglandins. Ketoprofen has a short biological half-life (1 - 3 h), and its chronic use is associated with gastric irritation / bleeding [15]. The present study was aimed at developing biodegradable implants loaded with ketoprofen for prolonged and localized drug release for improved analgesic and anti-inflammatory effects. The prepared implants were analyzed for drug loading, thickness, hardness, swelling, drug release (*in vitro*), and *in vivo* analgesic and anti-inflammatory activities.

EXPERIMENTAL

Materials

Ketoprofen, PLGA, and chitosan (75 % deacylated) were purchased from Sigma Aldrich, Japan.

Preparation of implants

Direct compression method was used to prepare ketoprofen implant tablets. Three formulations of implant tablets (IKT1, IKT2 and IKT3) were prepared by using PLGA, chitosan and ketoprofen as biodegradable polymers at the ratios of 5:3:2, 3:5:2 and 4:4:2, respectively at room temperature. A total of 100 mg was mixed and kept in a cylinder of diameter 10 mm. Tableting was effected by applying a constant pressure (50 kg/cm^2) for 10 sec using hand press single punch machine. All the implant tablets were sterilized by gaseous sterilization method and then kept in a vacuum desiccator until further use.

Physicochemical evaluations

The thickness of all formulations of implant tablets was measured at three different locations with the help of screw gauge (n = 3). Hardness was tested by using Monsanto hardness tester (US). Twenty tablets of each batch were evaluated for weight variation (WV) [16]. Individual and average weights were calculated. Weight variation (WV) was determined using Eq 1.

$$WV\ (\%) = \frac{Wt - Wi}{Wt} \times 100 \quad \text{..... (1)}$$

where *Wt* is total weight of 20 tablets, and *Wi* is the sum of individual weight of 20 tablets.

The friability test was done by placing ten tablets (pre-weighed) in a friabilator. The friabilator was rotated at a speed of 25 rpm for 4 min. Then the tablets were taken out, dusted and again weighed. Friability (F) was calculated as in Eq 2.

$$F\ (\%) = \frac{Wo - Wt}{Wo} \times 100 \quad \text{..... (2)}$$

where *Wo* was the initial weight (before rotation), and *Wt* was the final weight (after rotation).

For the estimation of drug loading, implant tablet (n = 3) was dissolved in methanol (100 ml) with continuous stirring on a magnetic stirrer (400 rpm; temperature 37 ± 1 ^{o}C) for 4 h. The resultant solution was analyzed spectrophotometrically for drug content.

The morphologies of the surfaces of the implants were studied using scanning electron microscopy (SEM). The implants were subjected to SEM (JEOL, model no.5600, Japan) after hydration with phosphate-buffered saline, pH 7.4 for 1 h.

In vitro drug release studies

In vitro drug release studies on the ketoprofen implants were carried out in 100 ml bottle (5 cm in inner diameter, and 11 cm in height) containing phosphate-buffered saline (PBS, pH 7.4) at a temperature of 37 °C ± 1 °C. The bottle was kept on the magnetic stirrer and held in place with the help of burette stand. Aliquots (3 ml) were taken out at different time points (up to 5 days), and were immediately replaced each time with an equal volume of fresh, pre-warmed medium. The withdrawn samples were analyzed spectrophotometrically at 256 nm.

Swelling and polymer erosion studies

After 120 h of the drug release study, the wet weight and dried weight of the implant tablets were determined. The amounts of water absorbed and the amount of polymer eroded were calculated and expressed as percentage swelling.

In vivo studies

Animals

Male Wistar rats (150-225 g, aged about 4 weeks) were used for the study. They were housed in cages in standard conditions with food and water *ad libitum*. The rats were used after a resting period of 2 days post-procurement. The protocols of the animal study were approved by the Animal Ethical Committee of Sichuan University, Chengdu (approval no. 2016; 015) and were performed according to the guidelines 2010/63/EU on the handling of animals used for scientific purposes [17].

In vivo analgesic activity

The rats were divided into five groups (6 rats per group) viz: control, standard and 3 test groups (one each for one single type of formulation). The rats were put on a hot plate analgesiometer (MK-350 D, Japan) maintained at 50 ± 1 °C. The three types of implant tablets were surgically placed subcutaneously (1.0 mg/kg body weight) on the shaved dorsal surface of the rats in the respective test groups 30 min before the beginning of the test. Rats in the control group received blank implants while the standard group received oral ketoprofen suspension at the same dose. The time it took the rats to start of jumping or licking their forepaws was determined as reaction time. The maximum response (MR), time of the maximum response (TMR) and the duration of drug action (DA) were recorded.

In vivo anti-inflammatory activity

The anti-inflammatory activity (*in vivo*) was assessed against carrageenan-induced edema in hind paw (using carrageenan) of the rats. One control, one standard and three test groups were prepared. The hind paw volume of each rat was measured for all rats before treatment, using mercury plethysmometer. The control group received blank implants, while the standard group received diclofenac (1.0 mg/kg, p. o.). Rats in the test groups received implants (1.0 mg/kg, s. c.) which were surgically administered. After 1 h of implant administration, hind paw edema was induced by injecting 0.1 mL of 1 % w/v aqueous suspension of carrageenan in the right hind paws of the rats. The percent inhibition of edema relative to the controls was studied. Inhibition of edema (H) was calculated using Eq 3.

$$H\,(\%) = \left[\frac{Ec - Et}{Ec} \times Ec\right] \times 100 \quad \ldots\ldots\ldots\ldots (3)$$

where *Ec* is % edema in control and *Et* is % edema in test.

Statistical analysis

The results are presented as mean ± standard deviation (SD). Statistical analysis was performed by ANOVA using GraphPad Prism© 4.0. Values of $p < 0.05$ were considered to be indicative of statistical significance.

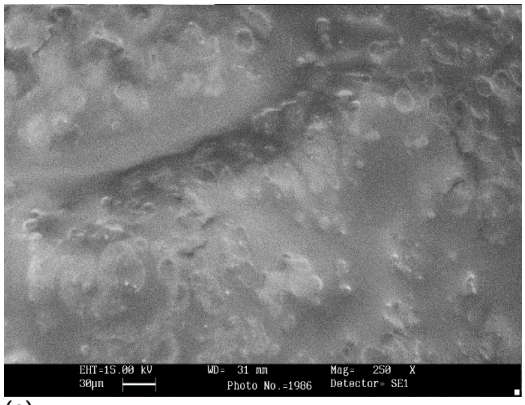
RESULTS

The physical properties of the three ketoprofen implants such as drug content, thickness, weight uniformity, hardness and friability are shown in Table 1.

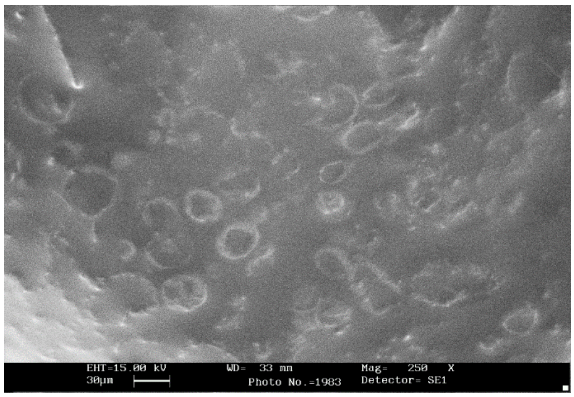
Physicochemical characteristics of the implants

The implant tablets were round, smooth in appearance, uniform in thickness and showed no cracks or physical defects on the surface. The friability was below 1 %, and the drug content ranged from 89.98 ± 2.06 to 92.95 ± 1.65 %. The implant tablets absorbed water three times their weight before drug release. Hardness value was intermediate for IKT1 implants. The surface

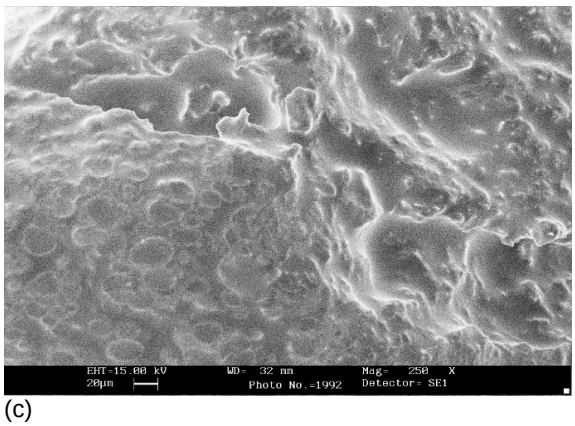
morphology of the implant tablets studied by SEM is shown in Figure 1.



(a)



(b)



(c)

Figure 1: Scanning electron micrographs of the hydrated surface of the implants: (a) IKT1; (b) IKT2; (c) IKT3

In vitro drug release

Results from *in vitro* drug release studies showed cumulative drug release of 70.23, 89.24, and 92.04 % for IKT1, IKT2 and IKT3, respectively at the end of 5 days (Figure 2).

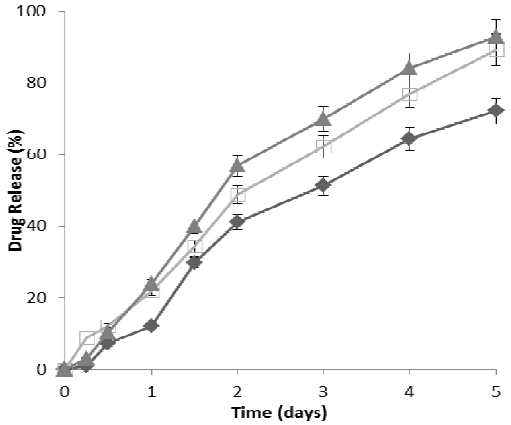


Figure 2: *In vitro* release of ketoprofen in phosphate buffered saline (pH 7.4) from implants IKT1 (♦), IKT2 (□), and IKT3 (▲)

In vivo analgesic and anti-inflammatory activities

The prepared implants showed very significant *in vivo* analgesic and anti-inflammatory activities (Table 2). The best analgesic activity was produced by IKT3.

Table 2: *In vivo* analgesic activity of ketoprofen SR implants

Formulation	MR (s)[a]	TMR (h)	DA (h)
IKT1	7.25±1.25	2.5	>24
IKT2	5.50±1.25	2.75	>24
IKT3	7.75±1.00	2.5	>24
Standard (ketoprofen tablet)	1.25±0.50	3.0	8

[a]Values are mean ± SD (n = 6); MR = maximum analgesic response; TMR= time of maximum analgesic response; DA = duration of analgesic action

Table 1: Physical characteristics of ketoprofen implants prepared with PLGA and chitosan

Code	Thickness (mm)	Hardness (kg/cm^2)	Weight uniformity	Friability (%)	Drug content (mg)	Swelling (%)
IKT1	3.24± 0.53	5.0±0.03	4.7±0.034	0.84±0.05	92.95± 1.65	40.24±1.08
IKT2	3.40± 0.02	5.2±0.02	5.0±0.012	0.77±0.02	89.98± 2.06	28.70±0.02
IKT3	3.30± 0.32	4.5±0.01	4.8±0.001	0..78±0.02	90.74± 1.31	31.4±1.01

On the other hand, there was no appreciable difference between the anti-inflammatory

activities of IKT1 and IKT3 implants, although they showed better activity than IKT2 or the standard (Figure 3).

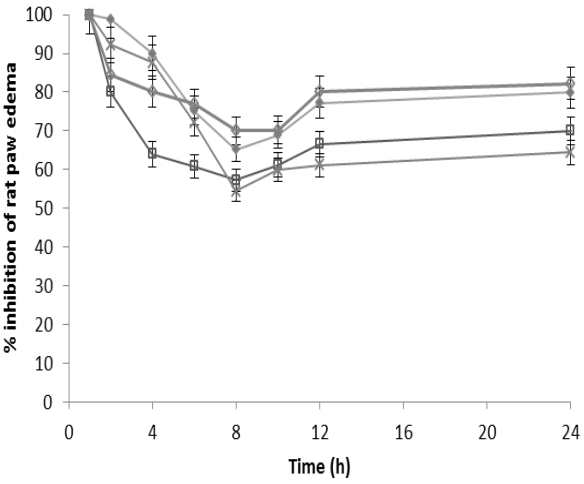


Figure 3: *In vivo* anti-inflammatory activity in carrageenan-induced rat paw edema of the ketoprofen sustained-release implants: IKT 1 (♦), IKT2 (□), IKT3 (◊), Standard (×)

Rat paw edema was inhibited 79.95, 69.98 and 82.24 % by IKT1, IKT2 and IKT3, respectively at the end of 24 h, while 64.45% inhibition was exhibited by the standard. The IKT3 implant showed longer inhibitory activity than any of the other two implants.

DISCUSSION

Implants are drug delivery systems which provide the most intimate and direct contact of the drug at the site of action. Localized high concentration of drug is very effective in RA and OA [18-21]. In the present study, the implants of ketoprofen were prepared with PLGA and chitosan for the prolonged drug release and effective analgesia and anti-inflammatory effect in osteoarthritis.

The implants containing PLGA in larger proportion (IKT1) showed high swelling, high drug content and high friability, while the implant with equal ratio of the polymers (IKT3) showed rougher surface than IKT2 or IKT1. The rough surface is a quality expected to pave way for better drug release. The implants showed good qualities. Good swelling is an essential feature for the slow release of drug from an implant. The selection of polymer, and the amount of polymer play a vital role in swelling. Polymeric chains generally need to be unfolded for easy drug diffusion out of the polymeric matrix of the implant. The implant IKT1 containing higher amount of PLGA showed the best swelling. It was observed that *in vitro* release showed improved drug release with matrix diffusion mechanism (assisted by the swelling of the polymeric matrix). Thus, the formulation which showed better swelling exhibited more prolonged drug release [19-22]. It was evident that due to better swelling, the drug release was higher with IKT1and IKT3 than with IKT2. This might be due to the presence of PLGA in high proportion.

In the *in vitro* analgesic activity, IKT3 showed the best analgesic response and the shortest time of maximum analgesic response. The ketoprofen implants delayed reaction time on the hot plate test. The ketoprofen implants were effective at 1 mg/kg with significantly increased hot-plate latency, and their anti-inflammatory effects were very effective with long duration of action. However, the IKT3 implant provided relatively quick onset of action with prolonged duration due to its better swelling as well as the prolonged drug release. Results from various studies indicate effective and prolonged drug delivery from polymeric implants in orthopaedics [22-25]. The anti-inflammatory activity model of carrageenan-induced inflammation showed that IKT2 was well balanced in composition, and the best-suited preparation for relatively fast onset of activity and prolonged drug release.

CONCLUSION

The findings of this study that the ketoprofen-loaded implant IKT3 (4:4:2 ratio of PLGA, chitosan and ketoprofen) demonstrate quick onset and prolonged duration of action. Thus, these implants possess potentials for therapeutic use in the management of osteoarthritis, but extensive preclinical and clinical studies need to be done first.

DECLARATIONS

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them. This manuscript was written by Jian Zhang. Jian Zhang, Xiaoping Xu and Lin Zhu did all experiments together. The whole experiments were designed by Xiaofan Fei and Zhaohui Jin.

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