PLGA Implant Tablet of Ketoprofen: Comparison of in Vitro and in Vivo Releases

Hiraku Onishi,* Mari Takahashi, and Yoshiharu Machida

Department of Drug Delivery Research, Hoshi University; 2-41 Ebara, Shinagawa-ku, Tokyo 142–8501, Japan.
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An implant tablet of ketoprofen (KP) was developed in order to achieve its sustained supply for approximately one week, and its release was evaluated in vitro and in vivo. Implant tablets (30 mg) containing 1 and 5 mg of ketoprofen, prepared using poly(ω-lactic acid-co-glycolic acid) copolymer (PLGA; MW 10000; lactic acid : glycolic acid=1 : 1 (mol/mol)) as a matrix, exhibited similar week-long sustained release in vitro. Plasma concentration was monitored after the implant tablet (5 mg of KP) and a KP solution (0.5 mg of KP) were administered subcutaneously to rats, and in vivo release rate was analyzed by deconvolution. The release rate from the implant tablet was faster in vivo than in vitro in the initial phase, but much lower in vivo than in vitro in the later phase. The plasma level decreased to the level less than the minimal effective concentration at 96 h after administration. However, the calculated plasma concentration given by convolution based on in vitro release rate was more than 7 times greater than the minimal effective concentration even at 96 h after administration. As the implant displayed the discrepancy between in vitro and in vivo release rates, the improvement of the in vivo release rate is required.

Key words implant tablet; in vitro release; in vivo release; ketoprofen; poly(ω-lactic acid-co-glycolic acid) copolymer (PLGA)

Implant dosage forms are useful for patients having difficulty in taking drugs orally, and their prolonged drug release can relieve patients of frequent dosage. For time-dependent drugs, it is important to maintain the drug concentration at the diseased site above the minimal effective concentration.1–3) So far, implants have been developed mainly for such time-dependent drugs, and many of them have dealt with the prolonged release for a period of several weeks or months.4–6) However, these implants cannot cover drugs for which the dose amount required per day is medium or large because such situations require a very bulky dosage form, leading to a burden on patients. Also, there is an attempt to use implants for the local treatment, in which the drug concentration around the implanted site is important and a supply of drugs to the systemic circulation is not required.9,10

In a previous study, a poly(DL-lactic acid-co-glycolic acid) copolymer (PLGA) tablet containing phenol red (PR) as a water-soluble model drug allowed a week-long sustained release of the model drug in vivo.7) Considering that many drugs are prescribed for one week or so, it is suggested that implants showing a week-long sustained release might be useful for patients who have difficulty in taking drugs orally or lacking in gastrointestinal absorption due to esophageal ulcers, a surgical operation of the digestive system etc. I.v. infusion causes the patient to become bedridden during the treatment period, leading to a lack of normal activities, while treatment with implants can relieve the restriction on activities and improve quality of life. In the present study, an attempt was made to apply such a week-long sustained release system to a real drug, ketoprofen (KP). KP is a non-steroidal anti-inflammatory drug, and usually applied at a medium dose.12–14) The preparation of a KP-loaded implant tablet has been performed according to a previous report, and the in vitro and in vivo releases have been examined and evaluated.

MATERIALS AND METHODS

Chemicals Poly(ω-lactic acid-co-glycolic acid) copolymer with a ω-lactic acid/glycolic acid ratio of 1 : 1 (mol/mol) (PLGA5010, MW 10000) was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Ketoprofen (KP) was purchased from Sigma Chemical Co. (St. Louis, U.S.A.). All other chemicals were of reagent grade.

Animals Male Wistar rats (6–7 weeks old, 220 g) were purchased from Tokyo Laboratory Animals Science Co., Ltd. (Tokyo, Japan). Soon after purchase, they were used for the animal experiments. The experimental protocol was approved by the Committee on Animal Research of Hoshi University, Japan. Also, all the animal experiments were performed in compliance with Guiding Principles for the Care and Use of Laboratory Animals, Hoshi University, Japan.

Preparation of Implant Tablets Implant tablets weighing 30 mg were prepared by direct compression at room temperature. As KP and PLGA were fine powder, they were used without treatment. Briefly, KP and PLGA powders with a total weight of 30 mg were mixed mechanically, put into a cylinder with a diameter of 10 mm, and compressed at a constant pressure of 50 kg/cm² for 10 s using a Shimadzu Hand Press HP-10 (Shimadzu, Japan).

In Vitro Release Studies The implant tablet was put in 100 ml of phosphate-buffered saline, pH 7.4, (PBS) contained in a bottle (4.5 cm in inner diameter×10.5 cm in height), which was warmed at 37 °C beforehand. The bottle was shaken at 60 strokes per min at 37 °C. Aliquots (4 ml) were taken out at predetermined time points. Immediately after each sampling, fresh PBS (4 ml) was added to the incubated medium. The sample was measured spectrophotometrically at 256 nm using a HITACHI 220A spectrophotometer (HITACHI, Japan) to determine the amount of KP released.

Swelling and Polymer Erosion After the above in vitro release test for 120 h, the implant tablet was taken out, the excess medium on the surface was removed by brief absorption with paper, and the weight of the wet tablet (Ww) was measured. Then, the wet implant tablet was dried completely using a vacuum pump, and the weight of the dried tablet (Wd) was measured. The amount of KP released (Wkr) was...
calculated from the results of the in vitro release stated above. The weight \((W_S)\) of the salts contained in PBS absorbed by the tablet was calculated from the salt concentration (1.13%, w/w) and amount of water by assuming the densities of water and PBS to be 1.0. The water absorbed by the tablet was calculated using the following equation:

\[
\text{water absorbed} (\%, \text{w/w}) = 100 \times \frac{(W_0 - W_p) - (W_0 - W_3)}{(W_0 - W_3)}
\]

When the initial polymer amount before the release test was \(W_{pp}\), the polymer erosion from the implant tablet was calculated as follows:

\[
\text{polymer erosion} (\%, \text{w/w}) = 100 \times \frac{(30 - W_{pp}) - (W_0 - W_3)}{W_{pp}}
\]

**Animal Experiments** The rats weighing 220 g were anesthetized by the i.p. injection of a pentobarbital saline solution (40 mg/2.5 ml/kg). One milliliter of PBS containing 0.5 mg of KP (KP solution) was injected subcutaneously per rat as a single bolus, and the plasma concentration was monitored for 24 h. The implant tablet was administered subcutaneously as follows. Namely, the hair on the center of the back (3 cm × 3 cm) was removed with a shaver, a skin incision (1.5 cm) was made, one implant tablet containing 5 mg KP was inserted subcutaneously per rat, and the incision was sutured with surgical string. Then, the plasma concentration was investigated for 120 h. At appropriate time points, blood samples (0.4 ml) were withdrawn from the jugular vein with a heparinized syringe, and centrifuged at 3000 rpm for 10 min to obtain the plasma. One hundred microliters of 1 M HCl aqueous solution was added to 100 μl of the plasma and shaken vigorously for 1 min. Then, 2.5 ml of diethyl ether was added, and the mixture was shaken vigorously for 1 min. After centrifugation of the mixture at 2000 rpm for 5 min, 2 ml of the organic layer was taken and dried at 40 °C under nitrogen gas. The residue was dissolved in 0.2 ml of the mobile phase of high performance liquid chromatography (HPLC).

The amount of KP in each sample was analyzed at room temperature by HPLC as follows.

A Shimadzu LC-6AD pump with a Shimadzu SPD-10AV spectrophotometric detector adjusted to 256 nm and a Shimadzu C-R7A-Plus chromatopac was used as an HPLC system, each component being from Shimadzu Co. (Japan). A Lichrosorb RP-18 column (4.6 mm in inner diameter × 150 mm in length; Sumika Chemical Analysis Service, Ltd., Japan) was used as an analytical column. A mixture of 0.02 M phosphate buffer of pH 3.0 and acetonitrile (55 : 45, v/v) was used as a mobile phase, and the flow rate was 1.0 ml/min. Twenty microliters of the sample was injected to the column. The determination of KP was performed based on the absolute calibration method.

**RESULTS**

**In Vitro Release, Swelling and Polymer Erosion** Two formulations, IT-1 and IT-2, were prepared as shown in Table 1. The implant tablets containing 1 and 5 mg of KP possessed almost the same thickness of 3.2 mm. Both implant tablets displayed almost the same release profiles as shown in Fig. 1. The release rate became slower in the later phase. The cumulative amount of KP released was nearly proportional to the square of time. However, both the implant tablets containing 1 and 5 mg of KP showed almost a week-long sustained release of KP efficiently in vitro.

Immediately after the release test for 120 h, the implant tablets were measured as to their wet weight and dried weight. Considering the amount of KP released together with the weights, the amounts of water absorbed and PLGA eroded were calculated (Table 1). Each implant tablet absorbed an amount of water that was approximately three times the amount of the tablet before the release test. The amounts of PLGA eroded were 15.1 and 27.8% for IT-1 and IT-2, respectively.

**Plasma Concentration–Time Profiles** After s.c. administration of the KP solution (0.5 mg of KP), plasma concentration was 10.22 μg/ml at 15 and 30 min, then lowered gradually to 0.33 μg/ml at 24 h.

The plasma concentration was monitored for 120 h after s.c. administration of the implant tablet. The plasma concentration was maximal and 41.3 μg/ml at 1 h after administration, then decreased gradually to the plasma levels of 4.70, 0.96 and 0.27 μg/ml at 24, 48 and 96 h after administration, respectively. The plasma level could be detected until 96 h.

![Fig. 1. In Vitro Release of KP from the Implant Tablets Containing 1 and 5 mg of KP](image)

The small figure shows the relation of the cumulative released amount with the square of time \(t\). The results are expressed as the mean±S.D. (n=4).

### Table 1. Characteristics of Implant Tablets after the Dissolution Test for 120 h

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Initial weight [PLGA; KP] (mg)</th>
<th>Wet weight(^a) (mg)</th>
<th>Dried weight(^b) (mg)</th>
<th>Amount of absorbed water(^c) (mg)</th>
<th>Amount of polymer eroded(^d) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IT-1</td>
<td>30 [29; 1]</td>
<td>115.8±2.3</td>
<td>25.8±1.2</td>
<td>89.0±6.0</td>
<td>15.1</td>
</tr>
<tr>
<td>IT-2</td>
<td>30 [25; 5]</td>
<td>111.1±1.6</td>
<td>19.7±0.3</td>
<td>90.3±1.5</td>
<td>27.8</td>
</tr>
</tbody>
</table>

\(^a\) The results are expressed as the mean±S.D. (n=4). \(^b\) The value was calculated with the mean amounts of dried weight, trapped salt and released drug.
The release amount were calculated as described in Fig. 3. The released at 96 h after the administration was less than the in the initial phase, but in the later period, was much slower.

The in vivo release rate was much faster than the in vitro release rate. Further, the cumulative amount released at 96 h after the administration was less in vivo than in vitro, and decreased to 0.27 μg/ml at 96 h. When the plasma concentration was simulated by convolution by assuming that the in vitro release rate occurred in vivo, the calculated plasma concentration at 96 h after administration was 2.26 μg/ml, which was more than 7 times greater than the minimal effective concentration from the early stage (6 h) to the last time (120 h) (Fig. 4).

As a result, the in vivo release rate and in vivo cumulative release amount were calculated as described in Fig. 3. The in vivo release rate was much faster than the in vitro release rate in the initial phase, but in the later period, was much slower than the in vitro release rate. Further, the cumulative amount released at 96 h after the administration was less in vivo than in vitro.

Analysis of Plasma Concentration
A linear absorption from the injection site to the systemic circulation and two-compartment model were applied to the pharmacokinetic analysis of KP after s.c. administration of the KP solution (Fig. 2). The tri-exponential curve, given by that model, was fitted to the mean plasma concentration–time curve given by the KP solution by the non-linear least squares method using the program MULTI. As a result, the following equation was obtained as the best fitted curve.

\[
C(t) = 11.51 \exp(-0.304t) + 1.07 \exp(-0.0504t) - 12.58 \exp(-8.19t) \tag{3}
\]

where \(C(t)\) was the plasma concentration at time \(t\) after s.c. administration of the KP solution (0.5 mg of KP per rat). The calculated curve is described with a broken line in Fig. 2.

The in vivo release rate of the implant tablet (5 mg of KP per rat) was calculated by deconvolution. Namely, when \(C_1(t)\), \(g(t)\) and \(R_n\) are the plasma concentration at time \(t\) after s.c. administration of the implant tablet, 2\(C_1(t)\) and the mean in vivo release rate from time \(t_{k-1}\) to \(t_k\), they are related to one another as follows.

\[
C_1(t_n) = \sum_{i=1}^{n} \left[ R_i \times \int_{t_{i-1}}^{t_i} g(z)dz \right] \tag{4}
\]

Therefore, \(R_n\) was calculated by the following equation.

\[
R_n = \left[ \frac{C_1(t_n) - \sum_{i=1}^{n} \left[ R_i \times \int_{t_{i-1}}^{t_i} g(z)dz \right]}{\int_{0}^{t_n} g(z)dz} \right] \tag{5}
\]

As a result, the in vivo release rate and in vivo cumulative amount released were calculated as described in Fig. 3. The in vivo release rate was much faster than the in vitro release rate in the initial phase, but in the later period, was much slower than the in vitro release rate. Further, the cumulative amount released at 96 h after the administration was less in vivo than in vitro. After s.c. administration of the implant tablet, the plasma concentration was observed to be 0.96 μg/ml at 48 h, and decreased to 0.27 μg/ml at 96 h. When the plasma concentration was simulated by convolution by assuming that the in vitro release rate occurred in vivo, the calculated plasma concentration was 0.3 μg/ml at 96 h. When the plasma concentration was simulated by convolution by assuming that the in vitro release rate occurred in vivo, the calculated plasma concentration was 0.3 μg/ml at 96 h.
ate period of time such as one week. Also, as to the drugs administered at a medium dose per day, such a very slow drug release is unpractical, because a large amount of drug must be implanted. Thus, an approximately week-long sustained release system was developed as reported previously,\(^1\) which demonstrated that the simple PLGA tablet containing a small amount of phenol red (PR) displayed almost a zero-order release rate. In the present study, KP was chosen as a drug because it is often prescribed at a moderate dose per day for approximately one week, and its implant tablets were prepared in the same manner as reported before.\(^1\)

Both the implant tablets (30 mg) containing 1 and 5 mg of KP exhibited similar \textit{in vitro} release profiles. The cumulative amount released was almost proportional to the square root of time. These \textit{in vitro} characteristics were different from those of the PR-loaded implant tablets reported before.\(^1\) The release of KP was considered to involve a mechanism different from that for the release of PR. However, both the implant tablets of KP displayed almost a week-long sustained release. As both the implant tablets showed a similar \textit{in vitro} release rates, the implant tablet containing 5 mg of KP was used for \textit{in vivo} experiment.

According to the literature, the pharmacokinetic profile of KP in the systemic circulation apparently follows a bi- or more exponential equation.\(^2\) Further, the dose dependency of the pharmacokinetics of KP appears to be small.\(^2\) Thus, the plasma concentration after the KP solution (0.5 mg of KP) was analyzed with the assumption that KP was absorbed from the injection site into the systemic circulation with the first order kinetics and the KP absorbed was eliminated according to the two-compartment model (Fig. 2). The calculated curve, given by Eq. 3, was fitted well to the observed one. The implant tablet did not sustain the plasma level of KP efficiently. The \textit{in vivo} release rate was analyzed by deconvolution and convolution techniques, which are sometimes used for analysis of \textit{in vivo} release.\(^2\)

The \textit{in vitro} release rate calculated by deconvolution was fairly different from the \textit{in vitro} one (Fig. 3). Namely, in the early stage, the implant tablet exhibited a faster release rate \textit{in vivo} than \textit{in vitro}. In contrast, the \textit{in vivo} release rate was lowered to less than one-tenth of the \textit{in vitro} release rate from 24 h after the administration. When the plasma concentration was simulated by convolution with the \textit{in vitro} release rate, the plasma concentration simulated at 96 h after administration was 2.26 \(\mu g/ml\), which was more than 7 times greater than the minimal effective concentration of 0.3 \(\mu g/ml\) (Fig. 4).\(^1\) Therefore, if the \textit{in vitro} release rate were realized in \textit{vivo}, the implant would act fairly well. Further, when a zero-order release for 120 h was presumed for the implant tablet (5 mg of KP), the plasma concentration was simulated. In this case, the release rate was 0.0417 mg/h, and the plasma levels were expected to be kept at the level of more than 10 times the minimal effective concentration from 6 h after administration (Fig. 4). As reported previously,\(^1\) the implant tablet might heighten the initial release \textit{in vivo} by change in tablet shape such as mechanical breakdown at the administration site. Also, the body fluid might influence the drug release \textit{in vivo} due to the existence of various biological components.\(^2\) Further, the present implant tablet (30 mg) contained KP at 5 mg, which was larger than the content (1 mg) of the previous implant tablet (30 mg) of PR. An increase in the drug content tends to accelerate the initial release rate.\(^1\) These situations appeared to cause the difference between \textit{in vitro} and \textit{in vivo} releases.

As described above, the \textit{in vivo} release rate is essential so that the effective concentration can be maintained for an intended period. The present implant displayed the discrepancy between \textit{in vitro} and \textit{in vivo} release rates. As discussed above, a zero-order release appears to be better for the maintenance of high plasma level of KP. Addition of other additives\(^2\) combination of PLA or PLGA of different molecular weights\(^9\), utilization of micro- or nano-particles in tabeting,\(^2,8,29\) design of a special dosage form shape\(^9\) etc. are useful to modify the release from the implant tablet. Improvement of \textit{in vivo} release with these approaches will be needed to allow the implant tablet of KP to be of practical use. Further, the evaluation of side effect is important for the practical use. As the implant displays a higher concentration near the administration site,\(^9,10\) its local side effect will have to be examined as well as the systemic one.

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