Evaluation of in vivo–in vitro release of dexamethasone from PLGA microspheres

Banu S. Zolnik 1, Diane J. Burgess *
Department of Pharmaceutical Sciences, University of Connecticut, Storrs, CT 06269, United States

Received 11 October 2007; accepted 8 January 2008
Available online 24 January 2008

Abstract

Two poly(lactic-co-glycolic acid) (PLGA) microsphere formulations, with different polymer molecular weights were investigated to determine whether an in vitro and in vivo relationship could be established for dexamethasone release. A USP apparatus 4 was used for in vitro testing. The in vivo release kinetics and pharmacodynamic effects of dexamethasone were evaluated using a Sprague Dawley rat model. The in vitro release from both formulations followed the typical triphasic profile of PLGA microspheres (initial burst release, followed by a lag phase and a secondary zero-order phase). The in vivo release profiles differed in that the lag phase was not observed and drug release rates were faster compared to the in vitro studies. It is speculated that the lack of lag phase in vivo may be a result of different PLGA degradation mechanisms in vivo as a consequence of the presence of enzymes as well as other in vivo factors such as interstitial fluid volume, and local pH. This may result in degradation of the PLGA microspheres proceeding from the surface inward in vivo. Whereas, in vitro an “inside out” degradation is thought to occur in some PLGA microsphere systems as a result of the autocatalytic degradation process where build up of acidic oligomeric units can occur within the microspheres. A linear in vitro–in vivo relationship was established after normalization of the time required to reach plateau for the in vitro and in vivo data and the in vitro release data were predictive of the in vivo release. Inflammation was significantly reduced in the tissue surrounding the dexamethasone microspheres compared to the positive control (empty microspheres) and the number of inflammatory cells was similar to that of normal tissue within one to three days.

© 2008 Elsevier B.V. All rights reserved.

Keywords: Drug delivery; In vivo–in vitro relationship; PLGA microspheres; Dexamethasone; USP apparatus

1. Introduction

Microsphere systems have been used for localized drug delivery to reduce side effects as well as to improve the therapeutic response at the local site [1,2]. Dexamethasone, a synthetic steroidal anti-inflammatory drug, has been incorporated into microspheres and liposomes to control the inflammatory reaction at the site of implantable devices such as biosensors and metallic stents, respectively [3–5]. If unchecked such devices can result in severe tissue response including acute (one to five days) and chronic inflammation (two to four weeks), as well as fibrosis [6]. In another application, microspheres containing both dexamethasone and bupivacaine have been prepared where dexamethasone acts as a vasoconstrictor to extend the pharmacological activity of bupivacaine [7,8].

In order to evaluate the performance of such microsphere systems, in vitro release methods with in vivo relevancy must be developed [9]. In vitro release data can be used for in vivo and in vitro correlation (IVIVC) to aid in setting dissolution specifications and to act as a surrogate for bioequivalence studies (for example, in the case of formulation, equipment, process and manufacturing site changes) [10]. A variety of in vitro release testing methods (dialysis sac diffusion, reversed dialysis sac, sample-and-separate, and continuous flow) have been used for injectable dispersed systems [11–13]. The dialysis sac method involves suspending microspheres or other dispersed systems in a dialysis sac with a semi-permeable membrane that allows diffusion of the drug, and then drug concentration is...
monitored in the receiver chamber. Disadvantages of this method include: i) potential for microsphere aggregation due to the lack of agitation, and ii) violation of sink conditions may result when drug release from the microspheres is faster than drug diffusion through the membrane [11]. The reversed dialysis sac method, where the dispersed system is placed in the chamber and the dialysis sacs contain only media, has been utilized to overcome the problem of violation of sink conditions that can occur with the traditional dialysis sac method [11]. The sample-and-separate method utilizes USP apparatus 2, and determination of release requires sampling and separation of the microspheres from the media. Disadvantages of this method include aggregation of the microspheres and the potential for loss of microspheres during the separation step leading to erroneous release profiles [13]. The continuous flow method utilizes flow-through cells, where microspheres are contained, and the media is continuously pumped from a reservoir through the microsphere bed [14]. This method has the advantage that sampling and separation are not required, and media volume is easily adjusted. In addition, a modification of this method has been reported where the microspheres are interspersed with glass beads in the flow-through cell to avoid aggregation [13].

Ideally, in vitro methods should be selected with the view to attaining a correlation between in vivo and in vitro release data [9]. To achieve this, researchers have investigated miniaturized methods considering that the volume at the subcutaneous site is low [15,16]. However, such methods can suffer from: violation of sink conditions, due to the small volumes used; and microsphere aggregation, as a result of limited agitation [17]. To overcome these problems the flow-through method has been used. This method avoids aggregation as discussed above. USP apparatus 4 also allows the use of small volumes since media replenishment can be achieved easily and without microsphere loss thus facilitating the maintenance of sink conditions [13]. In addition to the choice of testing method, media conditions may be varied to aid in the development of a relationship between in vivo and in vitro release data. To this end, the use of co-solvents, the addition of surfactants and enzymes, as well as variation in pH, ionic strength and temperature have been investigated [18–25]. For example, acidic media has been used to mimic drug release from PLGA microspheres in vivo [25,26]. The in vitro and in vivo performance of drug loaded PLGA microspheres has been evaluated [25,27–37]. However, there are a few examples in the literature where a correlation between in vitro and in vivo release data of single controlled release parenteral formulations has been reported [25,36,37]. Moreover, in order to establish a relationship between in vitro and in vivo release, more than one formulation must be investigated [38]. In addition, the in vitro method should be able to discriminate between formulations with different in vivo release characteristics.

In vivo release from controlled release parenterals such as microspheres may be affected by the environment at the site of administration. In vivo factors that affect drug release can be classified as, delivery system independent and delivery system dependent. Delivery system independent factors include: barriers to drug diffusion (e.g. fluid viscosity, and connective tissue); drug partitioning at the site (for example, uptake into fatty tissue); the fluid volume available at the site; and in the case of intramuscular injection muscle movement may also be an important factor. Delivery system dependent factors are those specific to a particular delivery system and include: enzymatic degradation of susceptible polymers; protein adsorption; phagocytosis as well as any possible inflammatory reaction to the delivery system. For example, the acute phase of inflammation results in edema and an influx of phagocytic cells at the local site. Whereas, the chronic phase of inflammation can lead to fibrosis which in turn results in isolation of the delivery system and a consequent reduction in the fluid volume.

In vitro–in vivo correlation (IVIVC) can be categorized as Levels A, B, C, D and multiple Level C. Level A is a point-to-point correlation over the entire release profile. For Level B, the mean in vitro dissolution time is compared to either the mean residence time or the mean in vivo dissolution time. Level C is a single point correlation between a dissolution parameter (for example, the amount dissolved at a particular time or the time required for in vitro dissolution of a fixed % of the dose) and an in vivo parameter (for example, Cmax or AUC). A multiple Level C correlation is also possible and this involves a correlation at several time points in the release profile. Level D is a rank order correlation (qualitative) [10]. IVIVC can be achieved either by mathematical modeling (e.g. time variant non-linear modeling) of the in vitro and in vivo data [38] or by alteration of the in vitro release method to obtain in vitro data that mimics the in vivo data [17,24]. In order for the in vitro method to be used as surrogate for bioequivalence studies, a Level A IVIVC is required.

In the present study, two PLGA formulations with different in vivo release characteristics were chosen in order to investigate the relationship between in vivo and in vitro release. To develop an in vitro release method that is able to discriminate between formulations which have different in vivo release characteristics, the USP apparatus 4 method was selected since it offers advantages, as discussed above, and this method has been recommended for controlled release microspheres [9]. The in vivo pharmacodynamic affect of dexamethasone (i.e. control of inflammation at the site) was also investigated in this study as this may affect the in vivo release kinetics.

2. Materials and methods

Poly(D,L, lactic-co-glycolic acid) (PLGA) polymers, PLGA Medisorb 50:50 DL 2A and 2.5A (Mw: 13,000 and 28,000, respectively), were gifts from Astra Zeneca. Methylene chloride and tetrahydrofuran (Optima grade) were obtained from Fisher Scientific (Pittsburgh, PA). Dexamethasone, poly(vinyl alcohol) (PVA), carboxymethylcellulose sodium salt and sodium chloride were obtained from Sigma (St. Louis, MO).

2.1. Preparation of microspheres

An oil-in-water emulsion solvent evaporation technique was used for dexamethasone microsphere formulation. 2 g of PLGA was dissolved in 8 mL of methylene chloride, and 200 mg of dexamethasone was dispersed in this solution using a homogenizer at 10,000 rpm for 1 min. This organic phase was added
slowly to 40 mL of a 1% (w/v) aqueous PVA (Av. Mw 30,000–70,000) solution and homogenized at 10,000 rpm for 3 min. This emulsion was added to 500 mL of a 0.1% (w/v) aqueous PVA solution and stirred at 250 rpm under reduced pressure for 6 h at 25 °C. The resulting microspheres were filtered (Durapore Membrane Filter, 0.45 μm, Fisher Scientific, Pittsburgh, PA), washed three times with double distilled deionized sterile water and vacuum dried for 24 h.

2.2. Characterization of microspheres

2.2.1. Particle sizing

An Acusizer (optical particle sizer model 770, Santa Barbara, CA, USA) was used to determine the mean particle diameter and distribution. Microspheres were suspended in 0.1% (w/v) PVA solution in water. A 500-μL portion of this suspension was diluted with 25-mL deionized water prior to detection.

2.2.2. High performance liquid chromatography (HPLC)

The concentration of dexamethasone was determined using HPLC. The HPLC system consisted of a Constametric 4100 pump (Thermseparation), an automatic sample injector (Bio-Rad) and a UV absorbance detector (Bio-Rad) set at 242 nm. The mobile phase consisted of acetonitrile:water:phosphoric acid (30:70:0.5 (v/v/v)). The analytical column was a NovaPack® C18 (9 mm × 150 mm, pore size 4 μm) (Millipore Corp, Waters, Milford, MA). The flow rate was set at 1 mL/min. The retention time of dexamethasone was 5 min, and total run time of HPLC analysis was 15 min. The chromatogram was analyzed by PeakSimple Chromatography System (Model 203, software 3.29, SRI instruments, Torrance, CA). This HPLC method is adapted from that of Spangler and Mularz [39].

2.2.3. Drug loading

10 mg of microspheres were dissolved in 10 mL of THF, filtered (Milllex-HV, 0.45 μm, Fisher Scientific, Pittsburgh, PA) and analyzed using the HPLC method described above for dexamethasone content. All measurements were conducted in triplicate and the mean values and standard deviations are reported.

2.2.4. Differential scanning calorimeter (DSC)

Samples were analyzed using a TA Instruments 2920 DSC. Samples were heated to 150 °C and cooled to −30 °C at a rate of 5 °C/min. The second cycle was used to determine the glass transition temperature (Tg) for characterization of the microspheres as prepared. Samples were analyzed in aluminum pans with pinhole lids.

2.2.5. Gel permeation chromatography (GPC)

The Mw of the PLGA microspheres was determined using GPC (Waters) with an evaporative light scattering detector (ELSD, Polymer Laboratories PL-ELS 1000). Four Jordi Flash (high-speed) columns were connected in series. The mobile phase was THF with a flow rate of 3 mL/min. The columns were held isothermally at 40 °C. Microspheres (10 mg) were dissolved in 10 mL in THF and filtered (Whatman Paradisc PVDF(Polyvinylidene Fluoroide) Syringe Filters with 0.22 μm pore size). All glass equipment (vials and syringes) was used to minimize possible contamination from plastic materials. The polymer solution injection volume was 100 μL. The data collection and analysis were performed using Waters Millennium software. Weight average molecular masses were calculated based on polystyrene standards (1,250,000; 400,000; 200,000; 43,000; 17,600; 6930; 2610; 982; and 472 Da).

2.2.6. In vitro release studies

In vitro release studies were conducted using a modified USP apparatus 4 (Sotax CE7 smart, or CY 7 piston pump, Sotax, Horsham, PA) with flow-through cells (12 mm diameter) packed with glass beads (1 mm) (to prevent microsphere agglomeration and to achieve laminar flow [13]) in a closed system mode at 37 °C. 45 mg of microspheres were dispersed in the flow-through cells and 250 mL of 0.1 M phosphate buffered saline (PBS) pH 7.4 (prepared according to the USP monograph) with 0.1% (w/v) sodium azide was circulated through a fiberglass filter (0.45 μm). A flow rate of 20 mL/min was used. This flow rate was chosen since this is the standard flow rate for USP apparatus 4 and we have previously reported [1] that the flow rate does not affect the release rate for PLGA microspheres of high molecular weight (such as the formulations reported here) where release is erosion/diffusion controlled.

1.3-mL samples were withdrawn and replenished with 1.3 mL of fresh media. The samples were analyzed by HPLC (as explained above) and any drug degradation was accounted for in the cumulative release data reported. When the drug concentration reached 5% (w/v) of the solubility of dexamethasone, half of the total media volume was replenished. This media replenishment was taken into account in the calculation of the cumulative percent release. All measurements were conducted in triplicate and the mean values and standard deviations are reported.

2.2.7. In vivo release studies

Dexamethasone PLGA microspheres were prepared under aseptic conditions. Microspheres were suspended in a viscosity enhanced diluent (30 mg/mL carboxymethylcellulose sodium salt, 9 mg/mL NaCl and sterile water) [40] in order to prevent any sticking of the microspheres in syringes/needles during injection. Rats were anesthetized in an induction chamber filled with a 4.5% (v/v) mixture of isofluorane in oxygen. The back of each animal, where microspheres were injected, was shaved with a 4.5% (v/v) mixture of isofluorane in oxygen. The back of each animal, where microspheres were injected, was shaved prior to injection. PLGA Microspheres with Mw 13,000 and 28,000 formulations known herein as formulations 13K and 28K, respectively were injected subcutaneously using 18 gauge needles. Microspheres were administered at 4 mg dexamethasone encapsulated dose per animal for both the 13K and 28K formulations. Five rats were sacrificed at each of the following time intervals: 1, 3, 5, 8, 11, 12, 14 days for 13K formulations and 1, 3, 5, 8, 11, 12, 14, 17, 22, 26, 30 days for 28K formulations and 1, 3, 5, 8, 14, 17, 22, and 26 days for control. Subcutaneous tissues including the microspheres were removed from the injection site. The microspheres were separated from
the tissue samples. The drug content of the degraded microspheres in vivo was determined according to the HPLC method described above. The released drug amount was calculated by subtraction of the drug content in the degraded microspheres from the total drug loaded into the microspheres. All animal studies were conducted at the University of Connecticut in accordance with Institutional Animal Care and Use Committee guidelines using an approved protocol (number E2901201).

2.2.8. Pharmacodynamic study of dexamethasone microspheres

Tissue samples from the various rat studies described above were fixed in 10% formalin and sections were immersed in paraffin and cut using a microtome. The pharmacodynamic effect of the dexamethasone microspheres was determined via histological examination using hematoxylin and eosin (H&E) staining. Empty microspheres (28K) were used as a positive control and untreated subcutaneous tissue samples were used as a negative control. Photomicrographs of the histology slides were taken and digitally stored using an Olympus microscope (model AX70, Olympus America, Melville, NY) at a 10× magnification. Visual counting of the purple stained nuclei of the inflammatory cells was performed to reduce error that might occur as a result of the software counting two or more cells in close proximity as one. The magnified images of the photomicrographs were quantified visually using Microsoft® Power Point® 2002 program which allowed placement of 42 uniformly sized boxes for ease of counting. This method of evaluating the number of inflammatory cells has been previously reported [41,42].

3. Results

3.1. Polymer selection and microsphere characterization

In order to investigate whether an in vitro–in vivo relationship could be established, two microsphere formulations were prepared and characterized for molecular weight, glass transition temperature (Tg), and drug loading (Table 1). Although the molecular weights were different (13,000 and 28,000) the Tg values were similar (44 °C and 45.6 °C) for the formulations known herein as formulations 13K and 28K, respectively. The particle size was similar for both formulations (number-weight average particle size 20 μm). The drug loading for the 13K and 28K formulations were 7.64±0.28 and 8.25±0.25%, respectively followed by an apparent zero-order release phase. The time to reach plateau was different for the formulations (8 and 12 days for formulations 13K and 28K, respectively followed by an apparent zero-order release phase.

3.2. In vitro studies

Dexamethasone release from both formulations 13K and 28K showed a triphasic release profile, with an initial burst release, followed by a lag phase and then a secondary zero-order release phase. Such triphasic release profiles are typical of PLGA microspheres [43]. The 13K and 28K formulations exhibited an initial burst phase of approximately 30% and 20% with a lag phase until days 5 and 9, respectively. The observed decrease in the initial burst with increase in polymer Mw may be a result of decreased diffusion pathway in the higher Mw microspheres (28K). We have previously reported similar trends for other PLGA microsphere formulations of differing MW (5 kDa, 25 kDa, 70 kDa) [18]. The longer lag phase with the 28K formulation compared to the 13K formulation is considered to be due to the higher Mw which requires more time for degradation to take place (Fig. 1).

3.3. In vivo studies

The in vivo release profiles showed an initial burst release of approximately 48 and 15% for formulations 13K and 28K, respectively followed by an apparent zero-order release phase. The time to reach plateau was 8 and 12 days for formulations 13K and 28K, respectively (Fig. 2).

3.4. Evaluation of relationship between in vitro and in vivo release

It has been recommended that IVIVC should be conducted on formulations with the same in vitro mechanism of release [44]. When more than one mechanism of release occurs for a single formulation, each release phase should be correlated separately [38,44]. It has also been recommended that burst release from systems such as microspheres should be investigated separately [9,45]. The secondary zero-order phase of both formulations was used to establish a relationship between the in vitro and in vivo release data (IVIVR). As shown in Figs. 1 and 2, the time to reach a plateau was different for the in vitro and in vivo data. Therefore, the time required to reach plateau was normalized for the in vitro and in vivo data for both formulations. Normalization was achieved by plotting in vitro time on the x-axis and in vivo time on

<table>
<thead>
<tr>
<th>Table 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLGA microsphere formulation and physico-chemical characterization</td>
</tr>
<tr>
<td>Ratio of LA:GA</td>
</tr>
<tr>
<td>13K</td>
</tr>
<tr>
<td>28K</td>
</tr>
</tbody>
</table>

Fig. 1. Dexamethasone release from PLGA microspheres at 37 °C in PBS buffer (pH 7.4) using USP apparatus 4: (♦) 28K formulation, and (■) 13K formulation (mean±SD, n=3).
the y-axis for both formulations. A linear relationship between the in vitro time and in vivo time was obtained where the time scaling parameters (slope) were 0.4984 and 0.5008 and the time shifting parameters (intercept) were 1.4276 and 3.8255 for formulations 13K and 28K, respectively. Time shifting/scaling allowed the in vitro data to be on the same time scale as the in vivo data. This, in turn made it possible to establish a relationship between in vitro and in vivo release data by plotting the in vitro release data on the x-axis and the in vivo release data on the y-axis, while time points (z-axis, not shown) were on the same scale. Consequently, a linear relationship between the in vitro and in vivo data was established for both the 13K and 28K formulations (Fig. 3).

3.5. Prediction of in vivo release based on in vitro and in vivo relationship

The in vitro and in vivo relationship established for the 28K formulation was utilized to predict in vivo release of dexamethasone from the 13K formulation. The relationship between in vitro and in vivo release of the 28K formulation was obtained via linear regression (circles in Fig. 3) as follows:

\[
\% \text{ cumulative in vivo release} = 1.6381 \times (\% \text{ cumulative in vitro release}) - 24.457
\]

with a correlation coefficient of 0.9862. This relationship was used to predict % cumulative in vivo release of the 13K formulation using the experimental in vitro data obtained for the 13K formulation. Time scaling was obtained from the time scaling/shifting parameter of the 28K formulation since time scale differences between in vivo and in vitro release were observed. Experimental and predicted percent cumulative release versus time in vivo data were plotted (Fig. 4). A model independent approach using a difference factor \( f_1 \) and a similarity factor \( f_2 \) was utilized to compare experimental and predicted percent cumulative in vivo data. The difference factor was defined in the FDA Guidance for Industry: Dissolution Testing of Immediate Release Solid Oral Dosage Forms [46] as “calculation of the percent difference between the two curves at each time point and is a measurement of the relative error between the two curves”:

\[
f_1 = \left\{ \frac{\sum_{i=1}^{n} |R_i - T_i|}{\sum_{i=1}^{n} R_i} \right\} \times 100.
\]

The similarity factor is a measurement of the similarity in the percent release between the two curves and is defined as the “logarithmic reciprocal square root transformation of the sum of the squared error” in the FDA guidance document [46].

\[
f_2 = 50 \times \log \left\{ 1 + \left( \frac{1}{n} \sum_{i=1}^{n} (R_i - T_i)^2 \right)^{-0.5} \right\} \times 100
\]

where \( n \) is the number of time points, \( R_i \) is the experimental data at time \( t \), and \( T_i \) is the predicted in vivo percent cumulative release at time \( t \).

The experimental and predicted in vivo cumulative release data from days 1, 3 and 8 were used since a model independent approach requires the use of the same time points. The difference factor \( f_1 \) and similarity factor \( f_2 \) were calculated as 16 and 58, respectively. It is noted in the FDA guidance document that generally, \( f_1 \) values of 15 and \( f_2 \) values greater than 50 suggest equivalence of the two profiles.

3.6. Histology studies

Figs. 5 and 6 are representative micrographs of histology sections taken at different times representative of acute and chronic inflammation for the 13K and 28K formulations together with a positive control (empty PLGA microspheres, Sprague Dawley male rat model, \( n=5 \) for each data point): (○) 13K formulation, and (□) 28K formulation.

Fig. 2. Dexamethasone release obtained from PLGA microspheres in vivo. Sprague Dawley male rat model, \( n=5 \) for each data point): (○) 13K formulation, and (□) 28K formulation.

Fig. 3. An in vitro–in vivo relationship for dexamethasone release from PLGA microspheres: % released in vivo was plotted against % released in vitro for formulations: (●) 28K, and (■) 13K.

Fig. 4. Experimental (in vivo) and predicted % cumulative release data of 13K formulation. (■) in vivo experimental, (○) predicted.
28K formulation) and a negative control (untreated tissue section). Hematoxylin–eosin (H&E) stains the nuclear material of the inflammatory cells purple, while the normal tissue remains pink. The inflammatory cells were predominately monocytes and with a small percentage of polymorphonuclear leukocytes (PMNs). The change in the number of inflammatory cells with time is shown numerically in Table 2. The positive control shows a severe inflammatory response due to the presence of the PLGA microspheres and the tissue trauma resulting from the needle injection. The inflammatory response to the PLGA microspheres may be partially due to PLGA degradation resulting in release of acidic oligomeric units in the immediate vicinity of the microspheres. The typical inflammatory response of acute followed by chronic inflammation is clearly seen in the positive

Fig. 5. Pharmacodynamic changes in representative tissue sections on days 1, 3, 5, 8 and 14. Sprague Dawley rats were subcutaneously injected with 13K and 28K formulations. The rats were sacrificed and the implant site was dissected, processed and stained using hematoxylin and eosin (H&E). Inflammation is evident in the positive control (blank PLGA microspheres — left column) while normal tissue can be seen in untreated tissue sections (negative control — top panel). Minimal inflammation is seen in those animals treated with 13K formulation (middle column) and 28K formulation (right column).
control with the numbers of the inflammatory cells peaking at
day 1 (approximately 2300) and gradually decreasing to
approximately 950 by day 28 (Table 2). The inflammatory
response to the formulation 13K is much less severe than that to
the positive control, with an initial number of inflammatory cells
(560) which is only slightly greater than the negative control
(456). In addition, the 13K formulation is able to control
inflammation to values below that of the negative control for the
remainder of 14 day study period. The 28K formulation also
showed a less severe inflammatory response compared to the
positive control. The number of inflammatory cells at day 1 was
higher (753) than the negative control (456) but reduced over the
study period and by day 5 the number of inflammatory cells was
comparable to the negative control.

4. Discussion

The major differences between the in vivo and in vitro
release from the 13K and 28K dexamethasone microsphere
formulations are the lack of lag phase and the faster release
kinetics for the apparent zero-order release phase of the in vivo
data. In addition, the in vitro and in vivo burst phases appeared
not to correlate, however the number of in vivo data points may
well be sub-optimal to fully characterize the burst release. The
faster release kinetics in vivo may be a result of enhanced
degradation due to the presence of enzymes as well as
other in vivo factors such as interstitial fluid volume, and local
pH. Accelerated enzymatic PLGA degradation in vivo has also
been reported by other researchers [17,47]. For example, Clark
et al. have reported faster in vivo compared to in vitro release
kinetics for PLGA microspheres [17]. However, there is some
controversy in the literature with respect to role of enzymes in
PLGA degradation [48].

The release kinetics from the 13K formulation were faster,
both in vivo and in vitro, compared to the 28K formulation due
to the rapid degradation of the low Mw PLGA. The lack of lag
phase in vivo for both the 13K and 28K formulations may be
due to a different polymer erosion mechanism compared to
in vitro. It is considered that in vitro degradation of PLGA
microspheres occurs as a result of autocatalysis due to the build
up of acidic oligomers within the microspheres and this has
been reported to result in “inside out” erosion [49,50]. Whereas,
in vivo enzymatic degradation is more likely to occur from the
surface in and therefore, a lag phase might not be expected to
occur. In a previous publication from our laboratory, it has been
reported that release kinetics of vascular endothelial growth
factor (VEGF) from PLGA microspheres was slower in vivo
compared to in vitro [51]. A possible explanation for this is the
severe inflammatory reaction that occurred in the presence of
these microspheres. This is considered to be a result of both
tissue reaction to the PLGA microspheres as well as to the
foreign protein (human VEGF was used in a rat model). In the
present study, dexamethasone release from the microspheres
reduced the number of inflammatory cells down to that of
normal tissue (negative control) by day 3 for the 13K formulation
and by day 5 for the 28K formulation. The initial level of
inflammation for both formulations was significantly less
than the positive control of blank microspheres. The number
of inflammatory cells at day 1 was less for the 13K formulation
compared to the 28K formulation and this may be attributed to
higher burst release of dexamethasone from the 13K formulation
(48%) compared to the 28K formulation (15%).

A linear in vitro and in vivo relationship was established for
formulations 13K and 28K after normalization of the time scales
of the in vitro and in vivo data. A bio-relevant in vitro release

![Positive Control](image1.png)

![28K](image2.png)

Fig. 6. Pharmacodynamic changes in representative tissue sections on days 17,
22 and 26. Sprague Dawley rats were subcutaneously injected with 28K
formulations. The rats were sacrificed and the implant site was dissected,
processed and stained using hematoxylin and eosin (H&E). Inflammation is
evident in the positive control (blank PLGA microspheres — left column) while
normal tissue can be seen in untreated tissue sections (negative control — Fig. 5
top panel). Minimal inflammation is seen in those animals treated with 28K
formulation.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Control</th>
<th>13K</th>
<th>28K</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2300**</td>
<td>560</td>
<td>753</td>
</tr>
<tr>
<td>3</td>
<td>1297</td>
<td>208</td>
<td>620</td>
</tr>
<tr>
<td>5</td>
<td>1150</td>
<td>282</td>
<td>455</td>
</tr>
<tr>
<td>8</td>
<td>701</td>
<td>287</td>
<td>448</td>
</tr>
<tr>
<td>14</td>
<td>1766**</td>
<td>397</td>
<td>377</td>
</tr>
<tr>
<td>17</td>
<td>794</td>
<td>NA</td>
<td>153</td>
</tr>
<tr>
<td>22</td>
<td>754</td>
<td>NA</td>
<td>426</td>
</tr>
<tr>
<td>26</td>
<td>950**</td>
<td>NA</td>
<td>469</td>
</tr>
</tbody>
</table>

Table 2

Effect of dexamethasone on the inflammation mediation cell population at the
vicinity of the PLGA microspheres

Positive control, blank microspheres (no dexamethasone); 13K formulation; and
28K formulation. The numbers are counted from 42 boxes across the entire
tissue section. **The numbers are estimated from the representative boxes
across the tissue section since some parts of the section could not be counted due
to the density of inflammatory cells causing overlap and therefore, the number
maybe slightly underestimated. NA: not applicable.
method (USP 4 method) and Sprague Dawley rat model were utilized to obtain this relationship. In vitro and in vivo correlation (IVIVC) for microspheres and implants have been previously reported, however these correlations were made on single formulations or different types of correlations were established on formulations with different release characteristics (e.g. diffusion and erosion controlled) [36,37].

The current study is one of a few reports showing the same in vitro and in vivo relationship for more than one controlled release parenteral formulation [52,53]. The in vivo release profile of the 13K formulation was predicted using IVIVR and time scaling/shifting data obtained using the 28K formulation in vitro and in vivo data. The equivalence of the experimental and predicted in vivo data was assessed using a model independent approach. The difference and similarity factors indicated that the percent cumulative release in vivo prediction of the 13K formulation was comparable to that of the experimental in vivo data based on the IVIVR established for the 28K formulation. A possible explanation for the calculated $f_1$ and $f_2$ values, 16 and 58, respectively being close to the threshold values of 15 and 50, respectively is that only three time points were used since this model independent approach requires the use of the same time points. When the complete profiles of both curves are compared as shown in Fig. 4, it is apparent that both predicted and experimental curves are in agreement. In addition, it should be noted that the USP apparatus 4 method was able to discriminate between two PLGA microsphere formulations with different in vivo release characteristics.

5. Conclusions

The in vitro and in vivo release characteristics of dexamethasone from two PLGA microsphere formulations (13K and 28K) were investigated to determine whether an IVIVR could be established. In vitro release of dexamethasone from both formulations exhibited a typical triphasic profile with an initial burst release, a lag phase, followed by an apparent zero-order release phase. A modified USP apparatus 4 method that eliminated problems of microsphere aggregation, which typically occur with other in vitro release methods, was used for in vitro release testing. Such a method is important for the development of an in vitro–in vivo relationship since the microspheres are not aggregated in vivo as is apparent from the histology data presented. In vivo, these microspheres exhibited a biphasic release profile with an initial burst followed by an apparent zero-order release phase. The lack of a lag phase in vivo is speculated to be a result of enzymatic degradation which leads to “outside in” erosion compared to the typical “inside out” erosion that is considered to occur in vitro. In addition, drug release was faster in vivo compared to in vitro. It is speculated that this may be a result of enzymatic degradation as well as the lack of a severe inflammatory response to these microspheres. Histology data revealed that both formulations were able to control inflammation at the local site. The 13K formulation was superior to 28K formulation with respect to the initial inflammatory response and this is considered to be a result of the higher burst release from the 13K formulation. A linear relationship between the in vivo and in vitro data post burst phase was established after normalization of the time required to reach a plateau.

Acknowledgments

The authors are thankful for the support from the US Army Medical Research and Material Command (W81XWH-04-1-0779 and W81XWH-05-1-0539); Office of Testing and Research CDER, FDA; CPPR-NSF; and Sotax Corp. The awarding of a USP fellowship to BSZ is greatly appreciated. The authors wish to thank Dr. David Young, AGI Therapeutics, Inc. for useful discussions.

References
