Modulating β-Lapachone Release from Polymer Millirods through Cyclodextrin Complexation

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ABSTRACT: β-Lapachone (β-lap) is a novel anticancer agent that kills tumors over-expressing the NADP(H): quinone oxidoreductase enzyme. However, poor aqueous solubility and low bioavailability hinder its therapeutic applications. Herein we describe the development of poly(D,L-lactide-*co*-glycolide) (PLGA) polymer millirods for local delivery of β-lap. The objective was to investigate the use of β-lap inclusion complexes with cyclodextrins (CDs) to control β-lap release kinetics from PLGA millirods. Differential scanning calorimetry was performed to measure drug/polymer interactions, complexation efficiency with different CDs, and complex/polymer interactions. β-Lap was found to have a solid-state solubility of 13% in PLGA. β-Lap dissolution in PLGA matrix lowered the glass transition temperature of PLGA from 44 to 31°C, and led to a slow release of β-lap ($8.8 \pm 1.2\%$ release after 22 days). For β-lap and CD interactions, increasing complexation efficiency was observed in the order of α-CD, γ-CD, and β-CD. β-Lap complexation with hydroxypropyl-β-cyclodextrin (HPβ-CD) prevented drug dissolution in PLGA, and led to fast release ($79.6 \pm 2.1\%$ after 2 days). Sustained drug release was achieved when β-lap was complexed with α-CD or γ-CD. These data demonstrate the ability to tailor β-lap release kinetics via CD complexation, providing exciting opportunities for the use of β-lap-millirods for intratumoral drug delivery.
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INTRODUCTION

A rising trend in cancer chemotherapy involves site-specific, controlled release of cytotoxic agents from biodegradable polymer depots. This strategy often proves advantageous when compared to traditional administration regimens, such as intravenous injection of drugs, where low water solubility of many anticancer drugs limits their bioavailability and anticancer efficacy *in vivo*. Controlled release strategies are superior since tumors are exposed to therapeutic levels of drug for a prolonged time period, all the while reducing toxicity to healthy cells.[1] Drug inclusion in a polymer depot also allows for potential tailoring of release kinetics, adding the benefit of being able to design the most efficacious delivery regimen. Taking these advantages into consideration, our laboratory developed a polymeric drug depot in the form of a cylindrical millirod composed of poly(D,L-lactide-*co*-glycolide) (PLGA), designed

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specifically for intratumoral delivery of anticancer agents. Research efforts have been devoted to the fabrication and mechanical characterization of drug-loaded polymer millirods,[2] as well as the control of drug release characteristics from these devices.[3,4] Direct implantation of doxorubicin-millirods has shown successful antitumor efficacy in rabbit VX-2 liver tumors *in vivo*.[5] In addition, local delivery of dexamethasone through millirod implants considerably improved drug effectiveness in decreasing fibrous capsule formation in ablated liver tissues compared to a systemic delivery of the drug.[6]

β-Lapachone (β-lap) is a novel antitumor agent with specific anticancer activity against human lung, prostate, and breast tumors.[7] β-Lap has a unique mechanism of action, which relies on bioactivation by the cytosolic enzyme, NAD(P)H:-quinone oxidoreductase-1 (NQO1).[8] This enzyme is endogenously elevated in tumor cells (up to 20-fold) compared to adjacent normal cells.[9] Furthermore, β-lap has distinct advantages over other chemotherapeutic agents in that it kills tumors independent of p53 status, cell cycle state, caspases, all the while inducing a novel μ-calpain-mediated apoptotic response.[8,10,11] In spite of the therapeutic potential of β-lap, its low solubility (0.04 mg/mL[12]) severely limits its applications *in vivo*. Moreover, our initial work with β-lap and other hydrophobic agents (e.g. dexamethasone[6]) have shown that direct incorporation of hydrophobic drugs into PLGA matrices leads to an extremely slow release of small quantities of drugs that may limit drug availability throughout the tumor treatment.

Research from many groups has shown that drug complexation with cyclodextrins (CDs) can effectively enhance the solubility and bioavailability of otherwise water insoluble drugs.[13,14] CDs are cyclic oligosaccharides in the form of truncated cones consisting of a hydrophobic core and a hydrophilic outer surface.[15,16] These oligosaccharides have the ability to form inclusion complexes with drug molecules (Fig. 1), which subsequently increase the apparent water solubility of the drug. CDs are differentiated mainly in the number of glucopyranose units (6, 7, and 8 for α,β, and γ-CD, respectively).[15–17] Hydroxypropyl-βcyclodextrin (HPβ-CD) is obtained by treating a base-solubilized solution of β-CD with propylene oxide, resulting in a CD with greater solubility (~500 mg/mL vs. 18.5 mg/mL for β-CD). Our laboratory recently utilized CDs to solubilize β-lap in aqueous solutions, and we were able to

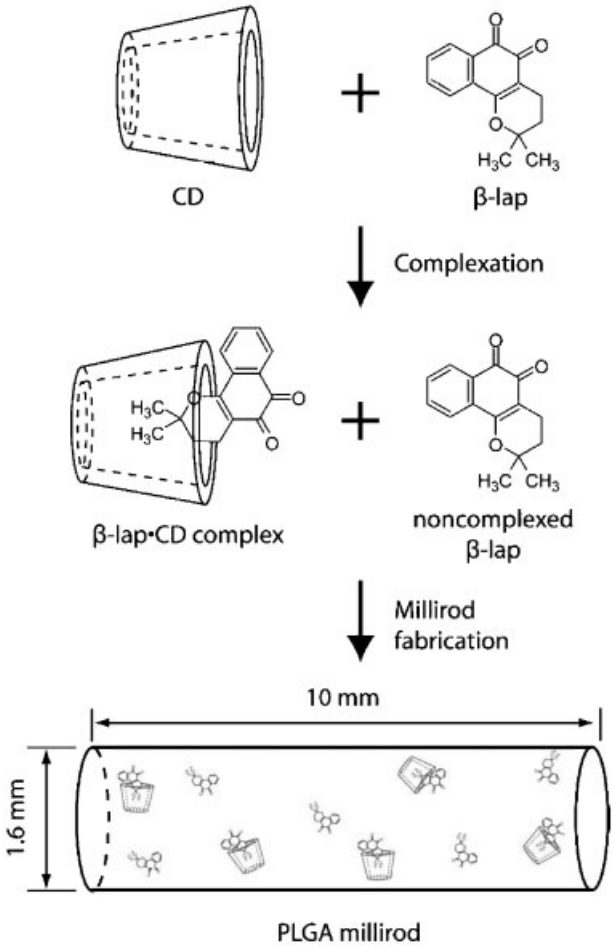

Figure 1. Schematic of β-lap · CD complexation, and incorporation of β-lap and β-lap · CD complex into the polymer millirod.

determine the binding affinities of the drug with the different CDs.[12] HPβ-CD and β-CD had the highest binding affinity to β-lap ($K_c = 1.1 \times 10^3$/M), followed by γ-CD and α-CD (160/M and 20/M, respectively). In the case of β-lap complexed with HPβ-CD, a 400-fold increase in drug solubility was observed (16.0 mg/mL compared to 0.04 mg/mL), with increased β-lap solubility also arising through complexation with γ-, β-, and α-CD.[12]

In the present work, we formed solid-state inclusion complexes of β-lap with different CDs and examined the interaction of free drug (as well as complexed β-lap) with PLGA. We hypothesized that CDs with different complexation affinities toward β-lap would provide an effective strategy to modulate the release kinetics of β-lap from PLGA millirods. To test this hypothesis, differential scanning calorimetry (DSC) experiments were

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performed to determine the degree of interaction between the polymer and drug in the presence and absence of different types of CDs. Release studies of various β-lap · CD complex formulations were then conducted to examine the influence of different polymer-drug interactions on release kinetics. Data from this study show that drug incorporation within the CD core prevents the drug from molecular dissolution within the PLGA matrix, which in turn, results in a dramatic increase in release from the polymer depot.

MATERIALS AND METHODS

Materials

PLGA (lactide:glycolide = 50/50, MW 50000 Da, inherent viscosity 0.65 dL/g) was purchased from Birmingham Polymers, Inc. (Birmingham, AL). β-Lap was synthesized following a previously reported procedure.[10] α-CD, β-CD, γCD, and HPβ-CD were obtained from Cyclodextrin Technologies Development, Inc. (CTD, High Springs, FL) with >98% purity. Glucose anhydrous was obtained from Fisher Scientific (Pittsburgh, PA).

Preparation of β-lap · D Inclusion Complexes

Accurately weighed quantities of CDs (α-CD, γ-CD, and HPβ-CD) and β-lap were dissolved in a methanol/water mixture (3/1 v/v). The β-lap · β-CD complex was prepared by dissolving the drug and β-CD in a 1/2 methanol/water mixture (v/v). Following solvent evaporation, the mixture was resuspended in deionized water, stirred for 24 h, and lyophilized. The drug · CD complex was then ground to produce a fine powder and filtered through a 100 mesh sieve.

Preparation of β-Lap-Loaded PLGA Millirods

β-Lap-containing millirods were prepared by means of a compression-heat molding procedure previously described.[2] Briefly, the β-lap · CD complex powder and PLGA microspheres (5 μm) were accurately weighed and mixed with a mortar and pestle. The contents were then inserted into a Teflon tube with an inner diameter of 1.6 mm. The Teflon tube was placed inside a stainless steel mold, which was placed in an iso-temp oven at 90°C ± 2°C (Fisher Model 282A) for 2 h to allow for PLGA polymer annealing. A compression pressure of 4.6 MPa was applied by means of a copper weight during the annealing process. After 2 h, the polymer millirod was removed from the Teflon tubing and cut to a length of 10 mm for subsequent analyses. A schematic diagram of the complex formation and millirod preparation is shown (Fig. 1).

To examine the effect of formation of inclusion complex on β-lap release kinetics, millirods of four different formulations were fabricated. Formulation 1 consisted of a millirod composed of 40% β-lap · HPβ-CD complex (1.2% β-lap) and 60% PLGA with a molar ratio of β-lap and HPβ-CD of 1:5.5. Formulation 2 consisted of 1.2% β-lap mixed with 38.8% HPβ-CD (physical mixture from β-lap particles and HPβ-CD powder) and 60% PLGA, while Formulation 3 consisted of 1.2% β-lap mixed with 38.8% glucose and 60% PLGA. The final formulation, Formulation 4, consisted of millirods composed of 1.2% β-lap and 98.8% PLGA. To examine the effect of different CDs on release kinetics, millirods that consisted of β-lap complexed with α-CD, β-CD, γ-CD, and HPβ-CD were produced. All millirods share the same composition of 40% β-lap · CD complex (1.8% β-lap) and 60% PLGA. The molar ratios of β-lap and CD are 1:5, 1:4.5, 1:4, and 1:3.5 for α-CD, β-CD, γ-CD, and HPβ-CD, respectively.

Differential Scanning Calorimetry (DSC) Analysis

DSC measurements of the solid-state solubility of β-lap in PLGA were performed using a Shimadzu Differential Scanning Calorimeter (DSC-60, Columbia, MD) with samples under a nitrogen atmosphere. The procedure was adapted from a method previously published by Panyam et al.[18] to determine the solid-state solubility of dexamethasone in PLGA. Briefly, known quantities of β-lap (10.8 mg) and PLGA (19.5 mg) were separately dissolved in methylene chloride. Different amounts of drug were mixed with polymer, and transferred to aluminum pans. The solvent was then allowed to evaporate, and the pans were crimped and weighed. Samples were heated to 180°C at a heating rate of 10°C/min. The heats of melting of β-lap were obtained using the peak integration calculation method provided by the DSC software. The solid-state solubility value of β-lap was determined by plotting enthalpy values as a function of β-lap loading percentage. The X-intercept resulting from a linear regression of the data represents the percentage solubility value of β-lap in PLGA.

DSC analyses of CD · drug complex or physical mixture, as well as drug or drug · complex powder with PLGA microspheres, were carried out using a Perkin Elmer Differential Scanning Calorimeter (DSC-7, Boston, MA) with samples under a nitrogen atmosphere. Contents were weighed (approximately 10 mg) and placed in an aluminum pan. For analysis of the CD/drug complex or physical mixture, data was recorded from 20°C to 180°C at a rate of 10°C/min. In drug or drug · complex powder mixed with PLGA microspheres, the samples were pretreated at 90°C for 2 h before DSC analyses to mimic the millirod fabrication process. The sample was cooled to 20°C before a subsequent heating run from 20 to 180°C at a rate of 10°C/min. Values for glass transition temperature (Tg) and melting enthalpy were obtained using a half Cp extrapolation method and peak integration calculation method, respectively.

In vitro Release Characterization of β-lap from Polymer Millirods

Millirods were placed in glass scintillation vials that contained 10 mL phosphate buffered saline (PBS) at 37°C. The sample vials were placed in an orbital shaker (C24 model, New Brunswick Scientific, Edison, NJ) with a rotating speed of 100 rpm. At different times, the millirod was removed from the solution and placed into a new scintillation vial containing 10 mL of fresh PBS at 37°C. The concentration of released β-lap was measured using a UV-Vis spectrophotometer (PerkinElmer, Lambda 20 model, Boston, MA) at the maximum adsorption wavelength of the drug ($\lambda_{max} = 258.0$ nm). The percentage of cumulative β-lap released was obtained by normalizing the released amount to the total amount loaded in the millirods. All experiments were performed in triplicate ($n = 3$).

SEM Analysis of Millirod Microstructure

Scanning electron microscopy (SEM) was used to characterize β-lap-loaded millirod morphology. Millirods pertaining to all three previously mentioned formulations were placed in 10 mL of distilled water at 37°C for 12 h, after which time they were freeze-dried overnight. Millirods were then cut into two sections for both lateral and cross-sectional analyses. A thin film of palladium (Pd, ~2 nm in thickness) was sputter-coated to minimize electron charging on the sample surface. A voltage of 5 kV was used during sample examination with a Hitachi S-4500 scanning electron microscope (Hitachi, Ltd., Tokyo, Japan).

RESULTS

DSC Characterization of β-lap · CD Complexes

Figure 2A shows the DSC thermograms of β-lap complexed with HPβ-CD. Pure β-lap had a melting point of 159.7°C, with a melting enthalpy (ΔH°_{m}) of 94.1 J/g. When β-lap was complexed with HPβ-CD at molar fractions of β-lap ranging from 67% to 33%, a decrease in melting enthalpy was observed (Fig. 2B). At 67% and 50% molar fraction, lower melting points were also observed at 156.3°C and 155.5°C, respectively. The values

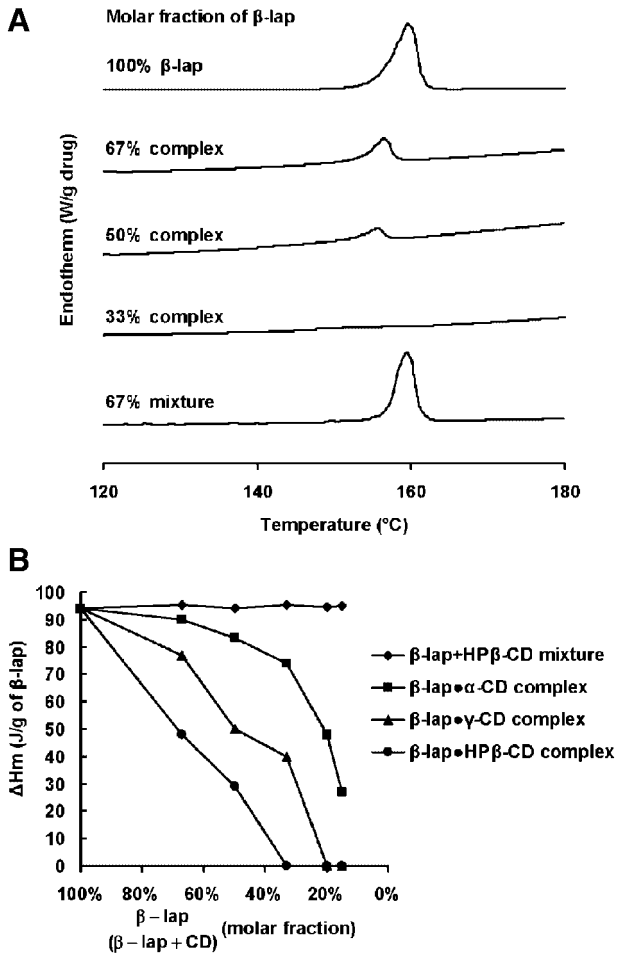

Figure 2. DSC thermograms of β-lap complexed or physically mixed with CDs. Figure A depicts DSC thermograms of β-lap complexed or mixed with HPβ-CD at various molar fractions of β-lap. Figure B shows β-lap melting enthalpy (ΔH_m), when complexed or mixed with CDs, as a function of drug molar fraction.

of ΔH_m for β-lap recorded at these two molar fractions were 48.1 and 29.2 J/g, respectively, and were used to determine the percentage of β-lap in crystalline form ($\chi_{\beta\text{-lap}}$) using the following equation:

$$\chi_{\beta-\text{lap}} = \frac{\Delta H_m}{\Delta H_m^\circ} \times 100 \quad (1)$$

where ΔH_m is the recorded enthalpy of the β-lap when complexed with CD and ΔH_m° is the enthalpy of β-lap alone. Utilizing Eq. 1, we found that the percentage of β-lap in the crystalline form at 67% and 50% molar fractions was 51% and 31%, respectively. Also evident in Figure 2A was the disappearance of the β-lap crystalline peak at a molar fraction of 33%. In stark contrast to the β-lap · HPβ-CD complex, the physical mixture of the drug with HPβ-CD at a β-lap molar fraction of 67% had an enthalpy similar to that of pure β-lap (Fig. 2A), with the drug melting peak occurring at the same temperature (159.2°C for the mixture compared to 159.7°C for the pure drug). In fact, the values of ΔH_m for the β-lap and HPβ-CD mixture remain constant at varying molar fractions of the drug, with approximately 100% of the drug in the crystalline form (Fig. 2B).

The enthalpy data for inclusion complexes formed with α and γ-CD is shown in Figure 2B. Similar to the HPβ-CD · drug complex, the values of ΔH_m decreased with decreasing molar fraction of the drug, with the β-lap · γ-CD complex showing a larger decline in drug crystallinity than the β-lap · α-CD complex. At a molar fraction of 20%, 50% of β-lap was in crystalline form in the β-lap · α-CD complex, whereas there was no drug in the crystalline form in the β-lap · γ-CD complex. Taken together, at the same molar fraction, the normalized enthalpy follows the order: HPβ-CD < γ-CD < α-CD.

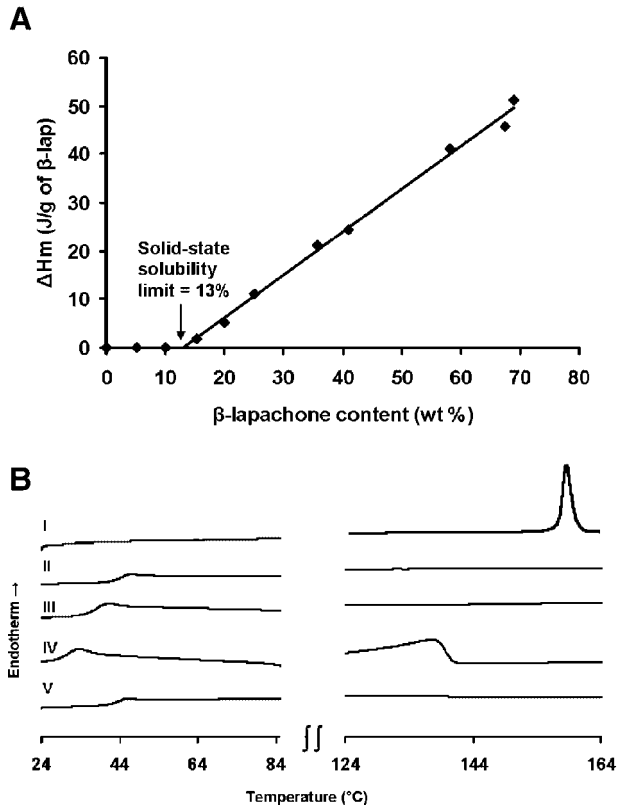

Figure 3. (A) β-Lap melting enthalpy (ΔH_m) as a function of β-lap loading percentage. The X-intercept indicates the solubility of β-lap in PLGA. (B) Effect of HPβ-CD complexation on the β-lap interactions with PLGA matrix. Thermograms representing the following: (I) β-lap alone; (II) PLGA alone; (III) 10% β-lap in PLGA; (IV) 20% β-lap in PLGA; and (V) β-lap · HPβ-CD complex (5.7% β-lap, 34.3% HPβ-CD) in PLGA.

Solubility of β-Lap in PLGA Polymers

In order to gain insight into interactions between β-lap and the PLGA polymer, solid-state solubility studies were carried out using DSC. Figure 3A plotted the values of ΔH_m (J/g) as a function of β-lap loading percentage. The X-intercept, provided by the linear regression of the data, yields the solid-state solubility of β-lap in PLGA, which was 13%. It should be noted that the β-lap melting temperatures were lower than that of pure drug alone (data not shown). The dissolution behavior of β-lap within the PLGA helps to explain the change in thermal properties of the polymer, and more importantly, discrepancies in subsequent release studies.

DSC Characterization of β-Lap Interactions with PLGA Polymer

As previously described, the millirod fabrication process involves heat treatment in order to allow for polymer annealing. This process may lead to interactions between drug and various components of the millirod, specifically between the polymer and the drug. To determine these interactions, the millirod preparation process was mimicked and DSC analyses conducted.

The PLGA polymer has a glass transition temperature of 44°C,[19] observed in thermogram II in Figure 3B. However, when 10% β-lap was

physically mixed with PLGA, the Tg of the polymer decreased to 35.4°C (III). A further decrease in glass transition occurred when drug loading was increased to 20%, yielding a Tg of 31.7°C (IV). Since a β-lap loading of 20% surpassed the solid-state solubility limit in PLGA, the drug melting peak shifted to a lower temperature ($T_m = 138$°C) as observed in the endotherm. When β-lap (5.7%) was complexed with HPβ-CD (34.3%), the Tg of the PLGA was not affected as shown in thermogram V, in which the glass transition of the polymer was approximately 44°C.

β-Lap Release from Polymer Millirods

Figure 4 illustrates the effect of formation of inclusion complexes on β-lap release kinetics from PLGA millirods. First, release of free β-lap from PLGA millirods was extremely slow, with only $8.8 \pm 1.2\%$ of the drug released after 22 days (Fig. 4A). When an excipient molecule, such as glucose, was added to the polymer millirod, the release of drug was faster, but the amount released was not dramatically increased ($37.0 \pm 2.0\%$ after 22 days). Significant facilitation of β-lap release was observed when the drug was either physically mixed or complexed with HPβ-CD. Approximately $55 \pm 2.2\%$ of the drug was released after 2 days when HPβ-CD was included in the millirod as an excipient molecule (Fig. 4A). In comparison, when β-lap was complexed with HPβ-CD, $79.6 \pm 2.1\%$ of the drug was released after the first 2 days, yielding the fastest release among the different formulations. Figure 4B highlights the difference in average release rates among the different millirod formulations. In the first two time periods, the average β-lap release rate was significantly faster for millirods containing β-lap complexed with HPβ-CD, with the release rate decreasing during the 12–48 h time period. Average release rates from millirods composed of β-lap mixed with HPβ-CD, as well as from millirods containing glucose as an excipient, remained fairly constant throughout the time periods, demonstrating a more sustained pattern of release. The release rate of millirods with drug alone was minimal throughout the time periods, reflecting the slow release of β-lap from these millirods.

β-Lap complexation with different CDs leads to modulated differences in drug release kinetics (Fig. 5). While all the release profiles showed a noticeable improvement over free β-lap (Fig. 5A), α-CD provides the slowest release rates among all

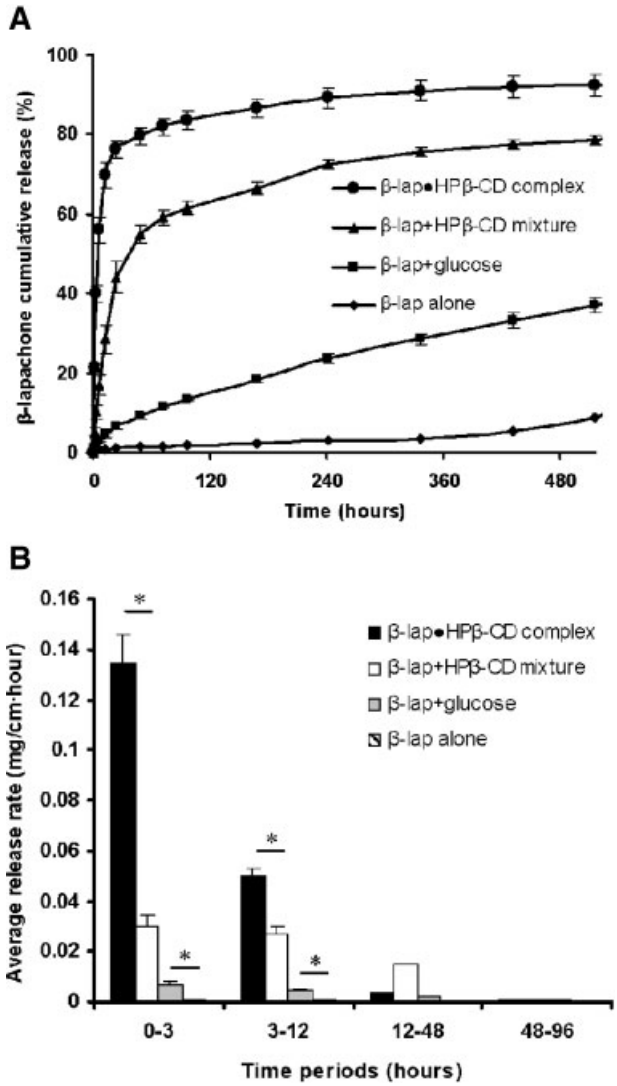

Figure 4. (A) Cumulative release of β-lap from millirods that contained β-lap (1.2%) either complexed or physically mixed with HPβ-CD, and in the absence or presence of glucose. (B) Average release rates of β-lap from the millirods in (A) during various time periods. Error bars were calculated from triplicate samples. Statistical analysis between groups was performed using a Student's two-tailed t-test ($^*p < 0.05$).

the CDs. After 22 days, $66 \pm 4.6\%$ of the drug was released, which was less than the amount of drug released (~90% after 22 days) when β-lap was complexed with γ-, β-, or HPβ-CD. Figure 5B compares average drug release rates during different initial time periods. β-Lap complexed with HPβ-CD demonstrated the fastest release rate in the first 3 h. When β-lap was complexed with β-CD, drug release lacked the initial high burst with approximately half the release rate in

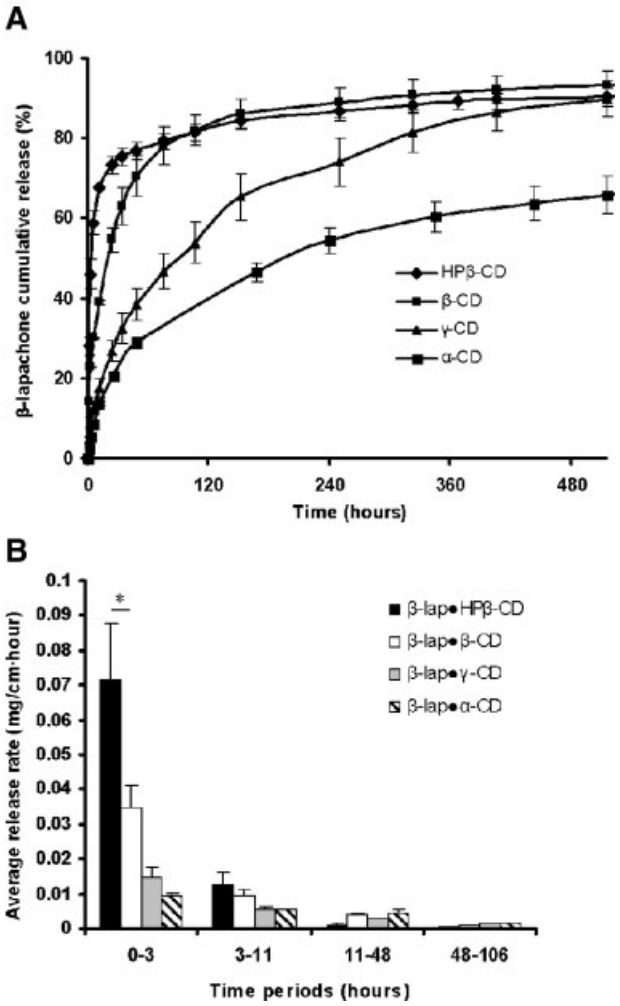

Figure 5. (A) Cumulative release of β-lap from millirods when the drug (1.8%) was complexed with different types of CDs at the same CD loading density (38.8%). (B) Average release rates of β-lap during various time periods. Error bars were calculated from triplicate samples. Statistical analysis between groups was performed using a Student's two-tailed *t*-test (*$p < 0.05$).

the initial 0–3 h-period. The release kinetics from β-lap · γ-CD millirods was much more sustained ($38.8 \pm 4.0\%$ of the β-lap released after 48 h) than in the previous examples.

Morphology Studies of Millirods before and after β-Lap Release

In an effort to examine the disparity in β-lap release from different millirod formulations as shown in Figure 4, SEM images were obtained from millirods that were incubated in PBS for 12 h. Following incubation in PBS, the cross-sectional morphology of a millirod containing β-lap alone appeared very smooth (Fig. 6A). In contrast, when β-lap was complexed with HPβ-CD and incubated for 12 h, there was a significant formation of pores in the millirod microstructure (Fig. 6B), a result akin to millirods containing noncomplexed β-lap, glucose, and PLGA (Fig. 6C). As observed from these figures, the latter two millirod formulations were both morphologically similar, with comparable porosity in the PLGA matrix.

DISCUSSION

Solid-State Inclusion Complex Formation Highlights Differences in Complexation Efficiency among CDs

Inclusion complexes of β-lap and four CDs (α-CD, β-CD, γ-CD, and HPβ-CD) in aqueous solution were investigated previously by our laboratory. In the previous report, we demonstrated the formation of a 1:1 inclusion complexes through hydrophobic binding of the dimethyl moiety of β-lap inside the CD cavity.[12] More importantly, an enhanced water solubility of β-lap (16 mg/mL as compared to 0.04 mg/mL) was observed following HPβ-CD complexation. In order to incorporate these complexes into PLGA millirods, it was necessary to form solid-state inclusion complexes of the drug with CD. In the present study, we demonstrated that β-lap and CD physical mixtures showed almost no interaction, as evidenced by the presence of a drug melting peak and near 100% crystallinity of drug (Fig. 2B), even at high molar fractions of CD. Upon formation of a solid-state complex, a decreased drug melting peak appeared, indicating a decline in melting enthalpy and loss of β-lap in its crystalline phase. This phenomenon was apparent at 67% and 50% molar fractions of β-lap (Fig. 2A). Moreover, at lower molar fractions of β-lap (33%), the drug's melting peak disappeared altogether, indicating inclusion of the drug within the CD cavity and formation of the complex. These data agreed well with previously published reports by Manosroi et al.[20] and Jug et al.,[21] in which the absence of melting endothermic peaks of azelaic acid and piroxicam, respectively, indicated the incorporation of these agents within the HPβ-CD cavity.

From the DSC data, a significant difference in solid-state binding affinities with β-lap was demonstrated by different CDs. These differences in interactions observed between β-lap and the

distinct CDs were previously observed,[12] and are due primarily to the cavity size of the CD. β-Lap has a low binding affinity to α-CD mainly because the drug molecule cannot fit in the small hydrophobic cavity (diameter ~ 5 Å). Increased binding affinities are observed in β- and γ-CDs because of their larger cavity size (diameter ~ 6.25 Å and 7.9 Å, respectively). HPβ-CD and β-CD are better hosts than γ-CD for β-lap because their cavity size is small enough to allow for intramolecular bonding, which in turn endows the CDs with more rigidity (i.e., a more suitable host environment). γ-CD, on the other hand, has a larger cavity size and is more flexible, and binding of β-lap inside the hydrophobic cavity leads to decreased solubility of the inclusion complex.[12] In summary, HPβ-CD and β-CD had a higher efficiency of forming a complex with β-lap, followed by γ-CD, which in turn was superior to α-CD.

β-Lap Complexation with CDs Led to Modulation of Release Kinetics

DSC analysis showed that β-lap had a solid-state solubility of 13% in PLGA matrix (Fig. 3A). Below this limit, β-lap forms a homogeneous molecular-level mixture inside the PLGA matrix. For β-lap and many other hydrophobic drugs, this formulation from a binary mixture typically leads to extremely slow drug release kinetics due to limited water penetration into the hydrophobic millirod (as corroborated by the limited porosity observed by SEM analyses in Figure 6A), favorable drug/polymer interactions, and low aqueous solubility of the drug (therefore a small concentration gradient across the polymer/solution interface). The above factors provide a reasonable explanation for the slow release of β-lap from PLGA millirods, where only $8.8 \pm 1.2\%$ of drug was released after 22 days.

Pore-forming hydrophilic molecules (e.g., glucose, NaCl) have been frequently used to facilitate drug release from polymer depot.[22,23] In this study, water dissolution of glucose led to an increase in porosity inside the PLGA matrix as evidenced by SEM analysis (Fig. 6C). Increased porosity effectively enlarged the aqueous contact surface area for β-lap release from the PLGA matrix, leading to faster release kinetics over the β-lap-loaded millirods without glucose. In spite of this increased porosity, favorable hydrophobic interactions between β-lap and PLGA matrix as well as the low water solubility of β-lap are still limiting factors for a facile drug release, where only

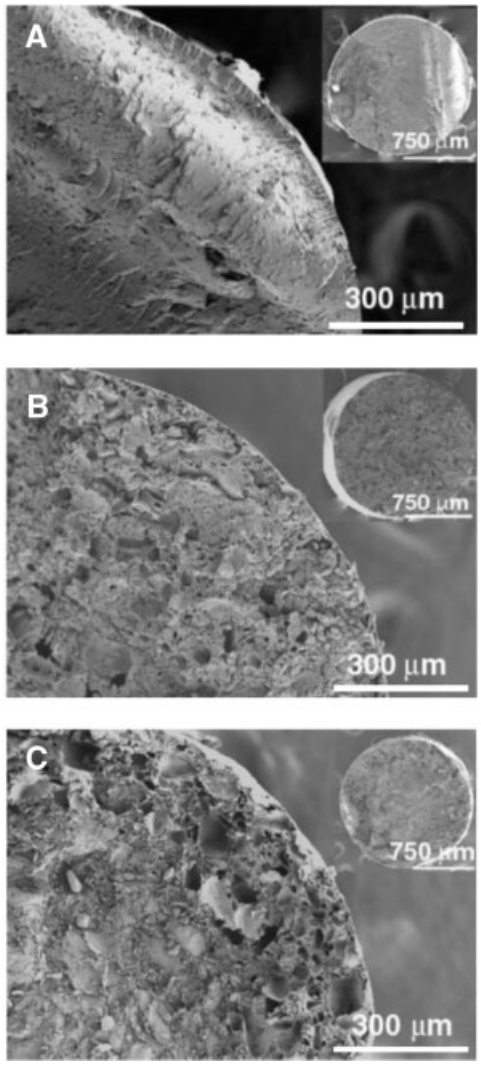

Figure 6. SEM analyses of microstructures of β-lap-loaded polymer millirods following 12 h incubation in PBS. Cross sections represent the following (A) β-lap alone (1.2% β-lap in 98.8% PLGA); (B) β-lap · HPβ-CD complex (1.2% β-lap, 38.8% HPβ-CD, 60% PLGA); (C) 40% glucose excipient (1.2% β-lap, 38.8% glucose, 60% PLGA). Figure insets represent SEM micrographs of the entire cross section of the millirod.

$37.0 \pm 2.0\%$ of β-lap was released after 22 days. The remainder of the drug was released when the PLGA matrix was clearly degraded (data not shown), leading to a "dose dumping" of drugs that is frequently observed with polyester-based drug delivery systems.[24–26]

Drug complexation with different CD molecules provides a useful strategy to control the release kinetics of hydrophobic drugs from a polymer depot. The faster release of the drug from

β-lap · HPβ-CD millirods can best be explained by a combination of factors. Due to the lack of interactions with the PLGA, the hydrophilic CD readily dissolves in aqueous environments given its solubility of 500 mg/mL.[12] After rapid release from the millirod, the CD leaves behind pores in the matrix, which facilitates water permeation into the PLGA matrix. This increased water permeation into the millirod results in a more rapid release of β-lap from the β-lap · HPβ-CD mixture millirods due to the increased porosity of the matrix. In addition to pore formation and rapid release of CD, instant *in situ* formation of drug inclusion complexes with the CD increases the water solubility of β-lap, enhancing drug release from millirods containing a mixture of β-lap and HPβ-CD. This release rate is much faster than that from glucose-loaded millirods despite their similar matrix morphology. However, the fastest release is seen in millirods containing drug that is complexed with HPβ-CD, due to rapid dissolution and the prevention of the formation of a molecular-level mixture between the drug and PLGA, as previously demonstrated by DSC. This solid-state solubilization of free β-lap in PLGA would otherwise hinder the drug from diffusing out of the hydrophobic environment provided by the PLGA microstructure. Panyam et al.[18] showed that dexamethasone was soluble in PLGA nanoparticles, which in turn increased drug encapsulation but decreased release kinetics. Previous studies by Miyajima et al.[27] also showed that basic drugs interacted with PLGA by shielding the carboxyl residues of the matrix, making the millirod more rigid and less hydrophilic, affecting both polymer erosion and drug diffusion. Therefore, the rapid solubilization and diffusion of the β-lap · HPβ-CD complex, as well as the absence of drug interaction with PLGA, leads to a faster release of β-lap from millirods.

It is important to note that the rate of drug release was also determined by CD complexation efficiency and the solubility of the CD. The fastest release occurred when the drug was complexed with HPβ-CD, while the slowest release occurred when β-lap was complexed with α-CD, which had the lowest binding affinity with the drug. The lower drug release rate resulting from the β-CD formulation compared to the HPβ-CD formulation stems primarily from its considerably lower water solubility (18 mg/mL for β-CD vs. 500 mg/mL for HPβ-CD). The slower drug release kinetics observed in the γ-CD formulation is most likely caused by its lower binding constant (~160/M), which may lead to an increase in the molar fraction of the drug "dissolved" in the polymer matrix.

A more comprehensive understanding of the different kinetic processes during drug release is necessary to rationally design optimal millirod formulations with controllable release kinetics. Key factors may include distribution of drug among different states (e.g., "dissolution" state within PLGA matrix, complexed state with CDs, or crystalline state), drug binding constants with different types of CDs, solubility and diffusivity of drug · CD complexes, dissolution kinetics of drug and drug · CD complexes, pore formation, and water permeation rates inside the polymer. This is clearly a complex system and a mathematical model that incorporates the aforementioned processes is currently under development in order to provide a quantitative understanding of the drug release behavior from the polymer implant system.

Implications for Intratumoral Drug Delivery

The long-term goal of this research is to design β-lap-loaded polymer millirods with optimal release rates of β-lap for intratumoral drug delivery applications. Based on the results from this study, we found that an effective modulation of β-lap release rates was achieved through complexation with different CDs. For therapeutic applications where an initial "burst" release is desirable, a millirod formulation with β-lap · HPβ-CD complex can be implemented to provide a maximal drug release rate in the first 3 h (Figs. 4B and 5B). In contrast, to examine the influence of prolonged drug release on therapeutic efficacy, the use of other CD molecules (e.g., γ-CD or α-CD) can provide a more moderate, sustained release of drug. In addition to their abilities in modulating drug release kinetics, CD complexation may also prove advantageous in that they it may facilitate drug penetration inside tumor tissues. A high initial drug concentration will bring about tumor cell apoptosis more rapidly, reducing the cell density and increasing subsequent drug distribution.[28] Additionally, β-lap is a small and hydrophobic molecule such as carmustine (BCNU) that can be easily eliminated by blood perfusion through capillaries in solid tumors.[29] A stable inclusion complex with a hydrophilic excipient, such as CD, may prevent this loss through an increase in molecular weight.[29] This would lead to longer retention times in the tumor and longer penetration distances. Current work is in

progress to evaluate the antitumor efficacy of PLGA millirods with different β-lap-CD complexes in solid tumors.

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