AAPS PharmSciTech

AAPS PharmSciTech 2000; 1(4) article 32
(http://www.pharmscitech.com/)

Effect of Isopropyl Myristic Acid Ester on the Physical Characteristics and In Vitro Release of Etoposide from PLGA Microspheres

Submitted: April 3, 2000; Accepted: November 10, 2000

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ABSTRACT The purpose of this paper was to study the effect of the isopropyl myristic acid ester (IPM) on the physicochemical characteristics of etoposide-loaded poly(lactic-co-glycolic acid) (PLGA) microspheres-specifically, the effects on the size and drug loading of the microspheres, the polymer matrix and surface morphology, and the release of etoposide from the microspheres. The experiment was structured to examine 2 IPM concentrations (25% and 50%) and 1 control (no IPM) at 2 different etoposide-loading percentages (10% and 5%). The microspheres were prepared using a single-emulsion solvent-extraction procedure. Samples from each batch of microspheres were then analyzed for size distribution, drug-loading efficiency, surface characteristics, in vitro release, and in vitro microsphere degradation. The incorporation of 50% IPM significantly increased ($P < .05$) the size of the microspheres when compared with the control and 25% IPM microspheres. However, incorporation of 25% or 50% IPM did not change ($P > .05$) the drug-loading efficiency in comparison with the microspheres prepared without IPM. The microspheres containing 50% IPM were shown to significantly increase ($P < .05$) the release of etoposide from the microspheres at both etoposide concentrations. The microspheres prepared incorporating 25% IPM and 5% etoposide increased the in vitro release ($P < .05$) in comparison with the microspheres prepared without IPM. The 5% etoposide-PLGA microspheres showed a smooth, nonporous surface that changed to a dimpled, nonporous surface after addition of 25% IPM. During the in vitro degradation study, the IPM-containing microspheres slowly became porous but retained their structural integrity throughout the experiment.

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KeyWords: Etoposide, PLGA microspheres, Isopropyl myristic acid ester, In vitro release, Scanning electron microscopy.

INTRODUCTION

The standard chemotherapy regimen in use today involves introducing a large systemic dose of a cytotoxic agent. Although this dose is effective in creating local therapeutic conditions toxic to cancer cells, the concurrent high systemic level leads to the death of normal cells. A targeted delivery system would improve the treatment regimen by increasing local concentration and concurrently decreasing systemic concentration, which would lead to decreased incidence of side effects, increased efficacy of the drug, and improved patient compliance [1]. Microparticles have been used for drug delivery and targeting [2-4]. Passive targeting of microspheres can exploit the fact that particles reasonably large in size (more than 7 μm) are retained after intravenous (IV) administration by mechanical entrapment in the lung's capillary beds [5, 6]. This process has been employed for many years in diagnostic imaging [7]. It also has been used to treat respiratory diseases and cancer [8]. The drug-loaded biodegradable microspheres lodge within the capillary networks of the lung and release their bioactive agent into neighboring tissue by a process of diffusion and biodegradation [9].

Etoposide is effective in the treatment of acute myeloid leukemia, Hodgkin's disease, non-Hodgkin's lymphoma, lung cancer (small cell and nonsmall cell), AIDS-related Kaposi's sarcoma, gastric cancer, breast cancer, and ovarian cancer [10-12]. Research has shown that chemotherapy regimens that use etoposide are more effective when the drug is given over an extended period of time [10, 13]. For the past decade, poly(lactic-co-glycolic acid) (PLGA) has been used

extensively for controlled drug delivery systems [14-19]. In general, the bulk erosion mechanism has been considered the main degradation pathway for PLGA [20]. The mechanism and the rate of release are highly dependent on the physicochemical properties of the polymer and drug and on the properties of the microspheres such as size, drug content, and porosity. Additives in the microspheres can be used to modify the release profile of the active component. The literature reports several instances of using some plasticizers (tributyl citrate and glycerin) and fatty substances to increase the rate of release of drugs from microspheres [21-23]. The goal of the present research was to study the effect of isopropyl myristic acid ester (IPM) on the size, drug loading, surface characteristics, and in vitro release of etoposide from PLGA microspheres.

MATERIALS AND METHODS

Materials

PLGA 50:50 (IV = .61) was obtained from Birmingham Polymers Inc, Birmingham, AL, and etoposide from ICN Pharmaceuticals, Aurora, OH. The poly-vinyl alcohol, methylene chloride, and isopropyl myristic acid ester were obtained from Sigma Chemical Company, St Louis, MO. The isopropyl alcohol, Tween-80, methanol, acetonitrile, and acetic acid were obtained from Fischer Chemical Company, Fair Lawn, NJ.

Synthesis of Etoposide-Loaded PLGA Microspheres

A measured amount of etoposide and PLGA 50:50 was dissolved in 5 mL of methylene chloride. The solution containing drug and polymer was then dispersed in 10 mL of continuous phase (1% PVA unless otherwise noted) while being stirred at 1300 rpm (L4RT Homogenizer, Silverson Machine LTD, Chesham, United Kingdom) and maintained at 60°C for 10 minutes to evaporate the solvent. After 10 minutes, the heat was removed and stirring continued at a reduced rate of 500 rpm for 50 minutes to further evaporate the solvent. Residual methylene chloride was removed by a wash of 10% isopropyl alcohol (IPA), and the microspheres were collected by suction filtration (Aspirator Pump, Cole-Parmer, Chicago, IL) and washed 2 more times with 10% IPA. The microspheres produced with IPM were manufactured in the same manner with the added step of the addition of IPM to the initial drug-polymer solution.

Microsphere Size Determination

A sample from each microsphere batch was dry mounted on a microscope slide with a cover slip. A Cole-Parmer Video Caliper (Model 49910-20, Cole-Parmer, Chicago, IL) under a X10 lens (Meiji Microscope, Osaka, Japan) was used to size a representative number of microspheres (100). The video caliper is a microprocessor-controlled video-based reticle generator that is projected onto a standard video monitor (CT-2086YD, Panasonic, Sacaucus, NJ). The caliper system was calibrated and used to size microspheres projected onto the video monitor through the video microscope system. Microspheres were photographed using a Polaroid Microcam (331 film, Polaroid Corporation, Cambridge, MA) at a magnification of X40.

Scanning Electron Microscopy

The shape and surface morphology of the microspheres were examined with a scanning electron microscope (SEM) (JOEL SEM, Peabody, MA). Dry microspheres were mounted on an adhesive stub and then coated with gold palladium under vacuum using a ion coater. The coated specimen was then examined under the microscope at 10 kV and photographed. The degradation of microspheres during the in vitro release studies was also examined. The microspheres were sampled at 0, 10, 20, and 30 days during an in vitro release test and the surface characteristics were studied by SEM as described above.

Drug Content

A measured amount (≈ 5 mg) of microspheres was dissolved in 1 mL of methylene chloride, to which 5 mL of methanol was added to precipitate the polymer. The resultant solution was then centrifuged (RC-5 Superspeed Centrifuge, Sorvall, Newtown, CT) at 7000 rpm (SS-34 Rotor, Sorvall, Newtown, CT) for 10 minutes. The supernatant was then collected and analyzed by high-performance liquid chromatography (HPLC).

In Vitro Release

A known amount of microspheres (≈ 2 mg) was added to a standard screw-top test tube. Ten milliliters of phosphate buffered saline (pH 7.4) containing 0.1% Tween-80 was added to each tube. Tween-80 was added to the dissolution fluid to maintain sink condition for etoposide during in vitro release. The samples were incubated in a water bath (Model 50 Reciprocal Shaking Water Bath, Precision Scientific, Winchester, VA) at 37°C, shaken at 100 rpm, and sampled at selected time intervals (1, 5, 10, 15, 20, 25, and 30 days). The samples were then centrifuged at 2000 rpm for 1 minute, and the supernatant was removed. Next, the remaining microspheres were washed in 5 mL of distilled water, centrifuged at 2000 rpm for 1 minute, and the supernatant removed. Finally, the samples were frozen at -80°C and lyophilized overnight. The microspheres were then analyzed for residual drug content by HPLC. The amount of etoposide released was then calculated by subtracting the residual content from the initial amount present in the microspheres.

HPLC Method for Determination of Etoposide

The method of Chow et al [24] with slight modifications was used to analyze the content of etoposide in the sample. The samples were analyzed using a Shimadzu HPLC system (Shimadzu Scientific Instruments, Inc, Kyoto, Japan) consisting of an SPD-10AV UV detector, SCL-10A system controller, SIL-10A auto-injector, dual LC-10AD pumps, and a CR-501 integrator. A mobile phase of 70% H_2O:acetic acid (100:1)-30% acetonitrile passing through a 5 µm phenyl column (Phase Sep, Deeside, United Kingdom) at a flow rate of 1.4 mL/min. The effluent of the column was then analyzed for etoposide at a detection wavelength of 239 nm. The amount of etoposide in the sample was determined from the peak area correlated with a standard curve. The standard curve was determined from a best-fit line of peak area versus amount (r2 = .9999) of standard solutions (40, 20, 10, 5, 2.5, and 1.25 µg/mL) with a 10 µl injection. The detection limit of this method is 10 ng of etoposide on the column.

Data Analysis

Statistical comparisons were made using the Student *t*-test and analysis of variance. The level of significance was taken as $P < .05$.

RESULTS AND DISCUSSION

Table 1 shows the composition of batches used to examine the effect of IPM on the size and drug-loading efficiency. Batches of microspheres were prepared with 10% and 5% etoposide and with no IPM, 25% IPM, and 50% IPM. With respect to the 10% etoposide-loaded microspheres, the size was significantly increased with increased IPM concentration. The 25% (batch A2) and the 50% (batch A3) IPM-containing microspheres were significantly larger than the no IPM (batch A1) microspheres. This size difference is contrary to 2 reports in the literature that show no significant effect of IPM on the size of the microspheres [21, 23]. The drug-loading efficiency of the 10% etoposide-loaded microspheres was not significantly ($P > .05$) affected by an increase in IPM concentration. This finding is in accordance with published results that also suggest no relation between IPM concentration and drug-loading efficiency [21-23].

Table 1. The Effect of Isopropyl-Myristic Acid Ester on the Size and Drug-Loading Efficiency of Etoposide-Loaded PLGA Microspheres (n = 4)

Batch Designation	Polymer (mg)	Drug (mg)	Drug (% wt/wt)	IPM (%wt/wt)	Drug-Loading Efficiency [a] (mean ± SD)	Size (mean ± SD)
A1	200	20	10.0	0	58.41 ± 2.84	18.11 ± 1.59
A2	200	20	10.0	25	56.43 ± 3.20	20.19 ± 1.25*
A3	200	20	10.0	50	61.76 ± 7.92	21.76 ± 0.98**
B1	400	20	5.0	0	71.32 ± 5.78	25.92 ± 2.83
B2	400	20	5.0	25	73.51 ± 2.72	25.68 ± 1.94
B3	400	20	5.0	50	74.09 ± 3.05	27.96 ± 2. 38

[a] Drug-loading efficiency = Drug loading actual / Drug loading theoretical x100. * Indicates significant difference ($P < .05$) from microspheres with no IPM.** Indicates significant difference ($P < .05$) from microspheres with no IPM and 25% IPM.

The effect of IPM on the release of etoposide from 10% etoposide-loaded PLGA microspheres is shown in **Figure 1**. The IPM was shown to increase the release of etoposide from PLGA microspheres. The 50% IPM microspheres significantly increased ($P < .05$) the overall release of etoposide from both the no IPM and the 25% IPM microspheres. The release of etoposide from the 25% IPM microspheres was increased, but not significantly from the microspheres without IPM.

The effect of IPM on the size and drug-loading efficiency of 5% etoposide-loaded PLGA microspheres is shown in **Table 1**. The increase in IPM concentration had no effect on either the size or the drug-loading efficiency of the microspheres. The release of etoposide from the 5% etoposide-loaded microspheres is shown in **Figure 2**. The microspheres containing 25% and 50% IPM significantly increased ($P < .05$) the release of etoposide from the microspheres when compared to microspheres prepared without IPM.

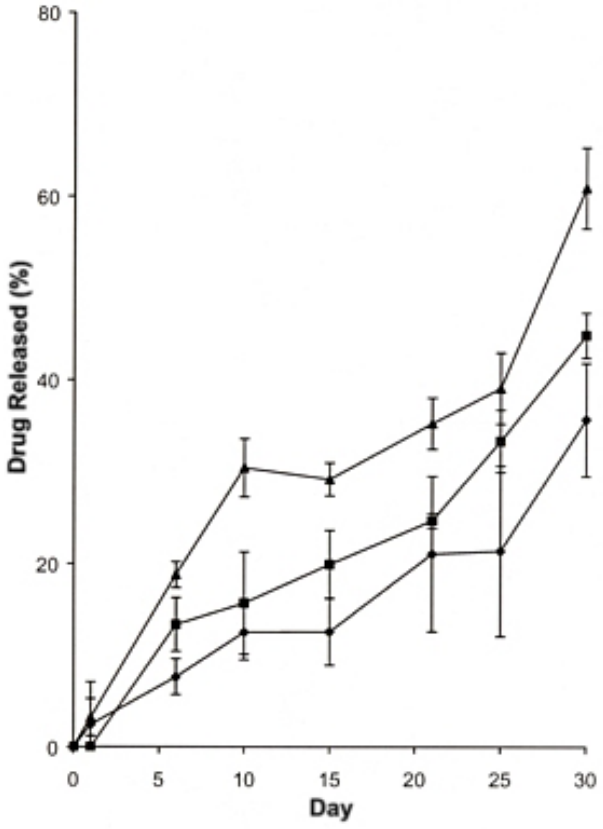

Figure 1. The effect of isopropyl myristic acid ester on the release of etoposide from 10% etoposide microspheres. Key: ◆no IPM, ■25% IPM, and ▲50% IPM.

The scanning electron micrographs of 5% etoposide-loaded microspheres without and with 25% IPM are shown in **Figure 3**. The 5% etoposide-loaded microspheres show a smooth, nonporous surface whereas the microspheres with IPM show a dimpled, nonporous surface (**Figure 3 A, B**). The microspheres following 30 days of degradation during the in vitro release studies show that the polymer matrix of both samples has significantly degraded (**Figure 3 C, D**). The 5% etoposide-loaded microspheres without IPM have broken down completely and the microspheres have lost most of their structural integrity (**Figure 3 C**). The microspheres containing IPM are also significantly degraded but retain their structural integrity (**Figure 3 D**).

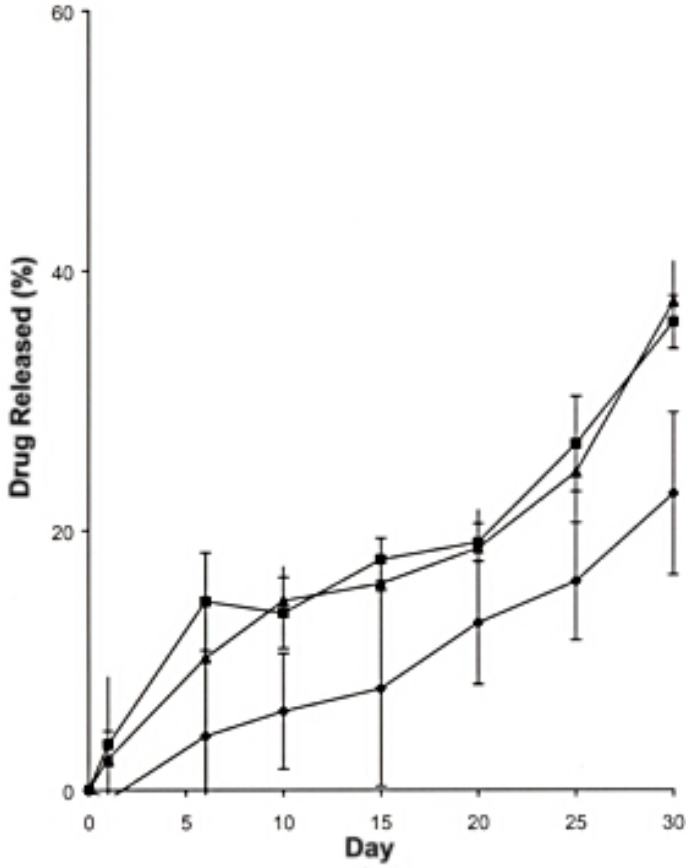

Figure 2. The effect of isopropyl myristic acid ester on the release of etoposide from 5% etoposide-loaded microspheres. Key: ◆no IPM, ■25% IPM, and ▲50% IPM..

The increased release of etoposide from the microspheres can be explained by the effect that the addition of IPM has on the polymer matrix and surface morphology of the microspheres. The release of etoposide can occur by diffusion through the polymer matrix, dissolution after solubilization in connected channels through the microparticle, and release after matrix degradation. As evidenced by SEM, the addition of IPM produced dimpled surface microspheres. During in vitro release, the dimpled surface became porous, providing greater surface area for drug release. The microspheres that do not have the added IPM released the drug by polymer erosion. The result is that the plain microspheres released less etoposide over a given time. It is also important to note that the microspheres produced with IPM maintained their structural integrity, whereas the plain microspheres did not. This structural integrity is an important consideration given the desire to target these microspheres to the lung and to entrap them for long periods based on their size. Thus, the plain etoposide microspheres without IPM did not become porous during in vitro release, and therefore did not provide greater surface area for drug release than microspheres prepared with IPM.

CONCLUSION

In conclusion, the release of etoposide from the microspheres proved to be unsuitable for a therapeutic dosage regimen. A fatty acid ester, IPM, was used to increase the release of etoposide from the microspheres. The incorporation of 50% IPM significantly increased ($P < .05$) the size of the microspheres, but did not affect the drug-loading efficiency. The release of etoposide from the microspheres was increased by the addition of 50% IPM. The addition of IPM to microspheres changed their surface characteristics from smooth and nonporous to dimpled and nonporous. The changes in surface characteristics of microspheres during in vitro release explain the difference in the release profiles between the plain and the IPM-containing etoposide microspheres. Thus, we have prepared etoposide-loaded PLGA microspheres with an appropriate physicochemical characteristic suitable for passive targeting to the lung. Further studies are warranted to examine the behavior and the effectiveness of the system in vivo, however.

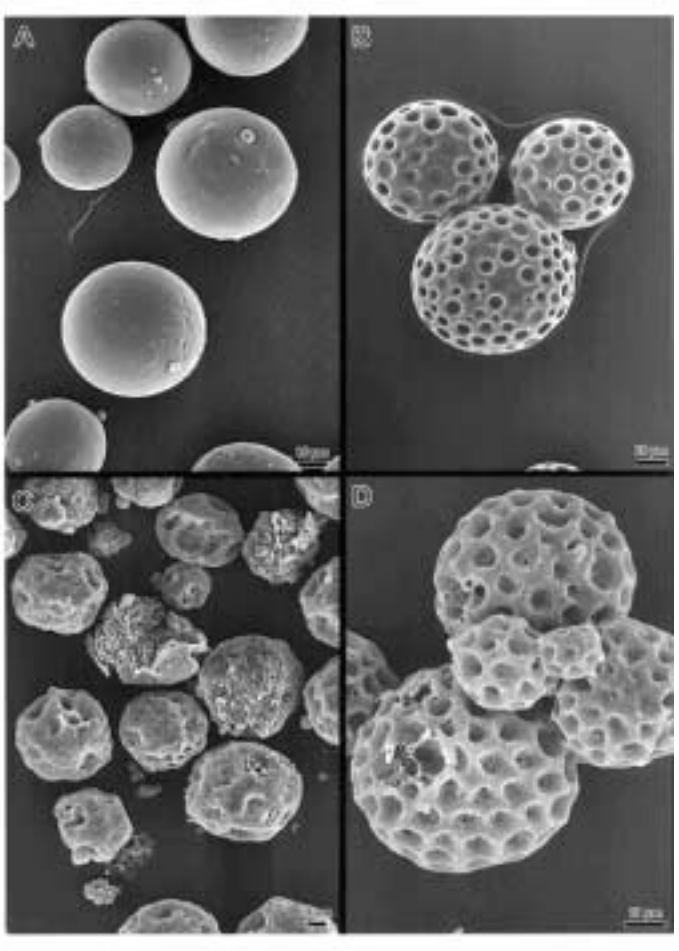

Figure 3. The effect of in vitro degradation on microsphere surface morphology. (A) 5% etoposide microspheres (B1); (B) 5% etoposide microspheres containing 25% IPM (B2); (C) surface characteristics of microspheres (B1) after 30 days of in vitro release; and (D) surface characteristics of microspheres (B2) after 30 days of in vitro release.

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