Drug Development and Industrial Pharmacy, 28(3), 339–344 (2002)

COMMUNICATION

Development of Biodegradable Microcapsules as Carrier for Oral Controlled Delivery of Amifostine

T. K. Mandal,* L. A. Bostanian, R. A. Graves, S. R. Chapman, and I. Womack

College of Pharmacy, Xavier University of Louisiana, New Orleans, Louisiana 70125

ABSTRACT

The primary objective of this project was to develop a biodegradable, orally active controlled-release formulation of amifostine. Development of such a formulation will mark an important advancement in the areas of chemoprotection and radioprotection. Biodegradable microcapsules of amifostine were prepared using poly(lactide/glycolide) (PLGA 50:50). The microcapsules were prepared by solvent evaporation technique. Amifostine-loaded microcapsules were evaluated for particle size, surface morphology, thermal characteristics, and drug release. Particle size and surface morphology were determined using scanning electron microscopy (SEM). Thermal characterization was conducted using differential scanning calorimetry (DSC). In vitro release study was performed at 37°C using phosphate buffer (pH 7.4). Amifostine release was calculated by measuring the amount of drug remaining within the microcapsules at a specific sampling time. The amount of amifostine in the samples was determined by high-performance liquid chromatography (HPLC) using an electrochemical detector. The yield of microcapsules was 75%. Scanning electron microscopy pictures revealed that the particles were nearly spherical and smooth with an average size of 54 μm. Differential scanning calorimetry thermograms showed that microcapsules loaded with amifostine have a glass transition at 39.4°C, and the melting endotherm of amifostine was absent. The absence of a melting endotherm for amifostine was an indication that amifostine was not in the crystalline state in the microcapsules, but rather in the form of a solid solution in PLGA. Approximately 50% amifostine was released during the first 6 hr of the in vitro release study.

*Corresponding author. E-mail: tmandal@xula.edu

The drug, however, continued to release over the observed period of 12 hr during which 92% amifostine was released.
Key Words: *Amifostine; Microcapsules; Controlled delivery; Biodegradable*

INTRODUCTION

Amifostine (Ethyol: Alza Pharmaceuticals, Palo Alto, CA/U.S. Bioscience, West Conshohocken, PA) is an organic thiophosphate, which has been studied extensively as a cytoprotective agent. Amifostine has demonstrated the ability to protect the kidney against the cytotoxicity of repeated doses of cisplatin without interfering with the desired anticancer effects of the latter (1). The drug has recently been approved by the U.S. Food and Drug Administration to reduce the renal toxicity associated with repeated administration of chemotherapy in patients with advanced ovarian cancer. Amifostine is being investigated further in other tumor types utilizing different chemotherapeutic combinations (2), as well as in radiation therapy (3). However, amifostine is not orally active (1), and must be injected in order to be effective. Amifostine is also rapidly cleared from the body and has a short distribution half-life of 0.9 min when administered as a bolus dose or as 15-min i.v. infusion (1,4,5). Attempts have therefore been made to develop various formulations of amifostine such as transdermal (6,7), pulmonary, and oral sustained-release microsphere (8) preparations. For example, a significant amount of amifostine absorption was observed following transdermal administration along with dimethyl sulfoxide (DMSO) as an absorption enhancer (6). Additionally, oral administration of ethyl cellulose microcapsules containing amifostine was also shown to provide radiation protection along with decreased toxicity in mice (8). It has thus been demonstrated in principle that an orally effective preparation of amifostine may indeed be possible.

The objective of the present investigation was to develop oral controlled-release formulations of amifostine using biodegradable polymer. Controlled-release formulations using biodegradable polymers have been developed for numerous therapeutic agents (9). The most widely investigated polymers are the aliphatic polyesters based on lactic acid and glycolic acid. These copolymers have attracted much attention because the biodegradation rate of the copolymer is easily controlled by altering its composition. These polymers have been used with numerous drugs for parenteral (10,11) as well as oral delivery (12,13). A wide variety of drugs have been successfully encapsulated into poly(lactide/glycolide) (PLGA) for controlled delivery (9).

MATERIALS AND METHODS

Chemicals and Reagents

The copolymer poly(DL-lactic/glycolic acid), PLGA 50:50 (RG 506; inherent viscosity 0.8) was obtained from Boehringer Ingelheim (Ingelheim, Germany). The surfactant, L-α phosphatidylcholine was obtained from Avanti Polar-lipids, Inc. (Birmingham, AL, USA). Amifostine, polyvinyl alcohol (PVA), chloroform, and dichloromethane were obtained from Sigma Chemical Co. (St. Louis, MO, USA).

Preparation of Biodegradable Microcapsules

Controlled-release biodegradable microcapsules of amifostine were prepared using poly(DL-lactic/glycolic acid) by the solvent evaporation technique (14). A specific amount (50 mg) of amifostine powder was dissolved in 400 μL of deionized water and then emulsified in 5 mL of dichloromethane containing 500 mg of PLGA. The polymer solution was previously mixed with 500 μL of lipophilic surfactant L-α-phosphatidylcholine in chloroform (10 mg/mL). The emulsification was carried out by sonication at output 4 (50 W) for 40 sec (ultrasonic probe, Sonic & Materials Inc., Danbury, CT). The resulting emulsion was further emulsified in 1 mL of an aqueous solution of PVA (1%) by vortexing for 25 sec and then diluted in 100 mL PVA aqueous solution (0.3%). The system was stirred magnetically (at 500 rpm) for 4 hr to allow complete evaporation of the solvent.

Amifostine microcapsules were finally collected by centrifugation at 3000 rpm and washed four times with deionized water to remove any residual PVA on the surface of the microcapsules. The

microcapsules were later freeze-dried (−70°C; 6×10^{-4} mbar) (Labconco, Kansas City, KS) to obtain a free-flowing powder. Each formulation was prepared in triplicate.

Thermal Analysis

Differential scanning calorimetry (TA DSC 2920, New Castle, DE) of PLGA, amifostine, unloaded microcapsules, and amifostine-loaded microcapsules was performed in order to characterize their physical state after microencapsulation. About 5 mg of a sample was weighed, crimped into an aluminum pan, and analyzed at a scanning rate of 3°C/min. The glass transition temperature (T_g) was calculated using TA universal analysis software by extrapolating the linear portion of the thermograms above and below the glass transition point and determining the midpoint.

Particle Size and Morphology

Size, morphology, and surface appearance of microcapsules were examined by scanning electron microscopy (SEM) (Amray AMR 1000A, Bedford, MA). Samples for SEM were mounted on metal stubs and coated with gold to a thickness of 200–500 Å. Pictures were taken and the microcapsule sizes determined according to a reference scale. Particle size distribution was also determined by a Coulter LS130 analyzer (Beckman Coulter Inc., Fullerton, CA). This technique measures the size of particles dispersed in a medium by the scattering pattern of a laser light shown through the medium. The size calculations assume the presence of spherical particles. Therefore, percentage volume distributions assume the volumes of spheres. The samples were analyzed in a water medium and the Frauenhofer method was utilized to calculate the size distributions. For each sample a background run of deionized water was performed. A sample of microcapsules (2 mg) was added to the deionized water in a micro-sample cell and counting was performed for 120 sec. After subtraction of the background, the particle size distribution calculation was performed.

Determination of Amifostine Content

The amount of amifostine present in microcapsules was determined by extraction into acidified water. Evaluation of the efficiency of the extraction procedure was performed in triplicate. Two milligrams of amifostine was thoroughly triturated with 100 mg PLGA. An aliquot (10, 20, and 30 mg, respectively) of the mixture was dissolved in 1 mL of dichloromethane. Amifostine, present in each sample, was extracted into 4 mL of the mobile phase (5 mM heptane sulfonic acid, 0.1 M chloroacetic acid, and 5% acetonitrile) by centrifugation for 15 min at 3000 rpm. The amount of amifostine in each sample was determined by high-performance liquid chromatography (HPLC).

Analysis

The analysis of amifostine was performed using a rapid and sensitive HPLC method (15). WR-149846 was used as the internal standard. The chromatography was performed using a μbondpack C-18 column; the mobile phase consisted of 5 mM heptanesulfonic acid and 5% acetonitrile in 0.1 M chloroacetic acid at pH 3.0; a flow rate of 1.2 mL/min; and electrochemical detection (Hg/Au electrode; oxidation potential set at +0.12 V). Retention times were 4.1 and 5.8 min for amifostine and WR-149846, respectively. Injection volume was 20 μL.

In Vitro Dissolution Studies

Dissolution studies of microcapsules were performed by measuring the percentage remaining (of amifostine) within the microcapsules at a predetermined sampling time. For each formulation, 15 samples (10 mg each) were placed in 1.5-mL tubes and incubated in 1 mL of phosphate buffer (pH 7.4; 0.1 M) with constant shaking (20 rpm) at 37°C. The total amount of amifostine remaining was determined at 0, 3, 6, 9, and 12 hr. Three of the 15 samples were centrifuged, freeze-dried, and extracted at each of the sampling times. The amount of amifostine was determined by HPLC.

Curve Fitting

The percentage of amifostine released was calculated from the data on percentage of amifostine remaining. Amifostine release data were used in curve fitting analysis. Curve fitting was performed using the SigmaPlot software package, Windows vers. 4.0 (Jandel Co., San Rafael, CA, USA). The

release data obtained between 0 and 12 hr were fitted to Eq. (1), and the best-fit parameters (k and n) were calculated:

$$M_t/M_\infty = kt^n \quad (1)$$

where M_t/M_∞ is the fractional release of amifostine in time t, k is the kinetic constant, and n is the diffusional exponent for amifostine release.

Statistical Analysis

The amount of amifostine released from the microcapsules during the in vitro study was compared using SAS software. A value $p < .05$ was considered as evidence of a significant difference.

RESULTS AND DISCUSSION

The yield of microcapsules was 75%. The yield was calculated based on Eq. (2):

$$\text{yield (\%)} = \frac{[\text{total weight of microcapsules}]}{\left[\begin{array}{c}\text{total weight of solids in the formu-}\\ \text{lation (i.e., PLGA + amifostine)}\end{array}\right]} \quad (2)$$

Differential scanning calorimetry thermograms (Fig. 1) showed that pure amifostine had a melting peak at 141.8°C and that PLGA had a glass transition temperature of 44.6°C for the unprocessed material and 43.6°C in the unloaded microcapsules. Microcapsules loaded with amifostine showed a glass transition at 39.4°C and the melting endotherm of amifostine was absent. The absence of a melting endotherm for amifostine was an indication that amifostine was not in the crystalline state in the microcapsules, but rather in the form of a solid solution in PLGA. This led to a lowering of the glass transition temperature for PLGA in the loaded microcapsules. The SEM pictures also showed the (Fig. 2) absence of any crystal on the surface of the microcapsules, revealing that the particles were nearly spherical and smooth. A description of the particle size distribution is given in Table 1. Eighty percent of the particles were between 4 and 86 μm, with a mean diameter of 54 μm.

The amount of amifostine released at a sampling time was calculated by determining the amount

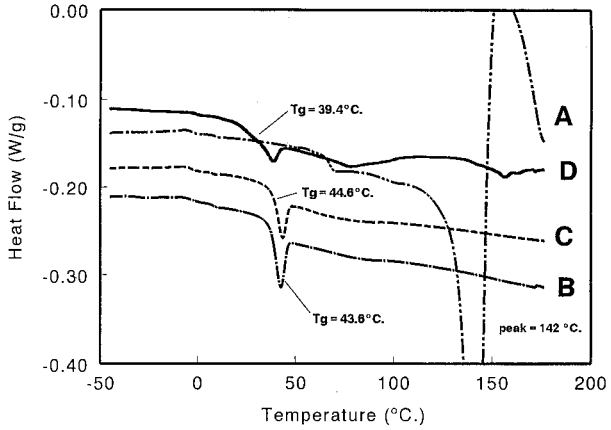

Figure 1. Differential scanning calorimetry thermogram of amifostine (A), PLGA (B), unloaded microcapsules (C), and amifostine-loaded microcapsules (D).

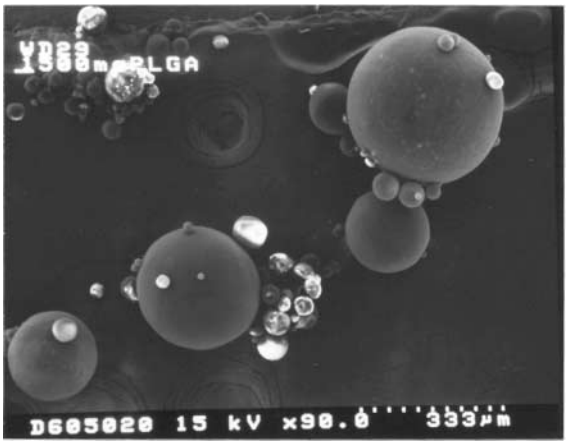

Figure 2. Scanning electron microscopy picture of amifostine microcapsules.

of amifostine remaining in the microcapsules. The efficiency of the extraction process was greater than 99%. Approximately 50% amifostine was released (Fig. 3) during the first 6 hr of the in vitro release study. The drug, however, continued to release over the observed period of 12 hr, during which 92% amifostine was released.

The release data were fitted to Eq. (1). The value of n indicates the drug release mechanisms. The value of n is 0.5 for fickian diffusion and 1 for case II diffusion. A value of n greater than 0.5 but less than 1 indicates a non-fickian or anomalous diffusion, which is a mixture of fickian and case II diffusion.

Table 1

Particle Size Distribution of Amifostine Microcapsules

	Particle Size			
Volume (%)	Diameter (μm)	Mean (μm)	Median (μm)	Mode (μm)
90	< 86	54	57	74
75	< 75			
50	< 57			
25	< 38			
10	< 4			

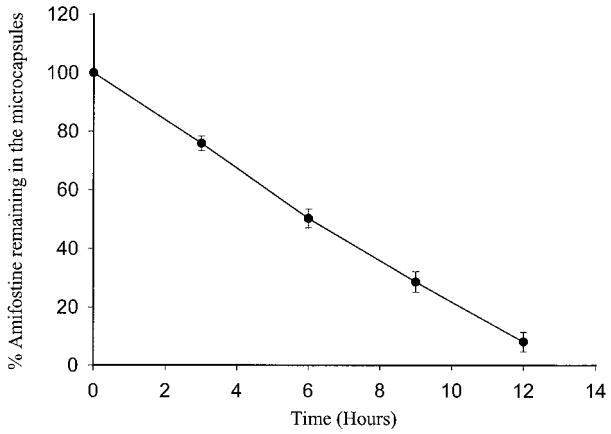

Figure 3. Dissolution of amifostine from PLGA microcapsules, n=3.

When n is greater than 1 the drug release occurs through the super case II diffusion (14). The coefficient of determination (R^2) for this fit was 0.999, which suggests that the equation provides a good fit for the amifostine release data. The value of the kinetic constant (k) was 9.17. The value of n was 0.93, which indicates that the drug release from the microcapsules followed the non-fickian or anomalous diffusion. In an effort to evaluate the degree of biodegradation of the matrix, release study microcapsule samples were collected, freeze-dried, and weighed (before amifostine extraction) to measure the weight loss. No significant weight loss was detected during the 3-day period. This result indicates that biodegradation did not play a significant role in amifostine release.

ACKNOWLEDGMENTS

The authors wish to thank Dr. Nikhil Sarkar from the LSU School of Dentistry for the DSC analysis. This work was funded in part by the NIH/NIGMS, grant #GM08008.

REFERENCES

1. Spencer, C.M.; Goa, K.L. Amifostine. A Review of Its Pharmacodynamic and Pharmacokinetic Properties, and Therapeutic Potential as a Radioprotector and Cytotoxic Chemoprotector. Drugs **1995**, *50*, 1001–1031.
2. Thompson, D.C.; Wyrick, S.D.; Holbrook, D.J.; Chaney, S.G. Effect of Chemoprotective Agent WR-2721 on Disposition and Biotransformations of Ormaplatin in the Fischer 344 Rat Bearing a Fibrosarcoma. Cancer Res. **1995**, *55*, 2837–2846.
3. Wasserman, T. Radioprotective Effects of Amifostine. Semin. Oncol. **1999**, *26* (7), 89–94.
4. Van der Vijgh, W.J.; Korst, A.E. Amifostine (Ethyol): Pharmacokinetic and Pharmacodynamic Effects In Vivo. Eur. J. Cancer **1996**, *32A*, S26–S30.
5. Coia, L.R.; Brown, D.Q. Protection of Bone Marrow by WR-2721 After Fractionated Irradiation. Int. J. Radiat. Oncol. Biol. Phys. **1989**, *17*, 908–909.
6. Lamperti, A.; Ziskin, M.C.; Bergey, E.; Gorlowski, J.; Sodicoff, M. Transdermal Absorption of Radioprotectors in the Rat Using Permeation-Enhancing Vehicles. Radiat. Res. **1990**, *124*, 194–200.
7. Sodicoff, M.; Lamperti, A.; Ziskin, M.C. Transdermal Absorption of Radioprotectors Using Permeation-Enhancing Vehicles. Radiat. Res. **1990**, *121*, 212–219.
8. Fatome, M.; Courteille, F.; Laval, J.D.; Roman, V. Radioprotective Activity of Ethylcellulose Microspheres Containing WR 2721, After Oral Administration. Int. J. Radiat. Biol. Relat. Stud. Phys. Chem. Med. **1987**, *52*, 21–29.
9. Lewis, D.H. Controlled Release of Bioactive Agents from Lactide/Glycolide Polymers. In *Biodegradable Polymers as Drug Delivery Systems*; Chasin, M., Langer, R., Eds.; Marcel Dekker: New York, 1990; 1–42.
10. Diaz, R.V.; Llabrés, M.; Ivora, C. One-Month Sustained Release Microspheres of 125I-Bovine Calcitonin In Vitro–In Vivo Studies. J. Contr. Rel. **1999**, *59*, 55–62.
11. Urata, T.; Arimori, K.; Nakano, M. Modification of Release Rates of Cyclosporin A From Poly-(L-Lactic Acid) Microspheres by Fatty Acid Esters and In Vivo Evaluation of the Microspheres. J. Contr. Rel. **1999**, *58*, 133–141.

12. Jones, D.H.; Partidos, C.D.; Steward, M.W.; Farrar, G.H. Oral Delivery of Poly(Lactide-Co-Glycolide) Encapsulated Vaccines. Behring Inst. Mitt. **1997**, *98*, 220–228.
13. Callender, P.P.; Jayaprakash, N.; Bell, A.; Petraitis, V.; Pettratienes, R.; Candelario, M.; Schaufele, R.; Dunn, J.; Sei, S.; Walsh, T.J.; Balis, F.M. Pharmacokinetics of Oral Zidovudine Entrapped in Biodegradable Nanospheres in Rabbits. Antimicrob. Agents Chemother. **1999**, *43*, 972–974.
14. Mandal, T.K.; Tenjarla, S. Biodegradable Microcapsules: Optimization of Formulations Using Factorial Design. Pharm. Sci. **1995**, *1*, 367–370.
15. Mandal, T.K.; Womack, I. High-Performance Liquid Chromatographic Analysis of Amifostine. Pharm. Pharmacol. Commun. **1999**, *5*, 541–543.