Preparation and Characterization of Carmustine Loaded Polyanhydride Wafers for Treating Brain Tumors

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INTRODUCTION

Bioerodible polymeric implants have been used for the delivery of a range of anticancer drugs (1–5). These devices are favorable for clinical use because the device is eliminated from the brain after the drug has been depleted. Most studies have been conducted by Brem and co-workers (4–15) using poly(1,3-p-carboxyphenoxypropane-sebacic acid) [P(CPP-SA)] as drug carrier.

The biocompatibility and elimination processes of this polymer carrier have been extensively investigated and found to be biocompatible and biodegradable in the brain (16–18). The inflammatory reaction elicited by these polymers when implanted in the brain of a rat, rabbit and monkey was compared with the clinically used implants of Surgicel (oxidized regenerated cellulose) and Gelfoam (absorbable gelatin sponge) (16–18).

The metabolic disposition and elimination processes of radiolabeled P(CPP:SA)20:80 implanted in the rat brain or rabbit brain were studied (19–20). Nearly all the radioactivity in the polymers was excreted by the four week time point and the device was completely eliminated from the implant site, four weeks after implantation.

This biodegradable polymer was used clinically in the brain. Poly(CPP-SA) wafers loaded with 1,3-bis-(2-chloroethyl)-1-nitrosourea (Carmustine) were studied for the treatment of glioma multiforma (21). A number of review articles have been published describing the developmental processes of this implant (4–8,22). The synthesis and characterization of poly(CPP-SA)20:80 and other biodegradable polyanhydrides has been investigated for over 10 years and enormous data is available (23–27). This polymer wafer in now produced for treating second surgery patients with glioma multiforma by a compression molding of a spray dried polymer-Carmustine powder. This is the first and only biodegradable delivery system that is produced by this multistep process which involves several steps: synthesis of polymer, purification by precipitation from solvent, spray dry of polymer-Carmustine chloroformic solution, and compression molding of the powder. Because of the high sensitivity of both the polymer and drug to hydrolysis and heat it is important to perform the production steps under controlled conditions to minimize degradation. The chemical properties of the polymer and the wafer during large scale production, storage and irradiation stability of this implant has not been published.

This report describes the preparation of Carmustine-polymer wafer under Good Manufacturing Practice (GMP), the release and polymer degradation properties, and the storage and irradiation stability of this implant.

EXPERIMENTAL

Materials

The following materials were purchased from Aldrich (Milwaukee, WI): sebacic acid, p-hydroxybenzoic acid, 1,3-dibromopropane, and acetic anhydride. All solvents were analytical grade. Sebacic acid (SA) and p-hydroxybenzoic acid were recrystallized twice from ethanol:water (95:50) and from acetone:water (1:1), respectively. Bis-1,3-p-carboxyphenoxy propane monomer (CPP) was prepared according to Conix (28) and purified by stirring in acetone for 24 hours. CPP and SA prepolymers and poly(CPP-SA)20:80 were prepared as previously described (29).

Instrumentation

Infrared (IR) spectroscopy was performed on a Perkin Elmer Series 1420 dispersive spectrophotometer. Prepolymers and polymer samples were cast on NaCl plates from solutions in CH_2Cl_2. Ultraviolet (UV) spectroscopy was performed using a Kontron Instruments Uvikon model 930. CPP concentration in the degradation studies was determined at 248 nm. Thermal analysis was determined on a Perkin Elmer DSC 7 differential scanning calorimeter, calibrated with Zn and In standards, at a heating rate of 20°C/min. Melting points were determined on an Electrothermal melting point apparatus (England). Molecular weights of the polyanhydrides were estimated on a gel permeation chromatography (GPC) system consisting of a Waters (MA, USA) 510 pump with UV detection (Applied Bioscience 759A Absorbance UV detector) at 254 nm or a Waters 410 Differential Refractive Index detector (RI), a Rheodyne (Coatati, CA) injection valve with a 20 μL loop, and a Waters 840 chromatography Waters Ultrastyrogel Linear column (7.8 × 300 mm) at a flow rate of 1 mL/min. The molecular weights were determined relative to narrow dispersed polystyrene standards (Polyscience, Warrington, PA) with a molecular weight range between 210 to 1,090,000. Samples for analysis were prepared by dissolving approximately 50 mg polymer in 25 ml chloroform. A validated method for molecular weight determination by GPC was used. In a typical analysis, a weight average molecular weight of 35,900 ± 800 and 35,400 ± 900 were obtained for UV and RI detection, respectively. The average molecular weight of the prepolymers was determined by GPC using 500A crosslinked polystyrene column. ^1H NMR spectra ($CDCl_3$/TMS/d/ppm) were obtained on a Varian 300 MHz spectrometer using solutions in deuterated chloroform. Polymer SA:CPP mole ratio was calculated from the ratio of the peak integration at δ1.2–2.1 ppm [$(CH_2)_6$, 12H, representing SA

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dyads] and δ7.0–8.0 ppm (phenyl protons, 4H, representing CPP dyads) (24).

The Carmustine content in wafers and in the release medium were determined by HPLC. A silica based uPorasil 30 cm column and a mobile phase of a mixture of methanol: dichloromethane in 1:9 v/v were used. A linear curve was obtained for the amount of Carmustine injected between 0.2 and 2 μg.

Preparation of Wafers

Powders of blank polymer and Carmustine loaded polymer were prepared by spray drying polymer solutions in dichloromethane using a Buchi Spray Dryer (Buchi, Japan). The spray dried powders were compression molded into 200 mg wafers using an automated single laboratory press (Carver Press, USA). The wafers were 14 mm × 1 mm in size with a flat surface. A measuring gun was used to charge precisely 200 mg of powder into the die. Wafers were pressed and immediately packed individually into aluminum foil pouches under a blanket of dry nitrogen. The spray drying, wafer preparation and packaging were conducted in a clean room under a controlled particle content and humidity (relative humidity—20%) to avoid contamination and condensation of moisture during operation. The wafers were packed into boxes containing dry ice and gamma sterilized at a dose of between 2.3 to 2.5 Mrad (Cobalt 60, Isomedix, USA) and kept frozen until use. Each preparation lot was of at least 100 wafers. The content uniformity of Carmustine-polymer wafers was determined on ten randomly selected single wafer pouches taken from four preparations and the appearance, weight, drug content, and molecular weight were determined.

In Vitro Release Studies

Wafers (n = 10) were individually placed in a 1L 0.1 M phosphate buffer solution pH7.4 at 37°C with constant mixing and the Carmustine released to the buffer was determined by HPLC using an automated system. The polymer degradation was estimated by monitoring the CPP release to the medium using UV detection at 248 nm.

Stability Studies

Wafers from 4 batches individually packed in double sealed aluminum pouches were stored immediately after irradiation in controlled temperature cabinets at −30, −20, and +8°C. At each time point, wafers (n = 5 for the 6 month study and n = 2 for the 2 year study) were taken for the determination of polymer molecular weight and Carmustine content.

RESULTS AND DISCUSSION

Polymer Synthesis and Wafer Preparation

Polymers were synthesized from purified CPP and SA prepolymers (29). Typical data analysis for the prepolymers and polymers are listed in Table 1. Blank and Carmustine loaded wafers were prepared from compression molding of spray dried polymer powder. Scanning Electron Microscope (SEM) micrographs of the spray dried material and the surface of a wafer showed spherical particles of an average particle size of about 10 μm with a uniform distribution. The surface and cross section of the compressed wafer was porous where the spherical particles remain intact. All wafers were off-white with smooth uniform surface and 14 mm × 1 mm in size. The mean wafer weight, the drug content, and the molecular weight were 199.6

Table 1. Data Analysis for CPP and SA Prepolymers and CPP-SA Polymer

Analysis	SA	CPP	Poly(CPP-SA)20:80
Appearance	white powder	white powder	white solid
IR (cm^{-1})[a]	1805, 1742	1790, 1730	1805, 1790, 1730
^1H NMR (ppm)	1.34 (s,8H), 1.62 (t,6Hz,4H) 2.21 (s,0.6H), 2.43 (t,6Hz,4H)	2.35 (s,8H), 4.24 (t,5Hz,4H) 6.96 (d,7Hz,4H) 7.99 (d,7Hz,4H)	1.31(s,6.3H), 1.64 (t,3.15H) 2.35 (m,0.43H) 2.43 (t,6Hz,2.3H) 2.59 (t,6Hz,0.85H) 4.24 (t,4Hz,0.86H) 6.97 (d,6Hz,0.86H) 7.98 (d,6Hz,0.62H) 8.08 (d,6Hz,0.23H)
Melting point (°C)	70 ± 3	102 ± 4	68 ± 4
Molecular weight[b]			
Mn	2200 ± 400	600 ± 150	18000 ± 4000
Mw	3200 ± 400	600 ± 150	50000 ± 20000
Solubility (g/100ml)			
in $CHCl_3$	>20	>10	>20
in hexane	<1	<1	<1
Yield (%)[c]	80 ± 10	45 ± 10	90 ± 5
CPP:SA ratio[d]	—	—	21.4:78.6

[a] Absorption peaks of the anhydride bond, no acid peaks at 1720 cm^{-1}.
[b] Molecular weight is weight average and was determined by GPC.
[c] Yield of the preparation and purification of the prepolymers and polymer.
[d] CPP content in the polymer was determined by ^1H NMR.

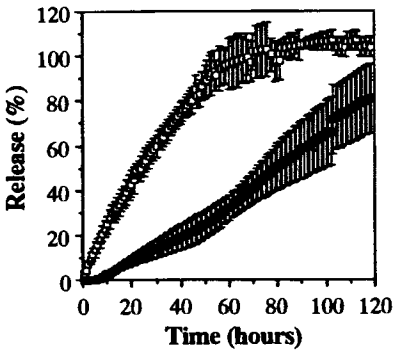

Fig. 1. Carmustine and CPP release from polymer wafers. Results are average of two preparations (n = 10). Wafers (200 mg, 14 mm × 1 mm, loaded with 3.85% Carmustine) were placed in phosphate buffer pH 7.4 at 37°C, Carmustine release was determined by a continuous HPLC analysis. Release of Carmustine (□), CPP (■).

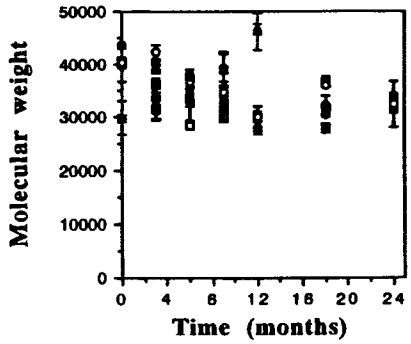

Fig. 2. Molecular weight change during 24 months of storage of 3.85% Carmustine loaded wafers. Wafers (n = 3 per point) from three batches stored at −20°C (□, ◆, ■, △) and −30°C (+, *, ○, ●).

± 6.60 mg, 7.63 ± 0.24 mg and 40330 ± 995 Daltons, respectively, which indicates uniformity within and between batches.

The molecular weight changes of the polymer during the process of manufacturing of the wafers was monitored. The initial molecular weight was 63,000 ± 6,000 which was slightly decreased to 57,000 ± 4,000 during the spray drying and wafer compression. Purification of the crude polymer by precipitation slightly increases the average molecular weight which is a result of the removal of the low molecular weight fraction. GPC chromatogram of the precipitated polymer shows a more symmetrical and narrower peak, where the contribution of low and high molecular weight fractions is reduced. The molecular weight was slightly decreased in the following steps as a result of self depolymerization in chloroform solution and hydrolysis (30).

In Vitro Polymer Degradation and Carmustine Release

The Carmustine release and polymer degradation of 10 wafers from two preparations is shown in Fig. 1. The degradation of the polymer was estimated from the CPP concentration in the releasing solution using UV detection. After 120 hours in buffer, all Carmustine and about 80% of the CPP were released. Both Carmustine and CPP were released constantly during the first 60 hours of degradation with a narrow standard deviation. The rapid release of Carmustine from the polymer can be explained by diffusion through the large surface area of the porous structure of the wafer as seen by the SEM. During that period, most of the polymer was degraded releasing the Carmustine and sebacic acid component (data not shown), leaving the slightly soluble CPP degradation product. The increase in the CPP concentration after 60 hours is a result of disintegration of the wafer which increases the surface area. The larger standard deviation is a result of random wafer disintegration. A similar CPP elimination profile was found in vivo (20).

Stability of Polyanhydride Devices

The stability of blank and Carmustine loaded wafers of poly(CPP-SA) was studied. Four preparations of blank and Carmustine-loaded poly(CPP-SA) wafers packed in double sealed aluminum foil bags were stored at −30, −20, and +8°C, and the molecular weight drug content and drug release rate were determined periodically for two years by GPC and HPLC, respectively. For both the blank and Carmustine loaded polymer the molecular weight remained between 30,000 and 40,000 throughout the test period at −30 and −20°C (Fig. 2) while the molecular weight dropped to below 30,000 at the +8°C storage condition (data not shown). The decrease in molecular weight at +8°C was inconsistent which indicates problems with packaging or vapor condensation on polymer during the production process resulting in polymer hydrolysis as evident from IR analysis (appearance of acidic peak at 1720 cm^{-1}). Under freezing temperatures (−20°C) there is minimal active water to cause anhydride hydrolysis. The Carmustine content and release rate (data not shown) did not change with time at all storage conditions, confirming stability.

Polyanhydrides may decrease in molecular weight during storage either as a result of hydrolysis caused by entrapped water during preparation or from penetration of water vapors into the package during storage, or as a result of spontaneous thermodynamic depolymerization to cyclic and shorter polymer chains [30]. To minimize hydrolysis or depolymerization during storage, a temperature of −20°C was selected for the storage of the clinical batches.

The effect of radiation on the Carmustine-loaded wafer finished product was evaluated from comparing the analysis of the wafers before and after radiation. No change in MW, Carmustine release, and stability of the wafers were found. The ^1H NMR and IR spectra of the wafers (dissolved in $CDCl_3$) before and after irradiation were essentially the same which indicate a safe process.

CONCLUSIONS

The Carmustine-polymer wafers were prepared in high reproducibility. Terminal sterilization by γ-irradiation did not affect the wafer properties. Carmustine was released constantly for about 3 days with 100% release after 120 hours, at which time 80% of the polymer was degraded and dissolved. The wafers remain unchanged when stored at −20°C or below. Storage at refrigeration is possible if more reliable packaging is available.

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