Assessment of in vitro release of carboplatin from six carrier media

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OBJECTIVE
To investigate in vitro carboplatin release from six carrier media.

SAMPLE
6 carboplatin-containing carrier media.

PROCEDURES
An in vitro release study was performed with six commercially available carrier media: a hemostatic gelatin sponge, a poloxamer copolymer gel, and two sizes (3 and 4.8 mm in diameter) of beads molded from two commercial calcium sulfate products. All carrier media contained 10 mg of carboplatin. Carrier media specimens were placed in 37°C PBS solution for 96 hours. Carboplatin concentrations in PBS solution were measured by use of high-performance liquid chromatography at 15 time points to calculate the amount and proportion of carboplatin released from each specimen.

RESULTS
Peak release of carboplatin from the poloxamer copolymer gel and hemostatic gelatin sponge were achieved after 4 and 20 hours, respectively. Maximum release did not differ significantly between the poloxamer copolymer gel and hemostatic gelatin sponge, but both released significantly more carboplatin within 96 hours than did both of the commercial calcium sulfate products. The poloxamer copolymer gel released 99% of the carboplatin, and the hemostatic gelatin sponge released 68.5% of the carboplatin. Peak release of carboplatin from the calcium sulfate beads was not reached within 96 hours.

CONCLUSIONS AND CLINICAL RELEVANCE
In this study, carboplatin release from the hemostatic gelatin sponge was incomplete. The poloxamer copolymer gel and hemostatic gelatin sponge released carboplatin rapidly in vitro, whereas calcium sulfate beads did not.


Chemotherapeutic agents involving platinum drugs (eg, cisplatin) have been used in local delivery systems in clinical patients following incomplete tumor resection.1–3 Open-cell polylactic acid1,2 and a biodegradable polymer implant delivery system3 have been investigated as vehicles. Other carrier compounds, including gelatin sponges,4–6 hydrogels,7,8 polymethylmethacrylate bone cement,4,9,10 and plaster of Paris,11–14 have been used for local delivery of antimicrobial or antifungal drugs.

Ideally, a carrier medium for drug delivery to soft tissues should be biodegradable and completely absorbed, which avoids the need for a second surgery to remove the carrier medium. The carrier should also induce no adverse effects on local tissue biological processes or wound healing. A secondary feature would be predictable and complete release of the chemotherapeutic drug from the carrier medium.

ABBREVIATIONS
HPLC High-performance liquid chromatography

Carboplatin is a second-generation, platinum-based antineoplastic agent. It is an ideal molecule for these delivery systems because it is an extremely hydrophilic molecule and highly soluble in aqueous media.

Several potential implantable media meet the requirements and can be considered as carrier media for use in soft tissue implantation and, potentially, as bone substitute. A nonionic polyoxyethylene-polyoxypropylene-polyoxyethylene copolymer is commercially available. It is unique because it undergoes reverse gelatification (ie, changes from a liquid to a gel when warmed to body temperature).7,15 This product has no apparent adverse effects on tissues when used in patients.7,16 Hemostatic gelatin sponges are frequently used to stop or prevent bleeding after collection of liver biopsy specimens, and they can be left in place without causing tissue reactions.17–19 Gelatin sponges impregnated with chemotherapeutics have been used in chemoembolization.15,20,21

Calcium sulfate is slowly absorbed from tissues, with radiographic disappearance of the beads by 5 weeks after implantation.22 Use of calcium sulfate is
generally limited to orthopedic applications to augment fracture repairs and prosthetic implants or to fill bone defects.\textsuperscript{22} The objective of the study reported here was to compare in vitro release of carboplatin from 6 carrier media over a 96-hour period. We assessed a hemostatic gelatin sponge, a poloxamer copolymer gel, and 2 sizes of beads molded from each of 2 commercially available calcium sulfate products; thus, we assessed 6 specimens (1 for each of the 6 carrier media). We hypothesized that there would be slower but complete release of carboplatin from the poloxamer copolymer gel over 96 hours, compared with carboplatin release from the hemostatic gelatin sponge, whereas calcium sulfate beads would release less carboplatin over 96 hours than would the poloxamer copolymer gel.

**Materials and Methods**

**Sample**

Six carrier media were evaluated. These comprised a poloxamer copolymer gel,\textsuperscript{a} a hemostatic gelatin sponge,\textsuperscript{b} and each of 2 sizes (3 and 4.8 mm in diameter) of beads molded from each of 2 commercially available calcium sulfate products\textsuperscript{c-d} (products A and B, respectively).

**Preparation of carrier media**

Each carrier medium was prepared so that it contained 10 mg of carboplatin. The poloxamer copolymer gel (25% [wt/vol]) was prepared in compliance with United States Pharmacopeia guidelines.\textsuperscript{23} In a negative-pressure biological safety cabinet, 1 mL of carboplatin solution\textsuperscript{e} (10 mg/mL) was added to 1 mL of refrigerated (6°C) poloxamer copolymer gel in liquid form. The solutions were mixed and immediately placed into a 1-inch dialysis tube (permeability, 12 to 14 kDa)\textsuperscript{5} so that gelatification could occur in situ in the tube. Two hemostatic gelatin sponges (12 X 6 mm) were folded and inserted into another dialysis tube. One end of that tube was clamped with a dialysis clamp,\textsuperscript{6} and 1 mL of the carboplatin solution was placed within the folded sponges by use of an 18-gauge, 1.27-cm needle. The other end of the dialysis tube then was clamped with a dialysis clamp.

Two commercially available calcium sulfate products were used. Each product was divided in half, and 1 mL of the carboplatin solution was added to each half. Brand-specific templates were used to create 3-mm-diameter beads with half of each calcium sulfate product and 4.8-mm-diameter beads with the other half of each product. Beads were allowed to sit undisturbed overnight in the templates; they were stored in a refrigerator (6°C) in a bag that shielded the beads from light. Subsequently, beads were placed into dialysis tubes, and both ends of the dialysis tubes were clamped adjacent to the beads.

**Experimental methods**

Beakers (400 mL) containing 300 mL of PBS solution\textsuperscript{b} (pH, 7.4) were warmed to 37°C on a magnetic hot plate.\textsuperscript{1} Dialysis tubes containing each of the carboplatin-calcium sulfate beads were placed into separate beakers, which was followed by preparation of the poloxamer copolymer gel and hemostatic gelatin sponge products. Immediately after the carboplatin was mixed with the poloxamer copolymer gel, the clamped dialysis tube was placed into a beaker. Similarly, the dialysis tube containing the hemostatic gelatin sponges was placed into a beaker immediately after addition of the carboplatin. The top of each beaker was covered with clear plastic wrap\textsuperscript{1} to prevent evaporative loss. Temperature of the PBS solution was maintained at 37°C throughout the experiment, and PBS solution was continuously stirred at 600 rpm by use of a magnetic stirrer.

**Collection of eluent samples**

Baseline samples were collected immediately before specimens were placed into the beakers (time 0). Two 500-μL samples of PBS solution were collected from each beaker at each of 15 time points (0, 1, 2, 4, 6, 8, 10, 12, 16, 20, 24, 36, 48, 72, and 96 hours). One milliliter of fresh PBS solution was placed back into each beaker after acquisition of the samples at each time point. The level of the PBS solution in each beaker was recorded before each time point to allow for approximate volume correction. Temperature of the PBS solution and room temperature were recorded before each sample collection. Room temperature ranged from 21° to 22°C, and mean ± SD temperature of the PBS solution was 37.1 ± 0.8°C (range, 36° to 39°C).

**Carboplatin analysis**

Carboplatin concentration in samples of PBS solution was measured by use of HPLC and a method developed by our laboratory group. Official analytic reference standard carboplatin\textsuperscript{8} (100 mg) was obtained. Potency of the reference standard was 0.999 mg/mg of material. The reference standard was weighed and dissolved in 100% HPLC-grade distilled water to create a stock solution (concentration, 1 mg/mL). Further dilutions of the stock solution were made with distilled water to prepare fortifying solutions for quality control samples, calibration samples, and samples for use in development of the analysis method. Fresh stock solution was made each day; stock solution was stored at 4°C in a tightly sealed dark vial when not in use. Fortifying solutions were added to PBS solution to create 8 calibration standards (range, 0 to 50 μg/mL).

The mobile phase consisted of 100% HPLC-grade distilled water (flow rate, 1 mL/min). Fresh mobile phase was prepared, filtered (0.45-μm filter), and degassed for each day’s assays. The HPLC system consisted of a quaternary solvent delivery system,\textsuperscript{1} autosampler,\textsuperscript{8} and UV detector\textsuperscript{8} set at a wavelength of 220 nm. Chromatograms were integrated with a computer program.\textsuperscript{9} The HPLC column was a reverse-phase, 4.6-mm X 15-cm C18 column\textsuperscript{8} maintained at a constant temperature.
of 40°C. Retention times for the peaks of interest were between 3.6 and 3.7 minutes.

All study samples, calibration samples, and blank (control) samples were loaded into HPLC vials for injection (injection volume, 40 μL). A fresh set of calibration, blank, and quality control samples was prepared for each day’s assays. Quality control samples were prepared with blank sample matrix fortified with carboplatin. Concentrations of quality control samples were back-calculated and were within 15% of anticipated values, which was within the range for our acceptance criteria. All calibration curves were linear ($R^2 \geq 0.999$). Limit of quantification was 0.05 μg/mL, which was determined with United States Pharmacopeia guidelines24 by use of the lowest point on a linear calibration curve that met our acceptance criteria.

Calculation of carboplatin concentration
A standard serial dilution series (50, 10, 5, 1, 0.5, 0.1, and 0.05 μg/mL) was created from the stock solution. The measured carboplatin concentration in a sample was multiplied by the total eluent volume at the time of sample collection to calculate the amount of carboplatin released. In addition, the amounts of carboplatin in previously collected samples were added to obtain the total amount of carboplatin released. Results were expressed as a percentage of the total amount of carboplatin instilled in each specimen (ie, 10 mg). Results were corrected at each time point on the basis of the measured amount of PBS solution in a beaker at that time point.

Statistical analysis
The maximum amount of carboplatin released (total amount and percentage of instilled amount) was compared among the various carrier media by use of ANOVA. Data for carboplatin release were logarithmically transformed. To determine the size and direction of significant effects, least squares means were compared by use of a Tukey adjustment. Significance was set at values of $P < 0.05$. All statistical analyses were performed by use of computer software.9

Results

Carrier media
Product A beads were 2.8 and 4.2 mm, respectively, at their widest point, whereas product B beads were 4.1 and 5.5 mm, respectively, at their widest point. The dialysis tube containing the poloxamer copolymer gel absorbed PBS solution to fill the tube to full capacity, which was not the case for the hemostatic gelatin sponge or any of the beads. Macroscopically, no evidence of bead disintegration was detected at any time point.

Volume of PBS solution
Volume of PBS solution in the beaker with the 3-mm-diameter beads decreased to 210 mL (product A) and 205 mL (product B). Volume of PBS solution in the beakers for the remaining 4 specimens decreased to only 290 mL.

Carboplatin release
Maximum release for the poloxamer copolymer gel (99.0%) was achieved at 4 hours (Figure 1). Maximum release for the hemostatic gelatin sponge (68.5%) was achieved at 20 hours. Maximum release for the calcium sulfate beads was not achieved by 96 hours. Maximum release for product A was 26.9% for 3-mm-diameter beads and 41.0% for 4.8-mm-diameter beads, whereas for product B, maximum release was 34.0% for 3-mm-diameter beads and 47.9% for 4.8-mm-diameter beads. Maximum carboplatin release did not differ significantly between the poloxamer copolymer gel and hemostatic gelatin sponge for the total amount released ($P = 0.098$) or the percentage amount released ($P = 0.088$). Total amount and percentage amount released for 4.8-mm-diameter beads of product A did not differ significantly from values for 3-mm-diameter beads of product B ($P = 0.256$ and $P = 0.252$, respectively) or 4.8-mm-diameter beads of product B ($P = 0.125$ and $P = 0.122$, respectively).

We attempted to measure the residual amount of carboplatin remaining in the gelatin sponge at the end of the experiment. However, to perform this analysis, the sponge had to be dissolved in an acidic solution. Because acidic solutions degrade carboplatin, this analysis was unsuccessful.

![Figure 1](image_url)
No nonacid solvents were able to adequately dissolve the sponge and allow us to measure the remaining amount of protein-bound carboplatin.

**Discussion**

Analysis of data of the present study indicated that carboplatin release from calcium sulfate beads was slow, whereas carboplatin release from the poloxamer copolymer gel and hemostatic gelatin sponge was rapid. Release from the poloxamer copolymer gel was nearly complete (99%) after 4 hours, whereas release from the hemostatic gelatin sponge was incomplete (68.5%) and peaked after 20 hours. Carboplatin release differed significantly between the poloxamer copolymer gel and hemostatic gelatin sponge; therefore, we rejected the first part of our hypothesis. Carboplatin release from calcium sulfate beads was incomplete, ranging from 27% to 48% after 96 hours for the 4 types of calcium sulfate beads evaluated in this study; therefore, we accepted the second part of our hypothesis.

A variety of methods have been used for studies on in vitro drug release. Investigators of prior in vitro drug release studies conducted to assess nanoformulations or poloxamer gels as carriers used dialysis tubes suspended in PBS solution, a Franz cell, or a gel-gel diffusion cell. In the study reported here, a dialysis tube suspended in PBS solution was used because volume limitations of Franz cells would have precluded us from evaluating the calcium sulfate beads. In previous studies, there was wide variation in the volume of PBS solution (range, 1 to 40 mL) and stirring speed (range, 50 to 600 rpm). A volume of 300 mL was used in the present study so that the calcium sulfate specimens could be completely submerged and the concentration gradient resulting from removal of 1-mL samples from the eluent would be minimized. A stirring speed of 600 rpm was chosen to ensure appropriate dispersion of carboplatin within the eluent as well as appropriate exposure of calcium sulfate beads to PBS solution. Although evaluating only 2 or 3 beads would have allowed better surface exposure to the PBS solution, we opted to use half of the calcium sulfate for each bead size because increasing the volume of carboplatin relative to the calcium sulfate beads would have altered structural properties of the beads and was in opposition to manufacturer’s recommendations. Beads would most likely be touching and not be fully separated in wound beds. Furthermore, completely submerging the beads in PBS solution and continuously stirring the PBS solution at 600 rpm were expected to provide sufficient flow within the fluid and ensure adequate exposure of all beads.

Carboplatin release from the poloxamer copolymer gel was more rapid than expected. Investigators of a previous study found a release of 60% after 12 hours. Similarly, vancomycin release from the poloxamer copolymer gel was assessed in another study, and investigators found that it was nearly complete after 150 hours. Both of these studies involved use of a Franz cell. Furthermore, doxorubicin release from poloxamer copolymer gel instilled in a dialysis bag reached a plateau after 72 hours.

Interestingly, there was release of 80% of a carboplatin solution across a membrane with a molecular-weight cutoff of 3,500 Da at 12 hours. There was complete (100%) release of carboplatin from the carrier medium in other studies. Therefore, it is possible that the carboplatin in the aforementioned study would have been completely released over a longer period.

Two hemostatic gelatin sponges, rather than 1, were used to ensure that the absorptive capacity of the sponge was not exceeded. Peak release of carboplatin from the hemostatic gelatin sponge was attained at 20 hours, but only 68.5% of the carboplatin was recovered from the PBS solution. Incomplete carboplatin release may have been caused by carboplatin binding irreversibly to proteins within the matrix of the hemostatic gelatin sponge. There can be a moderate percentage of protein binding to carboplatin in vivo, with reported values of 40% to 50% after 6 to 24 hours. The hemostatic gelatin sponge was manufactured from purified porcine skin, which made irreversible protein binding a likely explanation for the incomplete release. Because the hemostatic gelatin sponge was discarded after completion of the experiment, we duplicated the first 24 hours of the elution experiment for the hemostatic gelatin sponge to ensure that the finding of incomplete carboplatin release was repeatable. We also attempted to measure the residual amount of carboplatin contained in the hemostatic gelatin sponge at the end of the original 96-hour experiment, but acid digestion of the sponge resulted in degradation of carboplatin.

In another study conducted to investigate cisplatin release from porous gelatin particles, which was prepared from acidic gelatin similar to the hemostatic gelatin sponge used in the study reported here, investigators found a maximum release of 60% after 24 hours and suggested that there was conjugation of cisplatin to the gelatin. However, no attempts were made to confirm the amount of conjugated cisplatin in that study.

Carboplatin release from the smaller (3 mm in diameter) calcium sulfate beads (27% and 34%) was less than that from the larger (4.8 mm in diameter) beads (41% and 48%) during the time frame of the present study. This finding is in agreement with the higher dissolution of antimicrobials by a larger single bead than by a smaller single bead reported in another study. This finding was unexpected because the volume of the calcium sulfate products used resulted in more 3-mm-diameter beads than 4.8-mm-diameter beads, which thereby increased the exposed surface area. Beads were loosely packed in each dialysis tube, which should have allowed the PBS solution to reach all beads. In another study conducted to investigate the release of antimicrobials, investigators also found that smaller beads had a higher rate of release dur-
ing the first 48 hours, but it decreased to 0 at day 4, whereas larger beads had a higher total amount of release and longer duration of release of antimicrobials over 5 days.

Carboplatin release from calcium sulfate beads did not reach a peak within the time frame for the study reported here. Although this limits the conclusions that can be drawn, the time frame for the present study was intended to compare short-term release from the carrier media, including calcium sulfate beads and faster-dissolving compounds such as the poloxamer copolymer gel and hemostatic gelatin sponge. Evaluation of calcium sulfate beads under similar conditions for a prolonged period (14 to 21 days) would allow for a more thorough investigation of total release and determination of a release plateau.

The ideal therapeutic time frame for sustained local release of a therapeutic agent is not known, but most likely it will range between several days and several weeks. Follow-up studies are needed to investigate the duration of carboplatin release from various carrier media in vivo to further define an ideal compound for soft tissue applications.

In the study reported here, there was incomplete maximum release of carboplatin from the hemostatic gelatin sponge, but the amount released was likely sufficient to result in local concentrations with anti-tumor effects. The poloxamer copolymer gel and hemostatic gelatin sponge released carboplatin quickly in the present in vitro study, and they did not differ significantly with regard to the amount of carboplatin released. Peak release of carboplatin from calcium sulfate beads was not reached within 96 hours, and additional studies are needed to determine the duration necessary to obtain more complete release of the drug from calcium sulfate beads.

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**Footnotes**

b. Gelfoam absorbable gelatin sponge, USP, 12 X 7 mm, Pharmacia & Upjohn Co, Kalamazoo, Mich.
c. Osteoset resorbable mini bead kit, Wright Medical Technology Inc, Arlington, Tenn.
d. Stimulan Rapid Cure, provided by Biocomposites Inc, Wilmington, NC.
e. Hospira Inc, Lake Forest, Ill.
f. One-inch dialysis tubing, 12 to 14 kDa permeability cut off, Carolina Biological Supply Co, Burlington, NC.
g. Carolina Biological Supply Co, Burlington, NC.
h. Gibco by Life Technologies Corp, Grand Island, NY.
i. RT 5 power IKAMAG, IKA Works Inc, Wilmington, NC.
j. Target Corp, Minneapolis, Minn.
k. United States Pharmacopeia, Rockville, Md.
I. Agilent Technologies, Wilmington, Del.
m. 1100 series autosampler, Agilent Technologies, Wilmington, Del.

**References**


