Efficacy and Pharmacokinetics of Site-Specific Cefazolin Delivery Using Biodegradable Implants in the Prevention of Post-operative Wound Infections

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Purpose. The study objective was to evaluate the efficacy and pharmacokinetics of cefazolin delivered locally as a glyceryl monostearate (GMS) based biocompatible implant for prevention of post-operative wound infection in Sprague Dawley rats subcutaneously inoculated with *Staphylococcus aureus.*

Methods. For the efficacy and pharmacokinetic studies, 18 rats were subcutaneously inoculated with 4.5×10^7 CFU of *S. aureus* on the dorsum (6 per rat), and randomly assigned into three group of 6 rats each: (1) the control group, in which rats did not receive antibiotics, (2) the intermittent IM treatment group, in which rats received IM injections of 10 mg/kg cefazolin every 4 hr (total of 180 mg/kg in 3 days), and (3) the implant treatment group, in which rats were implanted subcutaneously with four Cefazolin-GMS implants in the vicinity of the inoculations. The implants were designed to deliver 180 mg/kg cefazolin over a 3 day period. For efficacy evaluation, the rats were euthanized one week post-inoculation and abscess count, weight and size were determined.

Results. Rats in the control group had developed 21 abscesses out of the 36 inoculations, indicating validity of the infection model. The local delivery of cefazolin resulted in complete eradication of the infection, since no abscesses formed in the rats in the implant group. In the IM treatment group, only one abscess was formed and no significant difference in efficacy between the two treatment groups was observed. The GMS implants sustained the release of cefazolin for a period of three days with only 3-fold fluctuations in plasma concentration (5.5–17.5 μg/ml). However, plasma concentrations after the intermittent IM administration of cefazolin fluctuated 110-fold between 44-0.4 μg/ml every 4 hr. The release rate of cefazolin from the implants was nearly zero order for the entire duration. Bioerosion of the implants was determined by examining the condition of the implants six weeks post-implantation. Two of the 12 implants had completely disappeared and the remaining implants were in a pasty form and had lost 20–80% of their weight. Absence of irritation or inflammation around the implants indicated biocompatibility of the GMS implants.

Conclusions. Implantable system that provided a prolonged delivery of cefazolin was found to be effective against *S. aureus* infection, and demonstrated suitable pharmacokinetics and biocompatibility with significant bioerosion.

KEY WORDS: wound infection; post-operative; site-specific; implants; efficacy; *S. aureus.*

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INTRODUCTION

Most of the 23 million patients who undergo surgery annually in the United States receive perioperative antimicrobial prophylaxis (1,2). Despite this practice, 500,000–920,000 surgical wound infections occur annually, accounting for 24% of all nosocomial infections. Such infections contribute substantially to the morbidity and costs associated with surgery. Thus, economical, physical and psychological impact of post-operative wound sepsis mandates the use of proper preventive measures to minimize infections (2). In addition to aseptic techniques, high systemic doses of antibiotics are administered perioperatively, and continued postoperatively for 2 to 3 days in patients who have undergone major surgical procedures in order to achieve an effective concentration of antibiotics at the site of the surgery (3,4).

Cefazolin is one of the most commonly used prophylactic antibiotic for the vast majority of operative procedures (4,5). One gram of cefazolin is administered IV or IM, one-half to one hr prior to the start of surgery in order to achieve adequate antibiotic levels in the serum and the tissues, followed by 0.5–1 g IV or IM every 6 to 8 hr for 24 hr postoperatively. In surgeries such as prosthetic arthroplasty, in which the occurrence of infections may be particularly devastating, the prophylactic administration of cefazolin may be continued for 3 to 5 days following the surgery (6). These high-dose administration of antibiotics can be potentially toxic because of the required large systemic doses in order to provide adequate levels at the surgical site. Furthermore, the systemic administration could be ineffective in certain cases in which blood supply to the surgical site is compromised due to the poor vascularity and/or the necrosis of the surgical site.

Systemic continuous infusion of antibiotics such as cefazolin and penicillin G has been demonstrated to be more effective in reducing infections than the intermittent administration of an equivalent dose since the continuous infusion provides a longer time period above the minimal inhibitory concentration (MIC) for the infecting organism (7–9). However, even with continuous systemic infusion, high doses of antibiotics are required because of the need to achieve effective concentrations at the site of surgery and the relatively short half-lives of antibiotics used.

The local administration of antibiotics into the surgical wound offers the opportunity of providing high local concentrations, and targeting only the tissues at risk of infection, concurrently minimizing the potential toxic side effects of the drug. Direct administration (10–12), and local infusion (13,14) of antibiotics to the surgical wound has been found to be more effective, however, the rapid absorption of the antibiotics from the wound site reduced the duration of protection against infections, and the local infusion is cumbersome and requires continuous medical attention. Therefore, the overall objective of this study was to develop a biodegradable implantable delivery system which will: 1.) provide a short term (1–3 days) local delivery of cefazolin for the prevention of post-operative wound infections, and 2.) erode and biodegrade shortly after release of the antibiotic and thus be biocompatible. A combination of four devices based on glyceryl monostearate (GMS) was shown earlier to provide a continuous delivery of cefazolin for a 3 day period, and each of the devices was designed to com-

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pletely disintegrate *in vitro* within 3–5 hr after 100% release of cefazolin (15). The disintegration of the GMS based devices was intended to facilitate the bioerosion and the subsequent bioabsorption of GMS from the implantation site *in vivo*. The specific aims of the present study were therefore, to evaluate the *in vivo* efficacy and pharmacokinetics of the locally delivered cefazolin from GMS implants in a subcutaneous abscess infection model with *Staphylococcus aureus* ATCC 29213 in male Sprague-Dawley rat, and also evaluate bioerosion and biocompatibility of the implants (3,7). Each rat in this study received six subcutaneous injections containing 4.5×10^7 colony forming units (CFU) of *S. aureus* to induce infection. The rats were then randomly assigned to three groups: control group (no antibiotic treatment), intermittent intramuscular group (intermittent IM injections of cefazolin) or implant group (cefazolin released from subcutaneous implants) for evaluation of efficacy and pharmacokinetics.

MATERIALS AND METHODS

Materials

Cefazolin USP and Cefoxitin USP were obtained from Marsam Pharmaceuticals Inc. and Merck and Co., Inc., respectively. GMS was a gift from Eastman Chemicals. Trichloroacetic acid (TCA) was purchased from Fisher Scientific. *S. aureus* ATCC 29213 was obtained from MUSC Microbiology Laboratory. Broth and Mueller Hinton II agar plates were obtained from Dilco Labs and BBL Labs, respectively. Ketamine HCl, xylazine HCl, povidone-iodine, non-absorbable sutures and normal saline for injection were obtained from MUSC hospital supplies. Male Sprague-Dawley (275–325 g) rats were obtained from Harlan Sprague-Dawley.

Preparation of Devices

Cefazolin loaded GMS based devices were prepared by compression as described previously and three of the devices were coated to delay the release, and combination of one uncoated and the three coated devices provide sustained release of cefazolin for 72 hr (15).

MICROBIOLOGICAL METHODS

Preparation of the Staphylococcus Aureus *ATCC 29213 Inoculum*

S. aureus was subcultured from agar plate into a tube containing sterile broth. *S. aureus* in broth was incubated at 35°C for 5 hr to reach logarithmic growth phase, and the concentration of the *S. aureus* inoculum suspension was adjusted to 1.5×10^8 CFU/ml by diluting the incubated broth with sterile normal saline. The inoculum concentration of bacteria was adjusted by matching the turbidity of the suspension to that of 0.5 McFarland standard (latex particle suspension, 0.5 equivalent to 1.5×10^8 CFU/ml) measured with a spectrophotometer at 625 nm.

Verification of the Inoculum Concentration

In addition to the bacteria in the log-phase of growth, other contributors to the turbidity of the suspension include dead bacteria cells and particulates. Therefore, concentration of the inoculum in CFU/ml was verified each time before inoculation as follows. A sample from the bacteria suspension was diluted with normal saline to obtain two suspensions at concentrations, $1/10^5$ and $1/10^6$ of the original bacterial concentration. Then, 20 µl aliquots from each suspension were cultured on agar (Mueller Hinton II) plates and incubated at 35°C for 24 hr. The colonies formed in each plate were counted and the concentration of the initial inoculum calculated and verified. The CFU concentration of *S. aureus* suspension was verified every time an inoculum was prepared before each experiment, and the average concentration of the inoculum was found to be $9.1 \times 10^7 \pm 3.2 \times 10^7$ CFU/ml (n = 6).

Minimum Inhibitory Concentration (MIC) of Cefazolin for S. Aureus *ATCC 29213*

In order to determine the susceptibility of *S. aureus* to cefazolin, the MIC was determined using the Microdilution Broth technique according to the National Committee for Clinical Laboratory Standards (NCCLS) guidelines (16). Cefazolin stock solutions (256 µg/ml) in broth and a suspension of *S. aureus* (1×10^6 CFU/ml) in broth were prepared separately. Cefazolin solutions (128-0.0625 µg/ml) were incubated with 5×10^5 CFU/ml *S. aureus* suspension for 18 hr at 35°C. The MIC was identified to be 0.5 µg/ml, as it was the lowest cefazolin concentration that completely inhibited the growth of *S. aureus* as detected microscopically. This value of MIC agreed with the MIC range (0.25–1 µg/ml) of cefazolin reported for *S. aureus* ATCC 29213 (16).

HPLC ASSAY OF CEFAZOLIN IN PLASMA

Preparation, Processing and Extraction of Samples and Standards

A stock solution of cefazolin in plasma was prepared by spiking blank plasma with a concentrated aq. solution of cefazolin, and further diluted with blank plasma to obtain standards with concentrations from 2–60 µg/ml. Blank rat plasma was obtained by centrifuging blood at 3000 rpm for 5 min. Blood samples from the rats were collected from its tail, allowed to clot (10 min) then centrifuged to separate plasma which was stored in a freezer until the assay time. Both cefazolin standards and samples in plasma were similarly treated as described below to prepare them for analysis by HPLC. Forty microliter of 5% TCA in methanol was added to 100 µl plasma (sample or standard), in order to precipitate plasma proteins. The mixture was vortexed for 10 seconds and 50 µl cefoxitin solution (internal standard, 30 µg/ml) was added. The resultant mixture was vortexed for 10 seconds then centrifuged at 5000 rpm for 10 min, and the supernatant was separated and was ready to be injected into the HPLC system. Three to four samples were processed at a time to avoid any drug degradation.

HPLC Analytical Conditions

Plasma extracts were analyzed for cefazolin by reversed phase HPLC using 3.9 mm × 30 cm Waters Microbondapak C18 column. The mobile phase was composed of 7% acetonitrile in 0.05M KH_2PO_4, and pumped at a flow rate of 3 ml/min.

Cefazolin and cefoxitin peaks were detected at 273 nm, with retention times of 14 and 11 min, respectively.

Validation of the Assay for Cefazolin in Plasma Samples

The method precision was determined by preparing, processing and analyzing six individual plasma standards at 9.52 μg/ml. Linearity was established by analyzing plasma standards ranging in concentration from 1.87–57.1 μg/ml. The peak area ratios of the cefazolin peak to the cefoxitin peak were plotted versus the concentration of cefazolin in the corresponding plasma standard. The extraction efficiency was estimated by spiking blank plasma with known amounts of cefazolin, processing and analyzing as described above, and cefazolin concentrations estimated using the calibration curve in plasma. The recovery of cefazolin was estimated at 2, 4, 10 and 40 μg/ml in plasma. Stability of cefazolin in plasma was estimated before extraction, and also in the extract at 4°C.

EXPERIMENTAL DESIGN OF THE *IN VIVO* EFFICACY, PHARMACOKINETIC AND BIOEROSION STUDIES

Based on the results of a pilot study, the animal study was designed and conducted as per the experimental design described below. The protocol was approved by the Institutional Animal Care and Use Committee based on the methods suggested in the "Principles of Laboratory Animal Care" (NIH publication #85-23).

Pharmacokinetic and Efficacy Study

Eighteen Sprague-Dawley male rats weighing 275–325 gm were obtained through Division of Laboratory Animal Resources (DLAR), and acclimated to the facilities for one week before the start of the experiments. The rats were housed at the DLAR facilities and allowed free access to food and water prior to and during the experiment. The rats were assigned at random to 3 groups of 6 rats each. Each rat in the three groups was anesthetized with an IM injection of 50 mg/kg ketamine HCl and 12 mg/kg xylazine HCl, then the dorsal fur was clipped, and the back was painted with povidone-iodine solution. Each rat received six 0.3 ml subcutaneous injections of *S. aureus* suspension (4.5×10^7 CFU per injection), at different sites on the back in the clipped area. The rats did or did not receive antibiotic treatment before and after the inoculation based on their group assignment as described below.

Group 1: The Control Group

The rats in this group did not receive any antibiotic treatment after the inoculations with *S. aureus*, and no blood samples were withdrawn from these rats.

Group 2: The Intermittent Intramuscular (IM) Treatment Group

The rats in this group were infected, and received IM cefazolin treatment before and after the inoculation. After the administration of anesthesia, the rats in this group were injected with 10 mg/kg cefazolin every 4 hr for 68 hr (total of 18 injections, 180 mg/kg/rat). The bacterial inoculations as described above were made 20 min after the first cefazolin injection (perioperative). Blood samples were collected after the 1st and the 14th IM injection of cefazolin as described below.

Group 3: The Implant Group

The rats in this group were infected and received cefazolin containing implants 20 min before the inoculation. A guide template (Figure 1) was used to assign the implantation and the inoculation sites. Four subcutaneous pockets on the back of each rat were made using a sterile pair of surgical scissors, and a device was implanted in each pocket. The four devices together were designed to deliver 54 mg (equivalent to 180 mg/kg) cefazolin over a period of 3 days. After the implantation of the devices, the subcutaneous pockets were sutured with nonabsorbable surgical sutures. Twenty min after the insertion of the first device, the bacterial inoculations as described above were made in the vicinity of the subcutaneous pockets. Blood samples were collected at different time intervals during the 3 day period.

Pharmacokinetics of Cefazolin Delivered by Multiple IM Injections (Gp. 2) and SC Implants (Gp. 3)

Blood samples of 200 μl were collected from the tail vein after securing the animal in a comfortable rodent restrainer. Blood sampling was performed by cutting 2 mm of the tail at zero time and gently squeezing the tail in order to collect 200 μl of blood, however, for subsequent samples, gentle squeezing of the tail was used to induced bleeding. The sampling schedule for rats in the intermittent IM group 2 was: 5, 15 and 30 min and 1, 2 and 3 hr after the 1st and the 14th cefazolin dose. In the implant group 3, the blood sampling schedule was: 0.5, 2, 5, 8, 16, 25, 32, 40, 48, 56, 64, 68, 72 and 76 hr after implantation.

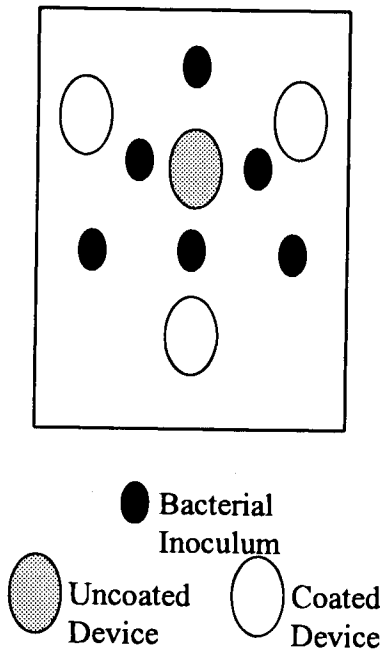

Fig. 1. Schematic of the guide template used to assign the locations of the implantation of the GMS devices and the bacterial inoculations on the dorsal side of the rats.

Each blood sample was allowed to clot (10 min) then centrifuged at 3000 rpm for 5 min. Plasma (100 μl) was separated and stored in the freezer until it was processed for the HPLC assay as described above.

Cefazolin plasma concentrations after the IM injections were fitted to noncompartmental pharmacokinetic model, and absorption and elimination rate constants, half-life, AUC, C_{max} and C_{min} were determined. Cefazolin plasma concentrations in the implant group were plotted versus time and the AUC, C_{max}, and C_{min} were determined. Wagner-Nelson method was used for the assessment of the extent of drug absorption as a function of the total drug released *in vivo* from the implants at various time intervals and correlate it to *in vitro* release (15,17).

In Vivo *Efficacy of Cefazolin Implants in Controlling the Infection Compared to the IM Intermittent and Control Groups*

The rats of all three groups were humanely euthanized with CO_2 seven days after the bacterial inoculation at the DLAR facilities. A dorsal incision was made to separate the skin and the abscesses from the underlying muscles. The encysted abscesses were dissected from the skin, counted, their dimensions measured with a microcaliper and individually weighed. The abscesses' count, diameters and weights were compared among the three groups, in order to evaluate the efficacy of the implants in controlling the infection. Also, the condition of the implants, the adjoining tissues, and the underlying skin of each rat in the implant group 3 were observed and photographed for any adverse reactions to the implants, and biocompatibility study.

Bioerosion of the GMS Based Implants

Three Sprague-Dawley rats were obtained through DLAR for this study. Each rat was implanted under anesthesia with four devices as described before, however the rats did not receive bacterial inoculation or cefazolin, and were allowed free access to food and water. Six weeks after implantation, the rats were euthanized and the skin in the implantation area was reflected away from the underlying musculature. The condition of the remnants of the implants and the tissues surrounding them was examined and photographed. The remnants of the implants were carefully excised and dried. The weight loss (indication of bioerosion) of the implants was determined by comparing the weight of the dried remnants of implants to their initial weight before implantation.

RESULTS

Validation of the Assay for Cefazolin in Plasma Samples

Method precision of the assay at 9.52 μg/ml of cefazolin resulted in peak area ratios of 1.23 ± 0.1 (7.3% RSD). The assay was linear from 2–60 μg/ml of cefazolin with $r^2 > 0.99$, and a limit of detection at 0.5 μg/ml. However, the sensitivity of the assay could have been further increased by increasing the injection volume. Extraction efficiency of cefazolin was 94 ± 11% (n = 3), 96 ± 5% (n = 3), 101 ± 8% (n = 8) and 100 ± 2% (n = 4) at concentrations of 2, 4, 10 and 40 μg/ml, respectively. Cefazolin was stable in plasma when stored at 4°C before extraction since the samples extracted 24 hr late yielded peak area ratios corresponding to 94–96% of the original concentration. Cefazolin was also stable in the extracts when stored at 4°C. Although cefazolin was found to be stable in both plasma and the extract, plasma samples were immediately frozen and stored at −10°C after obtaining them from the rats, and only 3–5 samples were extracted at the same time in order to prevent any degradation of cefazolin before, during and after the processing.

THE *IN VIVO* EFFICACY, PHARMACOKINETIC AND BIOEROSION STUDIES

Suitability of the Surgical Wound Infection Model

The concentration of *S. aureus* suspension in CFU/ml was verified every time an inoculum was prepared before each experiment, and was found to be $9.1 \times 10^7 \pm 3.3 \times 10^7$ CFU/ml (n = 6). The clinical isolate of *S. aureus* (ATCC 29213) used to induce infection was also sensitive to cefazolin with an MIC of 0.5 μg/ml, consistent with that reported in the NCCLS (16). In the control rats, 21 out of 36 inoculations of 4.5×10^7 CFU of *S. aureus* resulted in the formation of discrete abscesses (Table I). The subculturing of these abscesses resulted in the growth of *S. aureus* verifying that the bacterial inoculation was indeed responsible for the development of the abscesses. The formation of abscesses in all the rats of the control group signifies the ability of the *S. aureus* for developing subcutaneous infection in the rat model. Therefore, the ability of an antibiotic therapy for eradicating and inhibiting the development of the bacterial infection can be tested and evaluated using this model. The bacterial inoculation was chosen to be large (4.5×10^7 CFU), in order to evaluate the efficacy of the antibiotic therapy in preventing a high-risk infection. The examination of the inoculation sites for

Table I. Efficacy of Cefazolin Delivered Either From Implants or From Intermittent IM Injections in Controlling Infection with *Staphylococcus aureus* by Comparing the Number, Size and Weight of the Abscesses Formed at the Inoculation Site

Group (Number of rats)	Number of Abscesses Formed	Efficacy Compared to Control	Diameter (mm) of Abscesses Mean ± SD	Weight (mg) of Abscesses Mean ± SD
Control (n = 6)	21/36	—	2.67 ± 1.28 (n = 21)	32.47 ± 28.20 (n = 21)
Intermittent IM (n = 6)	1/36	95%	0.5 (n = 1)	8 (n = 1)
Implant (n = 6)	0/36	100%	—	—

abscesses was conducted 7 days after the inoculations, which corresponds to 4 days after the termination of the antibiotic therapy in the treatment groups to allow the bacteria that survived the therapy, if any, to re-establish the infection and form abscesses so one can distinguish between the bactericidal and the bacteriostatic efficacy of the antibiotic treatment.

Efficacy of Cefazolin Implants in Controlling the Infection Compared to the IM Intermittent and Control Groups

If the subcutaneously injected *S. aureus* were not eradicated (killed) by the antibiotic therapy, it should result in the formation of abscesses at the inoculation sites. The size, weight and number of the abscesses reflect the severity of the infection and the failure of the antibiotic therapy in controlling and containing the infection. Therefore, the number, size and weight of the abscesses formed was used as an indicator of the efficacy of the treatments (7). One week after inoculation, six rats in the control group (no antibiotic treatment) had collectively developed 21 abscesses out of the total 36 bacterial inoculations (6 per rat), however, the number of the developed abscesses varied from rat to rat. The average weight and diameter of the encysted abscesses were 32.5 ± 28.2 mg and 2.7 ± 1.3 mm (n = 21), respectively. In the implant group, none of the 36 inoculations resulted in the formation of abscesses, signifying the total eradication of the *S. aureus* injected due to the sustained localized delivery of cefazolin. The intermittent IM administration of cefazolin also resulted in the prevention of infection in five out of the six rats, the sixth rat had only one small abscess (8 mg, 0.5 mm). Statistically, there was no significant difference between the efficacy of the two treatments. The efficacy of the treatments was compared to the control group by comparing the number of abscesses formed in each treatment group to those in the control group as seen in Table I. Based on this comparison, the efficacy of cefazolin delivered from the implants and when administered as intermittent IM injections every 4 hr was 100% and 95%, respectively.

Pharmacokinetics of Cefazolin Delivered by Either SC Implants or IM Injections

Pharmacokinetics of Cefazolin Delivered from the Subcutaneous Implants (Gp. 3)

Cefazolin was continuously delivered from the implants with the plasma concentrations well above the MIC (0.5 μg/ml) for the entire 72 hr duration (Figure 2). Plasma concentrations were initially higher at 17.5 μg/ml for the first two hr due to the burst effect. Later, cefazolin concentrations started to decrease and then nearly plateau between the 8th and the 32nd hr, during which they ranged between 5.5 and 7.5 μg/ml. Afterwards, the plasma concentrations increased to range between 9 and 14.5 μg/ml, during the time period between the 40th and 64th hr, as cefazolin was being released from the coated implants. Cefazolin release from the first coated implant is expected to have started between the 25th and the 30th hr. After the 64th hr, the plasma concentrations started to decrease as cefazolin release from the implants approached termination. The overall plasma concentrations ranged between C_{max} = 17.5 μg/ml at 30 min to C_{min} = 17.5 μg/ml at 76 hr during the entire period, averaging 9.3 ± 4.9 μg/ml, suggesting very little fluctuations.

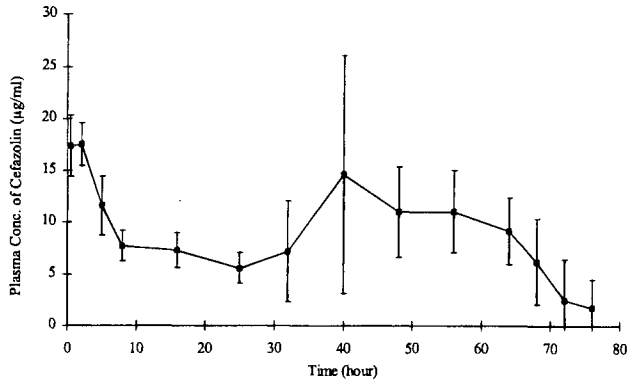

Fig. 2. Plasma profile of cefazolin delivered from GMS based devices, implanted subcutaneously in Sprague-Dawley rats (n = 6). MIC of cefazolin for *Staphylococcus aureus* is 0.5 μg/ml.

The area under the curve (AUC) was calculated to be 704 ± 131 μghr/ml (n = 6).

Pharmacokinetics of Cefazolin Administered as Multiple IM Injections (Gp. 2)

The plasma concentrations of cefazolin in this group fluctuated tremendously (Figure 3). After the first IM injection of 10 mg/kg cefazolin administered under anesthesia, the plasma concentrations ranged between 33 μg/ml (C_{max}) at 30 min and 6 μg/ml (C_{min}) at 3 hr (n = 6). At steady state, after the 14th IM injection, the plasma concentrations ranged between 37 μg/ml (C_{max}) and 2μg/ml (C_{min}), 30 min and 2 hr post-dose, respectively. Plasma concentrations at the end of 4 hr post-dose were not detectable and were estimated from curve fitting of the data to be 0.44 μg/ml. Thus, there would be time periods during which cefazolin plasma concentration would be lower than its MIC of 0.5 μg/ml. Cefazolin plasma profiles after the 1st and the 14th dose were fitted independently to the following non-compartmental pharmacokinetic model:

$$C = C_i e^{-k_e t} - C_i e^{-k_a t} \quad (1)$$

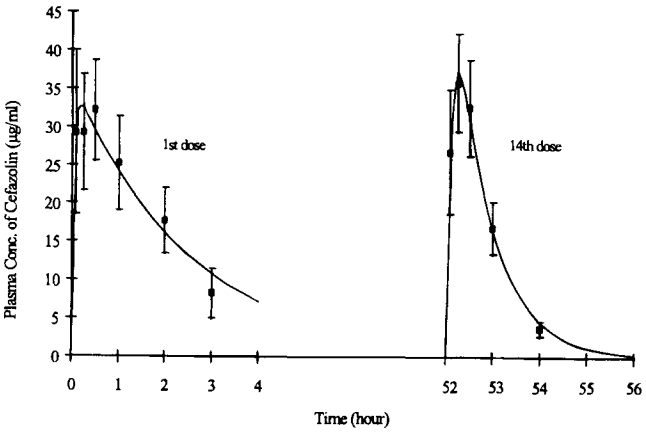

Fig. 3. Cefazolin plasma profiles after the administration of the 1st dose (under anesthesia) and the 14th dose of 10 mg/kg/dose to Sprague-Dawley rats (n = 6). MIC of cefazolin for *Staphylococcus aureus* is 0.5 μg/ml.

Where C is plasma concentration of cefazolin at time t, k_e is the apparent first-order elimination rate constant, k_a is the apparent first-order absorption rate constant, C_i is the Y axis intercepts.

The absorption rate constants were 15.8 ± 9.2 hr^{-1} and 11.9 ± 7.4 hr^{-1} after the first and the 14th dose, respectively (n = 6). The elimination rate constants were 0.46 ± 0.13 hr^{-1} and 1.27 ± 0.16 hr^{-1} after the first and the 14th dose, respectively (n = 6). These values are similar to those obtained by fitting the plasma concentrations reported by Intoccia *et al.* (18) (k_e = 1.3 hr^{-1} and k_a = 10.3 hr^{-1}) after a single IM injection of cefazolin 20 mg/kg to 30 Sprague-Dawley rats. The fitted plasma concentrations at steady state ranged from 44 μg/ml (C_{max}) to 0.4 μg/ml (C_{min}), 15 min and 4 hr post-dose, respectively, suggesting 110-fold fluctuations in plasma concentrations.

The elimination rate constant (k_e) of cefazolin was slower after the first dose compared to that after the 14th dose. The reduction in the elimination rate constant under anesthesia can be attributed to the combined effect of the anesthetic agents (ketamine/xylazine) on the blood flow (19). The administration of anesthesia reduces the blood flow through the kidneys and since 96% of cefazolin is renally eliminated in Sprague-Dawley rats, the anesthetic agents had resulted in an overall reduction in cefazolin clearance.

Cefazolin $t_{1/2}$ is about 30 min, thus steady state plasma concentrations were achieved in 2 hr (4 $t_{1/2}$). Therefore, the pharmacokinetic parameters obtained from the 14th dose were employed in predicting the plasma profile for all the doses except for the first dose (assuming no anesthesia effect on the 2nd dose), and compared to that of the implant group in Figure 4 and compare it to the MIC. The AUC for the whole profile (18 doses) was calculated to be 784 ± 122 μg hr/ml (n = 6), statistically similar to that of implants.

The Extent of Absorption and In Vivo *Release of Cefazolin from the Implants*

Extent of cefazolin absorption from the implants was identical to that from multiple IM injection since the AUC of cefazolin delivered by the implants (Gp. 3, 704 ± 131 μg hr/ml, n = 6) during the three day period was not significantly different (p = 0.3) from the AUC of identical dose administered as multiple IM injections (Gp. 2, 784 ± 122 μg hr/ml, n = 6). Therefore, all of the 100% loaded cefazolin was released and absorbed intact from the implants during the three day period. The slightly lower AUC from the implants may be due to still some residual cefazolin in the implants or at the implantation site, remaining to be absorbed into plasma.

The rate limiting step for appearance of cefazolin in plasma is the release of cefazolin from the implants rather than the absorption rate which was much faster based on the k_a from the IM data, and the *in vitro* release profile of implants (15). Therefore, the amount of cefazolin released and absorbed at the implantation site at various times was estimated from the plasma profiles of the implant Gp. 3 (Figure 2) by Wagner Nelson Method and considered to be *in vivo* release. Figure 5 shows the *in vivo* release of cefazolin from the GMS based implants compared to that obtained *in vitro*. The *in vivo* release of cefazolin from the combination of the four devices was nearly linear, indicating zero order release. The *in vivo* release profile paralleled the *in vitro* release profile for the first 26 hr. However, after 32 hr the *in vivo* release profile from the coated devices had a smaller slope than that *in vitro*, signifying a slower release rate *in vivo* from the coated devices. Overall, there was an excellent correlation (R^2 = 0.98) between the *in vivo* and the *in vitro* release profiles of cefazolin from the GMS based implants.

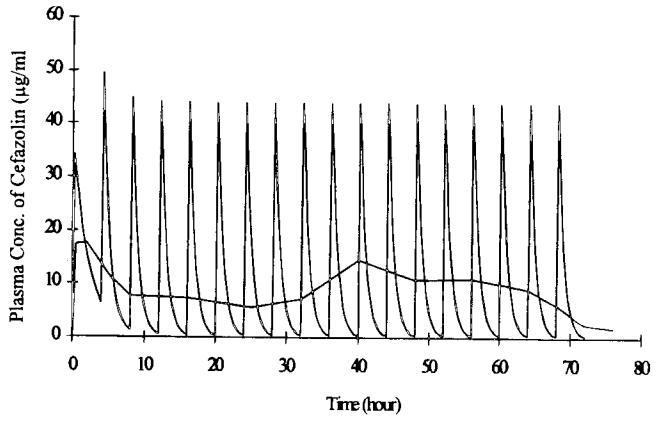

Fig. 4. Comparison of plasma profiles of cefazolin delivered by GMS implants to that obtained from multiple i.m. injections of 10 mg/kg cefazolin every 4 hr in Sprague-Dawley rat (n = 6). Plasma profiles of cefazolin were simulated for multiple i.m. injections, using pharmacokinetic parameters obtained from the 1st dose for simulating the 1st dose and parameters obtained form the 14th dose for simulating the rest of the 17 doses. MIC of cefazolin for *Staphylococcus aureus* is 0.5 μg/ml.

Bioerosion of the GMS Based Implants

None of the six rats in the implant group 3 died during the entire experiment. Even though, the rats were inoculated with *S. aureus*, they were healthy and the incisions made to insert the implants were completely healed within the first 3 days of implantation. The lack of inflammation at the implantation site even at the early stages, signifies the biocompatibility of the GMS based implants. During the entire 6 week period, the three rats employed in the erosion study were healthy, and upon opening of the implantation site (six weeks post-implantation), remnants of the implants were in a pasty form and no visible irritation nor significant fibrosis was found around them, suggesting biocompatibility of the GMS based implants. One of

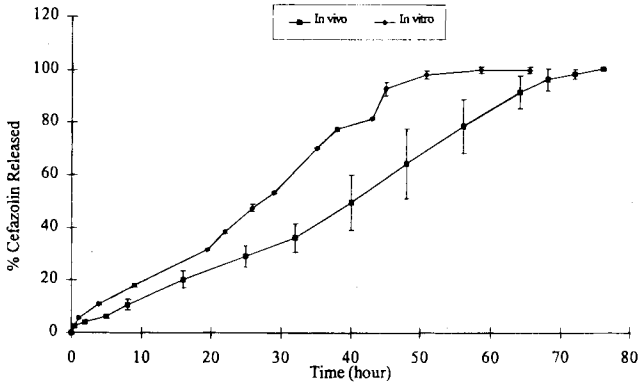

Fig. 5. *In vivo* release of cefazolin from GMS based devices implanted subcutaneously in Sprague-Dawley rats (n = 6) compared to the release profile obtained *in vitro*.

the four implants completely disappeared in two rats while the remaining implants had lost significant weight with weight loss ranging from 20–100% (Figure 6). These results are a good indication of the bioerosion and the bioabsorption of the GMS based implants from the implantation site.

DISCUSSION

The study demonstrated successfully the efficacy and improved pharmacokinetics of cefazolin delivered from biodegradable implants for the prevention of post-operative wound infection. The devices are to be implanted in the surgical wound before the closure, where cefazolin is released slowly for three days, providing high local concentrations to eradicate and kill any bacteria to prevent an infection and subsequently the devices would dissolve in the body into safe by-products. This site-specific antibiotic delivery would obviate the need for multiple systemic administration of antibiotics at high dose, which may not only be toxic but may be ineffective due to the lower concentrations at the site of surgery.

The implants prevented the development of abscesses, sign of infection from high-level inoculations with *S. aureus*, the most common pathogen isolated clinically in surgical wound infections (1,3). No abscesses were formed in the rats of the implant group, however one out of the 36 inoculations resulted in an abscess in the intermittent IM group. Although there was no significant difference ($p = 0.3$) between the efficacy of the two treatments, the failure of multiple IM injections of cefazolin in completely preventing infection demonstrate why systemic antibiotic prophylaxis is sub-optimal and why so many post-surgical infections do occur (1,2,4). Earlier studies have not shown significant superiority in efficacy of the continuous delivery of antibiotics over the intermittent administration of equal doses, when the antibiotics are administered in high doses (7–9). Vogelman *et al.* (9) showed that the maximum bactericidal efficacy of cefazolin against *S. aureus* occurred when the drug concentrations exceeded the MIC for 55% of the dosing interval. Since in this study, intermittent injections of cefazolin is expected to have provided concentrations at the inoculation site above the MIC for a period longer than 55% of the dosing interval, the efficacy results did not support the superiority of the continuous delivery of cefazolin. However, to prevent any infection, cefazolin is administered systemically in high doses. If cefazolin were to be administered at lower doses, the superiority of the local continuous delivery of cefazolin at equivalent doses can be easily predicted in terms of efficacy, since the local delivery from the implants provides higher concentrations of cefazolin at the surgical site where it is most needed. Furthermore, the local administration of antibiotic is designed to provide effective concentrations at the surgical site. In contrast, the systemic administration could fail to provide effective concentrations to the tissues, especially in the case of necrosis and when the blood supply is compromised such as in surgery.

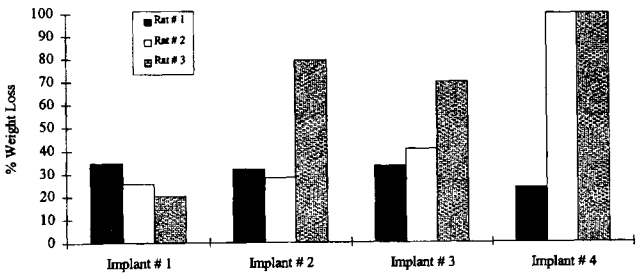

Fig. 6. Bioerosion of the four GMS based implants, as measured by the weight loss of the implants, six weeks post-subcutaneous implantation in three Sprague-Dawley rats.

Thus, with the local sustained delivery of an antibiotic, smaller doses are required for this prophylactic therapy to be effective. The sustained delivery of these smaller doses will also result in a less fluctuating plasma profile compared to multiple bolus delivery of identical doses of the antibiotic (Figure 4). The lower doses and lowered fluctuations in plasma profile can be a significant therapeutic improvement for aminoglycosides such as gentamicin and vancomycin whose side effects and systemic toxicity are more pronounced and drastic than those of cephalosporins since their therapeutic windows are much narrower and their MICs are closer to the toxic concentration (5). Thus use of the GMS based implants for the local delivery of an aminoglycoside such as vancomycin and gentamycin would be advantageous in protecting against nephrotoxicity and ototoxicity with an improvement in efficacy. The GMS based implants maintained plasma concentrations between 5.5–17.5 μg/ml, 3-fold fluctuation for 76 hr while the 18 IM injections of cefazolin resulted in a 110-fold fluctuating plasma profile, with the concentrations at steady state ranging between 0.4–44 μg/ml every 4 hr (Figure 3). Due to a 30 min half-life of cefazolin in rat, IM administration was required every 4 hr for a total of 18 injections in order to maintain a continuous presence of cefazolin in the plasma for 72 hrs. In case of implants, the antibiotic delivery system once implanted into the surgical wound at the time of surgery would release the antibiotic for the duration of the prophylactic therapy without the need for the inconvenient repeated systemic injections. Thus, in addition to patient's convenience, the use of implants will also reduce the patient's care costs by reducing the cost for administering the injections and the possible earlier patient mobilization and the reduction in hospital stay.

No adverse effects were observed to implants, and all the rats were alive and healthy for the six week duration of the study. The surgical wounds created for implanting the devices were healed with out an indication of inflammation at the implantation site, and upon opening, no visible irritation or inflammation was found in the subcutaneous tissues around the implants, and fibrous encapsulation of the implants was not observed. The well being of the rats and the absence of a visible irritation in the subcutaneous tissues as well as the erosion of the implants are good indicators of the biocompatibility and bioerodibility of GMS based implants, although more studies are needed at the cellular level to identify any adverse pathological effects of GMS. Natural glycerides, such as mono-, di-, and tri-palmitate have been shown to be biodegradable and biocompatible when investigated in rabbit (20), therefore, the biocompatibility and biodegradability of glyceryl monostearate (GMS) observed in this study is not surprising.

In vivo release of cefazolin from the combination of the four GMS based implants was maintained for three days at almost zero order rate, and correlated very well with its *in vitro* release profile (Figure 5). Since the AUC of cefazolin released

from the implants was not significantly different ($p > 0.05$) from that of cefazolin administered by multiple IM injections, assuming same extent of absorption from the IM and the subcutaneous administration sites, one can conclude that 100% of loaded cefazolin was released and absorbed intact from the implants.

The elimination (k_e) and absorption (k_a) rate constants of cefazolin were found to be 1.3 ± 0.2 hr^{-1} and 11.9 ± 7.4 hr^{-1}, respectively when it was administered without anesthesia. These values agree with those reported by Intoccia *et al.* (18) ($k_e = 1.3$ hr^{-1} and $k_a = 10.3$ hr^{-1}) after a single IM injection of cefazolin 20 mg/kg to rats. However, the elimination (k_e) and absorption (k_a) rate constants of cefazolin were 0.46 ± 0.1 hr^{-1} and 15.8 ± 9.2 hr^{-1}, respectively when it was administered under anesthesia. Thus, the elimination rate constant of cefazolin was significantly lower when administered under anesthesia (0.46 hr^1 vs. 1.3 hr^{-1}, $p = 2.04 \times 10^{-6}$). The combination of ketamine and xylazine for anesthesia has been reported to reduce blood pressure of rodents, which is accompanied by a reduction in blood flow, and since 96% of cefazolin is eliminated renally, the reduction in renal blood flow may be have resulted in the overall reduction of its elimination rate (19). The AUC with anesthesia (69.8 ± 14 μg hr/ml, 1st dose) was also significantly higher ($p = 0.002$) than that without anesthesia (44.7 ± 6.8 μg hr/ml, 14th dose) indicating a slower clearance under anesthesia, further supporting the above possibility. Since the first dose of cefazolin as surgical prophylaxis is usually administered under anesthesia, the reduced clearance may inadvertently provide higher levels for prophylaxis, although unnoticed so far.

Various antibiotic delivery systems using non-biodegradable material such as PMMA, and biodegradable materials such as bone grafts, demineralized bone matrix, polyanhydrides and poly(D,L-lactide) have been reported primarily for orthopedic infections, with antibiotic release for a period ranging from a week to a month (21–31). However, GMS based implants for cefazolin is the first antibiotic delivery systems designed solely for the prevention of post-surgical wound infection which releases antibiotic for a 3-day period. Therefore, the delivery system evaluated in this study is novel, unique and found to be ideally suited for prevention of post-surgical wound infection in terms of efficacy, pharmacokinetics and biodegradability.

CONCLUSIONS

Rat infection model was used to simulate a post-operative wound infection, in order to evaluate the efficacy of the cefazolin loaded GMS implants as a prophylactic measure. The local continuous release of cefazolin from the GMS delivery system successfully eradicated the *S. aureus* induced infection in all the rats. Although there was no significant difference in efficacy between the implant and the intermittent IM group, the local delivery from the implants provides higher concentrations of cefazolin at the implantation area than the systemic administration of the same dose. Thus, it is expected that lower dose of cefazolin is required when it is administered locally than required by a systemic administration. The implants delivered cefazolin at nearly zero order rate for a 3 day period *in vivo*, resulting in very little fluctuations in plasma levels for 76 hr. Cefazolin plasma levels in the implant group were maintained between 5.5–17.5 μg/ml for nearly 3 days, in contrast, the intermittent IM cefazolin resulted in a highly fluctuated plasma profile, in which the concentrations at steady state ranged between 0.4–44 μg/ml every 4 hr. The well being of the rats and the absence of a visible irritation in the SC tissues as well as the erosion of the implants are good indicators of the biocompatibility and bioerodibility of GMS based implants. Overall, the results demonstrates an effective, biodegradable, bioerodible delivery system based on GMS for the local delivery of antibiotics for the prevention of post-operative wound infection.

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