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Research Paper

Local Delivery of Vancomycin for the Prophylaxis of Prosthetic Device–Related Infections

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Purpose. To evaluate the *in vivo* efficacy and pharmacokinetics of vancomycin delivered from glycerylmonostearate (GMS) implants in a prosthetic-device based biofilm infection model.
Methods. A biofilm infection model was developed in male Sprague-Dawley rats by implanting a vascular graft on the dorsal side of each rat and infecting it with 1.5×10^8 cfu/ml *Staphylococcus epidermidis*. The rats were divided into 3 groups of 6 rats each: 1) the control group that received no antibiotics, 2) the IM group that received multiple IM injections of vancomycin at a dose of 25 mg/kg every 6 h for a total of 12 doses, and 3) the implant group that received GMS implants designed to deliver vancomycin at a total dose of 300 mg/kg for a period of 4 days. The pharmacokinetics of vancomycin was determined from IM and implant groups by analyzing for vancomycin in blood using HPLC. *In vivo* efficacy was studied by evaluation of the wound site and the prosthetic device upon excision, for evidence of infection in the form of purulent discharge at the wound site and yellowish discoloration of the prosthetic device and inflammation as sign of biofilm formation. Microbiological evaluation on the wound site and the prosthetic device was performed by culturing the swabs at the wound site and the prosthetic device in sterile tryptic soy broth for 36–48 h at 37°C.
Results. Vancomycin was successfully delivered in a sustained manner for 100 h from GMS implants and the resulting plasma profile showed that the concentrations, after an initial burst, plateaued at about of 4.77 ± 1.43 μg/ml with less fluctuations than the IM group in which the plasma concentrations fluctuated between 2.73 ± 0.94 μg/ml and 19.26 ± 3.67 μg/ml. Upon excision of the wound site, all the animals in the control group developed infection in the form of purulent discharge and yellowish discoloration of the prosthetic device. However, none of the rats in the implant group showed evidence of infection clearly demonstrating the efficacy of the local delivery system in preventing infection. Systemically delivered vancomycin by IM injections failed to prevent infection in four out of six rats. Microbiological evaluation of the wound site and prosthetic device resulted in isolation of biofilm-producing organisms such as *Staphylococcus epidermidis, Enterococcus faecalis,* and *Staphylococcus aureus*. These organisms were isolated in greater number of animals in the control group compared to the IM and implant groups.
Conclusions. The GMS implants as a delivery system for vancomycin were successful in preventing infection in all the animals compared to the IM and control groups demonstrating the efficacy of a local delivery system in a prosthetic device related biofilm infection model.

KEY WORDS: local delivery; prosthetic device; biofilm infection; surgical wound infection; vancomycin; implants; antibiotic; pharmacokinetics; efficacy; glyceryl monostearate.

INTRODUCTION

Advances in bioengineering have led to the development of a variety of materials that can be introduced into the body for a variety of therapeutic purposes (1). During the past few decades, a number of prosthetic implants have been developed for use in various parts of the body such as orthopedic implants, heart valves, vascular catheters, peritoneal implants, penile prosthesis, and so forth. However, their longevity and functionality is frequently limited by infection (2).

In spite of advances in surgical techniques and rigorous application of aseptic procedures, microbial infections have been occurring in many implant-related surgeries. In addition to tissue damage and inflammation at the site of implantation, device dysfunction and severe systemic infection have been observed with prosthetic device associated infections (3). The causative organisms in the majority of device-related infections are *Staphylococcus epidermidis, Staphylococcus aureus,* and *Escherichia coli* (3). Infections associated with prosthetic devices are deep-rooted, difficult to treat, and recalcitrant to high doses of antibiotics (4).

Prosthetic device–related infections are difficult to treat because bacteria associated with prosthetic implants form a biofilm that coats the device and dwells beneath as a colony,

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a highly networked unit of cells that resists host immune response and renders administered antibiotics ineffective (5). A biofilm is defined as an assemblage of microbial cells that is both enclosed by an extracellular polysaccharide containing polymer matrix and attached to a surface by means of glycocalyx (6,7). Biofilms are found in nature in great abundance and they have been implicated in the corroding of water pipes in chemical industries, fouling of industrial conduits, and malfunctions in computer chips (4). In the medical world, biofilm bacteria are responsible for the development and persistence of infection in the presence of prosthetic implants and cause local and systemic toxicity and frequently result in failure of implants.

A number of different reasons have been offered to explain the difficulty encountered in treating biofilm-related infections. It has been proposed that the reason for reduced efficacy of antibiotics toward biofilm bacteria is the hindered or reduced diffusion of antibiotics across the biofilm barrier (8). Nickel *et al.* (9) implicated the negatively charged glycocalyx in the antibiotic resistance of biofilms. It has been observed that bacteria growing as biofilms have lower growth rates and typically less fastidious nutrient requirements (10).

Conventionally, antibiotics for the prophylaxis of prosthetic device–related infections have been administered by intravenous or intramuscular routes (11). Due to the recalcitrant nature of prosthetic device–related infections, high concentrations of antibiotics are needed locally at the site of implantation, to prevent infections. However in most cases, due to poor vasculature of the wound site, conventional route of delivery fails to achieve optimal concentration of the antibiotics at the implantation site with unnecessary exposure of other organs in the body to high concentration of antibiotics (12). Attempts to overcome this problem have made use of local delivery systems, which deliver antibiotics at the site of implantation where they are most needed (13–18). High concentrations of antibiotics are achieved locally with minimal exposure to other organs in the body thus minimizing problems due to drug toxicity.

Allababidi *et al.* demonstrated the efficacy of glyceryl monostearate (GMS)-based implants delivering cefazolin for the prophylaxis of surgical wound infections in an animal model (18). Although cefazolin is the most commonly used antibiotic for the prophylaxis of surgical wound infections, increasing incidence of methicillin-resistant *Staphylococcus aureus* (MRSA) in hospitals has mandated the frequent use of vancomycin (19). Also, vancomycin is the drug of choice in the prophylaxis and treatment of prosthetic device–related infections, which are mainly caused by *Staphylococcus epidermidis*. Hence, a local delivery system of vancomycin would provide significant benefit as compared to conventional systemic delivery of vancomycin for prophylaxis and treatment of prosthetic device–related infections.

Previous studies indicated that vancomycin could be delivered from a combination of uncoated and coated implants into an *in vitro* system for a 4-day period with a near zero-order release (20). It is unknown however, if local delivery of vancomycin with such a delivery profile *is* effective in the prophylaxis of prosthetic device–related infections. Thus, the objectives of the research were 1) to evaluate and compare the *in vivo* efficacy of vancomycin delivered from the GMS implants in a biofilm animal model with conventional route of administration (intramuscular; IM); 2) to compare the pharmacokinetics of vancomycin delivered by the implant vs. IM dosing; and 3) to study the bioerosion and biocompatibility of the implants in rats.

MATERIALS AND METHODS

Materials

Vancomycin hydrochloride was obtained from the Medical University of South Carolina hospital pharmacy. Glycerylmonostearate was a gift from Eastman Chemicals (Barcelona, Spain). PEG 8000 was a gift from Union Carbide (Piscataway, NJ, USA), and Tween 80 was obtained from ICI Chemicals (London, England). Perchloric acid, tryptic soy broth, tryptic soy agar plates, high performance liquid chromatography (HPLC)-grade acetonitrile, monobasic sodium phosphate, and dibasic sodium phosphate were purchased from VWR Scientific (West Chester, PA, USA). Ketamine HCl was obtained from Fort Dodge Laboratories, Inc. (Madison, NJ, USA), and xylazine HCl was obtained from Bayer Corporation (Berlin, CT, USA). Normal saline for injection was obtained from Abbott Laboratories (Chicago, IL, USA). Male Sprague-Dawley rats were obtained from Harlan Sprague-Dawley (Indianapolis, IN, USA). *Staphylococcus epidermidis,* isolated from a blood specimen of a patient, was obtained from American Type Culture Center (ATCC 35883).

Methods

HPLC Assay and Stability of Vancomycin

A validated HPLC assay adapted based on a method developed by Luska *et al.* (21) was used. The assay method was validated and stability of drug in plasma confirmed. The stability of vancomycin in plasma and plasma processed with perchloric acid was studied for 24 h at two vancomycin concentrations, 50 and 10 μg/ml at 4°C and –20°C.

Microbiological Methods

Preparation of the Culture Suspension of Staphylococcus epidermidis

Staphylococcus epidermidis (ATCC 35983) was obtained from ATCC as a freeze-dried pellet and was reconstituted in sterile distilled water. The culture suspension was revived by immediately incubating an aliquot in sterile tryptic broth at 37°C for 48 h. The culture suspension of the organisms thus obtained was subcultured in sterile tryptic broth each day to maintain a viable culture. The concentration in the culture suspension was adjusted to 0.5 MacFarland (equal to 1.5×10^8 cfu/ml) by matching the turbidity of the test tube to 0.5 MacFarland's standard and also by measuring the absorbance of the culture suspension at 625 nm. The viability and inoculum concentration of the culture was verified on the day of the study before inoculating the animals.

In Vivo Efficacy and Pharmacokinetic Study: Experimental Design

The *in vivo* efficacy and pharmacokinetic study attempted to compare the relative efficacy of vancomycin delivered by local vs. systemic administration in preventing biofilm infection of an implanted prosthetic device challenged by inoculation with *Staphylococcus epidermidis*. The infection model was developed by injecting *Staphylococcus epidermidis* (1.5×10^8 cfu/ml) in the logarithmic phase of growth, on and around the implanted prosthetic device [a polytetraflouroeth-

ylene (PTFE) IMPRA Carboflo Vascular Graft, Bard Peripheral Vascular, Inc. (Tempe, AZ, USA), 1 cm in length] in rats to produce a biofilm infection. Based on the pilot experiment, the *in vivo* efficacy and pharmacokinetic studies were conducted using the experimental design described below.

Eighteen rats were used in the experiment, and each of the rats in the three groups were anesthetized with an intramuscular injection of a mixture of ketamine HCl (50 mg/kg) and xylazine HCl (15 mg/kg), the dorsal fur was clipped, and the back painted with povidone-iodine. Then the rats were randomly allocated to the three treatment groups of six rats each as described below. The animal protocol was approved for the institutional animal use and care committee (IUACC) prior to conducting the pilot study.

Group 1: The Control Group

Under aseptic conditions, one subcutaneous pocket was made on the dorsal side of each rat, a small piece of sterile plastic prosthetic device was placed in the pocket and multiple injections of *Staphylococcus epidermidis* (1.5×10^8 cfu/ml, in the logarithmic phase of growth) were made around the prosthetic device according to a guide template (Fig. 1). No antibiotic treatment was provided to the rats in this group and no blood samples were withdrawn from these animals to evaluate if a biofilm indeed is developed in the absence of antibiotic treatment.

Group 2: The Intermittent IM Group

The rats in this group were surgically implanted with a sterile prosthetic device and infected as the rats in the control group. This was immediately followed by an intramuscular injection of 25 mg/kg vancomycin in the thigh muscle, and dosed again with 25 mg/kg vancomycin, once every 6 h for 3 days (total of 12 injections, 300 mg/kg per rat) to simulate clinical dosing.

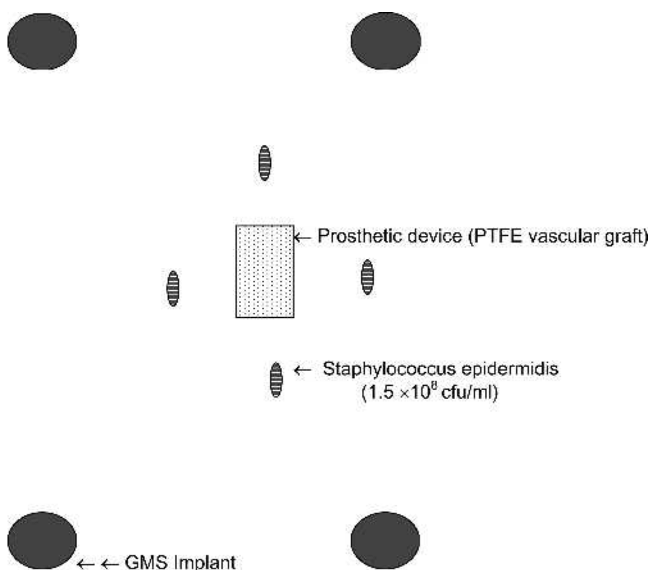

Fig. 1. Guide template used to determine the site of implantation of GMS implants and prosthetic device and subsequent microbial inoculations.

Group 3: The Implant Group

The rats in this group were surgically implanted with a prosthetic device and infected as the rats in the control group. Using a guide template (Fig. 1), the implants containing vancomycin were placed in the surgically created pocket, the implants were separated from each other by creating space within the pocket using forceps. Implants were designed to deliver a total vancomycin dose of 300 mg/kg per rat.

GMS Implants

A combination of 4 GMS implants (1 uncoated and 3 coated) designed to deliver a total vancomycin dose of 300 mg/kg per rat over a period of 96 h were prepared for *in vivo* implantation as previously described (20).

Evaluation of the Pharmacokinetic Parameters of Vancomycin Delivered by Either Intramuscular Injections or GMS Implants

Blood samples were collected from the tail vein of rats from group 2 at predetermined time intervals upon administration of first and ninth doses and analyzed for vancomycin. The sampling schedule in this group was 0, 15, 30, 60 min, 1.5, 2, 3, 4, 5, and 6 h after the administration of the first and ninth doses. Blood samples were allowed to clot for 10 min and centrifuged at 5000 rpm for 5 min to separate plasma. Vancomycin plasma concentration profiles from the ninth dose were fitted to a noncompartmental pharmacokinetic model and the pharmacokinetic parameters (elimination rates, half-life and AUC) were determined. The C_{max} and C_{min} were observed to study fluctuations in the plasma concentrations.

For animals in group 3, blood was withdrawn at predetermined intervals over 4 days. The sampling schedule for the animals in this group was 0, 1, 2, 3, 6, 9, 12, 24, 30, 36, 48, 54, 60, 72, 78, 84, 96, and 100 h after implantation of the GMS implants. Blood samples were collected as indicated earlier and analyzed using the HPLC assay. The plasma profile was obtained, and C_{max} and C_{min} for this group were observed both pre- and post-steady state and compared to those obtained from the IM group to evaluate the fluctuations in plasma concentration. In addition, vancomycin *in vivo* absorption profile from the implants was obtained using Wagner-Nelson's deconvolution method (22). The *in vivo* release thus obtained was compared and correlated to the *in vitro* release obtained for the implants.

Evaluation of the *in Vivo* Efficacy of Vancomycin Implants as Compared to the IM Delivery of Vancomycin and Control Group in Biofilm Infection

The rats from all three groups were euthanized seven days after the injection, a dorsal incision was made on the skin and the underlying tissues were exposed. The tissues surrounding the implanted site were examined for purulent discharge (pus), which is a known indicator of infection. The prosthetic device was examined for discoloration and gross changes in the morphology, which is evidence for the presence of a biofilm. In addition, swabs of the wound exudates and the prosthetic device were removed aseptically and cultured in sterile tryptic soy broth at 37°C for 36 h. The resultant liquid cultures were plated on sterile tryptic soy agar and incubated at 37°C for 36 h. The plates that showed evidence of colonies were typed and identified.

Evaluation of Bioerosion and Biocompatibility of GMS Implants with Vancomycin

Six rats were procured and anesthetized as described earlier and were implanted with the GMS implants loaded with vancomycin designed to deliver 300 mg/kg per rat over a period of 3–4 days. Each rat received four implants, one uncoated and three coated, and each implant was placed in an individual pocket on the dorsal side as illustrated by the guide template (Fig. 1). An additional pocket was made at the center into which a prosthetic device (PTFE vascular graft, 1 cm in length) was placed and a viable culture of *Staphylococcus epidermidis* (1.5×10^8 cfu/ml) was injected on and around the prosthetic device. The rats were placed in individual cages and provided free access to food and water. They were weighed regularly for the entire period of 6 weeks, as loss/gain in weight is an indirect measure of the biocompatibility or toxicity of the GMS implants. The rats were euthanized 6 weeks postimplantation, and the wound site was photographed and evaluated for presence of any gross symptoms of infection. The implants were carefully removed, dried to constant weight, to determine the extent of bioerosion, which was expressed as the percent GMS implant remaining at the end the 6-week period. The surrounding tissues were observed for purulence and the prosthetic device was noted for any discoloration, which indicates the formation of a biofilm.

RESULTS

HPLC Assay Validation and Stability of Vancomycin

A calibration curve of vancomycin in plasma with R^2 of 0.99 was obtained with vancomycin standards ranging in concentration from 1.25 to 50 μg/ml. The limit of detection was found to be 1.25 μg/ml and the day-to-day variation as measured by RSD on the slope of the calibration curve was found to be 6.5% (n = 8). Method precision on the assay was 2.22% (RSD, n = 6), determined by preparing, processing and analyzing six individual vancomycin plasma standards at a concentration of 50 μg/ml. Stability of vancomycin in plasma and plasma processed with perchloric acid was studied at two concentrations; 50 and 10 μg/ml at 4°C and –20°C. These results indicate that vancomycin is stable in plasma when stored at 4°C and –20°C for a period of 24 h and hence plasma was processed immediately prior to analysis.

In Vivo Efficacy and Pharmacokinetic Study

Pharmacokinetics from IM Administration

The average plasma profile of vancomycin obtained from the ninth dose was represented by an absorption phase followed by the elimination phase (Fig. 2). Pharmacokinetic parameters were calculated by fitting the individual plasma profiles to the noncompartmental pharmacokinetic model:

$$C = C_i e^{-ket} - C_i e^{-kat},$$

where C is the plasma concentration of vancomycin at any time t, C_i is the *y*-axis intercept, k_e is the apparent first-order elimination rate constant, and k_a is the apparent first-order absorption rate constant. The absorption and elimination rate constants were 0.0479 ± 0.0218 min^{-1} and 0.00647 ± 0.00134 min^{-1}, respectively (n = 6). The biological half-life of vancomycin in rats was calculated to be 105 ± 31.30 min with steady state attained in approximately 6 h (4 $t_{1/2}$) and thus, within the second dose. Therefore, the pharmacokinetic parameters obtained from the ninth dose represent those at steady state and were used in predicting the plasma profile for all doses except the first dose. The observed C_{max} and C_{min} ranged between 19.26 ± 3.67 μg/ml and 2.73 ± 0.94 μg/ml (n = 6), respectively in the 6 h indicating a 7-fold fluctuation. The AUC for the whole profile (12 doses) was calculated as 43294 ± 6876 μg min/ml (n = 6).

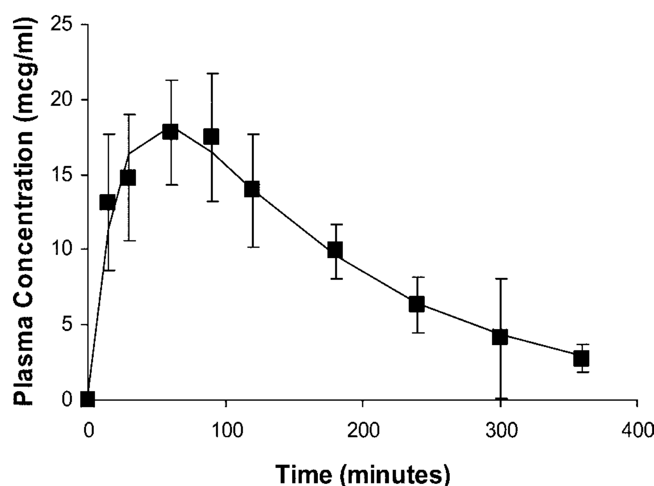

Fig. 2. Plasma profile obtained after the ninth IM dose (steady state) of 25 mg/kg vancomycin to male Sprague-Dawley rats in the prospective study with the fitted line representing first-order absorption and elimination fitted to the equation $C = C_i e^{-k_e t} - C_i e^{-k_a t}$ using the following parameters: elimination rate constant (k_e) = 0.00656 min^{-1} and absorption rate constant (k_a) = 0.0395 min^{-1} (n = 6). Data presented as mean ± SD.

The plasma concentration profile following the first dose was characterized by slow and incomplete absorption followed by plateauing of the concentrations and slow elimination. This is may be due to the effect of anesthesia, which lowers blood pressure leading to a lower renal blood flow and slower rate of renal elimination, primary route of elimination for vancomycin (22,23). This effect of anesthesia on reducing clearance of cefazolin was observed earlier in a similar animal model (17). The observed C_{max} and C_{min} ranged between 6.8 ± 0.23 μg/ml and 1.5 ± 1.04 μg/ml (n = 6) during the interval of 6 h, indicating a 5-fold fluctuation in the plasma profile.

Pharmacokinetics of Vancomycin Delivered by GMS Implants

Vancomycin was released continuously from the implants with the plasma concentrations remaining above the MIC (2.5 μg/ml) for the entire 4-day period (Fig. 3). The plasma concentrations of vancomycin were initially higher at 20 μg/ml during the first 6 h due to an initial burst. However, the plasma concentrations were maintained at an average of 4.77 ± 1.43 μg/ml later, for the entire 4-day period. As seen in Table I, the overall fluctuations in the concentrations (after the first 6 h) ranged between 9.30 μg/ml and 1.90 μg/ml suggesting a 5-fold fluctuation over a 4-day period. The AUC was calculated to be 34128 μg min/ml, which is approximately 80% of the AUC obtained for an equal dose of vancomycin delivered by multiple IM injections indicating more than 80%

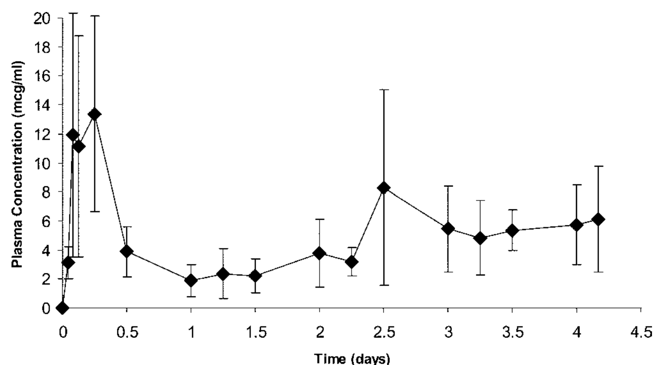

Fig. 3. Plasma profile of vancomycin obtained from a combination of GMS implants to deliver a total of 300 mg/kg dose over a 4-day period. (MIC of vancomycin to *Staphylococcus* epidermidis is 2.5 μg/ml). Data presented as mean ± SD.

bioavailability. The lower AUC might be due to some residual vancomycin in the implants or that, which remains to be absorbed at the site of implantation. Plasma profiles of vancomycin delivered from the GMS implants to the intermittent IM injections show lower fluctuations and concentration exceeding minimum inhibitory concentration (MIC) during the entire dosing interval without a single injection. (Fig. 4).

Although the plasma concentrations were only slightly above the MIC, the local concentrations of vancomycin at the wound site and in the vicinity of the prosthetic device would be significantly higher and therefore, the GMS implants achieved the objective of providing high local levels which will optimize prophylaxis; and low systemic levels which will reduce potential for side-effects. Also, the fluctuations in the plasma concentrations obtained from GMS implants were smaller and the initial burst in release of vancomycin is desirable as the potential for infections associated with surgery is maximum during the first few hours following the surgery. In summary, the plasma profile of vancomycin delivered by GMS implants suggests that the concentrations were sustained for over 4 days with little fluctuations during that period.

In Vivo Release and Absorption of Vancomycin from GMS Implants

The Wagner-Nelson method was used to measure the extent of vancomycin absorption from the GMS implants. The *in vivo* absorption profile of vancomycin from the implants is shown in Fig. 5, indicating a near-zero order release of the drug following a burst in the release. As seen in Fig. 6, the *in vivo* release of vancomycin from the combination of four implants closely matched the results obtained from *in*

Table I. Comparison of Vancomycin Pharmacokinetic Parameters in Rats Observed After Administration of IM Injections and GMS Implants

Pharmacokinetic parameter	IM administration	Implant
C_{max} (μg/ml)	19.26 ± 3.67	9.30 ± 5.05
C_{min} (μg/ml)	2.73 ± 0.94	1.90 ± 0.81
AUC (μg min/ml)	43294 ± 6876	34128 ± 9586
Relative bioavailability (%)	—	80
Fluctuation (C_{max}/C_{min})	7.05	4.89

Data presented as mean ± SD; n = 6.

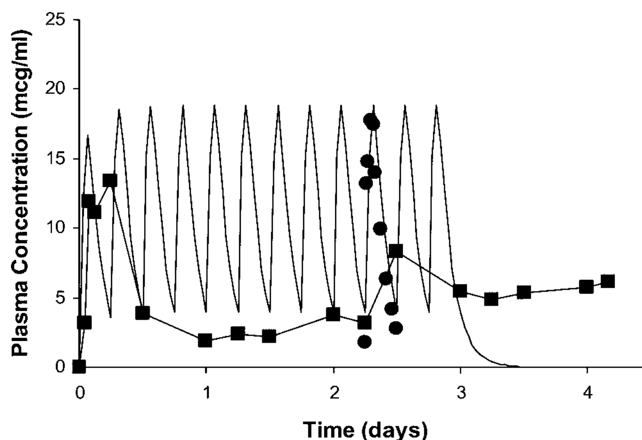

Fig. 4. Comparison of the plasma profiles in male Sprague-Dawley rats obtained after IM administration of 12 doses of 25 mg/kg vancomycin and GMS implants (■) designed to deliver 300 mg/kg over a period of 4 days. The actual plasma concentrations obtained after the ninth IM dose are also included ●.

vitro release. There was excellent correlation ($R^2 = 0.94$, p = 1.21×10^{-9}) between the *in vivo* and *in vitro* release of vancomycin from the GMS implants with a slope of 0.97 (Fig. 6). The close correlation of the *in vivo* results with *in vitro* results indicates that release of vancomycin from a combination of four implants occurs in a predictable manner and that the *in vitro* release methodology is appropriate for studying the *in vitro* release kinetics. The level A *in vitro–in vivo* correlation (IVIVC) is also similar to that obtained for cefazolin from similar implants (18).

In Vivo Efficacy of Vancomycin in the Prevention of Infection at the Implantation Site

Morphologic Observations of the Prosthesis and Wound

The animals were sacrificed at the end of 1 week, and the implantation site was carefully opened using aseptic technique. The presence of purulent discharge and the morphology of the prosthetic device were observed and photographed to evaluate the relative efficacy of the treatment groups as

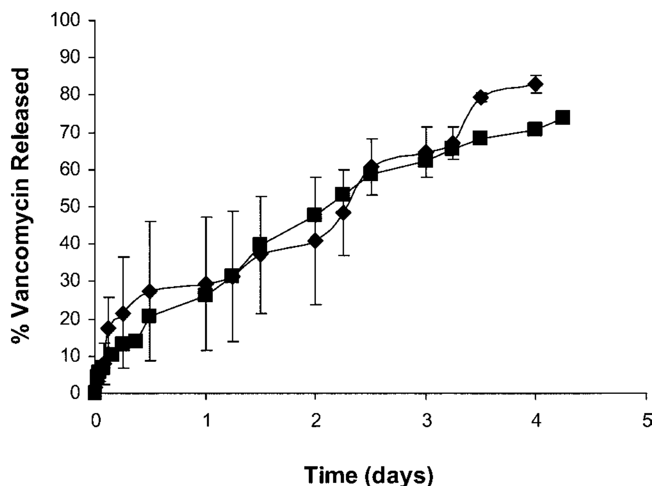

Fig. 5. Comparison of *in vivo* release (◆) of vancomycin from GMS implants in male Sprague-Dawley rats (n = 6) with *in vitro* release (■) conducted in pH 7.4 phosphate buffer solution at 37°C. Data presented as mean ± SD.

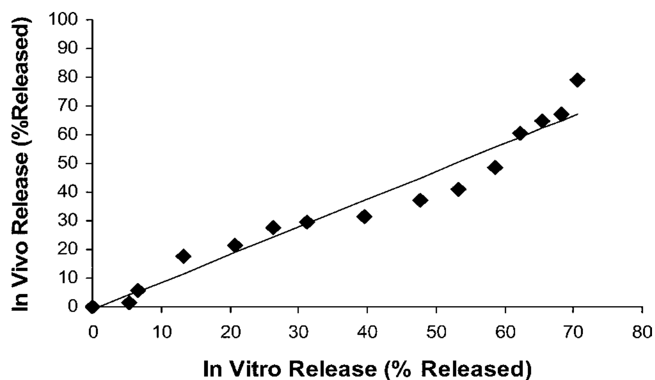

Fig. 6. Correlation of *in vitro–in vivo* release of vancomycin from GMS implants (R^2 = 0.97, slope = 0.94).

compared to the control group. All the rats in the control group showed signs of infection at the site of implantation characterized by presence of yellow to green pus and yellowish discoloration of the prosthetic device (Fig. 7A). Also, one of the rats displayed signs of hemorrhage and purulence characterized by foul odor at the site of implantation.

As shown in Table II, 66% (4/6) rats in the IM group showed evidence of pus and discoloration of the prosthetic device suggesting that IM delivery of vancomycin was not completely successful in preventing development of a biofilm infection (Fig. 7B). In contrast, the implant group of rats showed no sign of infection and the prosthetic device isolated from 83% (5/6) rats was completely clean and devoid of any visible signs of biofilm (Fig. 7C). In one rat, the prosthetic device was slightly yellowish in color, indicative of presence of biofilm. Overall, the site of implantation appeared clean and there were no visible signs of irritation, inflammation and hemorrhage at the site of implantation and all the implants disintegrated at the time of sacrifice except for one implant in rat no. 1. Statistical analysis of the incidence of biofilm infection by χ^2 test indicated that there is a significant difference between the treatment groups ($p < 0.05$) and between implant and control groups ($p < 0.005$) but not between IM and control groups suggesting that the implants were more effective at preventing infection in the rats than IM delivery of vancomycin.

Microbiological Results for the Prosthetic Device

In order to confirm the gross morphologic observations of the results of the efficacy study, further microbiological evaluation of the wound site and the prosthetic device was performed. The swabs taken from the wound site and the prosthetic device were cultured for 36 h and organisms isolated from the liquid cultures were plated on tryptic soy agar medium. The plates that showed positive growth (characterized by appearance of colonies) after 36 h were identified. As shown in Table III, a variety of organisms, both gram-positive and gram-negative were isolated from the wound site and the prosthetic device from all three groups. The results indicate that the number of gram-positive organisms isolated from the wound site and prosthetic device in the control group is higher than in the IM and implant groups suggesting that in the treatment groups, vancomycin may have prevented growth of gram-positive organisms. The frequency of occurrence of gram positive organisms was higher (80%) for the

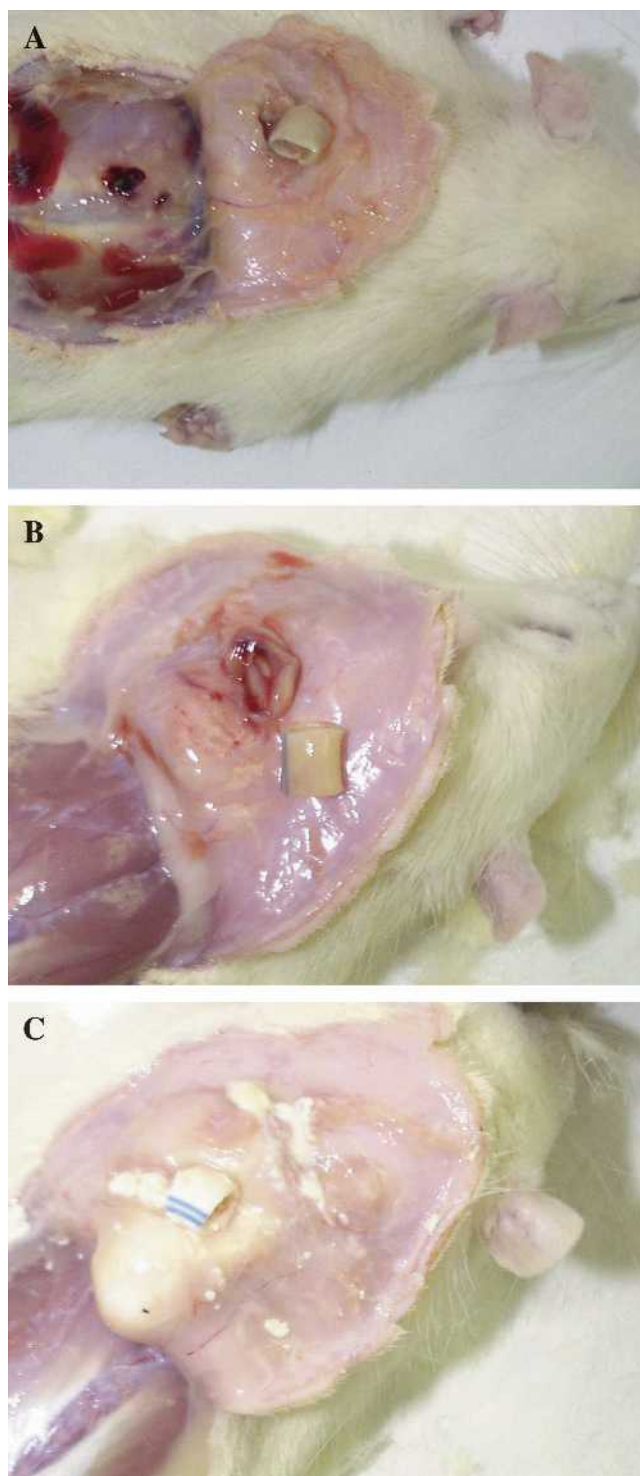

Fig. 7. (A) Representative picture of the rat in the control group taken at the time of excision of the wound site showing clear evidence of infection in the form of purulent discharge and yellowish discoloration of the prosthetic device due to biofilm. (B) Photograph of the rat in the IM group taken at the time of excision of the wound site showing evidence of infection in the form of purulent discharge and yellowish discoloration of the prosthetic device due to biofilm. (C) Photograph of the rat in the implant group taken at the time of excision of the wound site showing no evidence of infection characterized by lack of purulent discharge and a clean prosthetic device indicating the absence of biofilm. The remnants of the GMS implants are seen around the prosthetic device in the form of white pasty material.

Table II. Results Based on Gross Morphological Observations of the Wound Site and Prosthetic Device at the Time of Excision in the *in Vivo* Efficacy Study

Group	Number of rats showing evidence of purulent discharge (pus)	Number of rats showing evidence of device discoloration (presence of biofilm)
Control (n = 6)	6/6	6/6
IM (n = 6)	4/6[a]	4/6[a]
Implant (n = 6)	0/6[b]	1/6[b]

[a] χ^2 analysis showed that the number of rats with purulent discharge and discolored prosthetic device was not significantly different between the control group and IM group.
[b] The number of rats with purulent discharge and discolored prosthetic device was significantly different between the control group and the implant group ($p < 0.005$) and between the implant group and IM group ($p < 0.05$)

control group compared to the implant group (60%) but not higher than IM group (80%) suggesting that IM delivery of vancomycin may not have successfully prevented biofilm infection. In addition, *Staphylococcus epidermidis* was isolated from five animals in the control group (83%) but only from two animals in the implant and IM groups (33%).

The microbiological results further suggest that complete eradication of infection was not achieved in the implant group despite the presumed high local concentrations of vancomycin and this indicates the inherent recalcitrant nature of prosthetic device–related infections. A variety of organisms, both gram-positive and gram-negative organisms were isolated from the wound site and the prosthetic device. The biofilm-producing gram-positive bacteria isolated from the wound site include *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Enterococcus faecalis,* and *Bacillus* species. The biofilm-producing gram-negative organisms include *Pseudomonas aeruginosa, Alcaligenes xylosidans,* and *Acinetobacter calcoaceticus* (6).

In summary, the above results suggest that vancomycin delivered from GMS implants appears to be effective in preventing infection at the wound site in the presence of a prosthetic device. Subsequent regrowth of organisms was observed in all three groups but a significant difference was observed in the number of gram-positive organisms among the three groups suggesting the efficacy of vancomycin delivered from the GMS implants.

Bioerosion and Biocompatibility of GMS Implants

The rats remained healthy throughout the duration of the experiment with no visible signs of infection and the wounds made at the time of surgery healed with no apparent swelling or evidence of infection. Upon examination of the implantation site, it was observed to be devoid of any signs of infection or purulence and the surrounding tissues were observed to be healthy with no apparent signs of inflammation. The weight gain of the rats was monitored throughout the study and was found to be normal. This indicated the lack of any apparent deleterious effects of the presence of GMS implants.

At the end of the six-week period, all the implants were converted to a pasty and pliable mass and were observed to have lost significant weight (Fig. 8). The extent of biodegradation of the implants was variable and was found to be between 50% and 94% and in one rat; there was complete disappearance of the implant (rat no. 5). These results indicate that the GMS delivery system is biocompatible and the implants undergo bioerosion.

DISCUSSION

Although many *in vivo* models for biofilm infections have been described in the literature, a vascular graft infection model with *Staphylococcus epidermidis* was chosen for the study since vascular grafts are very prone to biofilm formation (24–26). Vascular grafts are usually made of PTFE, one of the most commonly used biomaterials and are known to be affected by biofilm causing bacteria (24,27) and any morphologic changes on the prosthetic device are detectable

Table III. Types and Classification of Organisms Isolated from the Wound Site and the Prosthetic Device Obtained by Subculturing the Wound Swabs

Treatment group (number of rats)	Gram-positive		Gram-negative	
Control	***Staphylococcus epidermidis***	**5**	*Acinetobacter calcoaceticus*	2
(n = 6)	*Enterococcus faecalis*	6	*Escherichia coli*	1
	Staphylococcus aureus	3	*Morganella morganii*	1
	Bacillus species	1		
IM	***Staphylococcus epidermidis***	**2**	*Proteus mirabilis*	1
(n = 6)	*Enterococcus faecalis*	2	Other gram-negative bacteria	1
	Staphylococcus aureus	2		
Implant	***Staphylococcus epidermidis***	**2**	CDC enteric group	1
(n = 6)	*Enterococcus faecalis*	3	*Flavobacterium gleum*	1
	Staphylococcus aureus	1	*Acaligenes xylosidans*	1
			Acinetobacter calcoaceticus	1

Vancomycin is active only against gram-positive organisms. Biofilms are caused by *Staphylococcus epidermidis, Enterococcus faecalis,* and *Staphylococcus aureus.*

by marked discoloration and capsule formation (27). By inoculating the vicinity of the implanted prosthetic device with 1.5×10^8 cfu/ml of *Staphylococcus epidermidis* in log phase, a clinical infective episode was simulated, as it is known that a majority of the surgical wound infections are caused at the time when the first surgical incision is made (28). One of the parameters by which the suitability of the model can be tested is whether the wound site and the prosthetic device get infected upon inoculation. The evidence of infection in all the animals of the control group in the form of purulent discharge and discolored prosthetic device indicates that infection did develop in the absence of antibiotics, and device becoming discolored suggests that infection can be predictably developed by organisms in the presence of prosthetic device. The subsequent recovery of *Staphylococcus epidermidis* from the wound site and the prosthetic device after one week post-implantation indicate the viability of the organisms in the wound site and the distinguishing morphologic feature of the biofilm suggest the predictability of the model.

The problem of prosthetic device–related infection has been known to be challenging, requiring prolonged administration of antibiotics (29). Systemic administration of antibiotics for the prophylaxis of these infections has been practiced by surgeons but has often failed to protect the wound site and prosthetic device from biofilm infection (5). Among the reasons to explain the failure of this mode of delivery are reduced diffusion of antibiotics across the biofilm and failure to achieve optimal concentrations at the wound site due to poor vasculature of the surgical site (9). Thus, recent efforts have been aimed at the development of implantable delivery systems based on biodegradable materials which, when implanted at the wound site, release antibiotics locally for the desired period of time and are subsequently absorbed into the body (15–18,30). However, in this study we have investigated the efficacy and pharmacokinetics of vancomycin from a biocompatible nonpolymeric lipid (GMS)-based implants for the prophylaxis of prosthetic device–related biofilm infection. Due to the known nephrotoxicity and ototoxicity of vancomycin (31) and the anticipated fluctuations in peak and trough plasma levels with the clinically used IM dosing regimen, we also expected to demonstrate the increased safety by reducing fluctuations.

The plasma concentration of vancomycin in the implant group were indeed maintained at 4.77 ± 1.93 μg/ml for a 5-day duration after an initial burst with reduced fluctuations compared to the IM group in which the plasma concentrations fluctuated between 2.73 ± 0.94 μg/ml and 19.26 ± 3.67 μg/ml. Despite the low plasma levels observed in the implant group throughout the 5-day period, the local levels will be much higher and these high local levels are important in prevention of infection. The high local levels are likely to prevent infection because of increased likelihood of penetration of the antibiotic by diffusion through the biofilm causing increased bacterial kill. It is highly unlikely that bacteria will be able to survive and form a biofilm in the presence of such high local concentrations. In contrast to an implantable delivery system, following systemic administration, despite the high concentrations in the blood, it may fail to achieve optimal concentrations at the wound site due to poor circulation of the site by blood vessels. This leads to failure of the therapy and subsequent dysfunction of the prosthetic device due to the uninhibited formation of biofilm. In addition, the fluctuations in the plasma concentrations result in manifestation of the toxic effects of the antibiotics. This is particularly true for drugs such as vancomycin and gentamicin, which are known to be associated with nephrotoxicity and ototoxicity (31). The improved pharmacokinetics of the GMS implant group as compared to the IM group illustrate the advantage of a local delivery system over systemic delivery particularly for a drug such as vancomycin with a low margin of safety. Thus, local delivery of antibiotics not only optimizes therapy by concentrating the antibiotic where it is most needed; it also minimizes the exposure of the other organs in the body to toxic concentrations. Though this study attempts to compare the pharmacokinetics of vancomycin delivered by systemic vs. local routes by comparing the concentrations in the central compartment, it may be better to measure the actual local levels of the drug by a method such as microdialysis since these local levels are important in determining the efficacy of the GMS implants.

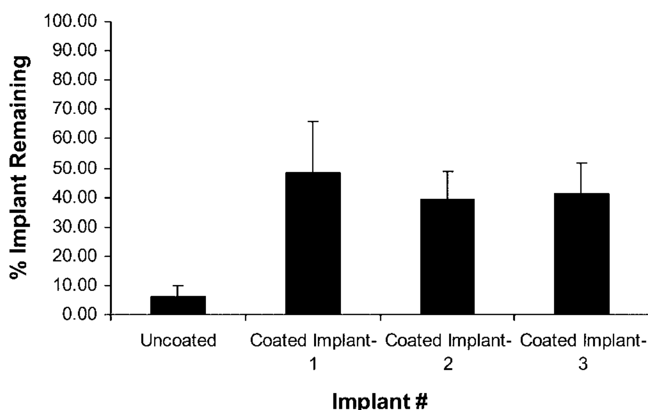

Fig. 8. Bioerosion of the four GMS implants measured by loss of weight of the implants upon excision (mean ± SD; n = 6).

Another advantage with a local delivery system is that with the low systemic levels that are achieved, even when the dose of the antibiotic is doubled, it is not likely to produce any side-effects because systemic levels at best may increase 2-fold. However, doubling the dose by systemic administration results in exposure of the body to peak concentrations of the antibiotics, which may be toxic and even though this may or may not achieve optimal concentrations at the wound-site. In this study, equal doses of the antibiotic were administered in the implant and IM groups to compare the relative efficacy of the treatments. However, further studies are needed to determine if lower doses of the antibiotics from the implants are effective in preventing infection.

One obvious disadvantage of these local delivery systems is that prolonged exposure of the body to low concentrations of the antibiotics may result in the development of resistant strains of the bacteria. This is a subject of recent interest as vancomycin-resistant strains of *Staphylococcus* species have been isolated from some patients in hospitals all around the world (32). Traditionally, vancomycin is considered as a last line of defense against tough staphylococcal infections (31). However, in the recent times, resistance to vancomycin has been observed and this has motivated physicians into using antibiotics with greater caution. Considering the overall risk-to-benefit ratio of providing targeted therapy at the site with reduced exposure of the body to toxic antibiotics, it appears

that there is a definite benefit of local antibiotic prophylaxis when the risk of serious infection is considered.

The observations made at the time of excision of the wound site suggested that in the absence of antibiotics, that is, in the control group (Fig. 8), there is a high probability of infection in the form of purulent discharge and a biofilm. This is perhaps due to the absence of antibiotics that would prevent infection and the formation of biofilm at the site of implantation. In contrast, as is evident in the implant group of animals (Fig. 7C), because vancomycin was delivered locally at the site of implantation, effective prophylaxis was achieved. This may be due to the fact that high local concentrations were effective in penetrating into the biofilm. The group of animals that received systemic antibiotics showed clear evidence of infection in four out of six animals (Fig. 7D), suggesting that systemic delivery was perhaps unable to achieve high local levels at the site of implantation and hence this mode of delivery was unable to prevent the formation of a biofilm.

The gross morphologic observations were supported by microbiological studies in which wound exudates were cultured and the resulting organisms were identified. A number of different gram-positive and gram-negative organisms were isolated from the wound site of all the three groups of animals. A significant difference was found between the control and implant groups with respect to the recovery of *Staphylococcus epidermidis* from the wound site. Also, *Enterococcus faecalis,* a biofilm-producing organism, was isolated from all the six rats in the control group. However, both the organisms were isolated from only two out of six rats in both implant and IM groups, suggesting that the treatment with vancomycin was effective in preventing growth of the organisms at the wound site. It is not surprising that the organisms were isolated from the rats in the IM group, as this correlates well with the gross morphologic observations made at the time of excision. Though *Staphylococcus epidermidis* was isolated from the wound site of the implant group, the absence of any visible signs of infection in the form of purulent discharge and yellowish discoloration of the prosthetic device indicate that the GMS implants were indeed successful in preventing infection and biofilm formation but unable to achieve sterilization at the wound site perhaps due to the high level of live innoculation. The isolation of *Staphylococcus epidermidis* and other gram-positive organisms at the wound site and on the prosthetic device from the implant and IM groups illustrates the recalcitrant nature of the biofilm infection. The regrowth of these organisms indicates the viability of biofilm-associated bacteria despite presence of high local concentrations of antibiotics. This also indicates the need for a delivery system with a combination of antibiotics, each, acting by a different mechanism to provide a lethal mixture of antibiotics that could be released locally, which may prevent any organisms from surviving at the wound site. In summary, the overall morphology of the prosthetic device remained unchanged in the implant group and absence of purulence at the wound site suggests that locally high concentrations of vancomycin were successful in preventing biofilm infection.

The general well-being of the rats during the *in vivo* and bioerosion studies and lack of any visible inflammation in the surrounding tissues indicate the biocompatibility of the GMS implants.

Allababidi *et al.* (18) earlier investigated the use of GMS-based implants for the prophylaxis of surgical wound infections. It was found that the implants loaded with cefazolin were effective in preventing surgical wound infection in a group of rats that were infected with *Staphylococcus aureus*. This current research applies the same principle of GMS-based local delivery system but for a different purpose, the prophylaxis of prosthetic device–related infections. This research has demonstrated successful use of the technology developed previously for preventing infection in the presence of a prosthetic device. From the earlier results of Allababidi *et al.* and this current study, it is clear that antibiotics like cefazolin, ciprofloxacin, and vancomycin, each with a different clinical use, could be successfully delivered from the GMS implants. This illustrates the applicability of the GMS-based local delivery system for a variety of antibiotics of different physical and chemical properties for prevention of all types of infections related to surgery. Further research into optimizing the delivery system to demonstrate the cellular level biocompatibility is needed prior to conducting tests for its potential for use as a delivery system in humans.

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