DRUG DEVELOPMENT AND INDUSTRIAL PHARMACY, 15(14-16), 2339-2357 (1989)

RELEASE KINETICS OF TOBRAMYCIN SULFATE FROM POLYMETHYLMETHACRYLATE IMPLANTS

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ABSTRACT

The effects of various formulation factors on the in vitro release characteristics of spherical polymethylmethacrylate implants were studied. Physical and mathematical models were proposed to describe observed in vitro release profiles. The in vitro release data could be described by a biexponential equation of the type: fraction of tobramycin remaining in the implant at time $t = Ae^{-\alpha t} + Be^{-\beta t}$, where α and β represent the rate constants for the initial rapid and subsequent slow phases of release. The influence of drug loading, volume of dissolution medium, implant size, type of commercial cement and the incorporation of water soluble additives on the release profiles as well as the α and β rate constants is described.

INTRODUCTION

Antibiotic-impregnated polymethylmethacrylate (PMMA) bone cement has been used for localized drug delivery in osteomyelitis since 1970.[1-3] More recently, antibiotic-bone cement composites have been fabricated as spherical, nonbiodegradable implants.[4] The primary advantage of localized drug delivery with implants is that relatively high local tissue antibiotic concentrations are obtained without corresponding high serum levels and associated toxicity. Implants containing various antibiotics have been extemporaneously prepared for clinical use. However, many aspects regarding the preparation and release characteristics of these implants have not been clearly defined.

The purpose of this study was to adopt a systematic pharmaceutical approach to optimize formulation and characterize the properties of tobramycin-PMMA implants. Tobramycin was selected as it is active against both Gram-positive and Gram-negative bacteria, including gentamicin-resistant Pseudomonas. The specific aims of the study were to:

1. Study the *in vitro* and *in vivo* release characteristics of tobramycin sulfate from the PMMA implant delivery system.
2. Develop physical and mathematical models which describe the drug release profiles *in vitro* and account for the observed changes in rate and extent of release between formulations.

THEORETICAL

(a) Mathematical Model

Guy et al., derived an equation for the diffusion controlled release of a drug from a sphere, radius r, by applying the three

dimensional form of Fick's second law of diffusion after transformation to spherical coordinates.[5] This equation can be rearranged as:

$$1 - \frac{M_t}{M_\infty} = \frac{6}{\pi^2} \sum_{n=1}^{\infty} \frac{1}{n^2} \exp(-n^2 \pi^2 \tau) \qquad \text{Equation 1}$$

The term $(1 - M_t/M_\infty)$ represents the fraction of drug remaining unreleased while τ is a function of the diffusion constant, D, as defined by the equation $\tau = D\ t/r^2$. Therefore, the decline in the fraction of drug remaining (fraction released) can be described by a multi-exponential equation of the type:

$$\text{fraction of drug remaining} = Ae^{-\alpha t} + Be^{-\beta t} + \ldots \qquad \text{Equation 2}$$

Since r is constant for a nonbiodegradable carrier such as PMMA, the slopes α, β etc. should reflect changes in the diffusion coefficient D, and therefore differences in porosity, between formulations. The first order drug release rate constants can be calculated from the slopes of the α and β phases of the release profile.

(b) Physical Model

Since PMMA is non-biodegradable and resistant to erosion, the dimensions of the spherical implant will not change for the duration of the release process. It was proposed that a biphasic release profile would be observed. An initial, relatively rapid release phase would be observed due to the diffusion and dissolution of drug on, or near the surface of the implant. A second, more prolonged release phase would then follow due to the

slow diffusion of the drug from within the PMMA implant matrix. Further, the rate and extent of drug release from the implant during the second prolonged phase in particular, could be significantly altered using formulation additives which modify the porosity of the PMMA implant matrix.

METHODS

Materials

Tobramycin sulfate (Nebcin^R, lot no. 1CE30C) was donated by Eli Lilly Co., Indianapolis, IN. Palacos^R cement (lot no. 608/7796) was donated by Richards Medical, Memphis, TN. Simplex^R cement (lot no. HR203-4) and Zimmer Low Viscosity^R cement (lot no. 51738000) were donated by Howmedica, New York, NY and Zimmer, Warsaw, IN respectively. Disodium phosphate (lot no. 0201ML) and polyethylene glycols PEG 3400 (lot no. 01224MP) and PEG 400 (lot no. 97901DM) were purchased from Aldrich Chemical Co. Inc., Milwaukee, WS. Potassium dihydrogen phosphate (lot no. 7100KBS) and methylene chloride (lot no. 4881KBCL) were obtained from Mallinckrodt Inc, St. Louis, MO. O-phthaldialdehyde reagent solution (lot no. 58F-5985) and isopropanol (lot no. 78F-6185) were purchased from Sigma Chemical Co., St.Louis, MO. Lactose was obtained from Amend Drugs and Chemical Co., Irvington, CO.

Preparation of Implants

Spherical PMMA implants containing tobramycin sulfate were prepared using a teflon-coated stainless-steel mold. All

chemicals and equipment were cooled to 4°C before use to the retard polymerization rate and facilitate easier preparation of the implants. Known quantities of tobramycin sulfate were mixed with solid PMMA using geometric dilution, then the liquid monomer added to initiate polymerization. Where applicable, solid additives (lactose and PEG 3400) were added to the solid PMMA before the addition of monomer while the liquid additive PEG 400 was added after the addition of monomer. Immediately after mixing thoroughly, the liquid dispersion was injected into the lubricated mold. After solidifying, the implants were removed, allowed to dry at room temperature for 24 hours, then stored for at least four days before testing to ensure loss of residual monomer and completion of polymerization. Initial studies indicated that polymerized methylmethacrylate bone cement attained constant weight after about 24 hours.

Weight Variation and Diameter Variation

The mean weight and standard deviation of ten samples from each batch were determined for weight variation studies. Variation in diameter of beads was determined using a micrometer. The mean diameter (n=6) and the standard deviation was calculated for each batch.

Assay of Drug Content

Three implants from each batch were assayed separately for tobramycin sulfate content by dissolving the PMMA in 4 mL of

dichloromethane and extracting the tobramycin sulfate five times with 5 mL of deionized distilled water. The aqueous extracts were mixed and the volumes adjusted to 50 mL.

Analysis of Tobramycin Sulfate

Tobramycin sulfate was determined spectrophotometrically using a derivatization procedure.[6] A 0.5 to 1 mL aliquot of the extracted solution was mixed with 1 mL of o-phthaldialdehyde reagent solution followed by addition of 1.5 mL of isopropanol to prevent precipitation of derivatized tobramycin. The volume was adjusted to 5 mL with distilled water and the absorbance was determined after 45 minutes on a Beckman DU-7 spectrophotometer at the wavelength maxima of 333 nm. The concentrations were obtained from a calibration curve of o-phthaldialdehyde derivatized tobramycin. The assay had a coefficient of variation of less than 3% and a detection limit of 0.5 μg/ml.

In Vitro Dissolution

Dissolution kinetics were studied under sink conditions by placing one implant in varying volumes (usually 100 mL) of phosphate buffer pH 7.4, while agitating in a horizontally shaking water bath (50 ± 1 rpm) at 37 ± 1°C. Samples were withdrawn at varying time intervals for a duration of 21 days (a realistic estimate of the expected duration of clinical use) and the amount of tobramycin sulfate released determined spectrophotometrically as described above. Equal volumes of fresh medium were added to

replace aliquots removed for assay and the amount of drug release corrected for dilution. Triplicate measurements were performed for each batch of implants prepared.

Formulation Factors

The influence of the following formulation factors on the release of tobramycin sulfate from PMMA carrier were studied:

1. Drug loading: that is, the drug to carrier ratio (tobramycin sulfate:PMMA). Drug:carrier ratios (dry weight) of 1:33 (clinically used), 1:20, 1:10 and 1:5 were used.
2. Influence of water-soluble formulation additives: PEG 400, PEG 3400 and lactose.
3. Type of commercial PMMA bone cement: The cements studied were Simplex, Zimmer and Palacos.
4. Effect of dissolution volume: 100 mL, 10 mL, 5mL and 2 mL of dissolution medium were employed for the study.
5. Effect of size: implants in two sizes of 2.9 mm and 6.2 mm diameter were studied.

Palacos bone cement was used for studies in 1,2,4 and 5 above. A drug to carrier ratio of 1:10 was used for studies in 2-5. Implants of diameter 6.2 mm were used in studies 1-4.

In Vivo Release

The rabbit osteomyelitic model described by Norden,[7] was used to assess *in vivo* release of tobramycin sulfate from PMMA implants. The implants contained a mean tobramycin sulfate

content of 6.1 mg per implant (which corresponds to almost double the drug loading used clinically). Osteomyelitis was induced in the tibia of nine New Zealand rabbits using Pseudomonas aeruginosa. One implant was surgically placed immediately adjacent to the tibia infection site in each rabbit 23 days post infection. Implants were then surgically removed from each of three rabbits 1, 3 and 7 days after implantation and the amount of drug remaining unreleased determined spectrophotometrically after extraction as described above.

Scanning Electron Microscopy

A Philips, Model 515, scanning electron microscope was used to study the changes in surface topography of implants both before and after *in vitro* dissolution and after implantation in the rabbit.

Data Analysis

Non linear regression analysis of the *in vitro* release data was performed using the JANA multiexponential curve stripping computer program. The mono, bi and triexponential fits were generated. Statistical analysis of computer generated parameters was performed using ANOVA and Student's t test. Fisher's LSD was used for the multiple comparison of statistically significant groups.

RESULTS AND DISCUSSION

The mean diameters of the large and small beads were 6.2 mm and 2.9 mm respectively with the percent coefficients of variation less than 2% within a batch. Mean weights of the batches of the larger beads ranged from 151.8 - 162.5 mg and the smaller beads had a mean weight of 19.9 mg. In no instance was the coefficient of variation for within batch variation greater than 3%.

The correlation coefficients generated for mono-, bi- and triexponential fits obtained by nonlinear regression analyses are summarized in table 1. Wilson et al., reported that rate of tobramycin release from Simplex PMMA beads could be fit to monoexponential and power functions however they obtained r^2 values < 0.9 for both fits.[14] Our results show that although the monoexponential fit is poor, both biexponential and triexponential fits provided r^2 values > 0.9. Since the biexponential relationship in equation 2 is proposed to fit our physical model, this approach was adopted in analysis of computer fits to release data. The rate constants, α and β, represent an initial, rapid surface release and a prolonged matrix diffusion-controlled release respectively.

Several investigators have reported that Palacos PMMA cement releases antibiotics at higher concentrations over more prolonged periods than other commercially available cements[8-10] ; others have suggested that there are no significant differences.[11] Von Frauhofer et al. noted that the rates of leaching of tobramycin

TABLE 1

Determination Coefficients for the Fitting of In Vitro Data to Multiexponential Equations

Formulation Factor	Tobramycin[a] Content (mg) Mean ± S.D.	R^2 Values for Exponential Fits		
		Mono	Bi	Tri
	A. Effect of Drug Loading			
1:33	2.9 ± 0.009	0.4565	0.9432	0.9810
1:20	4.73 ± 0.269	0.5052	0.9650	0.9908
1:10	9.41 ± 0.423	0.6430	0.9731	0.9860
1:5	17.95 ± 0.069	0.7796	0.9856	0.9927
	B. Effect of Additives			
10% PEG 400	9.13 ± 0.139	0.9064	0.9944	0.9974
20% PEG 400	9.34 ± 0.386	0.9295	0.9951	0.9980
30% PEG 400	10.21 ± 0.190	0.6585	0.9841	0.9793
10% PEG 3400	8.21 ± 0.294	0.8222	0.9799	0.9883
10% Lactose	8.72 ± 0.322	0.6667	0.9796	0.9916
	C. Effect of Size			
2.9 mm diam.	1.29 ± 0.01	0.2225	0.9103	0.9782
	D. Effect of Type of PMMA Bone Cement			
Zimmer	10.38 ± 0.446	0.7560	0.9445	0.9790
Simplex	10.31 ± 0.218	0.6277	0.9552	0.9902

a: Drug content determined by spectrophotometric analysis

from three commercial brands of cement were similar, but the initial amount of drug released differed.[12] Our studies indicated that statistical comparison of the rate constants obtained using Palacos, Simplex and Zimmer cements (at similar levels of drug loading) revealed no significant differences ($p > 0.1$). Also all

three cements showed similar extents of release for the duration of study. Palacos bone cement was used for all subsequent studies.

Table 2 summarizes the effect of various formulation factors on the fast and slow phase rate constants influence of various formulation factors. For all subsequent discussion, the criteria for statistical significance was $p < 0.1$.

Figure 1 and Table 2 summarize the effect of drug loading on the release profiles of tobramycin. During the rapid initial phase, release rates were all of the same order of magnitude although at the highest drug loading (17.9 mg / implant), differences were observed in the extent of release. Similarly for the slow phase, significant differences in β rate constant occurred only at the highest drug loading. At the tobramycin sulfate : PMMA ratio commonly used clinically, i.e. 1:33 the release is incomplete (18-20%) with essentially no drug released after three days. Our studies confirmed the work of Goodell et al., who found that over a period of 12 weeks less than 20% of the theoretically available tobramycin was released from beads prepared using a ratio of 1:33 of drug to polymer (dry weight).[13] Studies by Wilson et al., also showed that substantial amounts of tobramycin remains in the beads after 28 days.[14]

Our studies indicated that the inclusion of water soluble additives could alter both the rate and extent of tobramycin release from Palacos PMMA. The three additives PEG 3400, PEG 400 and lactose significantly affected the slower diffusion phase of

TABLE 2

Effect of Formulation Factors on Computer Generated Fast (α) and Slow (β) Phase Rate Constants

Formulation Factor	α (hrs^{-1}) Mean ± S.D.	β (hrs^{-1}) Mean ± S.D.
A. Effect of Drug Loading		
1:33	$3.47 \times 10^{-1} \pm 1.11 \times 10^{-1}$	$1.29 \times 10^{-4} \pm 1.38 \times 10^{-5}$
1:20	$1.93 \times 10^{-1} \pm 1.01 \times 10^{-1}$	$1.67 \times 10^{-4} \pm 1.92 \times 10^{-5}$
1:10	$9.65 \times 10^{-2} \pm 6.63 \times 10^{-3}$	$1.82 \times 10^{-4} \pm 3.10 \times 10^{-5}$
1:5	$1.01 \times 10^{-1} \pm 1.65 \times 10^{-2}$	$1.17 \times 10^{-3} \pm 6.32 \times 10^{-4}$
B. Effect of Implant Size		
6.2 mm	$9.65 \times 10^{-2} \pm 6.63 \times 10^{-3}$	$1.82 \times 10^{-4} \pm 3.10 \times 10^{-5}$
2.9 mm	$5.35 \times 10^{-1} \pm 3.63 \times 10^{-1}$	$1.19 \times 10^{-3} \pm 1.03 \times 10^{-4}$
C. Effect of Type of Cement		
Palacos	$9.65 \times 10^{-2} \pm 6.63 \times 10^{-3}$	$1.82 \times 10^{-4} \pm 3.10 \times 10^{-5}$
Zimmer	$1.85 \times 10^{-1} \pm 1.30 \times 10^{-1}$	$3.17 \times 10^{-4} \pm 9.90 \times 10^{-5}$
Simplex	$1.75 \times 10^{-1} \pm 1.14 \times 10^{-1}$	$1.83 \times 10^{-4} \pm 8.23 \times 10^{-5}$
D. Effect of Soluble Additives (10%)		
Control[a]	$9.65 \times 10^{-2} \pm 6.63 \times 10^{-3}$	$1.82 \times 10^{-4} \pm 3.10 \times 10^{-5}$
PEG 400	$7.29 \times 10^{-2} \pm 4.02 \times 10^{-2}$	$3.02 \times 10^{-4} \pm 3.51 \times 10^{-6}$
PEG 3400	$1.41 \times 10^{-2} \pm 1.74 \times 10^{-2}$	$9.01 \times 10^{-4} \pm 8.70 \times 10^{-5}$
Lactose	$9.67 \times 10^{-2} \pm 5.01 \times 10^{-3}$	$6.30 \times 10^{-4} \pm 3.78 \times 10^{-5}$
E. Effect of Increasing Levels of PEG 400		
Control[a]	$9.65 \times 10^{-2} \pm 6.63 \times 10^{-3}$	$1.82 \times 10^{-4} \pm 3.10 \times 10^{-5}$
10%	$7.29 \times 10^{-2} \pm 4.02 \times 10^{-2}$	$3.02 \times 10^{-4} \pm 3.51 \times 10^{-6}$
20%	$3.52 \times 10^{-1} \pm 2.64 \times 10^{-1}$	$8.51 \times 10^{-3} \pm 1.50 \times 10^{-3}$
30%	$1.94 \times 10^{-1} \pm 3.20 \times 10^{-2}$	$8.38 \times 10^{-3} \pm 9.07 \times 10^{-3}$

a: No additives

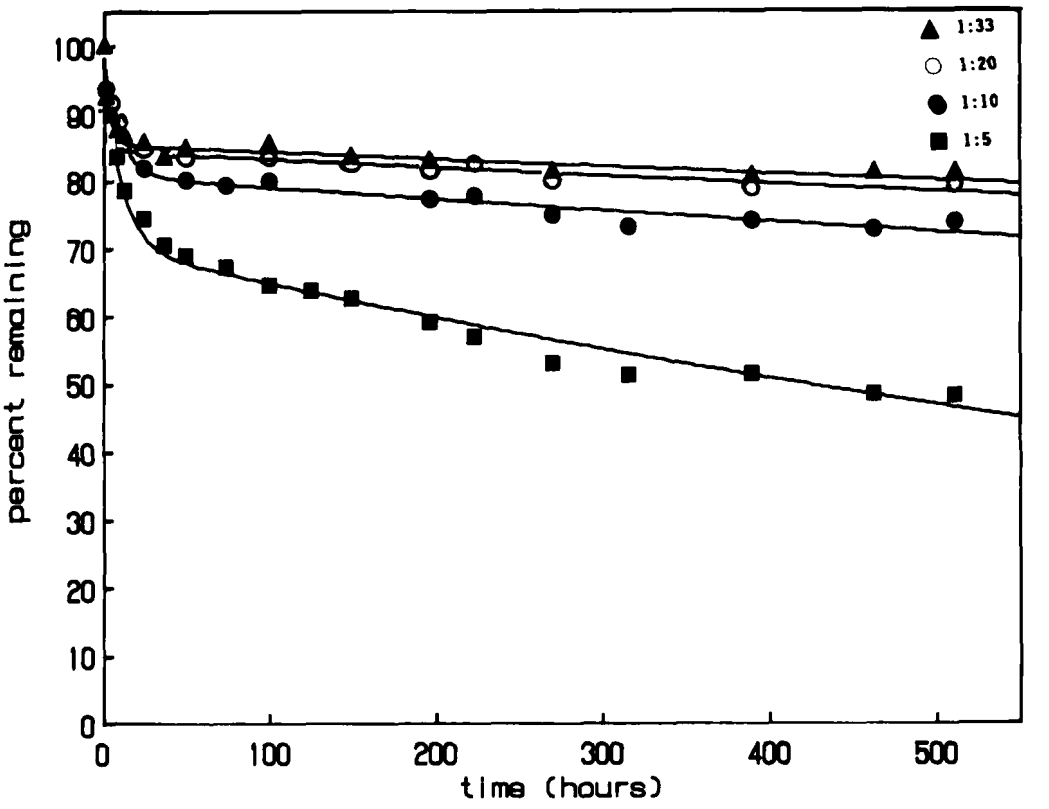

FIGURE 1
The effect of drug loading on tobramycin sulfate release profiles. The solid lines represent the lines of best fit. The symbols represent the observed values.

drug release possibly due to alterations in the porosity of the matrix (figure 2 and table 2).

Law et al determined the diffusion coefficient for benzyl penicillin in thin films of Palacos, Simplex and CMW cements assuming that antibiotic transport can be described by Fick's law using a finite difference approximation to quantify transient non steady state behaviour.[15] The investigators found that the

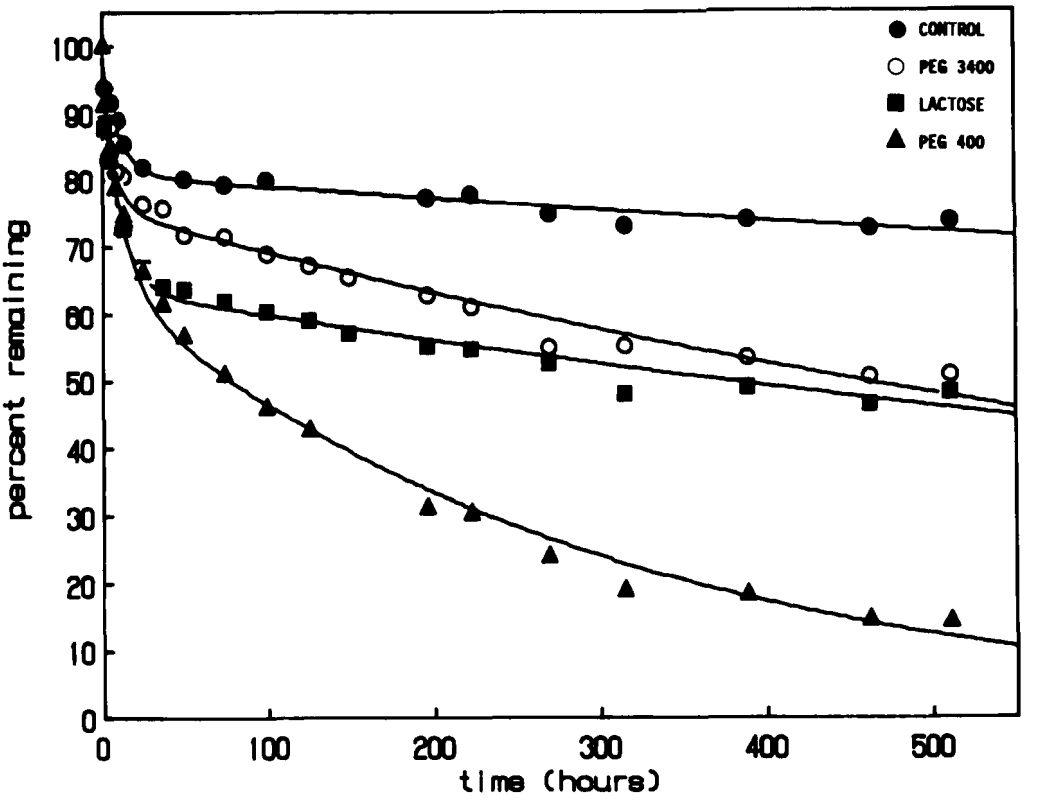

FIGURE 2
The effect of incorporation of 10% water soluble additives on tobramycin sulfate release profiles. The solid lines represent the lines of best fit. The symbols represent the observed values.

diffusion coefficient was increased in the presence of additives and proposed that the finite difference approach could be applied to determine release of antibiotic from preloaded PMMA beads. Dittgen and Stahlkopf showed that incorporation of amino acids of varying solubilities also affected release of chloramphenicol from polymethacrylic implants.[16] Our studies indicated that while the presence of lactose and PEG 3400 increased the extent of release

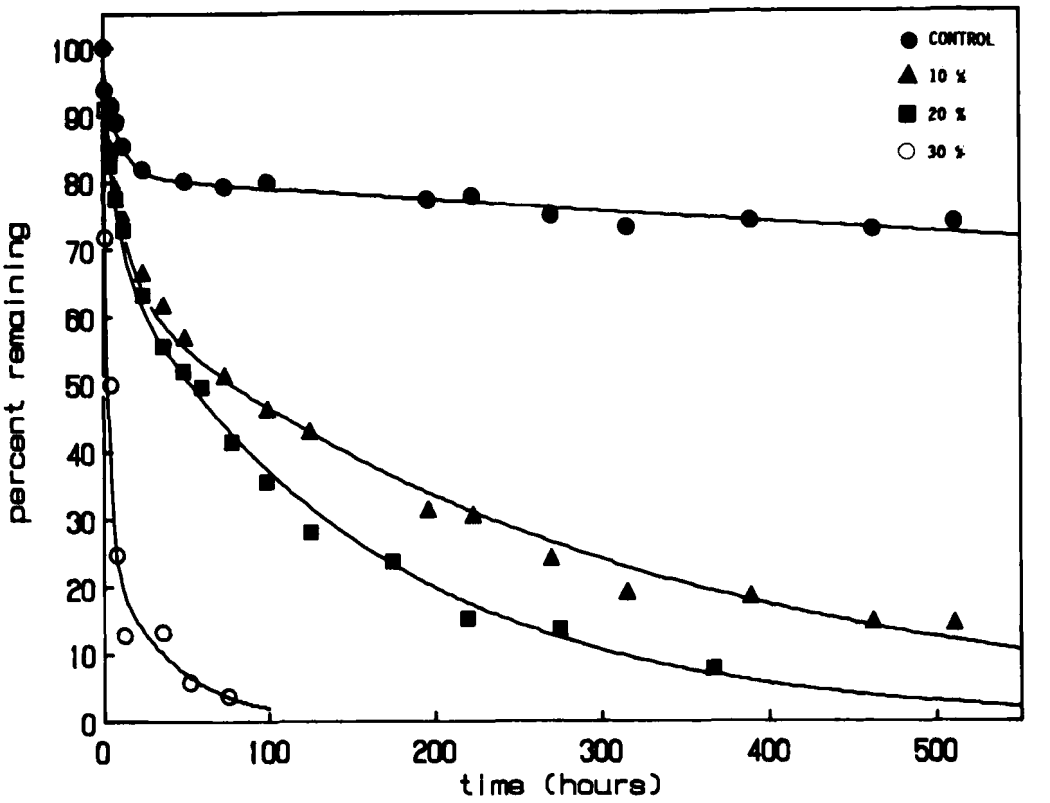

FIGURE 3
The effect of increasing levels of PEG 400 on tobramycin sulfate release profiles. The solid lines represent the lines of best fit. The symbols represent the observed values.

to about 50%, PEG 400 had a much more pronounced effect increasing the extent of release to about 85% after three weeks. Figure 3 illustrates the effect of increasing PEG concentrations on rate and extent of tobramycin release. Incorporation of 30% PEG resulted in almost complete release (> 95%) within 3 days.

Table 2 indicates that the initial rapid rate of release did not differ significantly while differences were observed with the

slower diffusion phase. The changes in the rates and extents of drug release due to increases in drug loading and incorporation of additives can be attributed to modifications in the porosity of the implants with changes in formulation.

Studies using smaller implants of 2.9 mm diameter indicated statistically significant differences in the two rate constants (table 2) and extent of drug released from the implant in comparison with implants of mean diameter 6.2 mm. This could be attributed to an increase in surface area per unit volume of the smaller implant.

The effect of varying dissolution volumes on the release rates from beads prepared using 1:10 ratio of drug:carrier indicated that no statistically significant differences were observed either in the rates and extent of drug release, when release studies were performed using different dissolution volumes. This is to be expected since the solubility of tobramycin was not a limiting factor in controlling release rate. This fact is of clinical significance since implants placed in the diseased tissue may be exposed to varying amounts of fluids.

The amount of tobramycin sulfate remaining unreleased from the PMMA implant after 1, 3, and 7 days in vivo is summarized in Table 3. The very low percent of tobramycin sulfate released in vivo (10.57%) after 7 days supports the in vitro findings (18 - 20 % released).

Scanning electron microscopic examination before and after dissolution and in vivo implantation show no significant changes

TABLE 3

Tobramycin Sulfate Released *in vivo* Using the Osteomyelitic Tibia Rabbit Model

Day post Implantation	Amount Remaining Unreleased (mg/implant)	Percent Released
0	6.140 ± 1.080	0.00
1	5.766 ± 0.220	6.10 ± 3.67
3	5.525 ± 0.592	10.39 ± 8.99
7	5.640 ± 1.258	10.57 ± 18.33

in surface topography for the implants prepared as they are clinically used, that is, without additives. However, distinct changes in surface characteristics and the appearance of pores are evident with the inclusion of water-soluble additives such as polyethylene glycol 400. Detailed results of the scanning electron microscopic evaluation of the changes in the implants will be reported elsewhere.

CONCLUSIONS

1. The release of tobramycin sulfate from non-biodegradable, spherical PMMA implants is biphasic and can be described by bi-exponential linear regression analysis.
2. Both *in vitro* and *in vivo* results indicate that drug release

is incomplete and poorly controlled from implants prepared at the drug:carrier ratio used clinically.

3. Although the release of tobramycin sulfate can be significantly improved with the addition of water-soluble additives such as polyethylene glycol 400, our current investigations are directed toward the design and development of biodegradable implants.

ACKNOWLEDGEMENTS

We gratefully acknowledge the assistance from Eli Lilly Co., for the donation of tobramycin; and Richards Medical, Howmedica and Zimmer for the donation of the bone cements used in this study. We also would like to thank Dr.D.Scott for supplying the molds.

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