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BIODEGRADABLE IMPLANTS CONTAINING GENTAMICIN: DRUG RELEASE AND PHARMACOKINETICS

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ABSTRACT

To estimate the releasing and pharmacokinetic features of antibiotics used in implantable delivery devices the implants based on stable carrier i.e. polymethylmethacrylate (Septopal) and biodegradable carriers i.e. copolymers of 2-hydroxyethylmethacrylate, N-vinylpyrrolidone and allylic alcohol, monocarboxylcellulose, collagen, alginic acid and its mixed sodium-calcium salt were studied comparatively. It was shown in vitro that the implants on the base of polymethylmethacrylate, collagen (especially cross-linked one), alginic acid and monocarboxylcellulose had the most pronounced prolongation effect. For the samples using alginic acid and monocarboxylcellulose the antibiotic release rate was closely connected with eluent content. The changes of genta-

micin levels in the implantation zones were of three-phase character. The drug concentration reached its maximum in phase I, was practically constant in phase II and slowly lowered in phase III. The comparison of the concentration levels and areas under cocentration-time curves showed that the sustained release and pharmacokinetic characteristics of the implants based on cross-linked collagen, alginic acid and monocarboxylcellulose were similar to those of polymethylmethacrylate.

INTRODUCTION

Many clinical situations exist in which it is advisable, sometimes necessary, to administer a drug in a controlled manner such that the concentration in the tissues of patient is high enough to be effective but in the bloodstream is not so high as to cause toxic or other undesirable side effects (1,2). If a drug incorporated in a polymer matrix is implanted at or near its intended site of action, it can be more effective, and can produce fewer unwanted side effects (3). For antibiotics a given approach have been successfully realized more than 15 years ago, when Buchholz and Engelbrecht used gentamicin-impregnated polymethylmetacrylate (PMMA) in surgical practice (4,5).It has been shown through in-vitro and in-vivo studies that the antibiotic is slow ly released from the PMMA-matrices by diffusion in concentrations far above the concentration necessary to inhibit the aethiologically important pathogens (5,6). While the antibiotic levels after injection are high in serum and comparatively low in tissues, after

implantation of the gentamicin-PMMA-matrices extremely low serum and urine concentrations can be observed, but high drug levels in the implantation zones leads to a good local therapeutic efficacy (7,8). However, these devices implanted in the body has to be removed after gentamicin is used up because the polymer remains intact. Obviosly, it would be far better if the systems for sustained release of the antibiotic involves carriers that gradually decompose. A major advantage of biodegradable implants is that, because the carrier totally disintegrated and is absorbed by the body, they do not have to be removed surgically after the drug supply is exhausted. The most frequently used biodegradable polymers are poly(ortho esters), poly(lactic acid), poly (glycolic acid) and copolymers of lactic and glycolic acids (3,9,10).

The objective of the present work are to test the possible application some other biodegradable polymers as carriers of gentamicin in the implantable devices capable of sustaining the drug release, providing its high local concentration and complete resolving after the action termination. In this paper the results of estimation the releasing and pharmacokinetic features of gentamicin implants based on the biodegradable carriers and comparison of them to those of gentamicin-PMMA-implants will be reported.

MATERIALS

A. Biodegradable Implants:

Following model biodegradable implants received from Sechenov Moscow Medical Institute were used.

1. Films based on copolymers of 2-hydroxyethylmethacrylate, N-vinylpyrrolidone and allylic alcohol.

were placed to Erlenmeyer flasks with 50 ml of eluent and allowed to stand without stirring at 37±0.5°C. At various time intervals, samples (0.5 ml) were collected using a glass pipette fitted with cotton wool. Fresh aliquots of the eluent at 37±0.5°C were immediately added to compensate the samples withdrawn.

B. Animal Experiments:

The implantable devices containing about 5 mg of gentamicin were inserted through a small incisions made on the back of 200-250 g Wistar rats anaesthetized with hexenal (i.p., 150 mg/kg) and pushed below the skin approximally 1 cm away from the incision site which was then sutured. The animals were killed at specified intervals and the specimens of blood and tissue from implantation zones sampled.

C. Gentamicin Assay:

The concentrations of the antibiotic in the samples of eluents, serum and tissue from implantation zones were estimated by the agar diffusion method using Bacillus subtilis ATCC 6633 as the standart strain (11).

D. Pharmacokinetics:

The following parameters were estimated from the serum and tissue concentration-time curves. The peak concentration (C_{max}) was the highest concentration measured, T_{max} denotes the time the peak concentration occured. Absorption rate constant (k_a), elimination rate constant (k_{el}) and elimination half-life were estimated by the method of back-projection. The areas under concentration-time curves were calculated by the trapezoidal method (12). Mole ratio of monomers in copolymers are 40:50:10

(CP1), 35:55:10 (CP2), 70:20:10 (CP3) and 90:0:10 (CP4). Gentamicin content is 10 w/w percent.

2. Lyophilized spherical granules on the base of mixed sodium-calcium salt of alginic acid (SCA). Gentamicin content is 3 w/w percent.

3. Tablets on the base of monocarboxylcellulose (MCC) containing ion-bonded gentamicin. The antibiotic content is 5 w/w percent.

4. Tablets on the base of alginic acid (AA) containing ion-bonded gentamicin. The antibiotic content is 5 w/w percent.

5. Lyophilized spongy lamellae based on nonmodified (NMC) and cross-linked (CLC) collagen. Gentamicin content is 25 w/w percent.

B. Biostable Implants:

As a model biostable implants the polymethylmethacrylate beads Septopal (E.Merck, FRG) containing 4.5 mg of gentamicin were used.

METHODS

A. Drug Release Study:

The sustained release characteristics of the implantable devices were estimated in vitro by gentamicin transfer from implant to distilled water (pH 6.0) and Sorensen phosphate buffer (pH 7.35, ionic strength 0.22). In additional experiments the eluents were isotonic phosphate buffer (pH 7.3, ionic strength 0.29) and saline solution (pH 6.15, ionic strength 0.15) with or without 3 percent whole rat blood.

For simulating the wound cavity conditions the implant samples containing about 5 mg of gentamicin

RESULTS AND DISCUSSION

In-vitro investigation showed that the prolongation properties of the implants based on mixed sodium-calcium salt of alginic acid and on the copolymers of 2-hydroxyethylmethacrylate, N-vinylpyrrolidone and allylic alcohol were from poor to moderate. It is seen from Tables 1 and 2 that the complete release of gentamicin from the implants required not more than 36 hours. These carriers were not used in the following experiments.

It is demonstrated that ionic binding of gentamicin to monocarboxylcellulose and alginic acid resulted in significant lowering of the antibiotic liberation to distilled water. Figure 1 shows that within 30 days only 22-30 percent of gentamicin released from these implants.

Under these experimental conditions the collagen implants had also a tendency for retarding the drug liberation (Figure 2). It is of important to note that the cross-linked collagen had a more pronounced prolongation effect in comparison to the nonmodified one. Thus, within 30 days about 50 percent and 90 per cent of gentamicin released from above implants respectively. During this period about 60 percent of the antibiotic released from the polymethylmethacrylate beads Septopal (Figures 1 and 2).

Replacing of distilled water by Sorensen buffer was accompanied by rapid increase in the rate of gentamicin liberation from the implants based on monocarboxylcellulose and alginic acid. From Figure 3 it seems that in these cases the liberation completed

Table 1. Percentage of Gentamicin Release from SCA-, CP1-, CP2-, CP3- and CP4-Implants into Distilled Water (pH 6.0)

Used Carrier	Releasing Time (hours)						
	0.5	1	3	6	12	24	36
SCA	60.3	78.2	98.1	*	*	*	*
CP1	98.1	98.5	99.1	*	*	*	*
CP2	97.2	98.3	99.0	*	*	*	*
CP3	26.2	43.1	64.2	80.6	95.4	102.3	*
CP4	15.1	25.5	34.2	55.1	71.2	92.2	99.3

* - No further sampling was performed

Table 2. Percentage of Gentamicin Release from SCA-, CP1-, CP2-, CP3- and CP4-Implants into Phosphate Buffer (pH 7.35, Ionic Strength 0.22)

Used Carrier	Releasing Time (hours)						
	0.5	1	3	6	12	24	36
SCA	96.4	95.9	99.8	*	*	*	*
CP1	99.3	101.5	100.8	100.2	*	*	*
CP2	97.6	98.5	99.5	99.2	*	*	*
CP3	27.3	41.7	64.5	78.9	92.2	99.5	*
CP4	16.7	25.4	36.2	57.2	73.6	92.3	100.6

* - No further sampling was performed

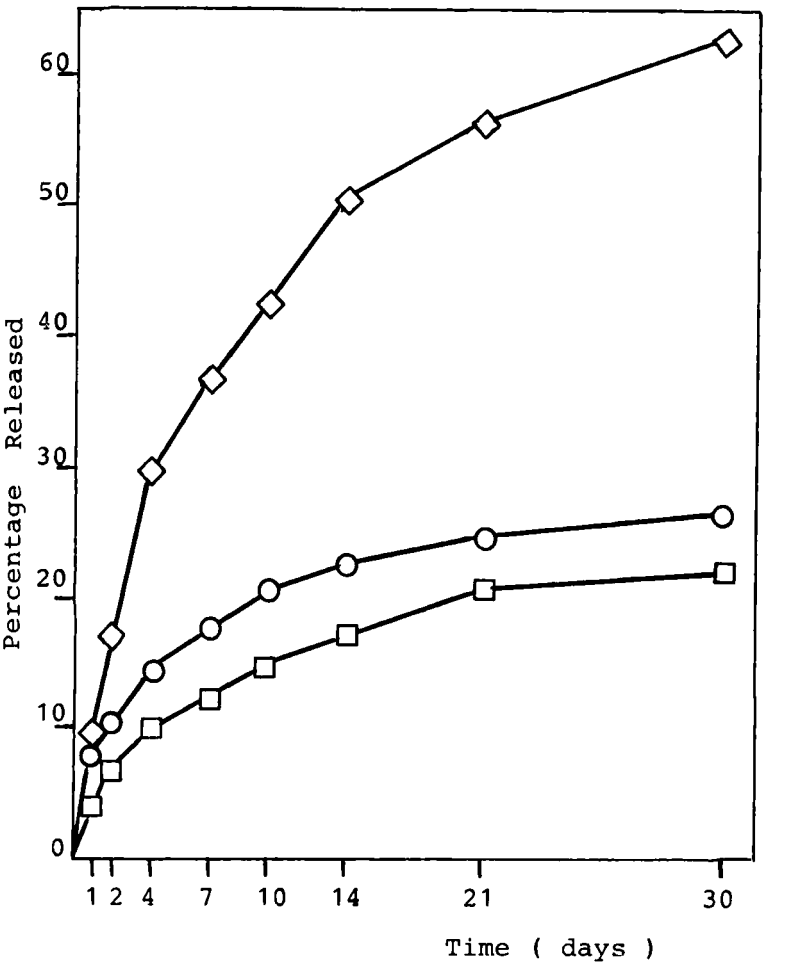

Figure 1. In-Vitro Release Profiles of Gentamicin for MCC-, AA- and PMMA-Implants Using Distilled Water (pH 6.0).

Keys: ○ - MCC-Implants Containing 5 mg of the Antibiotic.

□ - AA-Implants Containing 5 mg of the Antibiotic.

◇ - PMMA-Implants Containing 4.5 mg of the Antibiotic.

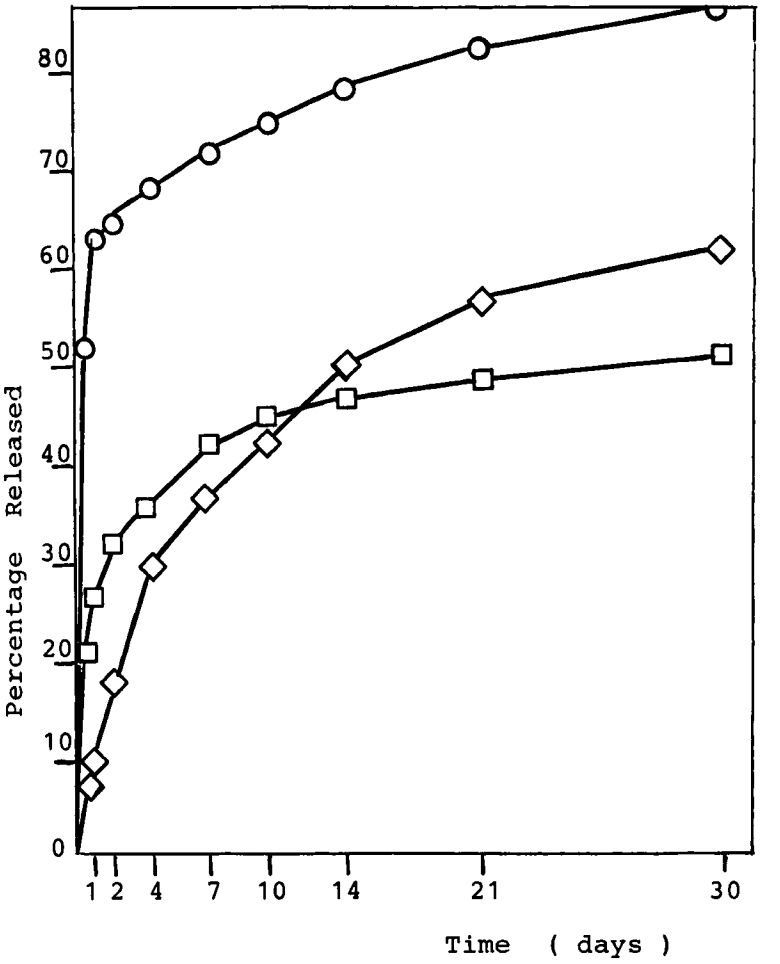

Figure 2. In-Vitro Release Profiles of Gentamicin for NMC-, CLC- and PMMA-Implants Using Distilled Water (pH 6.0).

Keys:

○ - NMC-Implants Containing 5 mg of the Antibiotic.

□ - CLC-Implants Containing 5 mg of the Antibiotic.

◇ - PMMA-Implants Containing 4.5 mg of the Antibiotic.

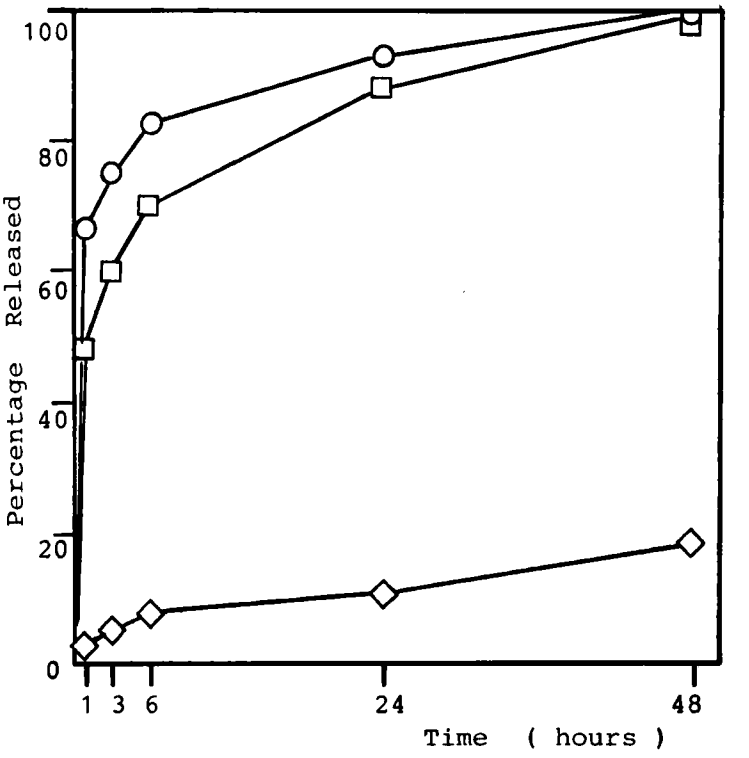

Figure 3. In-Vitro Release Profiles of Gentamicin for MCC-, AA- and PMMA-Implants Using Phosphate Buffer (pH 7.35, ionic strength 0.29).

Keys: ○ - MCC-Implants Containing 5 mg of the Antibiotic.

□ - AA-Implants Containing 5 mg of the Antibiotic.

◇ - PMMA-Implants Containing 4.5 mg of the Antibiotic.

within 2 days. For collagen implants only nonsignificant enhancement of the drug release was demostrated (Figure 4). Under these experimental conditions no changes in the rate of gentamicin release from the polymethylmethacrylate beads were observed (Figures 3 and 4).

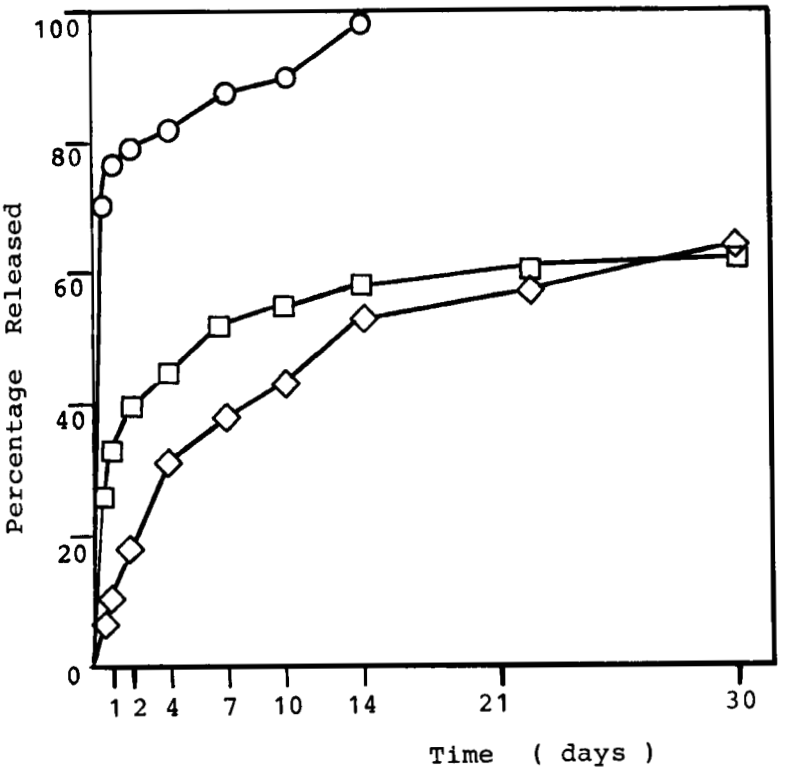

Figure 4. In-Vitro Release Profiles of Gentamicin for NMC-, CLC- and PMMA-Implants Using Phosphate Buffer (pH 7.35, ionic strength 0.29).

Keys: ○ - NMC-Implants Containing 5 mg of the Antibiotic.

□ - CLC-Implants Containing 5 mg of the Antibiotic.

◇ - PMMA-Implants Containing 4.5 mg of the Antibiotic.

Further investigations have demonstrated that acceleration of the antibiotic liberation from the monocarboxylcellulose and alginic acid implants into the media at higher pH and ionic strength values may be partialy compensated by addition of 3 percent fresh rat blood to the eluent (Figures 5 and 6 respectively). It suggested that hemostatic properties of monocarboxylcellulose and alginates might be an important factor responsible for sustention of the drug release from the implants based on these carriers.

The results of in-vivo studies demonstrated that for implants based on polymethylmethacrylate, nonmodified and cross-linked collagen, monocarboxylcellulose and alginic acid changes in the drug concentration in the implantation zones were of three-phase character (Figures 7 and 8). Table 3 summarized the tissue pharmacokinetic parameters of the antibiotic used in these implants.

With the use of implants based on polymethylmethacrylate, nonmodified and cross-linked collagen, monocarboxylcellulose and alginic acid in phase I within 1-6 hours the antibiotic concentration reached its maximum i.e. 89.0, 365.0, 210.0, 513.0 and 367.0 µg/g and rapidly declined to a certain intrinsic level. Therefore, during phase I most probable for infection development such implants provided antibiotic concentration in the implantation zone many times higher than those after systemic administration (13).

In phase II within 7-14 days the drug concentration for these implants was practically constant (C_{ss}) i.e. 4.6±0.6, 1.2±0.3, 5.2±1.9,

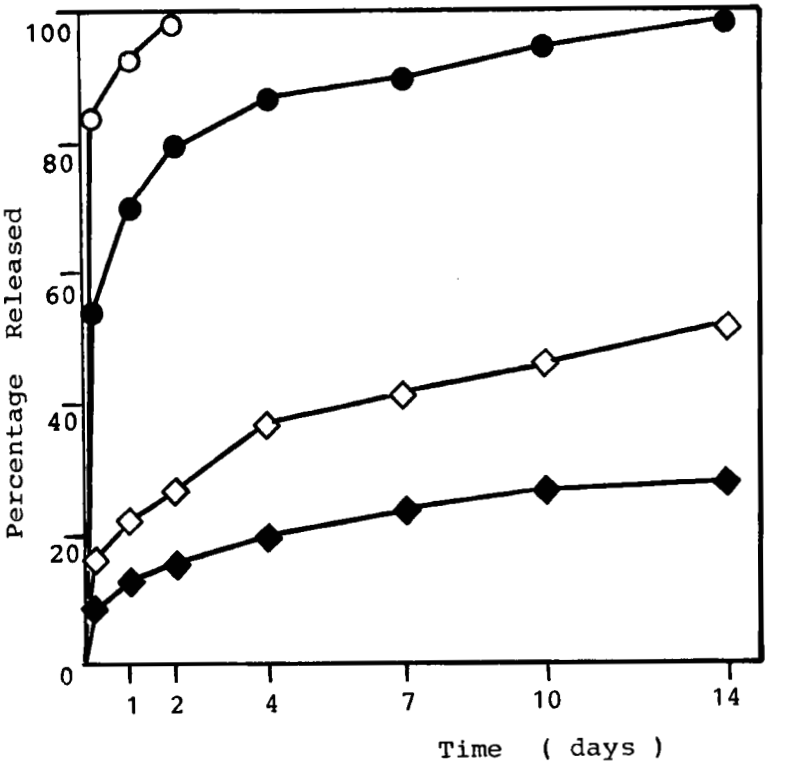

Figure 5. Effect of Eluent Content on the Release of Gentamicin from MCC-Implants.

Keys:

◇ - Saline Solution (pH 6.15, ionic strength 0.15).

◆ - Saline Solution + 3 percent Whole Rat Blood (pH 6.15, ionic strength 0.15).

○ - Phosphate Buffer (pH 7.35, ionic strength 0.22).

● - Phosphate Buffer + 3 percent Whole Rat Blood (pH 7.3, ionic strength 0.29).

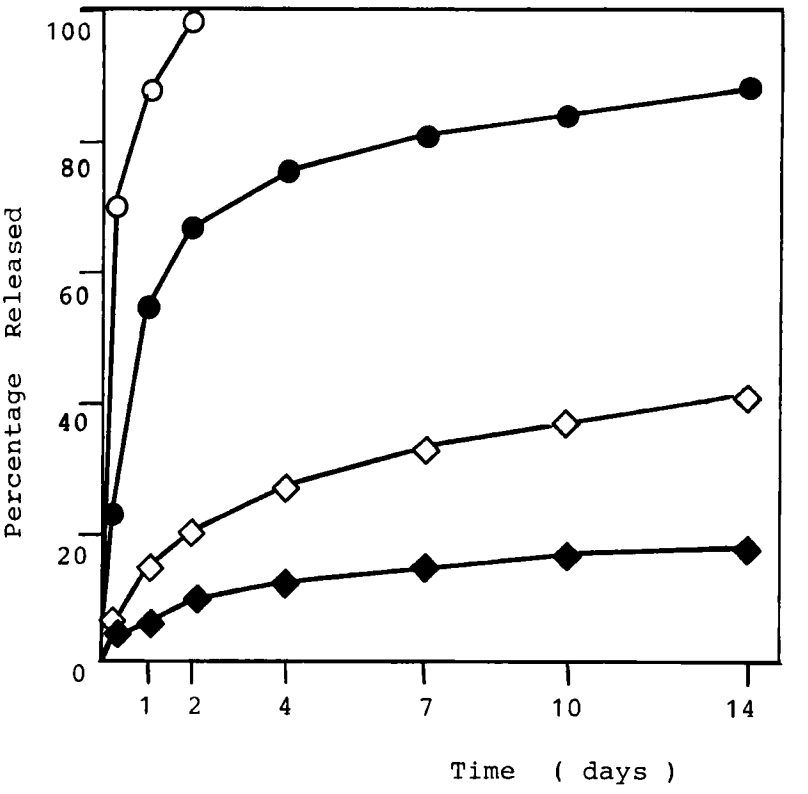

Figure 6. Effect of Eluent Content on the Release of Gentamicin from AA-Implants.

Keys:

◇ - Saline Solution (pH 6.15, ionic strength 0.15).

◆ - Saline Solution + 3 percent Whole Rat Blood (pH 6.15, ionic strength 0.15).

○ - Phosphate Buffer (pH 7.35, ionic strength 0.22).

● - Phosphate Buffer + 3 percent Whole Rat Blood (pH 7.3, ionic strength 0.29).

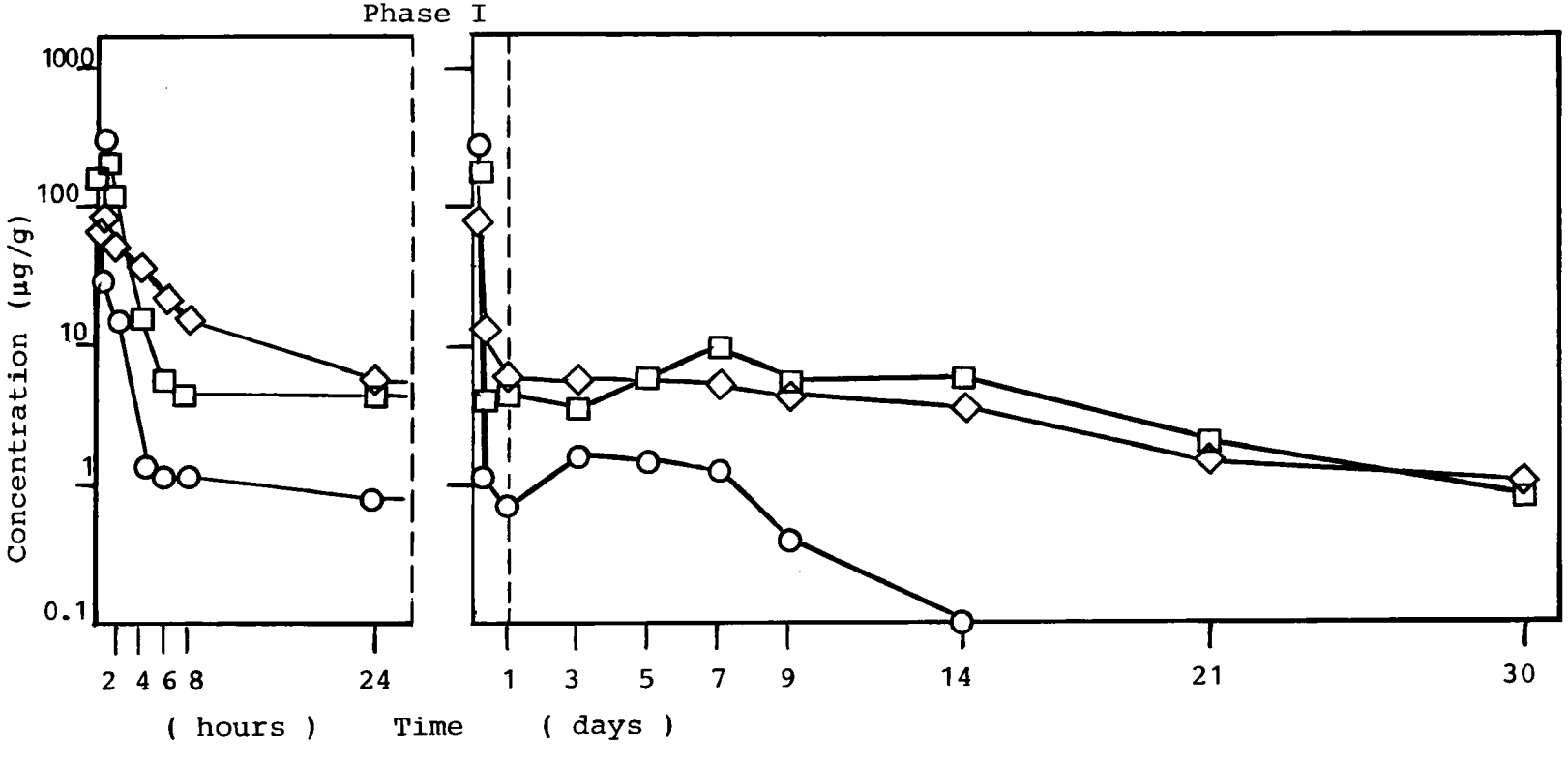

Figure 7. Tissue Pharmacokinetic Profiles of Gentamicin after Implantation of NMC-, CLC- and PMMA-Implants. Keys: ○ - NMC-Implants Containing 5 mg of the Drug. □ - CLC-Implants Containing 5 mg of the Drug. ◇ - PMMA-Implants Containing 4.5 mg of the Drug.

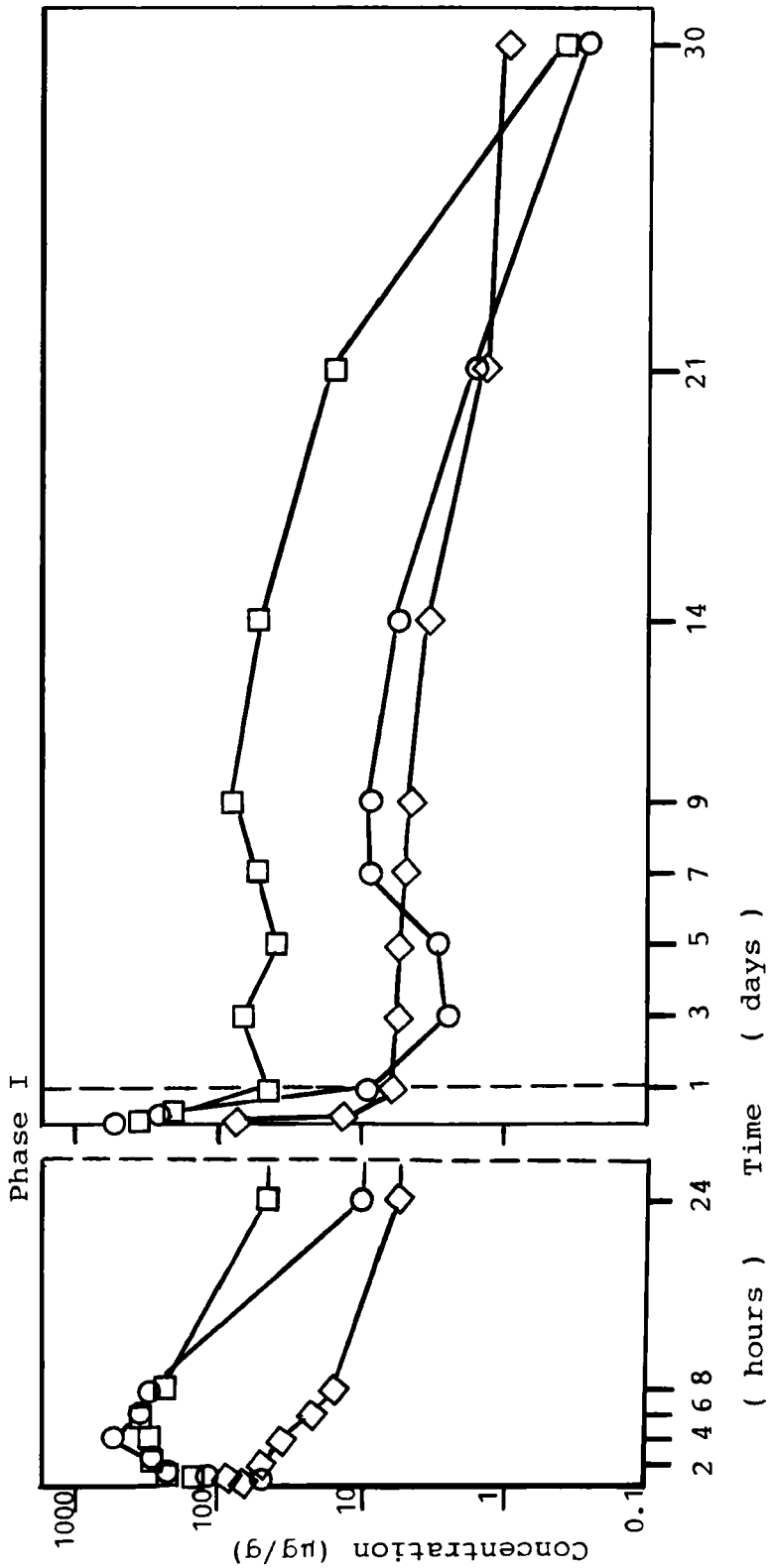

Figure 8. Tissue Pharmacokinetic Profiles of Gentamicin after Implantation of MCC-, AA- and PMMA-Implants. Keys: ○ - MCC-Implants Containing 5 mg of the Drug. □ - AA-Implants Containing 5 mg of the Drug. ◇ - PMMA-Implants Containing 4.5 mg of the Drug.

Table 3. Pharmacokinetics of Gentamicin in the Tissue from Zones of the Implantable Device Implantation

Parameters of pharmacokinetics	Used carrier				
	NMC	CLC	MCC	AA	PMMA
Phase I					
C_{max} (μg/g)	365.0	210.0	513.0	367.0	89.0
T_{max} (h)	1	1	4	6	1
k_a (h^{-1})	312.75	51.02	41.67	76.19	33.65
k_{el} (h^{-1})	1.93	0.70	0.20	0.12	0.12
$t_{1/2}$ (h)	0.36	0.99	3.46	5.92	5.66
Duration (h)	4	6	24	24	24
Phase II					
C_{ss} (μg/g)	1.2±0.3	5.2±1.9	6.2±3.0	55.0+14.7	4.6±0.6
Duration (h)	164	330	312	312	312
Phase III					
k_{el} (h^{-1})	0.005	0.012	0.008	0.013	0.003
$t_{1/2}$ (h)	58.21	148.52	89.30	54.18	215.96
Duration (h)	168	384	384	384	*

* - Not determined

6.2±3.0 and 55.0±14.7 μg/g respectively. Thus, for implants based on polymethylmethacrylate, cross-linked collagen, monocarboxylcellulose and especially alginic acid these levels were higher that the MICs of gentamicin for most pathogens causing wound and surgical infections (14).

During phase III gentamicin concentration in the zones of implantation of the implantable devices slowly lowered. For implants based on polymethylmethacrylate, nonmodified and cross-linked collagen, monocarboxyl-cellulose and alginic acid half-lives were 216, 58, 149, 89 and 54 hours.

It is interest to note that for biodegradable carriers phase II coincided in time with formation of fibrous capsule surrounded the implant and phase III coincided with its involution. The time of complete lysis of these implants and capsules was 3-4 weeks.

Figures 9 and 10 graphically portray the blood pharmacokinetic profiles of gentamicin observed for studied implants. Antibiotic blood levels observed at the period corresponding to phase I and equal to 3-10 percent of the drug levels in the implantation zones were demonstrated. In general, gentamicin maximum blood levels were similar to those after i.v. injection (excluded implant based on nonmodified collagen having higher value of maximum blood concentration). After 24 hours extremely low drug concentrations were detected in the blood).

The comparison of the areas under tissue concentration-time curves (Figure 11) showed that by prolongation effect in vivo the implants used cross-linked collagen, monocarboxylcellulose and especially

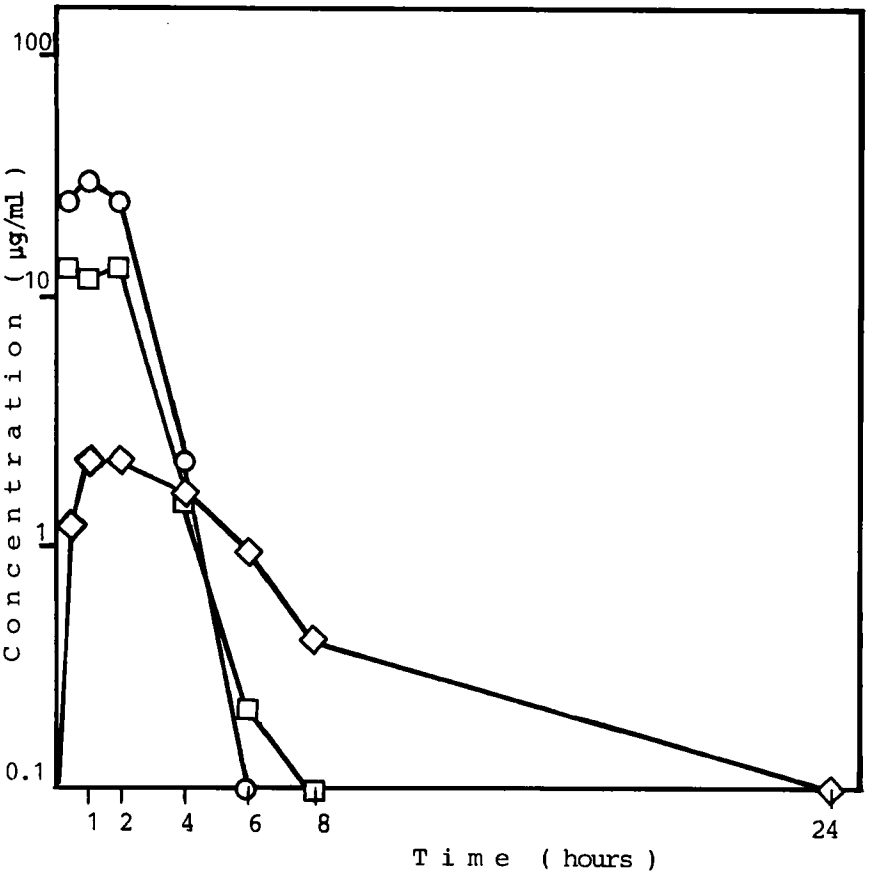

Figure 9. Blood Pharmacokinetic Profiles of Gentamicin after Implantation of NMC-, CLC- and PMMA-Implants.

Keys: ○ - NMC-Implants Containing 5 mg of the Antibiotic; C_{max} 33.6 µg/ml, T_{max} 1 h.

□ - CLC-Implants Containing 5 mg of the Antibiotic; C_{max} 17.0 µg/ml, T_{max} 0.5 h.

◇ - PMMA-Implants Containing 4.5 mg of the Antibiotic; C_{max} 2.1 µg/ml, T_{max} 1-2 h.

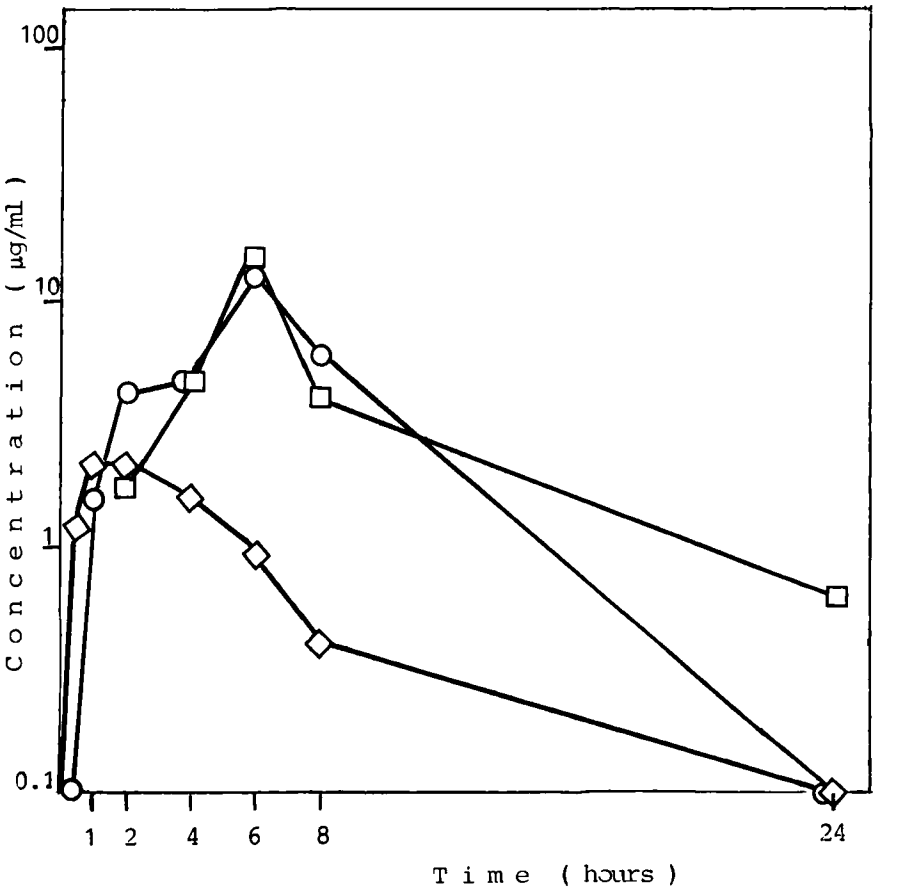

Figure 10. Blood Pharmacokinetic Profiles of Gentamicin after Implantation of MCC-, AA- and PMMA-Implants.

Keys:

○ - MCC-Implants Containing 5 mg of the Antibiotic; C_{max} 13.6 µg/ml, T_{max} 6 h.

□ - AA-Implants Containing 5 mg of the Antibiotic; C_{max} 14.0 µg/ml, T_{max} 6 h.

◇ - PMMA-Implants Containing 4.5 mg of the Antibiotic; C_{max} 2.1 µg/ml, T_{max} 1-2 h.

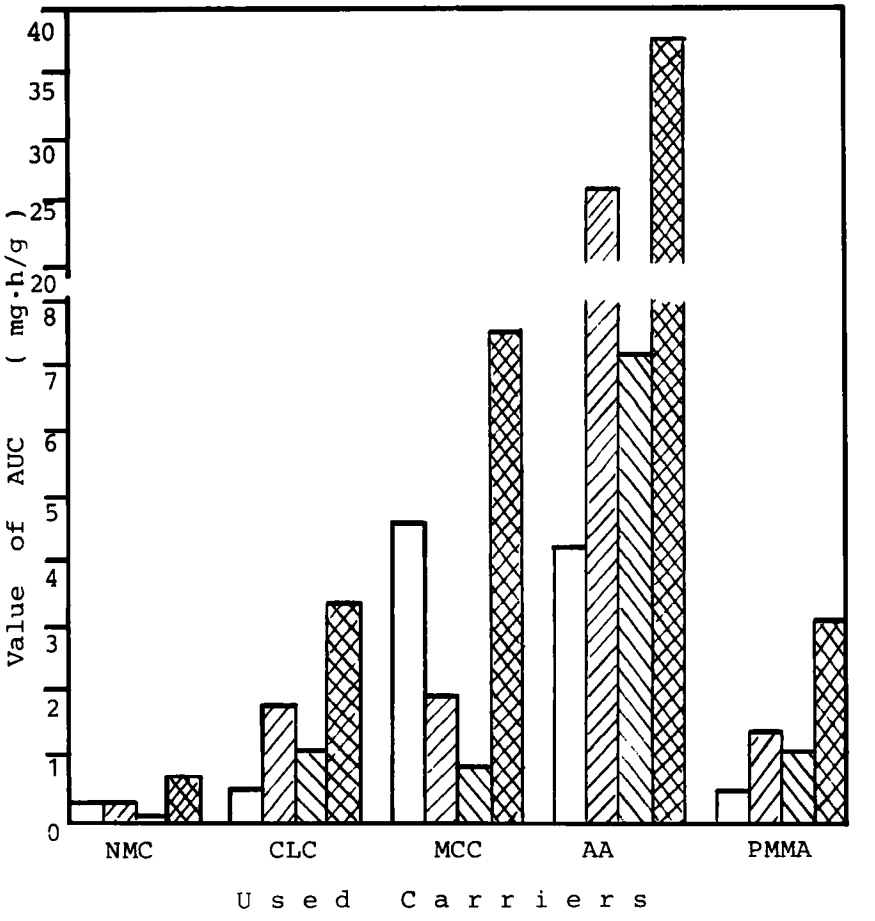

Figure 11. Comparison of the Areas Under Concentration Time Curves Realized after Implantation of NMC-, CLC-, MCC-, AA- and PMMA-Implants.

Keys: ☐ - AUC corresponded to Phase I.

▨ - AUC corresponded to Phase II.

▧ - AUC corresponded to Phase III.

▩ - Total AUC.

alginic acid were superior to polymethylmethacrylate implants. The implantable devices based on nonmodified collagen had no such advantage pharmacokinetic characteristics.

In conclusion, it is evident that gentamicin biodegradable implants based on cross-linked collagen, monocarboxylcellulose and alginic acid represent possible type of drug delivery systems providing high and prolonged antibiotic concentrations at the site of implantation.

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