Rapid release of gentamicin from collagen sponge

In vitro comparison with plastic beads

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The gentamicin-containing collagen sponge is a new product intended for local application in bone and soft-tissue infections. The release of gentamicin from the collagen sponges was compared in vitro to that from polymethyl-methacrylate (PMMA) beads. A static and kinetic experimental design was used. In the static model, pieces of collagen sponge or PMMA beads were added to 20 mL of distilled water, and during the following hours the gentamicin concentrations in the water were repeatedly measured. This simple model was extended to the kinetic model as the released gentamicin was removed from the water exponentially by means of an infusion-withdrawal pump. The gentamicin was released from the carrier substances with increasing half lives. During the first 4 hours, the half life increased from 0.2 to 1.5 hours for the collagen sponge and from 3 to 78 hours for the PMMA beads. After 1.5 hours, 95 percent of the gentamicin was released from the sponges, whereas only 8 percent was released from the beads.

During the last 10 years, gentamicin-containing polymethyl-methacrylate (PMMA) beads have been used successfully as a complement to the surgical treatment of bone and soft-tissue infections (Hedström et al. 1980, Wahlig 1982). The release of gentamicin from the beads is very slow. However, implantation of beads provides a high gentamicin concentration at the site of implantation, which persists for many days (Hedström et al. 1980, Wahlig et al. 1978, Wahlig 1982). The disadvantage of using the PMMA beads is the need for removal, necessitating a second operation. Bovine collagen prepared as a sponge and containing gentamicin is a new product intended for local antibiotic treatment. The collagen is resorbable, and therefore removal of this carrier substance is not necessary.

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The present study is an in vitro investigation comparing the release kinetics of gentamicin from the collagen sponge and from the PMMA beads.

Materials and methods

The collagen carrier of gentamicin (collagen sponge, Essex Pharma) is a pepsin-treated bovine collagen. It is produced as a sponge measuring 10 x 10 x 0.5 cm and weighing approximately 0.5 g. Each sponge contains 130 mg gentamicin. Before use, the sponges were cut into smaller pieces and the gentamicin content of the pieces was determined by weighing. The PMMA beads (Septopal®, Merck) were 0.7 cm in diameter, and each bead contained 4.5 mg gentamicin. The release of gentamicin from the carriers was measured in two experimental models.

In the *static model* the sponge or the beads were added to 20 mL distilled water, and during the following 2.5–5 hours, samples of 0.05 mL were repeatedly taken from the water for measurement of gentamicin concentration. During the experiments,

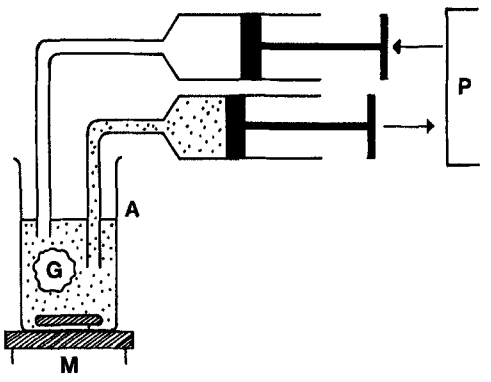

Figure 1. The kinetic model. P: infusion-withdrawal pump, A: flask, M: magnetic stirrer, G: gentamicin-carrying substance.

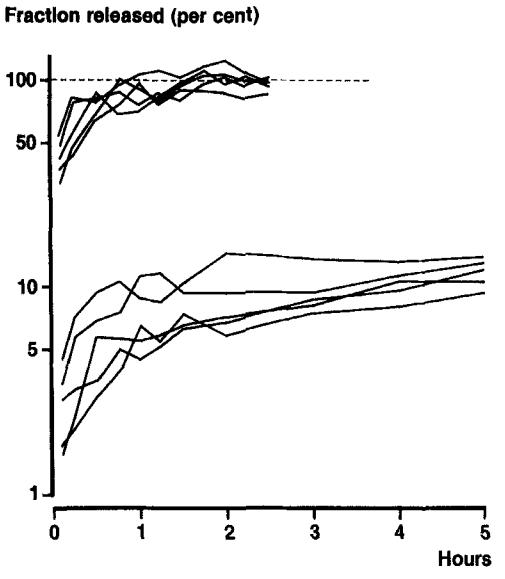

Figure 2. Fractions of gentamicin released from collagen sponges (upper five curves) and from PMMA beads (lower five curves) as found in the static model experiments.

continuous stirring was performed, and the temperature was kept at 37 °C. Five experiments were performed with each of the carriers in which an increasing amount of gentamicin was added. For the collagen sponge, this ranged from 0.027 to 0.17 g sponge (corresponding to 6.5–32 mg gentamicin), and for the PMMA beads from 2 to 7 beads (9–32 mg gentamicin).

The *kinetic model* is outlined in Figure 1. The flask A (simulation of the surgical wound) contained the volume V_A of distilled water and the PMMA beads or collagen sponge (G). Gentamicin-free water was infused to the V_A with a constant flow rate (H) and mixed with V_A with a magnetic stirrer. Simultaneously, gentamicin-containing water was withdrawn from V_A at the same flow rate. With this arrangement, gentamicin dissolved in the water will be eliminated from V_A exponentially with an elimination rate constant (K_E) determined by the formula $K_E = H/V_A$. The amount of gentamicin added to V_A was 9.0 mg (two PMMA beads or 0.035 g sponge). The volume V_A was 20 mL, and the flow rate (H) was adjusted to give an elimination rate constant at 0.023 min^{-1}, corresponding to a half life ($T_{1/2}$) at 30 min ($T_{1/2} = \ln(2)/K_E$). After adding the gentamicin, samples of 0.05 or 0.1 mL were repeatedly taken from V_A during the following 5–8.5 hours. The flow rate (H) was monitored by measuring the volume pumped out in 90–120-min periods throughout the experiments. During the experiments the temperature in V_A was kept at 37 °C. Two experiments were performed for each carrier.

Based on the measured concentrations, the fraction of gentamicin released at the individual sampling times was calculated. In the kinetic model the amount of gentamicin released (X^R) during a time period (Δt) from the time t_1 to t_2 is as follows:

$$X^R(\Delta t) = V_A[C(t_2) - C(t_1)] + K_E V_A \int_{t_1}^{t_2} C(t)$$

and it was calculated by the approximation:

$$X^R(\Delta t) = V_A[C(t_2) - C(t_1)] + \\ + K_E V_A[C(t_2) + C(t_1)](t_2 - t_1)1/2$$

The rate constants (K_R) for the release of gentamicin were calculated by linear regression, assuming a first order release kinetics, according to the formula: $\ln(G_t) = \ln(G_{t=0}) - K_R t$, where G_t is the amount of remaining gentamicin in the carrier substance at the time t.

For gentamicin concentration measurements, an agar diffusion method was used (Rosdahl et al. 1969), with filter-paper discs (diameter 9 mm) as diffusion centers, antibiotic medium no. 5 (DIFCO) as test medium, and *Pseudomonas aeruginosa* as the test strain. Standard concentrations were prepared in distilled water. To each disc was added 0.05 mL sample or standard solution.

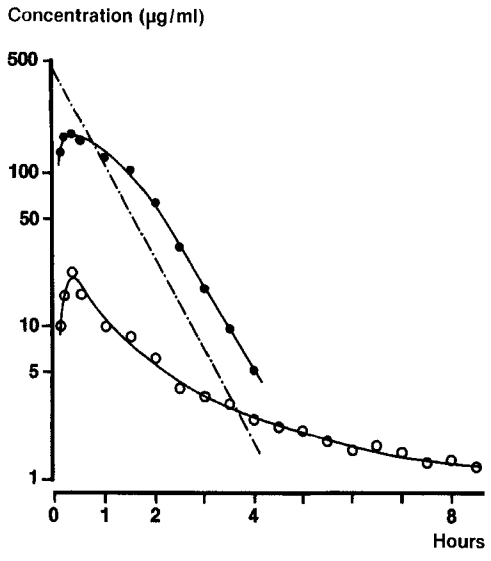

Figure 3. Measured concentrations of gentamicin in the kinetic model experiments. Nine milligrams of gentamicin was added bound to the collagen sponges (•) or to the PMMA beads (o). The dotted line (–·–·–) represents the expected concentration course after adding 9 mg unbound gentamicin.

Results

In the static model the fractions of gentamicin released from the carrier substances were not correlated with the total amount added ($P > 0.1$ Spearman test). However, the release of gentamicin from the collagen sponges was much more rapid than the release from the PMMA beads (Figure 2). After 5 minutes, 43 (32–55) percent of the gentamicin was released from the sponges, whereas only 3 (1.6–5.0) percent was released from the beads, and after 1.5 hours the corresponding figures were 94 (81–103) percent and 8.4 (6.3–11) percent.

In the kinetic model the highest concentration of gentamicin in the water was reached after 15 min for both carrier substances (Figure 3). However, this maximum concentration was 8 times higher for the sponge. The following decrease in concentration was likewise more rapid for the sponge gentamicin. By extrapolation, it was found that the concentration in the sponge experiments would fall below the concentration in the bead experiments after about 5 hours.

The rate constants for the release of gentamicin from the carriers were decreasing with time indicating that the release was not a simple first order reaction. However, by assuming first order release kinetics during shorter time periods, the corresponding release rate constants were calculated (Table 1).

Table 1. Release rate constants (in hours^{-1}) for the release of gentamicin from collagen sponges and PMMA beads. The corresponding half lives (hours) in italics

Periods (min)	Collagen sponges		PMMA beads	
	Static	Kinetic	Static	Kinetic
0–15	3.57 *0.2*	2.64 *0.3*	0.167 *4*	0.243 *3*
30–90	1.21 *0.6*	1.39 *0.5*	0.023 *30*	0.020 *33*
120–240	–	0.46 *1.5*	0.009 *73*	0.009 *78*
270–510	–	–	–	0.005 *133*

Discussion

The advantage of local application of antibiotics is high concentration in the infected area. This is of particular interest for gentamicin where systemic administration of therapeutic doses entails a risk of toxic side effects.

By local application of antibiotics bound to a carrier, the wound concentration achieved is determined partly by the release rate of the antibiotics to the wound fluid and partly by the absorption rate of the antibiotic from the wound fluid to the blood stream.

For both the gentamicin-carrying substances tested in the present study, the release rate constants were decreasing with time. However, the release rate constants were 10–50 times higher for the sponges than for the beads. The release rate for the PMMA beads found in the present study was in good agreement with the results published by other authors (Wahlig et al. 1978).

The absorption of gentamicin from surgical wounds has been investigated in only a few patients. After local application of gentamicin dissolved in saline just before wound closure in total hip replacements, the wound fluid concentration decreased, with a half life at approximately 3.5 hours, corresponding to an absorption rate constant of 0.2 hours^{-1} (Sørensen et al. 1984). The main indication for PMMA beads has been chronic osteomyelitis. The absorption rate in such wounds is probably lower than in total hip replacement wounds because of the low vascularization of infected bone tissue.

Presumably, the release rate of gentamicin found in the present study for the collagen sponges was considerably higher and the release rate for the PMMA beads was lower than the absorption rate in most—if not in all—surgical wounds. This difference in the release-to-absorption ratio will result in a marked difference in the pharmacokinetics of gentamicin (Gibaldi and Perrier 1975).

The use of gentamicin-containing collagen sponges in surgical wounds can be expected to give a rapid initial increase in wound-fluid concentration and a high peak level. However, the following decrease in wound-fluid concentration of gentamicin will be independent of the collagen, but determined by the rate constant for the absorption of gentamicin from the wound to the blood stream. This is clearly illustrated in the kinetic model experiments in the present study, even though (for technical reasons) a relative rapid elimination rate was used. The difference between application of gentamicin dissolved in saline and getamicin bound to the collagen sponge seems negligible. In contrast, the application of gentamicin-containing PMMA beads will give a slower initial increase in wound-fluid concentration with a lower peak level. The following decrease in fluid concentration will be determined by the rate constant for the release of gentamicin from the beads, which means the wound-fluid concentration will decrease with a half life of several days. Pharmacokinetic studies in patients with implanted PMMA beads seem to verify this kinetic profile for the gentamicin-containing PMMA beads (Härle and Ritzerfeld 1979, Hedström et al. 1980, Wahlig 1982).

Whether a short-lived high or a prolonged low wound-fluid concentration of gentamicin is preferable in the treatment of infected wounds or osteomyelitis is not known. However, should the latter be the case, the PMMA beads are preferable; and should the former be the case, it is difficult to see the advantages of the collagen sponges compared with gentamicin dissolved in saline. In addition, it has to be mentioned that the clinical use of the sponges entails a risk of adverse immunologic reaction to the bovine collagen, even though this risk seems low (Cooperman and Michaeli 1984).

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