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Controlled release of vancomycin from Poloxamer 407 gels

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Abstract

The purpose of this study was to investigate Poloxamer 407 25% (w/w) formulations aimed at prolonging the residence time of vancomycin, a time-dependent antibiotic, in a body site with a high infectious risk. Reversible thermal gelation of the formulations permitted their local injection in liquid form and in situ gelation as they warmed to body temperature. Neither the rheological properties of the Poloxamer matrices nor the antibacterial activity of vancomycin was altered by their combination. In vitro, the dispersed form exhibited prolonged release, with a lower diffusion coefficient of vancomycin compared to the solubilized form (4.7×10^{-8} vs 2.1×10^{-7} $cm^2\ s^{-1}$). In rats, a single dose was well tolerated and resulted in a high local concentration for 24 h (> 131 mg l^{-1} for the solubilized form), followed by lower but effective antibacterial levels for at least 8 days. Controlled-release profiles, good preservation of vancomycin activity, good tolerability in rats, and ease of administration suggest that Poloxamer 407 may be useful as a vancomycin delivery vehicle for local prophylaxis of infections, especially in prosthetic surgery.
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1. Introduction

Prevention and treatment of deep-seated infections, especially in poorly vascularised sites like bones and prosthetic devices remain difficult with conventional systemic antibiotic therapy. New systems adapted to local antibiotic administration are thus needed. The ideal matrix for local antibiotic delivery should meet the following criteria: carriage of sufficient drug, good tolerability, biodegradability (to avoid surgical removal), controlled release, and non traumatic when placed at a mobile site.

Poloxamer thermoreversible gels seem to fulfill the above conditions. Poloxamers are a broad group of compounds that were introduced commercially in the early 1950s, as food additives and for pharmaceutical preparations (Alexandridis

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and Hatton, 1995). These water-soluble, non toxic, inert surfactants are triblock copolymers with a central hydrophobic part (polyoxypropylene) and two identical lateral hydrophilic parts (polyoxyethylene). Different hydrophile–hydrophobe ratios and chemical and physical characteristics can be obtained by varying the block size and total molecular weight of the Poloxamer (Garcia Sagrado et al., 1994a). At concentrations above 20%, Poloxamer 407 solutions have unusual rheological characteristics (Schmolka, 1972), which permit them to be administered in liquid form via syringes and to gel in situ, sol-gel transition temperatures being lower than body temperature. The non toxic properties and stability of Poloxamer 407 aqueous solutions make them suitable as injectable vehicles (Johnston and Miller, 1985; Henry and Schmolka, 1989; Garcia Sagrado et al., 1994b). They have been tested as supports for topical application of various drugs and appear to be useful as controlled delivery systems (Mengi and Deshpande, 1992; Miyazaki et al., 1995; Paavola et al., 1995; Esposito et al., 1996). In addition, Poloxamer 407 exhibits non specific antiadhesive properties that can prevent the adherence of proteins and bacteria, and thereby hinder infections (Portolés et al., 1992; Reigel et al., 1993; Portolés et al., 1994).

Vancomycin utilization has increased markedly in the last few years, as a result of the increasing numbers of infections of bone and prosthetic devices by methicillin-resistant staphylococci. However, its restricted clinical efficacy in poorly vascularised body sites during parenteral use, and the risks of toxicity associated with high serum concentrations, warrant the search for new carrier systems for local vancomycin release.

The purpose of the present investigation was to evaluate two Poloxamer 407-based formulations designed for controlled delivery of vancomycin. Both are reversed temperature-sensitive gels. In the control formulation, Poloxamer 407 was prepared in saline solution, in the other one, it was prepared in pH 7.4 phosphate buffer. The pH value was set at 7.4 to ensure that the initial vancomycin loading dose was above the solubility limit in the gel. The study can be divided into two parts. The first was an in vitro physical characterisation of the formulations and their antibiotic release profiles. The second part was an in vivo pharmacokinetic evaluation of the formulations in healthy animals.

2. Materials and methods

2.1. Preparation of Poloxamer 407 formulations

Poloxamer 407 (Pluronic® F-127) was a gift from BASF (Levallois-Perret, France). This triblock copolymer consists, by weight, of approximately 70% ethylene oxide and 30% propylene oxide with an average total molecular weight of 12 500 (formula:

$$HO(CH_2CH_2O)_X(CH_2\overset{\overset{CH_3}{|}}{C}HO)_Y(CH_2CH_2O)_XH$$

where X and Y values are about 98 and 57, respectively). The Poloxamer 407 concentration in the two formulations was 25% (w/w). The gels were prepared by the cold method described by Schmolka (Schmolka, 1972). For the preparation of the control gel, a weighed amount of poloxamer 407 was gradually added to cold (5–10°C) sterile saline solution 9 g l^{-1} with gentle stirring. The container was left overnight in a refrigerator to ensure complete dissolution. Clear, viscous solutions formed. Gel formation was obtained by raising the temperature above the gelation temperature. The Poloxamer 407 gel pH 7.4 was prepared by means of the same method using a 0.01 M phosphate buffer pH 7.4 (Sigma, Saint-Quentin Fallavier, France) instead of saline. As the gels maintained their characteristics and properties after repeated cooling and heating, they were sterilized by autoclaving (120°C, 15 min, 1 bar). Gels loaded with vancomycin hydrochloride (molecular weight = 1485) (Lilly, Saint-Cloud, France) were prepared by using the same method and adding a weighed amount of the antibiotic to the previously prepared Poloxamer solution. The vancomycin concentration was 20 mg ml^{-1}. This value was lower than the solubility limit of vancomycin in the control gel, but was above the solubility limit (11.11 mg ml^{-1}) in the pH 7.4 gel. This latter gel was thus saturated with vancomycin, which was dispersed in the polymeric matrix.

2.2. Rheological properties of the gels

The rheological analysis was performed using a controlled-stress rheometer model Carri-Med CSL 100 (Carri-Med/Rhéo, 91160 Champlan, France). Flow measurements were performed with a cone-plate geometry (diameter 6 cm, angle 2°02, gap 60 μm). An imposed shear stress of 40 N m^{-2} was applied to the samples. The shear rate was measured as the temperature increased at a rate of 0.67°C min^{-1} from 10 to 20°C. At the minimum temperature the formulations were liquid, whereas at 20°C they had gelled. The sol-gel transition temperature was taken as the temperature at which the shear rate was below 10^{-2} s^{-1}. To better evaluate the mechanical properties of the gels, oscillation measurements were made with a cone-plate geometry (diameter 4 cm, angle 2°, gap 54 μm). An imposed shear stress of 59.68 N m^{-2} (in the linear region of all our samples) was applied. The frequency was 1 Hz and the temperature was reduced from 30 to 10°C at a rate of 1.18°C min^{-1}. The storage modulus G′ and the loss modulus G″ were recorded. The temperature at which G′ suddenly varied corresponded to the temperature of the gel-sol transition.

2.3. In vitro kinetic studies

We used a gel-gel diffusion system approximately mimicking in vivo conditions of drug transfer from the gel matrix to a prosthetic device or bone tissue. The diffusion cell comprised two compartments separated by a non limiting cellulose membrane which did not constitute a barrier against vancomycin diffusion (1.2-μm pores, Millipore® S.A., 78054 Saint-Quentin, France). The donor compartment was filled with formulation loaded with vancomycin, and the receptor compartment was filled with the same drug-free formulation. The length (h) and volume of each compartment were 1 cm and 1.3 ml, respectively. Diffusion cells were placed in a 37°C incubator with 100% humidity to avoid dessication of the formulations. At each time point (6, 24, 48, 96, 144 and 192 h), three systems were dismantled to assay the amount of vancomycin that had diffused into the whole receptor compartment.

2.4. In vivo kinetic studies

Male Wistar rats weighing 225–250 g were purchased from Charles River Laboratories (Saint-Aubin-les-Elbeuf, France). Animals were acclimatized for 1 week prior to use. They were housed in individual cages in air-conditioned room in accordance with guidelines for care and use of laboratory animals. To monitor local vancomycin concentrations, a multiperforated polytetrafluoroethylene (Teflon®) tissue cage (internal and external diameters of 10 and 12 mm, respectively; length, 32 mm; volume, 2.5 cm^3) was implanted subcutaneously at the nape of each rat, in aseptic conditions. After wound healing (2–3 weeks), the tissue cage was surrounded with a covering membrane and filled with fluid. Local treatment was then performed, consisting of an injection into the tissue cage of 2 ml of one of the two vancomycin/Poloxamer 407 formulations, or of a 20 mg ml^{-1} vancomycin aqueous solution (control rats). The tissue cage fluid could be aseptically aspirated and analyzed to determine the local drug concentration. Samples of fluid were analyzed over 8 days (192 h). Animals were weighed throughout the experiments to monitor their physiological status.

2.5. Vancomycin measurement

Vancomycin concentrations were determined using high-pressure liquid chromatography (HPLC) with a Shimatzu LC 6A pump connected to an SPD LC 6A detector (Touzart et Matignon, France) set at 229 nm. We used a 20-μl injection volume, obtained with a Wisp 717 automatic injector (Waters, Saint-Quentin, France), and a C18 Nucleosil column (Touzart et Matignon). The mobile phase was acetate buffer pH 4.5, and the flow rate was set at 1.7 ml min^{-1}. Biological fluids were deproteinised in acetonitrile, and aqueous samples were diluted in saline.

To control the remaining antibiotic activity, local concentrations were sometimes determined in parallel in a bioassay with *Bacillus subtilis* ATCC 6633 as the test strain.

Detection limits were 0.25 and 1 mg l^{-1} in the HPLC and bioassay methods, respectively.

2.6. Data analysis

In vitro, when the quantity released (M_t) into the receptor compartment represented less than 50% of the total quantity (M_0), its transfer rate was a function of the square root of time. Vancomycin release profiles were thus expressed in terms of the fraction released against the square root of time, and were analyzed by linear regression. The slopes of the linear profiles thus obtained were used to calculate the apparent diffusion coefficients of vancomycin, as follows:

$$\frac{M_t}{M_0} = \beta \cdot \frac{\sqrt{Dt}}{h\sqrt{\pi}} \quad (1)$$

in which M_t is the amount of drug released into the receptor compartment at time t, M_0 is the initial amount of drug in the donor compartment, D is the diffusion coefficient of vancomycin in the vehicle, t is time, and h is the thickness of the vehicle in the donor compartment. Coefficient β is a constant which only depends on the ratio C_0/C_S, where C_0 is the initial concentration of vancomycin in the donor compartment and C_S the solubility of vancomycin in the vehicle. When vancomycin was uniformly dissolved in the donor gel ($C_0 < C_S$), β was 1. When vancomycin was dispersed in the donor pH 7.4 gel ($C_0 > C_S$), β was 0.771 (Couarraze et al., 1989).

In the in vivo experiments, local vancomycin concentrations were determined at each time point for each rat in the various study groups. The mean values of local vancomycin concentrations in rats of the same group were calculated and plotted against the sampling time on a semilogarithmic scale to estimate elimination parameters. The area under the curve for each formulation was calculated after bilinear regression. The statistical significance of differences between mean pharmacokinetic values was determined by using Student's t-test.

Table 1
Gel-sol transition temperature of 25% (w/w) Poloxamer 407 formulations, expressed as mean ± standard deviation ($n = 6$). The various preparations were: unloaded control gel, unloaded pH 7.4 gel, and the same two formulations loaded with vancomycin 2%

Measurement	Gelation temperature ± SD (°C)	
	Flow	Oscillation
Control gel	12.7 ± 0.3	13.3 ± 0.2
pH 7.4 gel	12.9 ± 0.3	12.8 ± 0.2
Control gel + vancomycin	12.9 ± 0.7	13.2 ± 0.3
pH 7.4 gel + vancomycin	13.1 ± 0.5	12.8 ± 0.2

3. Results

3.1. Rheological analysis

Each Poloxamer 407 formulation with and without vancomycin was a viscous liquid at the storage temperature (4°C), formed a semisolid gel at the experimental temperature (37°C, above the gel-sol transition temperature), and returned to the liquid state below the gel-sol transition temperature. The gel-sol transition temperatures obtained by flow and oscillatory measurements were almost identical. The gelation temperatures were not modified in the presence of vancomycin (Table 1).

The apparent viscosities at 10°C of the various Poloxamer 407 formulations with and without vancomycin were similar (Table 2, Fig. 1). The different samples had Newtonian behaviour below the gelation temperature and non Newtonian behaviour above this temperature (Miller and Drabik, 1984; Lenaerts et al., 1987)

The dynamic rheological analysis showed the rheological changes as the temperature rose (Table 2, Fig. 2). The changes in rheological parameters can be divided into three phases. During the first, before the gelation point, elastic properties were negligible ($G' < G''$) and the samples were characterized by viscous liquid behaviour. The second phase corresponded to gelation. From this point onwards, G' and G'' values suddenly increased, and the samples were characterized by predominant elastic behaviour ($G' > G''$). The last phase consisted of stabilization, with G' values tending to stabilize while G'' values fell after having reached a maximum.

Table 2
Rheological parameters of 25% (w/w) Poloxamer 407 formulations, expressed as mean ± standard deviation ($n = 4$). All the samples were a viscous liquid at 10°C and an elastic gel at 30°C. The various preparations were: unloaded control gel, unloaded pH 7.4 gel, and the same two formulations loaded with vancomycin 2%[a]

	10°C			30 °C	
	μ (Pa s^{-1})	G′ (N m^{-2})	G″ (N m^{-2})	G′ (N m^{-2})	G″ (N m^{-2})
Control gel	0.17 ± 0.02	$(5.8 \pm 2.0) \times 10^{-3}$	0.74 ± 0.02	$23\,580 \pm 442$	817 ± 244
pH 7.4 gel	0.17 ± 0.02	$(7.5 \pm 2.6) \times 10^{-3}$	0.88 ± 0.02	$22\,710 \pm 1518$	686 ± 132
Control gel + vancomycin	0.14 ± 0.04	$(7.4 \pm 4.1) \times 10^{-3}$	1.06 ± 0.24	$24\,606 \pm 1609$	892 ± 406
pH 7.4 gel + vancomycin	0.15 ± 0.02	$(10.1 \pm 2.8) \times 10^{-3}$	1.27 ± 0.21	$21\,943 \pm 641$	740 ± 192

[a] Abbreviations: μ, viscosity; G′, storage modulus; and G″, loss modulus.

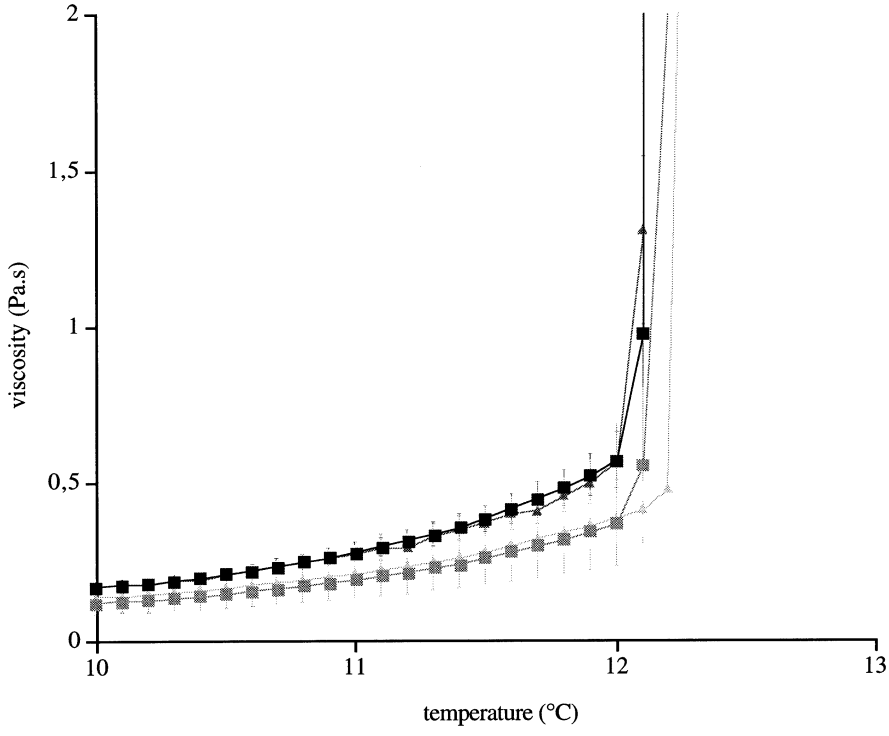

Fig. 1. Apparent viscosities of the various 25% Poloxamer 407 formulations. (■) control formulation ($n = 6$), (▲) pH 7.4 formulation ($n = 6$), (▩) control formulation loaded with vancomycin 20×10^3 mg l^{-1} ($n = 4$), (◬) pH 7.4 formulation loaded with vancomycin 20×10^3 mg l^{-1} ($n = 5$).

3.2. In vitro release experiments

Fig. 3 illustrates the in vitro release of vancomycin from the two Poloxamer 407 formulations at 37°C. The fraction of drug released was plotted against the square-root of time. With the two formulations the amount of vancomycin released increased linearly with the square-root of time ($r > 0.98$), so the diffusion coefficients were obtained with Eq. (1) (see above).

The formulation in pH 7.4 buffer, which was saturated with vancomycin, exhibited slower vancomycin release than the control formulation. Diffusion coefficients were 2.1×10^{-7} and 4.7×10^{-8} cm^2 s^{-1} for the control gel and the pH 7.4 gel, respectively (Table 3).

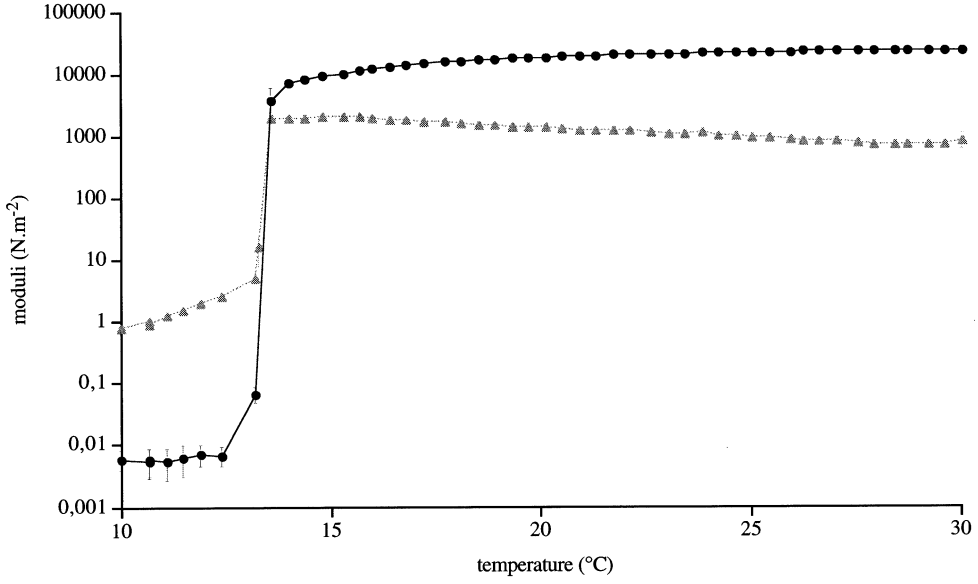

Fig. 2. Storage and loss modulus of the control Poloxamer formulation. (■) storage modulus, (▲) loss modulus.

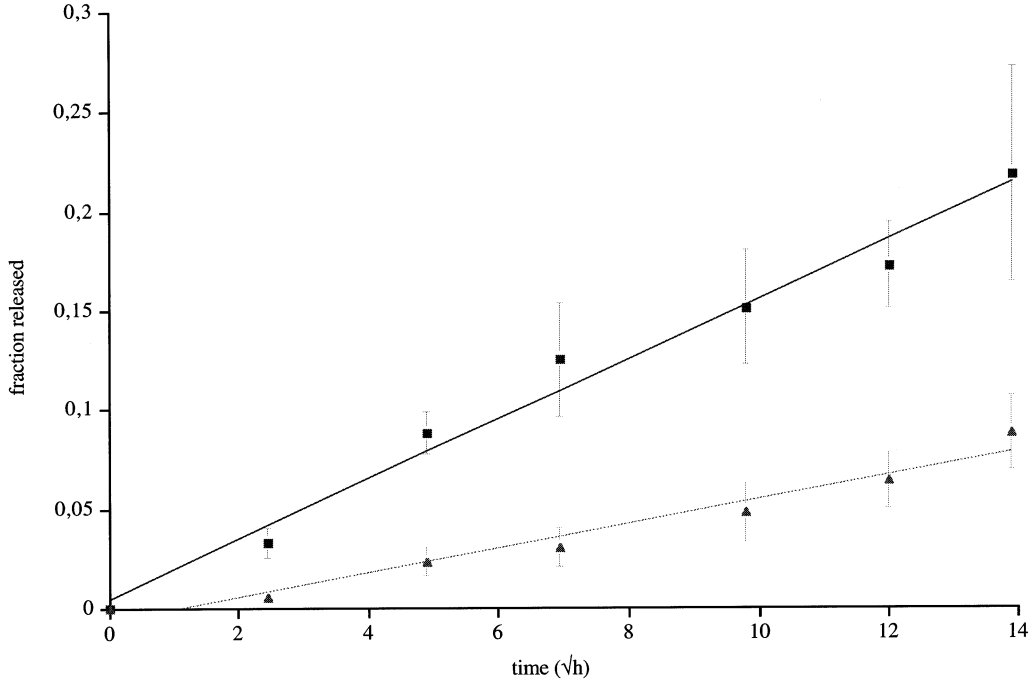

Fig. 3. In vitro vancomycin release from both Poloxamer 407 gels at 37°C. Values were measured by HPLC and are expressed as a fraction of vancomycin released in the receptor compartment versus the square-root of time. (▩) control Poloxamer 407 gel ($n = 9$), (▲) pH 7.4 Poloxamer 407 gel ($n = 12$). Initial amount of vancomycin in the donor compartment, 26 mg.

Table 3
Characteristics of in vitro vancomycin release from the two studied Poloxamer 407 formulations into a similar, unloaded system at 37°C. In the donor compartment, vancomycin was uniformly dissolved in the control gel and dispersed in the pH 7.4 gel. The initial concentration of vancomycin in the donor compartment was 2% (w/v) ($M_0 = 26$ mg)

	D ($cm^2\ s^{-1}$)[a]	Fraction released (%)
Control ge ($n = 9$)	2.1×10^{-7}	21.8
pH 7.4 gel ($n = 12$)	4.7×10^{-8}	8.8

[a] D, diffusion coefficient.

Table 4
HPLC and bioassay determination of local vancomycin concentrations after injection into the rat tissue cage of control Poloxamer 407 formulation loaded with 20×10^3 mg l^{-1} vancomycin ($n = 6$)

Time (h)	m ± SD (mg l^{-1})	
	HPLC	Bioassay
4	2900 ± 200	3040 ± 730
24	177 ± 86	104 ± 17
48	17.7 ± 6.0	17.2 ± 6.6
72	10.7 ± 2.9	4.1 ± 0.7
96	7.2 ± 1.3	3.2 ± 0.7
120	6.3 ± 0.7	2.0 ± 0.3
192	5.5 ± 1.3	1.9 ± 1.1

3.3. In vivo pharmacokinetic experiments

Data listed in Table 4 show that the combination of vancomycin with Poloxamer 407 retained antimicrobial activity. The local remaining vancomycin concentrations in Poloxamer 407 matrices assessed by both HPLC and the bioassay were in close agreement.

The weight gain was not hindered by any of the Poloxamer 407 treatments (data not shown).

The local pharmacokinetic profiles of vancomycin release from the solution and from the two Poloxamer 407 formulations in rats are shown in Fig. 4. The Poloxamer prolonged the local residence time of the antibiotic. The local vancomycin concentration reached 20 mg l^{-1} after 7, 40 and 26 h with the solution, the control gel and the pH 7.4 gel, respectively. After 8 days it was above 4 mg l^{-1} with the two Poloxamer 407 formulations. Local levels were higher than minimal inhibitory concentrations for most *Staphylococcus aureus* isolates (1 mg l^{-1}, MIC of sensitive strains ≤ 4 mg l^{-1}) throughout the experiment. The AUC0-∞ calculated with the vancomycin/Poloxamer 407 formulations were about fourfold greater than that observed with the vancomycin solution ($P > 0.001$), but no significant difference was observed between the two Poloxamer 407 gels (Table 5). Fig. 4 shows decrease in local vancomycin concentration profiles. For each of the preparations, two release half-lives were observed. The first release half-life ($t_{1/2æ}$) was more strongly lengthened with the control vancomycin/Poloxamer 407 formulation than with the pH 7.4 formulation, compared to the vancomycin solution. The second release half-life ($t_{1/2\beta}$) was also increased with the two Poloxamer 407 formulations but was greater with the pH 7.4 preparation than with the control.

4. Discussion

The purpose of this work was to investigate vancomycin release from two formulations aimed at prolonging the residence time of the drug at the injection site and thereby to increase its therapeutic efficacy. We observed that the combination of vancomycin with the biocompatible Poloxamer 407 vehicle slowed in vitro and in vivo release of the antibiotic.

Several attempts have been made to sustain local drug levels and thereby to increase their therapeutic efficacy, and a number of authors have recognised the potential of Poloxamer 407 gels for controlled topical drug delivery in various therapeutic indications. They have been tested as drug delivery systems for external application of anticancer drugs (Miyazaki et al., 1984), burn treatments (Henry and Schmolka, 1989), anti-inflammatory drugs (Chi and Jun, 1991; Mengi and Deshpande, 1992; Miyazaki et al., 1995), and antibiotics (Esposito et al., 1996); and they have also been investigated as injectable formulations with peptides such as urease (Pec et al., 1992), interleukin-2 (Johnston et al., 1992; Morikawa et al., 1987), Melanotan-I (an α-MSH analog)

interleukin-2 (Johnston et al., 1992; Morikawa et al., 1987), Melanotan-I (an α-MSH analog)(Bhardwaj and Blanchard, 1996) and lidocaine (Paavola et al., 1995).

Our first finding was the good compatibility between Poloxamer 407 matrices and vancomycin. The main characteristic of gels formulated with Poloxamer 407, is that they behave in a thermoreversible fashion. At low temperatures the Poloxamer forms micellar subunits in solution, and when temperature rises, micellar desolvatation and swelling give rise to large micelles and the creation of a cross-linked network (Chen-Chow and Frank, 1981; Attwood et al., 1985; Lenaerts et al., 1987; Alexandridis and Hatton, 1995). The result of these phenomenon is a sharp increase in viscosity upon heating. Vancomycin did not interfere with the gelation process or with the physicochemical characteristics of the Poloxamer 407 solutions. In particular, the gel-sol transition temperature and rheological properties (viscosity, storage modulus and loss modulus) of the studied

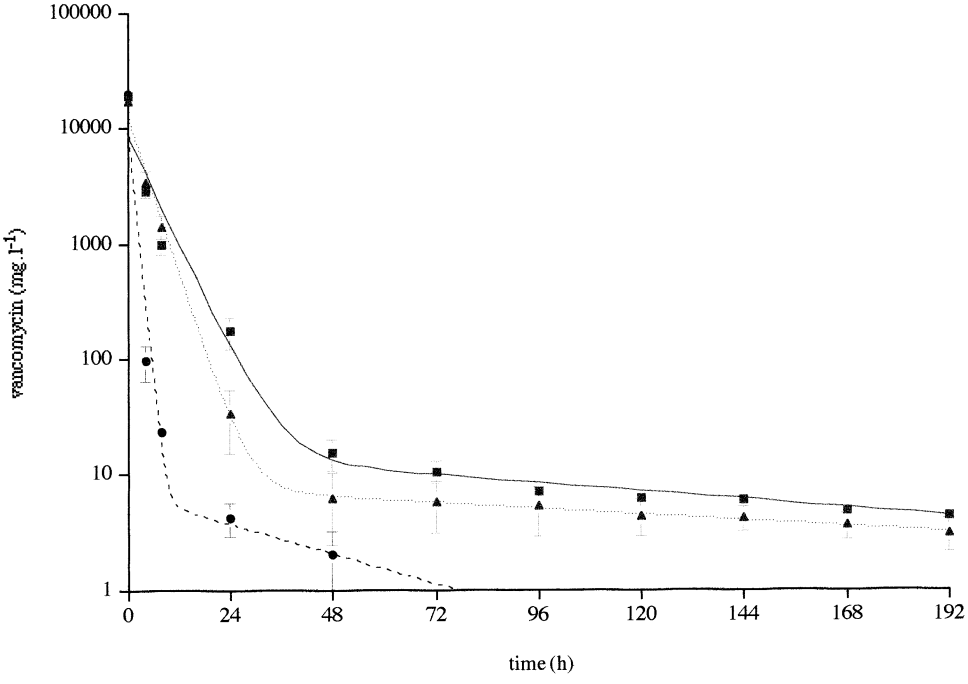

Fig. 4. Local concentration of vancomycin, measured by HPLC, following injection of either vancomycin/Poloxamer 407 formulation or vancomycin solution in the rat tissue cage. (●) solution ($n=10$), (▩) control Poloxamer 407 formulation ($n=9$), (▲) pH 7.4 Poloxamer 407 formulation ($n=6$). Initial vancomycin concentration, 20×10^{3} mg l^{-1} (40 mg dose).

Table 5
Comparison of local vancomycin pharmacokinetics after a single injection into the rat tissue cage of the control Poloxamer 407 formulation, the pH 7.4 Poloxamer 407 formulation, or the solution. The initial dose was 40 mg of vancomycin[a]

	AUC (g h l^{-1})	$t_{1/2\alpha}$ (h)	K_{α} (h^{-1})	$t_{1/2\beta}$ (h)	K_{β} (10^{-3} h^{-1})
Solution ($n=10$)	12.15	0.8	0.87 ± 0.28	28.2	24.6 ± 2.3
Control gel ($n=9$)	49.09*	3.9	0.18 ± 0.05*	101.3	6.8 ± 1.0*
pH 7.4 gel ($n=6$)	50.84*,**	2.7	0.26 ± 0.02*,***	241.1	2.9 ± 0.1*,***

[a] Values are expressed as means ± standard deviation. *$P<0.001$ vs solution; **no significative difference vs control gel; and ***$P<0.001$ *vs* control gel. Abbreviations: AUC, area under the curve; $t_{1/2\alpha}$, first release half-life; $t_{1/2\beta}$, second release half-life; K_{α}, first constant of decrease in local vancomycin concentration; and K_{β}, second constant of decrease in local vancomycin concentration.

formulations were not modified by adding the antibiotic.

Vancomycin was not altered either, as persistent antimicrobial activity was shown by the good correlation between the results of HPLC and bioassay measurements. Similar findings have been obtained with tetracycline in the treatment of periodontitis (Esposito et al., 1996). Other drugs have also been shown to retain their therapeutic activity, such as the antiinflammatory agent flurbiprofen (Mengi and Deshpande, 1992), the anesthetic lidocaine (Paavola et al., 1995) and the immunomodulator interleukin-2 (Pec et al., 1992).

In vitro diffusion of vancomycin through the Poloxamer 407 gel was slowed by setting the pH at 7.4; this step allowed the initial loading dose of vancomycin to be above the solubility limit in the gel and led to the formation of small particles of antibiotic dispersed throughout the matrix. Thus, saturation of the gel appeared to favour prolonged release of vancomycin. As the Poloxamer gels are considered to consist of large populations of micelles in aqueous phase, the incorporated solute may be released by diffusion through aqueous extramicellar channels of the gel matrix. The size of the aqueous channels, the arrangement of the drug between the micelles and the aqueous phase, and the microviscosity of the extramicellar fluid can affect the release of the drug (Chen-Chow and Frank, 1981; Rassing and Attwood, 1983). However, the most plausible explanation for the slowing of vancomycin diffusion at pH 7.4 is the involvement of a limiting factor, i.e. the dissolution rate of the antibiotic particles that form when the gel is saturated.

Other authors have already reported the non toxic properties and stability of Poloxamer 407 solutions, which make them suitable as injectable vehicles (Johnston and Miller, 1985; Henry and Schmolka, 1989; Garcia Sagrado et al., 1994b). In our work, despite receiving a large amount of gel relative to their body weight, the rats appeared to tolerate the gels well, as their general health and weight gain were not different from those of rats that received the aqueous solution.

Our in vivo experiments showed the sustained-release properties of Poloxamer 407 after a single injection into an implanted tissue cage. This was suggested by the decreased release slopes and the increased areas under the local vancomycin concentration-time curves observed with the gels compared to the solution. The observed release profiles are especially suited to vancomycin activity, which is both concentration-dependent and time-dependent. Very high local concentrations were measured for at least 24 h, being above 20 mg l^{-1} for 40 and 26 h with the control gel and the pH 7.4 gel, respectively. Afterwards, local concentrations were lower but still effective against *Staphylococcus aureus* throughout the 8-day experiment. This release profile should optimize therapeutic efficacy. The high initial local concentrations are aimed at inhibiting early bacterial adhesion to prosthetic devices, at a time when the risk of infection by exogenous bacteria is highest after surgery. The prolonged lower concentrations would protect the prosthetic device against endogenous infection. Moreover, antibiotic activity should be reinforced by the non specific antiadhesive effect of Poloxamer 407, that can limit protein and bacterial adhesion (Reigel et al., 1993; Portolés et al., 1995).

As the gels remain at the injection site, the duration of active vancomycin levels is determined by the dilution rate and diffusion through extramicellar aqueous channels, and by the biological degradation of the gel matrix itself (Gilbert et al., 1986). Local vancomycin elimination involved two release half-lives. The first could represent the vancomycin directly available for diffusion and release, while the second could be as a result of the combination of dilution and diffusion of the remaining vancomycin and the decomposition of the gel matrix. Furthermore, a local release probably takes place, it would result from the steady state between the inside of the tissue cage and the environment (tissue cage surface and surrounding tissues). In vivo, we failed to observed the expected slower vancomycin release from the pH 7.4 gel compared to the control gel early in the experiment. Even though the first slope was lower with the two gel formulations than with the aqueous solution, reflecting a lower elimination rate, it was higher with the pH 7.4 gel than with the control gel. This could be explained by the presence of vancomycin particles at the

periphery of the gel matrix, which are thus directly available for dissolution and release. In contrast, the second slope (lower with the pH 7.4 gel than the control gel) could result from slower dissolution of vancomycin in the aqueous channels and, possibly, slower decomposition of the pH 7.4 gel matrix.

In summary, we observed controlled-release profiles, good preservation of vancomycin's biological activity, and good tolerability in rats. The liquid state of Poloxamer 407 solutions at low temperatures facilitates administration and permits intimate contact between the drug preparation and tissues. In addition, these preparations do not need to be removed from the body at the end of therapy, as the gel matrix gradually dissolves. Taken together, these properties make Poloxamer 407 particularly attractive for the preparation of vancomycin delivery systems. Such a device, which could increase the benefit/risk ratio of low-therapeutic-index antibiotics, and which also exhibits non specific antiadhesive activity that might limit bacterial adhesion, should find applications in the prevention or treatment of infections of bone and prosthetic devices, and should now be tested in models of infection.

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