Collagen with gentamicin for prophylaxis of postoperative infection

Staphylococcus aureus osteomyelitis studied in rabbits

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In 34 rabbits, both tibiae were inoculated with *Staphylococcus aureus*. 14 legs received no treatment and served as controls. In 12 legs, the wound was treated with pure collagen and in 18 legs, collagen with gentamicin (Gentacoll®) in a dose of 10 mg/kg body weight was applied to the wound before closure. Postoperatively 12 received 10 mg/kg body weight gentamicin intravenously and no local treatment. The animals were killed 7 days after inoculation and evaluated macroscopically and microbiologically for infection. 6 rabbits (12 legs) were used for pharmacokinetic studies only and they were killed after 2, 4, and 18 hours, respectively.

11/14 untreated legs developed a macroscopically acute osteomyelitis. No infection was found in the 18 legs treated with Gentacoll® and 1/12 treated with gentamicin systemically had growth of the inoculated bacteria in tissue biopsies. The concentrations of gentamicin in the serum as well as locally reached peak values were well above the MIC value in all groups, with a maximum after 1–2 hours. No gentamicin could be detected after 18 hours, independently of the mode of administration.

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Submitted 94-07-18. Accepted 94-10-20

For local treatment of bone infections, local application of gentamicin-impregnated polymethylmethacrylate (PMMA) has been used for decades (Buchholz and Engelbrecht 1970, Wahlig et al. 1978, Ascherl et al. 1986). Experimental data suggest that slime or glycocalyx-producing bacteria may adhere to the PMMA, even when this still contains antibiotics to which the bacteria are sensitive (Gristina and Costerton 1985). This phenomenon may indicate the importance of a secondary operation for removal of the beads, although no clinical series has shown negative side-effects after long-term implantation of PMMA-beads (Henry et al. 1993).

A biodegradable carrier may release an antibiotic faster and in larger amounts thus eliminating the need for secondary surgical removal of the carrier. We studied the effect of gentamicin administered locally, with collagen as the carrier, and compared the results to those obtained with systemic gentamicin in an experimentally induced acute bone infection.

Animals and methods

34 rabbits (New Zealand white type SsC:CPH) weighing 2–3 kg were used for the experiments. The rabbits lived in laboratory cages with an ad libitum supply of water and standard food pellets. Daily veterinarian control was carried out.

Staphylococcus aureus, strain E 2371 isolated from a blood culture, and a MIC (Minimal Inhibitory Concentration) of 0.8 µg/mL for gentamicin (Espersen et al. 1984) was used as the infecting organism. The bacteria were grown overnight in Mueller-Hinton broth, washed at 2,000 × g for 10 min, 2 times in saline and adjusted to the desired concentration in saline by means of measurement of optical density at 540 nm. The cfu (colony-forming units)/mL was confirmed by serial dilutions and subculture on 5% blood agar plates, followed by colony counts.

All procedures were performed under general anesthesia, using intramuscular diazepam emulsion 2 mg/kg (Stesolid®, Dumex, Denmark), followed by intravenous fluanison 3 mg/kg (Hypnorm®, Janssen Pharma, Denmark). Both tibiae were exposed through a longitudinal incision under aseptic condi-

Table 1. The distribution of local versus no local treatment and systemic gentamicin. The treatment group consists of 28 rabbits (56 legs)

Group	n	Local treatment		Systemic gentamicin
		Right	Left	
1	6	C	G	–
2	6	G	G	–
3	6	0	C	–
4	4	0	0	–
5	6	0	0	+

C collagen, G Gentacoll®, 0 no local therapy.

tions. Four 1.1 mm holes were drilled in the anterior cortex at a distance of 0.3–0.5 cm and filled with one drop of 0.1 N NaOH for superficial bone necrosis. 1 min later, the bone and holes were soaked with 0.5 mL of the bacterial solution (2×10^8 cfu/mL). Kirschner wires measuring 1 × 6 mm were placed in the holes and another 0.5 mL bacterial solution was poured over the pins.

The animals were divided into 4 groups receiving different therapeutic measures.

12 legs were treated locally with collagen alone and in 18 legs Gentacoll® in a dose of 10 mg gentamicin per kg body weight (bovine collagen sponge, 5 × 5 cm with 32.5 mg gentamicin, Schering-Plough, Copenhagen, Denmark) was applied to the wound. 12 legs received no local therapy, but a single dose of gentamicin 10 mg/kg body weight was administered i.v. prior to wound closure. In 14 legs no treatment was given and they served as controls of the infection (Table 1).

During the study the rabbits received analgesics twice a day with 0.6 mg s.c. buprenorfin (Temgesic®, Reckitt & Colman, Hull, England).

The animals were killed 7 days after inoculation by an intravenous overdose of 10% pentobarbital. The tibiae were exposed under aseptic conditions and subcutaneous tissue, bone and bone marrow were inspected macroscopically and classified as +/- infection, defined as pus, necrosis, granulation tissue and inflammation. A swab was taken immediately after wound-opening, together with biopsies for quantitative cultures from muscle tissue, cortical bone and bone marrow.

Tissue biopsies and metal pins were placed in 1 mL of saline, vortex-mixed for 10 sec, and after serial dilutions in saline, cfu were determined. Blood cultures (Colorbact®, Statens Seruminstitut, Copenhagen, Denmark) were obtained in all rabbits 18 hours after inoculation and at the time of killing.

A further 12 legs (6 rabbits) were used for pharmacokinetic studies only. Infection was induced as mentioned above and in all legs Gentacoll® 10 mg/kg body weight was placed in the wound before closure. Biopsies were taken after 30 min (before termination of anesthesia) and after killing the animals 2, 4, and 18 hours after inoculation.

Table 2. Mean infection scores and ranges, according to different regimens for prophylaxis

	Legs	Score
Control	14	5 (1–8)
Collagen	12	6.5 (2–8)
Gentacoll® (10 mg/kg)	12	3 (2–3)
Gentacoll® (20 mg/kg)	18	0 (0–1)
Gentamicin (10 mg/kg) i.v.	12	0 (0–1)

Concentrations of gentamicin in blood and in the biopsies were measured by bioassays, using *S. epidermidis* (collection no. P903/76). Biopsies were placed in 0.5 mL saline and centrifuged at 3,000 × g for 10 min, and the supernatant was tested. Standard curves were obtained, using gentamicin diluted in normal human serum.

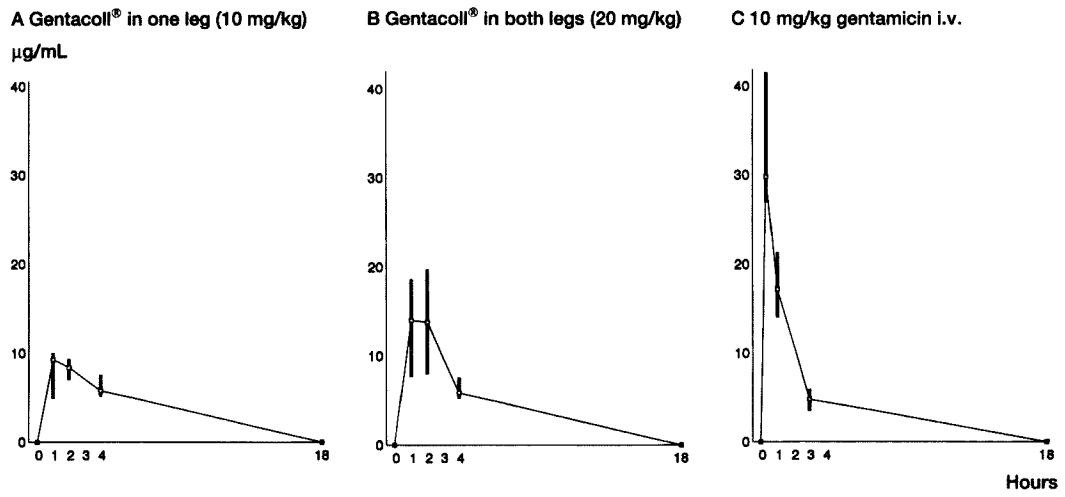
Infection was defined as growth of the same *S. aureus* in at least 1 of 5 samples (swab, tissue, bone, marrow or pins) and macroscopic signs of inflammation in at least one of 3 sites (tissue, bone or marrow). Identity of the infective strain was confirmed by phage typing (Rosendal et al. 1983). A score of 0–8 was calculated for each leg by assigning one point for growth of the same *S. aureus* in each of the 5 cultures and one point for inflammation of each of the 3 sites.

For statistical calculations Fischer's exact test and the Mann-Whitney U test were used.

Results

All but 3 untreated legs (n 14) developed acute osteomyelitis characterized by local swelling and different degrees of pus formation with necrosis and growth of the inoculated *S. aureus*, resulting in a score ranging from 1–8 at killing. A similar result was observed in the legs which were treated with collagen alone. In 1 case in each of the treatment groups (groups 1 and 2) we observed minor inflammation, but no bacterial growth. In 1 case treated with gentamicin systemically, we found a growth of the inoculated *S. aureus*, without any signs of inflammation (Table 2). Furthermore, the use of Gentacoll® and intravenous gentamicin gave lower infection scores as compared to the controls ($P <$

Figure 1. Concentration of gentamicin in serum, according to the prophylactic regimen.



0.01). The difference between pure collagen locally and controls was not significant (P 0.13). In the group treated with gentamicin intravenously, we furthermore found 1 rabbit with positive blood cultures, but without any sign of infection in the leg.

Rabbits receiving local gentamicin in 1 leg only had a peak value of 5–10 µg/mL, compared to 30–40 µg/mL in rabbits treated with systemic gentamicin (Figure 1). The concentration of gentamicin in the muscle tissue reached peak values of about 800 µg/mL 1/2 h after application of Gentacoll®. This concentration showed great variation, but the lowest value obtained at this time was 112 µg/g tissue. The peak values for bone and bone marrow reached a maximum 2 hours after the application of Gentacoll®. These figures presented also a great variation, with a minimal concentration of about 250 and 50 µg/g tissue, respectively. Substantial concentrations were still obtained in bone and bone marrow after 4 h but were not detectable after 18 h.

Discussion

In a pilot study we found a strong tendency to self-curing of the acute infection. We were only able to maintain the infection for at least three weeks after application of NaOH for local bone necrosis, together with an inoculum corresponding to 1×10^8 bacteria. The infection was then reproducible and, in the control group, 11/14 developed an acute osteomyelitis after a few days, which resembled a chronic infection after 2 weeks if no treatment was given. In our experimental series the animals were killed seven days after the inoculation when active infection could still be expected if the therapy had no effect.

Although collagen may interact with *S. aureus* (Liang et al. 1993), mimicking an antibacterial effect, we did not observe such an effect when pure collagen was applied to the wound as the only treatment.

The release of gentamicin from PMMA beads is slow and incomplete (Wahlig et al. 1978). Bovine collagen is degradable and a good carrier for gentamicin, which is fully released to the tissues with initially high concentrations (Ascherl et al. 1986, Sørensen et al. 1990). This was confirmed in our series; our peak values in bone and surrounding tissues were reached almost immediately and exceeded greatly the MIC values for most bacteria causing orthopedic infections. The results correspond to both in vitro findings (Sørensen et al. 1990) and clinical observations of gentamicin concentration in wound fluid (Ipsen et al. 1991, Jørgensen et al. 1991).

Furthermore, we observed a rapid disappearance of the gentamicin, which could not be measured after 18 h. This finding is in accordance with in vitro results (Sørensen et al. 1990) but contrary to the findings of Ipsen et al. (1991), who were able to detect gentamicin in wound exudate and serum for more than 6 days in patients with chronic osteomyelitis. The release of gentamicin from PMMA beads results in high initial and prolonged concentrations locally (Hedström et al. 1980, Sørensen et al. 1990), which probably then gives higher efficacy in cases of chronic infections (Evans and Nelson 1993).

Aminoglycosides have a concentration-dependent bactericidal effect, highest during the first hours after administration (McArthur et al. 1984, Sørensen and

Sørensen 1993). This phenomenon may explain that the rapid decline of gentamicin was able to prevent the development of postoperative bone-infection, in spite of a great inoculum. Even the application of Gentacoll® in one leg only resulted in a sufficiently high serum level to prevent the outbreak of infection in the contralateral leg. Furthermore, the study revealed that one intravenous injection of gentamicin, given at the time of wound closure, had a prophylactic effect which was equivalent to that of the application of Gentacoll® locally.

Collagen is not osseoinductive and disappears within 2 weeks in rabbit bone defects (Riegels-Nielsen et al. 1986). The rapid release of gentamicin results in initially high therapeutic concentrations and the following high clearance probably prevents toxic side-effects or bacterial resistance to the drug. This makes Gentacoll® valuable as prophylaxis against peroperatively acquired infections, as well as in the treatment of acute infections.

Acknowledgements

This investigation was financially supported by Schering-Plough, Copenhagen, Denmark.

The authors wish to thank Mrs. Lotte Corneliussen for her valuable help during the study period.

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