Eur J Orthop Surg Traumatol (1996) 6 : 97-100

European Journal of Orthopaedic Surgery & Traumatology

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Experimental implant-related osteomyelitis induced with *Staphylococcus aureus**

Ostéomyélite expérimentale par staphylocoque doré sur implants

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Abstract: *Experimental knee-implant infection was induced in the rabbit knee with a strain (1369,phage type 85,a laboratory strain) of Staphyloccus aureus. The experimental model was adapted from an experimental study in rabbits by Gudmund Blomgren, 1981. In these experiment's osteomyelitis was induced with an noculum of 100.000 bacteria in the tibia and femur. In one series we used gentamicin impregnated bone cement and in the other series dicloxacillin was given intravenously. The controlgroup, four rabbits had no supplemental antibiotics. The animals were monitored by clinical observation, microbiological, histological and antibody methods. Only in the controlgroup without supplemental antibiotics we would see manifest inflammation around the prosthesis with increased antibody titre. With supplemental antibiotics no bacteria was found around the prosthesis.*

The gentamicin concentration was measured in bone from femur, tibia and from the jointfluid. A high level of gentamicin was found in the bone but not in the joint fluid. The animal model was excellent to create a model for human total joint replacement to study the dissemination of localy and intravenously injected bacteria to the artificial joint. The use of either systemic or locally administered antibiotics as prophylaxis avoided development of infection.

Key words: *Osteomyelitis — Foreign body implant — Rabbit model*

Infections associated with prosthetic implants and medical devices are mostly caused by coagulase-negative staphylococci. Adhesion of bacteria to tissue surfaces of the host and to implanted artificial surfaces is considered to be an important step in the pathogenesis of infections.

Rabbits were used as an experimental model because they had proved themselves suitable for experimental purposes concerning osteomyelitis and septic arthritis [9, 14, 18].

Norden [13] injected sodium morrhuate and colony-forming staphylococci into the marrow of the tibia in young rabbits and showed that 89% of these animals developed osteomyelitis. He suggested that this model resembles the disease in humans. Schurman et al. [16] used a rabbit model for testing antibacterial efficacy in testing Palacos bone cement with and without gentamicin.

Blomgren [2] studied the dissemination of intravenously injected bacteria to total joint replacement in an rabbit model. The operation was either a fenestration of the femur or a total joint replacement using human finger joint prostheses. He concluded that a joint implant had a high risk of becoming infected during bacteremia. *Staph. aureus* results in acute bone infection, such as pus formation, bone resorption and periostial reaction.

A rabbit model used to study the effect of allogenic demineralized bone powder implants on the persistence of *Staph. aureus* osteomyelitis showed that infection persisted at a 7-to 10-fold higher level in animals receiving bone powder irrespective of the antibiotic status [17].

Mader and Adams [8] used a rabbit model to compare antibiotic treatment for methicillin-resistant *Staph. aureus osteomyelitis* and Winckler and Richter [19] used a rabbit model to investigate the correlation of direct radiographic and histopathological changes of bacterial osteomyelitis in the femora of rabbits. Mayberry-Carson et al. [11] induced experimental osteomyelitis in the rabbit tibia with *Staph. epidermidis* in the presence and absence of a foreign-body implant.

In the animals receiving an implant, osteomyelitis developed in 83% and bacteria were recovered by culture in 97% against 58% and none bacteria in animals without the implant.

This paper describes the model in 14 rabbits injected with *Staph. aureus* in femur and tibia before an implant was cemented in the rabbit knee.

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* EBJIS Congress, Copenhagen

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Material and methods

Technique of infection

New Zealand white rabbits, female, weighing 3-4 kg, age 15-20 weeks, were anesthetized with hypnorm/stesolid (given intramuscularly), the right hind leg was shaved and the skin was cleaned with chlorhexidin-alcohol.

The Prosthesis designed for replacement of human finger joints a St. Georg Fingermittelgelenk Endoprothese (Waldemar Link) were used in all cases (the model described by Blomgren [2]). An operating room, disposable sterile dressings and instruments for each rabbit were used.

Five rabbits were given supplemental dicloxacillin intravenously (experiment III), 5 were operated with gentamicin cement (experiment II) and the rest without supplementary antibiotics (controlgroup, experiment I).

The animals were monitored by clinical observation, biochemical, microbiological, histological and immunological methods.

After 2 weeks the knee was reopened and clinical observations were made especially concerning the prostheses.

The knee joints were approached through a medial parapatellar incision. The patella was dislocated laterally. The joint surfaces were resected and the medullary canals were opened. Commercial orthopaedic bone cement (Palacos, Schering Corp.) was used for fixation in experiment I and III and (Palacos with Gentamicin, Schering) in experiment II.

The inoculum of bacteria 0.1 ml was injected in the medullary canals of the femur as well as tibia in all experiment. After that the cement was injected with a syringe into the prepared medullary canals 1 ml in each and the prosthesis was inserted into the cement.

All capsules were closed with dexon and the skin with supramid.

Microorganisms

The organism used in most experiments was a strain of *Staph. aureus*, 1369 phage type 85. It is sensitive to Diclosil and gentamicin.

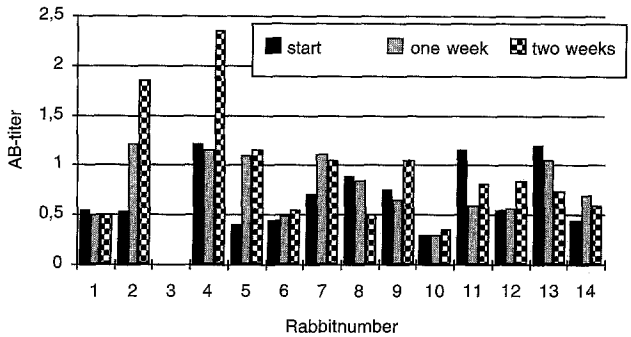

Fig. 1. Antibodyconcentration

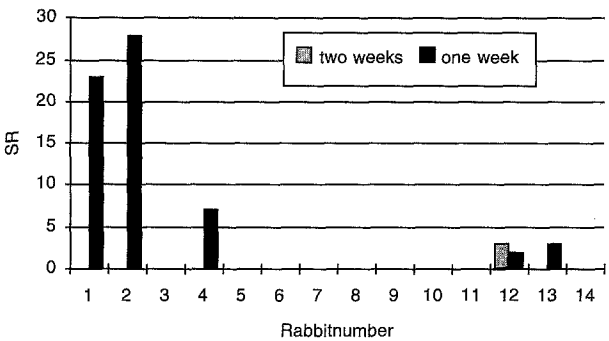

Fig. 2. Sedimentation rate. 1-4: control, 5-9: Gentamycin, 10-14: Diclosil

Experiment I: Four rabbits (1-4) were injected locally with *Staph. aureus* strain 1369, inoculum 100.000.

Experiment II: Five rabbits (5-9)were injected locally with *Staph. aureus* strain 1369, inoculum 100.000. They had supplemental antibiotics (gentamicincement).

Experiment III: Five rabbits (10-14) were injected locally with *Staph. aureus* strain 1369, inoculum 100.000. They had supplemental antibiotics (Dicloxacillin given intravenously, 50 mg/kg).

Monitoring

Blood samples for haemaglobin, white blod cell, sedimentations rate, antibody measuring and microbiology were collected preoperatively, after 1 week and before the animal were killed after 2 weeks.

The animals condition, the knee, temperature and weight as well as the appearence were monitored.

Autopsy

In order to prevent contamination of culture speciments, sterile instruments were used. A sterile syringe was used to aspirate pus from the joint in case of swelling of the kneejoint.

Tissue samples were taken from prosthesis-near tissues and examined histologically, using conventional microscopy and colouring in hematoxylen-eosin and gram-stains.

Results

No peroperative wound infection was seen. The operative procedure itself caused a weight loss of the animals and this together with a local infection gives a weight loss of about 15% and after that they never regained their initial body weight.

Postoperatively the rabbits moved freely in the cages although the function of the operated leg was affected to some degree. Only one rabbit died of manifest septicemia after one week (No. 3, controlgroup).

In the first experiment (I) inflammation and bacteria and loosening of the prosthesis was seen in the three rabbits with cement,without supplementary antibiotics. One died of septicemia (No. 3). >10.000.000 cfu were found in the femur, tibia and the joint-fluid in No. 1 and 1.000 cfu were found in No. 2 and 800 cfu in No. 4. The antibodytiter was increased 1.6 - 2.2 (>0.3) (Fig. 1).

The antibody titre was increased after one week and HGB and WBC changed after one week, showing that the animals respond to infection very fast.

The WBC was increased and the HGB was decreased. The sedimentation rates were increased after one week and normalized after two weeks (Fig. 2).

The histological changes showed osteoblast activity (Fig. 3) and bacteria in the area around the prosthesis (Fig. 4).

In the gentamicin cement group (II, Nos. 5-9) no bacteria were found in the knee and the antibody titre were not significantly increased (only one rabbit had antibody change more than 0.3).

There were only small changes in HGB, WBC and SR after one and two weeks.

The rabbits had no inflammation. Two of the rabbits had loosening of the tibiaprosthesis. Only a swelling of the knee were seen.

A high dose of gentamicin was found in the bone from femur and tibia between 1 and 10 (ref. 4.2-6.7) and in the joint-fluid the levels were <0.6.

In the last experiment (III) the five rabbits given antibiotics intravenously only had reactive histological changes with no bacteria in the tissue samples. No clinical, pharmacological or antibody changes were seen. In three animals the prosthesis was not loose. Knee swelling was seen but no other sign of inflammations (Figs. 1 and 2).

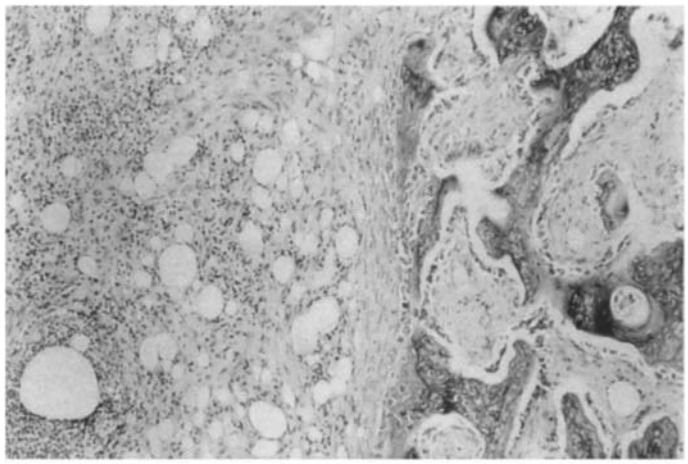

Fig. 3. Osteoblast activity in the interface membran

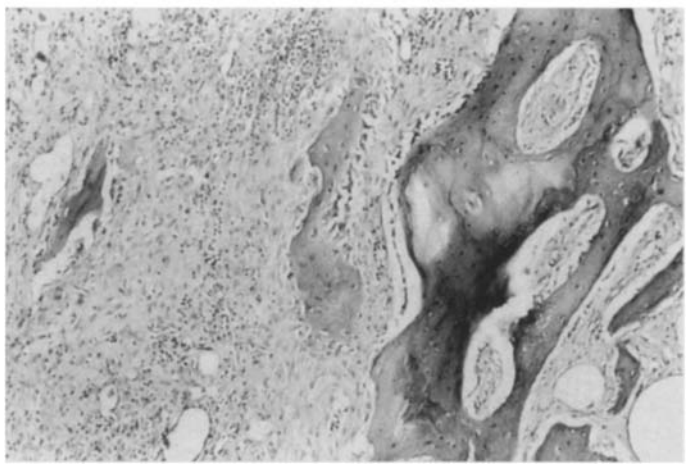

Fig. 4. Bacteria in the area around the prosthesis

Discussion

Accurate microbiological sampling is always difficult in infected implants because of the adhesive mode of growth of the infecting organisms.

Inoculum of 100.000 or more given localy without antibiotic supplement results in an infection with bacteria, increasing in cfu, around the prosthesis and an increased antibody titer after two weeks, found in this study and in others [15]. The prostheses were loose. The inflammatory antibody response was seen between the first and second week and it is well known that animals respond to infection after 10-12 days [2, 5].

With Dicloxacillin or Gentamicin supplement very little reaction with osteoblast activity was seen without bacteria. There was no histological or microbiological differences between the two sources of antibiotics. The gentamicin level was high in bone but could not be measured in the joint fluid after two weeks.

Mordenti [12] recognized that smaller animals eliminate drugs rapidly and showed that extrapolation of cephalosporin half-life data from animals to humans demonstrates the methodology employed in interspecies pharmacokinetic scaling and illustrates the relevance of kinetic studies conducted in animals.

Schurman et al. [16] showed that antibiotic concentrations in synovial fluid remained in the therapeutic range for three days and after that it leached out of the fluid but in the bones extremely small amounts of gentamicin left the cement daily,therefore it could be measured after two weeks.

The animal model was excellent to create a model for human total joint replacement to study the dissemination of locally injected bacteria as also found by others [1, 8, 10, 17, 19].

The total arthroplasty experiments demonstrate that when Gentamicin is added to Palacos bone cement or the animal are given Dicloxacillin intrave-

nously, protection against an intraoperative challenge of bacteria to the antibiotics is provided.

These studies establish the advantage of the use of antibiotic locally or parenteral in the prevention of early postoperative infection - there is no difference in the two ways of using antibiotics.

Acknowledgements. The Royal Veterinary and Agriculture University Copenhagen (blood-sample analysis). DEMA, Waldemar-Link Representative in Denmark (PIP-joint, St. Georg prosthesis). Schering-Plough A/S, Copenhagen (gentamicin cement).

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Received September 15, 1995 / Accepted in final form February 1, 1996

Ostéomyélite expérimentale par staphylocoque doré sur implants

Résumé : Une infection expérimentale sur implant a été provoquée sur des genoux de lapins avec une souche de staphylocoque doré (1369, phage type 85). Le modèle expérimental a été adapté de l'étude de Blomgren en 1981.

L'ostéite a été induite par un inoculum de 100.000 germes introduit dans le tibia et le fémur. Dans un groupe le ciment était imprégné de gentamicine ; dans le second, du Diclosil a été administré par voie intraveineuse ; un groupe témoin de 4 lapins n'a reçu aucun antibiotique. Les animaux ont été surveillés sur le plan clinique, biochimique, microbiologique, histologique et immunologique. Seul le groupe témoin sans antibiothérapie a présenté une inflammation nette autour de l'implant avec une augmentation du dosage des anticorps. Aucun germe n'a été retrouvé autour de l'implant dans les deux autres groupes. La concentration de gentamicine a été mesurée au niveau du tibia, du fémur et du liquide articulaire : il était élevé dans l'os mais non dans le liquide articulaire.

Le modèle animal est une fidèle reproduction d'une arthroplastue totale chez l'homme pour étudier la diffusion de germes inoculés localement ou par voie intraveineuse. L'utilisation d'antibiotique systémique ou local comme prophylaxie a empêché l'apparition d'une infection.

Mots-clés : Ostéomyélite — Staphylocoque