INFLUENCE OF MATERIALS FOR FIXATION IMPLANTS ON LOCAL INFECTION

AN EXPERIMENTAL STUDY OF STEEL *VERSUS* TITANIUM DCP IN RABBITS

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Resistance to infection may be influenced by foreign bodies such as devices for fracture fixation. It is known that stainless steel and commercially-pure titanium have different biocompatibilities. We have investigated susceptibility to infection after a local bacterial challenge using standard 2.0 dynamic compression plates of either stainless steel or titanium in rabbit tibiae. After the wounds had been closed, various concentrations of a strain of *Staphylococcus aureus* were inoculated percutaneously.

Under otherwise identical experimental conditions the rate of infection for steel plates (75%) was significantly higher than that for titanium plates (35%) ($p < 0.05$).

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Infection is a major complication after open reduction and internal fixation (ORIF) of a fracture. The incidence of infection is very low in good units, but the large number of implants used worldwide, some under inappropriate conditions of asepsis and surgical skill, results in a considerable number of cases. In addition to the effects on individual

patients, the economic cost of infection after ORIF justifies any attempt to improve the resistance to infection around such implants.

Robson (1979) described infection as the result of an imbalance between an overwhelming number of virulent bacteria and the local defence mechanisms. The capacity for defence is reduced by the damage of the primary injury, and by additional surgical trauma. In the classical experiment of Elek and Conen (1957) the implantation of foreign bodies was shown to increase the susceptibility to infection. Both Waldvogel and Vasey (1980) and Gristina (1987) have discussed the affinity and adhesion of bacteria to metal, in particular *Staphylococcus aureus*.

A number of implant-related factors can influence susceptibility to local infection. These include the size and shape of the implant (Melcher et al 1994), the technique and stability of fixation (Worlock et al 1994), the surface characteristics (Gristina 1987; Cordero, Munuera and Folgueira 1994), and the material and its biocompatibility (Gerber and Perren 1980; Petty et al 1985; Hierholzer and Hierholzer 1991). The type of metal seems to be important in tissue reaction and bacterial adhesion. An ideal metal should have good tissue compatibility, have optimal adhesion characteristics to reduce capsule formation and physical tissue irritation, contain no allergenic components and have a minimal rate of corrosion (Perren 1991). There are well-known differences between stainless steel and commercially-pure titanium, both of which are used for implants in ORIF.

The level of bacterial contamination varies in relation to the grade of open fractures, but even in clean procedures a low level must be assumed (Schneider 1979; Hansis 1990). The choice of implant and the surgical procedure are influenced by the extent of the damage and also by the degree of contamination. Reported rates of postoperative infection of up to 29% (Bach and Hansen 1989) have led to recommendations that severely contaminated fractures with much tissue damage should initially be treated by external fixation rather than primary internal fixation by plate or intramedullary nail. Prolonged external fixation is demanding for the patient; pin-track infection may develop and healing may be delayed when the definitive treatment is by external fixation. A change from external to internal fixa-

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Fig. 1

Fig. 2

Figure 1 – The operation site. Figure 2 – Radiographs after implantation of a DCP.

tion at a later stage may reduce overall infection rates (Hansis and Höntzsch 1988; Bach and Hansen 1989).

Based on recent experimental (Pascual et al 1992) and clinical (Matter and Burch 1990) studies it is suspected that in this situation the choice of an implant with increased biocompatibility, such as commercially-pure titanium, may reduce susceptibility to local infection. We have investigated this hypothesis. We simulated the clinical situation by using an animal model in which the proportion of infected cases could be adjusted and many possible confounding factors eliminated or taken into account.

MATERIALS AND METHODS

We modified the experimental model used by Worlock et al (1994) by inoculating various concentrations of bacteria directly on to dynamic compression plates (DCP) fixed to the tibiae of 46 White New Zealand rabbits. Their average weight was 3.53 ± 0.32 SD kg. The study was approved by the national animal protection authorities (no 3/1994; Kantonales Veterinäramt Graubünden).

Bacterial inoculum. We obtained a beta-haemolysing *Staphylococcus aureus* strain (V 8189-94) from an infected hip prosthesis (Department of Medical Microbiology, Kantonsspital, Lucerne) and prepared a broth inoculum suspension as described by Melcher et al (1994). We used bacterial concentrations from 4×10^3 to 4×10^6 colony-forming units (CFU) per 100 μ l.

Implants. Standard 6-hole 2.0 mm AO DCP plates (Synthes No 2/443.56; Mathys AG, Bettlach, Switzerland) of either stainless steel (ISO 5832-1) or commercially-pure titanium (ISO 5832-2) were fixed to the rabbit tibiae with six unicortical screws (Synthes No 21/401.006). The steel implants are electropolished and the titanium plates are anodically oxidised.

Operation. After premedication, endotracheal intubation and general anaesthesia by halothane and oxygen, the skin over the medial aspect of the midshaft of the tibia was incised under sterile conditions. The knife was changed before incision of the superficial fascia. Retraction of the muscles allowed bone exposure without damage to the periosteum. The plates were then fixed to the medial aspect of the bone. From 5 mm proximal to the incision a hollow metal needle (2.1 mm/14GA/5.1 cm; Intracath No 3932221/N2CD230; Becton Dickinson, Sandy, Utah) was introduced percutaneously and its tip placed alongside the implant (Fig. 1). The deep fascial layer was closed by continuous suture using 5/0 resorbable material (Polyglactin/Vicryl; Ethicon, Norderstedt, Germany) to cover the implant and the needle, and the skin was closed with interrupted 5/0 nylon sutures.

Still under otherwise sterile conditions a 1.6 mm sialography catheter was passed through the operatively placed needle and used to inject 100 μ l of bacterial suspension. The catheter was flushed with 100 μ l of sterile saline to reduce track infection and both catheter and needle were withdrawn. The insertion hole was closed with a single stitch. To standardise the surgical trauma, all the operations were done by one author (SA).

After radiography (Fig. 2), the animals were kept in separate hutches for four weeks and fed with antibiotic-free standard food. The wounds were examined daily; weight and temperature were checked three times weekly.

Evaluation. After 28 days the animals were killed by an intravenous injection of pentobarbital, and under sterile conditions the implants, the bone and the soft tissue surrounding the distal half of the implant were removed. The implants were rolled separately across tryptone soya agar (TSA) and agitated in a vortex mixer in 10 ml of thioglycollate. Bone was crushed in a sterile bone mill and soft-

tissue specimens were crushed in a mortar and pestle. The fragments were suspended in 50 ml of TSA. All samples were incubated for 24 hours. Bone and soft-tissue specimens were evaluated quantitatively as CFU/mg; implant cultures were qualitatively evaluated.

We defined infection as a positive bacterial finding for the bone or the implant. Isolated bacterial growth in the soft tissues without growth at the implant or bone was regarded as a negative result. All bacterial growths were lysotyped. **Experimental procedure.** We used a grouped sequential procedure (Ziegler, personal communication, 1994). This technique (Melcher et al 1994) reduces the necessary number of animals and allows the level of bacterial concentration to be determined at which the differences in infection rates will be most evident, usually around an infection dose of 50% (ID50). The use of an 'up-and-down' dosage technique allows the bacterial inoculum to be changed sequentially in each investigative phase. We used equal numbers of steel and titanium implants in each single phase and at each inoculum level. After a pilot series, we performed four experimental phases (Fig. 3), adapting the inoculum concentration towards the intended ID50 in subsequent phases. Finally, the number of animals used per phase was increased to gain statistical power without wasting animals by using too low or too high bacterial concentrations.

Statistical evaluation to assess the differences between the infection rates of both groups was based on the chi-squared test with $p < 0.05$ as the level of significance.

RESULTS

There were no problems with the implants, the anaesthetic or the surgical procedure. The four animals used in the pilot

series and two which had self-induced wound breakdown on the third day after operation were excluded from evaluation, leaving 20 in each group.

Observation time. One animal with a steel DCP in phase III was killed after ten days because of open wound infection after inoculation of 2×10^5 CFU. The infection was confirmed microbiologically. No other animal died or suffered from open wound infection.

Initial weight loss was directly related to the concentration of the inoculum, but was well compensated by the end of observation. There was usually an increase in temperature of 0.5 to 1°C in the two days after surgery. No animal had any greater increase in body temperature as a clinical sign of bacteraemia or systemic infection.

Microbiology. All the specimens with positive bacterial growth from the implant surface also had growth from the underlying bone or surrounding soft tissue. Bacterial growth from bone specimens but not from the implant was seen in six animals (three steel, three titanium), and two animals from each group had associated positive growth in the soft tissue. Bacterial growth only from the implant or only from the bone and the implant was not seen. Two animals from each group had isolated bacterial growth in the soft tissues, which we regarded as negative results. Lysotyping confirmed that only the inoculated strain of *Staphylococcus aureus* was present.

Evaluation. The overall infection rate for the 40 animals was 55%. That for titanium was 35% (7/20 animals) and for stainless steel 75% (15/20 animals). This difference is statistically significant ($p = 0.018$). Details are shown in Figure 3. The inoculum dose of 4×10^6 CFU in phase I was clearly too high, and was reduced in phase II; this showed a difference in infection rates but the lower range of the inoculum doses used was too low. In phase III we used 2×10^5 and 4×10^5 CFU which again resulted in a difference in the infection rates, and allowed us to estimate that the best range would be between 4×10^4 and 2×10^5 CFU. These inoculum doses were used in phase IV with a further increase in the number of animals and a group with a low inoculum dose of 4×10^3 CFU to confirm that this was below the critical range. The results in phases II, III and IV all showed a higher rate of infection associated with the stainless-steel plates. Calculations showed that the ID50 for stainless steel was 2×10^4 CFU, and for titanium 2×10^5 CFU. Figure 4 shows the most obvious differences in the infection rates between stainless steel and titanium in the range of the ID50 at inoculum doses of 4×10^4 and 2×10^5 CFU.

Fig. 3

Infection rates for stainless-steel and titanium DCP related to the experimental phase and the inoculum doses for each phase.

DISCUSSION

This animal model using the rabbit tibia is standard for experimental research on bone- and implant-related infection. The initial description by Norden (1970) and some of the later modifications aimed at a 100% infection rate. Investigations of implant-related infection in different

Fig. 4

Infection rate related to inoculum doses (CFU), showing the positive results as percentages of each group. The numbers indicate the number of infected and the total number of animals at each inoculum dose.

groups have used lower infection rates by graduating the inoculated bacteria (Petty et al 1985; Southwood et al 1985; Johansson et al, personal communication, 1992; Cordero et al 1994; Melcher et al 1994; Worlock et al 1994).

Johansson et al (personal communication, 1992) studied the infection rates of different plate materials (stainless steel, titanium) and designs (DCP, PCP (point contact plate)) after the intravenous injection of *Staphylococcus aureus*. Infection after ORIF of adult fractures is usually due to local inoculation, and we therefore used a local technique. Worlock et al (1994) used a local bacterial challenge after stability of fractured tibiae had been achieved with a standard stainless-steel DCP with bicortical screw fixation. Since we left the tibia intact, we used unicortical screws to avoid the high risk of fracture after bicortical screw fixation and also in view of possible future investigations. We considered that fracturing the bone would cause additional trauma and inevitably have made the groups less homogenous.

Different strains of *Staphylococcus aureus*, which are likely to have different pathogenetic potential, have been used as the bacterial challenge, and therefore the experimental results cannot be compared directly. This may explain the differences between our results and those of Worlock et al (1994) for the ID50 with stainless-steel plates. We did use the same strain of *Staphylococcus aureus* and the same technique of bacterial preparation as Melcher et al (1994), who studied the influence of different designs of intramedullary nails. Although doses of bacteria cannot be interpreted as absolute values it is of interest to compare the ID50 for slotted/solid intramedullary nails ($5 \times 10^4/2.8 \times 10^5$ CFU) with those for stainless steel and titanium plates ($2 \times 10^4/2 \times 10^5$ CFU).

Our results show that stainless-steel plates are associated

with a statistically significant greater infection rate than titanium plates of identical dimension and design, similar to the experimental results of Cordero et al (1994) who found a material-related difference of infection rates for intramedullary nails made of Co-Cr-Mo and Ti-Al-V alloy in the rabbit femur.

Stainless steel and titanium have different biocompatibility characteristics (Sutow and Pollak 1981; Williams 1981; Perren 1991), which may influence bacterial adhesion to their surface (Mayberry-Carson et al 1984; Gristina 1987). Soft tissue adheres firmly to a titanium implant surface (Gristina 1987; Matter and Burch 1990; Perren 1991); a known reaction to steel implants is formation of a fibrous capsule enclosing a dead space with a liquid film (Woodward and Salthouse 1986; Gristina 1987). In this space bacteria can spread and multiply and are less accessible to defence mechanisms.

Extrapolation of our experimental data to the clinical situation is unreliable. On one hand the quantification of bacterial contamination in clinical situations is impossible. On the other hand reported infection rates after ORIF with the DCP strongly depend on the degree of fracture and are between 1.1% (Matter et al 1994), 2.5% (Weise et al 1993) and 29% (Bach and Hansen 1989). We cannot demonstrate whether there would be a material-related difference in rate at an infection dose for example as low as ID10. Commercially-pure titanium does seem to offer some advantage after moderate bacterial contamination but the influence of the implant material is irrelevant when there is massive contamination.

Conclusions. We used a grouped sequential procedure to investigate local infection with a minimal number of animals. We found a significant difference in the infection rates after a local bacterial challenge depending on the

implant materials for DCP. The lower susceptibility to infection of titanium seems to be related to biocompatibility characteristics.

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