TRANSFORMING GROWTH FACTOR- β 1 STIMULATES BONE ONGROWTH TO WEIGHT- LOADED TRICALCIUM PHOSPHATE COATED IMPLANTS

AN EXPERIMENTAL STUDY IN DOGS

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Bone growth into cementless prosthetic components is compromised by osteoporosis, by any gap between the implant and the bone, by micromotion, and after the revision of failed prostheses. Recombinant human transforming growth factor- β 1 (rhTGF- β 1) has recently been shown to be a potent stimulator of bone healing and bone formation in various models in vivo.

We have investigated the potential of rhTGF- β 1, adsorbed on to weight-loaded tricalcium phosphate (TCP) coated implants, to enhance bone ongrowth and mechanical fixation. We inserted cylindrical grit-blasted titanium alloy implants bilaterally into the weight-bearing part of the medial femoral condyles of ten skeletally mature dogs. The implants were mounted on special devices which ensured stable weight-loading during each gait cycle. All implants were initially surrounded by a 0.75 mm gap and were coated with TCP ceramic.

Each animal received two implants, one with 0.3 μ g rhTGF- β 1 adsorbed on the ceramic surface and the other without growth factor. Histological analysis showed that bone ongrowth was significantly increased from $22 \pm 5.6\%$ bone-implant contact in the control group to $36 \pm 2.9\%$ in the rhTGF- β stimulated group, an increase of 59%. The volume of bone in the gap was increased by 16% in rhTGF- β 1-stimulated TCP-coated implants, but this difference was not significant.

Mechanical push-out tests showed no difference in fixation of the implant between the two groups. Our study suggests that rhTGF- β 1 adsorbed on TCP-ceramic-coated implants can enhance bone ongrowth.

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Transforming growth factor (TGF)-B and bone morphogenetic protein are the most potent bone stimulatory growth factors (Wozney et al 1990; Wozney 1992; Baylink, Finkelman and Mohan 1993). TGF- β 1 has been shown to stimulate bone healing in several animal models (Joyce et al 1990; Mackie and Trechsel 1990; Marcelli, Yates and Mundy 1990; Beck et al 1991; Aufdemorte et al 1992; Lind et al 1993) and recent studies have also demonstrated the ability of TGF-B to enhance bone healing with ceramic-coated implants in unloaded gap-healing models (Lind et al 1995; Sumner et al 1995).

The use of growth factors to induce and enhance bone healing could be helpful in orthopaedic surgery. In joint prostheses a major problem is late loosening of the prosthetic components (NIH 1994). This is especially so in young patients who will require their prostheses for many years (Cornell and Ranawat 1986; Malchau, Herberts and Ahnfelt 1993). In patients with osteopenic bone due to rheumatoid arthritis and osteoporosis and with revisions of loose prostheses, early enhanced biological fixation of cementless implants may improve the long-term clinical outcome.

A cementless technique for endoprosthetic fixation has been used for more than a decade, because it was considered that biological anchorage to bone would be better than fixation with cement. Experimental and clinical studies have shown, however, that there are problems with cementless implants, and in only very few clinical series have cementless techniques been able to match the results of those using cement (Heekin et al 1993; Hozack et al 1993). An early complication of a cementless implant is inferior mechanical fixation (Curtis et al 1992; Schwartz et al 1993), leading to micromotion of the implant. This has been shown in experimental studies to result in the formation of a fibrous membrane and inhibition of bony ingrowth (Curtis et al 1992; Søballe et al 1992a). There has been, however, an improve-

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ment in the survival of cementless acetabular components in primary arthroplasty, and for revisions (Cornell and Ranawat 1986; Turner et al 1993; NIH 1994).

Calcium phosphate ceramic coatings used alone and with porous coating have been shown to have potent stimulatory effects on implant fixation in experimental studies and promising results in clinical trials (Geesink 1990; Søballe et al 1992b; Søballe 1993). They have a different chemical stability and biological and biomechanical properties, but generally they are able to stimulate bone ongrowth (Uchida et al 1985; Klein et al 1991). Tricalcium phosphate (TCP) is more resorbable relative to hydroxyapatite and fluorapatite ceramics, which are chemically more stable and show less resorption in a biological environment (Winter et al 1981; Søballe et al 1990, 1991). We have studied the stimulatory effects of recombinant (rh) TGF- β 1 administered locally on bone healing and the mechanical fixation of TCP-coated implants subjected to physiologically-loaded conditions in trabecular bone using a canine model (Søballe et al 1992b).

MATERIAL AND METHODS

We used ten skeletally mature Labrador dogs with a mean weight of 23 kg (20 to 27). The protocol had been approved by the Danish animal research committee and all animal handling was performed according to Danish laws for research animals. A loaded device and a corresponding cylindrical grit-blasted titanium test implant coated with TCP ceramic were inserted bilaterally into the weight-bearing part of both medial femoral condyles (Fig. 1). Each animal received one implant without rhTGF- β 1 and one implant with 0.3 μ g rhTGF- β 1 adsorbed to the ceramic surface before insertion. The treatment groups were randomised to either the left or right knee, and blinded until statistical analysis. All implants were initially surrounded by a 0.75 mm gap. The observation period was six weeks.

Surgical procedures. Under halothane anaesthesia, we exposed the femoral condyles by a medial arthrotomy through a longitudinal prepatellar skin incision. The knee was flexed to show the weight-bearing portion of the medial femoral condyle. The site of implantation was selected in the central portion, which contacts the meniscus and tibia during the stance and walking phases. The technique of implantation has previously been described in detail by Søballe et al (1992b).

Initially, a 1.8 mm guide wire was inserted centrally into the condyle under fluoroscopic control. We used a cannulated hand-drill to create the hole for the implant device allowing firm fixation and permitting a gap of 0.75 mm around the test implant. Hand-drilling was performed to avoid thermal trauma to the bone. Using a custom-made tapping instrument the subchondral part of the drill hole was prepared for later placement of a threaded titanium ring centraliser.

The implant device was inserted into the drill hole and firmly fixed by the thread on the head. The centralising ring was then inserted subchondrally parallel to the piston and

Fig. 1

Diagram illustrating the implant device and test implant positioned in the weight-bearing part of the femoral condyles. A, the deep part of the implant device is fixed by the threaded titanium cylinder; B, the test implant is mounted on the threaded piston of the implant device surrounded by a 0.75 mm gap; C, a titanium ring inserted in the subchondral part of the condyle serves as support and centraliser for the polyethylene plug; and D, protrusion of the polyethylene plug which transmits the load from the tibial part of the knee to the implant.

the test implant screwed on to the threaded piston of the implant device. Finally, a polyethylene plug was screwed on to the most superficial part of the piston, so that the plug emerged 1 mm above the articular surface (Fig. 1). Prophylactic ampicillin (Anhypen; Gist-Brocades, Delft, Holland) was administered, with one gram given one hour before and a second immediately after operation. The animals had two implants inserted in both proximal humeri for another study. They were killed after six weeks by barbiturate sedation and an intravenous overdose of saturated KCl.

The implant. This is manufactured from titanium alloy and consists of a cylindrical head with self-tapping threads to ensure firm fixation in the bone (Søballe et al 1992b). A 3.0 mm diameter threaded piston allowed mounting of the test implant and polyethylene plug (Fig. 1).

We used cylindrical grit-blasted test implants of Ti-6Al-4V alloy with a diameter of 6.0 mm and a length of 9.0 mm. TCP ceramic coating was plasma-sprayed on to the surface. The coating had a thickness of 50 μ m, a purity of 95%, and a crystallinity of 93%. The mean roughness of the grit-blasted titanium surface was 8.20 Ra (SD 0.49) before TCP coating and 6.48 Ra (SD 0.62) after coating. The implants were sterilised by gamma irradiation.

Growth factor adsorption. RhTGF- β 1 was derived from a Chinese hamster ovary cells expression system and processed to more than 98% purity (Genentech Inc, South San Francisco, California). Administration was achieved by direct adsorption of the growth factor on to the TCP coating of the implants. Aseptic conditions were maintained throughout the adsorption process. To obtain the desired dose of 0.3 mg rhTGF- β 1, the implants were individually incubated with 2 ml of a solution of 1.6 μ g/ml rhTGF- β 1 in 20 mM sodium acetate buffer and 0.1% gelatin, at pH 5.0 for two hours at ambient temperature. The buffer contained gelatin to avoid non-specific binding of the growth factor to plastic tubes during handling. Any unbound rhTGF- β 1 on the implants was then removed by rinsing in the acetate buffer. The dose adsorbed was measured by determining the rhTGF- β 1 content in the buffer before and after incubation by ELISA assay. The control implants were incubated with the buffer without growth factor. The rhTGF- β 1-loaded implants were stored at 5°C in sterile containers and transported from the USA to Denmark. They were subsequently implanted between 8 and 12 days after the adsorption procedure.

We performed a separate study with a similar adsorption procedure using 125 I-iodine-labelled rhTGF- β 1 to evaluate the availability in vitro of the adsorbed growth factor. The total radioactivity of the TCP-coated implants was determined initially and subsequently when the implant was incubated in either serum or normal saline. The results showed that 90% of the adsorbed radiolabelled rhTGF- β 1 was released within four hours of incubation in serum and there was virtually no release in normal saline, indicating that the affinity of rhTGF- β 1 to TCP was strong and that the presence of serum proteins accelerates the desorption of rhTGF- β 1.

Preparation of the specimens. The distal femora were harvested, cleaned of soft tissues and stored at -20°C. Sections orthogonal to the long axis of the implants were cut on a water-cooled diamond band saw (Exact Apparatebau, Nordenstedt, Germany). The first cut was made 1 mm below the polyethylene plug. The first section, 4 mm thick, was stored at -20°C and used for mechanical testing. The second section was 1 mm thick, from which 50 μ m ground sections were prepared for histomorphometry.

Histological examination. Specimens were dehydrated in graded ethyl alcohol solutions from 70% to 100% containing 0.4% basic fuchsin. The sections were then embedded in methylmethacrylate (Technovit 7200 VLC, Exakt, Germany), mounted on acrylic slides, ground to 50 μ m using a micro grinding system (Exact Apparatebau, Nordenstedt, Germany) and finally counter-stained with 2% light green for 15 minutes. They were used for blinded quantitative histomorphometric evaluation of bone ongrowth and of woven bone volume in the initial gap between the implant and bone.

We performed quantitative histomorphometry using an image-analysis system (Grid, Olympus, Denmark) which is

based on user-specified grid counting on microscope fields captured on to a computer screen. Bone ongrowth was determined on the ground and stained sections by light microscopy at a magnification of X 100 using the line intercept technique (Gundersen et al 1988). The number of intersections with bone in contact with the implant surface was counted in successive adjacent fields around the entire implant circumference (approximately 250 intersections). Bone ongrowth was defined as the percentage of the implant surface that has bone in direct contact with the implant or the implant ceramic coating.

Bone-gap healing was determined as the percentage of woven bone in the initial gap and was quantitated by a point-counting technique. Twenty random fields (approximately 1500 points) were counted in the area from the implant surface to 750 μ m from the surface at a magnification of X 100. Resorption of TCP ceramic was evaluated qualitatively.

Mechanical testing. All implants were tested to failure by a push-out test using an Instron universal test machine (Instron Ltd, High Wycombe, UK). The specimens were placed on a metal platform with a central circular opening supporting the bone within 500 μ m of the bone-implant interface. A displacement rate of 5 mm/minute was used for all tests, and load-deformation curves were obtained by an X-Y recorder (PM 8043; Philips, Eindhoven, Holland) and a custom-made data-sampling computer program.

Ultimate shear strength (δ_u) was determined from the maximum force applied to the implant during the push-out procedure and calculated by $\delta_u = F/\pi DL$, where F is the maximal force applied to the specimen, D is the implant diameter, and L is the length of the implant tested. Apparent shear stiffness (E) was obtained from the slope of the straight part of the load-displacement curve and calculated as $E = (\Delta F/\pi DL)/\Delta L$. Energy absorption at the interface to failure was measured using a computer image analyser, as the area under the load-displacement curve until failure. The energy absorption was normalised by the surface area of the implant specimen tested.

Statistical analysis. All parameters were presented as mean values and standard error of means (Σ EM). All data sets were tested for approximation to normal distribution by probability analysis. A paired Student's *t*-test was used and *p* values less than 0.05 were considered significant.

RESULTS

Surgery. There were no immediate postoperative complications. All dogs were fully weight-bearing within two days of operation. In the first week two dogs had a small wound haematoma which was aspirated without any further complications. All ten dogs completed the observation period of six weeks and had a mean weight gain of 0.5 kg over that period. There were no signs of infection or of displacement of any of the implants.

Fig. 2a

Fig. 2b

Photomicrographs showing ongrowth to an implant without rhTGF- β 1 stimulation (a) and to an implant with 0.3 μ g rhTGF- β 1 stimulation (b). There is an increased amount of bone in contact with implant surface in the rhTGF- β 1-stimulated specimen (basic fuchsin and light green stain X 12).

Table I. The mean percentage ($\pm$ SEM) increase in bone ongrowth and gap healing in the two groups

	TCP/control	TCP/TGF-B	p value
Bone ongrowth	22.4 $\pm$ 5.6	35.6 $\pm$ 2.9	<0.05
Gap healing	23.2 $\pm$ 1.6	27.0 $\pm$ 3.3	NS

Table II. Mean values ($\pm$ SEM) of shear strength, shear stiffness and energy absorption in the two groups

	TCP/control	TCP/TGF- β
Shear strength (MPa)	0.67 $\pm$ 0.16	0.55 $\pm$ 0.16
Shear stiffness (MPa/mm)	5.73 $\pm$ 2.1	4.17 $\pm$ 1.1
Energy absorption (J)	104.0 $\pm$ 31	83.0 $\pm$ 24

Bone histomorphometry

Quantitative analysis. Bone ongrowth was significantly increased by 59% ($p = 0.04$) in the 0.3 μ g rhTGF- β 1 group compared with the TCP-coated control implants (Table I; Fig. 2). Gap healing was not significantly increased by the 0.3 μ g rhTGF- β 1 with a 16% increase in woven bone in the gap (Table I).

Qualitative analysis. Ceramic resorption was very evident after the six-week observation period with only few areas of ceramic granulate still present at the implant surface. Remnants of the ceramic were typically seen in areas of bone ongrowth.

Mechanical tests. The bone-implant interface shear strength of the TCP-coated implants stimulated with 0.3 μ g rhTGF- β 1 showed a slightly lower (22%) but not significant decrease compared with the TCP control implants ($p > 0.30$). The same trend was found for apparent shear stiffness, which was reduced by 35% compared with the TCP control implants ($p > 0.30$) and energy absorption which was reduced by 25% in the TCP-coated implants stimulated

with 0.3 μ g rhTGF- β 1 compared with the TCP control implants ($p > 0.30$). (Table II).

DISCUSSION

We have shown that bone ongrowth is enhanced significantly by rhTGF- β 1 adsorbed on to the ceramic surface of a non-porous-coated implant. The amount of woven bone in the 0.75 mm gap was increased in the rhTGF- β 1-stimulated group, but not significantly. Mechanical fixation was not enhanced by the improved bone ongrowth. This may be explained by the extensive resorption of the TCP coating, which probably weakens the bone-ceramic-implant interfaces. The remnants of ceramic coating are likely to provide poor mechanical fixation to the implant surface, which explains why increased bone ongrowth did not lead to increased mechanical fixation.

The TCP ceramic was largely resorbed. Use of a more stable ceramic such as hydroxyapatite (HA) would have provided a better correlation between the degree of bone ongrowth and mechanical fixation (Søballe et al 1993). HA, however, has a potent independent stimulatory effect on bone ongrowth which is formed by direct apposition on the ceramic surface (Geesink, de Groot and Klein 1988; Klein et al 1991; Søballe et al 1991). The independent effects of HA may reduce the possibility of demonstrating the stimulative effects of the growth factor. We therefore used TCP-coated implants because this coating favoured adsorption of growth factor and we had previously demonstrated the stimulatory effect of rhTGF- β 1 when used on TCP-coated unloaded implants (Lind et al 1995).

The use of smooth grit-blasted implants may have contributed further to the lack of correlation between bone ongrowth and mechanical fixation. Porous coatings provide a structural basis for bone ongrowth and thereby mechanical anchorage mediated by bone tissue (Cook, Walsh and Haddad 1985; Engh et al 1994). RhTGF- β 1 will also stimu-

late bone ongrowth into porous-coated implants (Sumner et al 1995), and if such implants had been used in the present study improved mechanical anchorage of the weight-loaded implant may have occurred. Additional experiments will be needed to answer this question.

We have demonstrated that growth factor will enhance bone healing to ceramic-coated implants. In another study Sumner et al (1995) implanted rhTGF- β 1 adsorbed on to porous-coated HA/TCP-coated implants into mature dogs and showed improved bone ongrowth and gap healing. Both of these studies used dog models in which the implants were not weight-loaded, in contrast to the clinical situation in which the patient is weight-bearing a few days after surgery.

TGF-B stimulation of bone ongrowth and gap healing was generally less pronounced in this study compared with an earlier investigation in which unloaded TCP-coated implants were used (Lind et al 1995); we have demonstrated a 59% increase in bone ongrowth and a 16% increase in gap healing compared with an increase of approximately 100% in these measurements in the unloaded state.

In the loaded implant model the implant gap had direct contact with the joint cavity and the synovial fluid. Bio-active substances in the synovial fluid may have interacted with the TGF-B activity in a negative manner. The operative trauma and the contact made with the tibial plateau by the loaded device and the polyethylene plug will create a level of synovitis with increased levels of cytokines in synovial fluid. Inflammatory cytokines, like interleukin 1, have been shown to reverse some of the stimulatory effects of TGF-B on mesenchymal tissues (Lorenzo, Sousa and Centrella 1988). The loaded experimental model used in this study is, however, clinically more relevant and contact between implant and joint cavity is also present in the clinical cementless prosthesis.

The differences between smooth-surfaced and porous-coated implants with TCP or HA-ceramic coatings may be considered. In an identical model using an observation period of four weeks, Søballe et al (1992b) found 42% bone ongrowth to porous HA-coated implants and only 8% ongrowth to porous titanium implants. We found 22% ongrowth to smooth TCP-coated implants without rhTGF- β 1 and 36% with rhTGF- β 1 stimulation. This suggests that the TCP coating has an ability to stimulate bone ongrowth to an extent less than that of HA but higher than that of titanium. Mechanical fixation in the earlier study was much stronger for the HA-coated implants, with a shear strength of 2.2 MPa compared with the 0.6 MPa for the smooth TCP-coated implants in this study. This large difference in mechanical fixation may be ascribed to both the porous coating and the chemical stability of the HA coating which gives an intact bone-ceramic interface, suggesting that porous HA-coated implants are to be favoured for cementless replacement.

The long-term survival of cementless implants in patients is strongly dependent on the degree of bone ongrowth and the resulting mechanical stability (Curtis et al 1992;

Schwartz et al 1993). We have demonstrated the ability of rhTGF- β 1 to stimulate bone ongrowth on ceramic-coated weight-loaded implants in mature dogs. Stimulation of bone healing by growth factor could improve the clinical outcome of cementless prostheses.

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