Transforming growth factor-ß stimulates bone ongrowth

Hydroxyapatite-coated implants studied in dogs

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Unloaded cylindrical grit-blasted titanium (Ti-6A-4V) implants (6 × 10 mm) coated with hydroxyapatite ceramic were inserted into the proximal part of the humerus of 20 skeletally mature Labrador dogs. The implants were initially surrounded by a 2 mm gap. In 10 dogs, HA-coated implants without growth factor were inserted in one humerus and implants with 0.3 µg rhTGF-ß1 adsorbed onto the HA coating were inserted in the contralateral humerus. In another group of 10 dogs, a dose of 3.0 µg rhTGF-ß1 was tested in a similar design. All dogs were killed at 6 weeks after treatment. Results were evaluated by histomorphometry and mechanical push-out testing. Bone ongrowth was increased by one third, using the 0.3 mg rhTGF-ß1 stimulation. Bone volume in the gap and mechanical testing showed no statistically significant differences between control and rhTGF-ß1 stimulated implants. RhTGF-ß1 only moderately enhanced bone ongrowth to hydroxyapatite-coated implants.

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Growth factors in the transforming growth factor ß superfamily have stimulated bone healing in several in vivo studies (Yasko et al. 1992, Beck et al. 1993, Lind et al. 1993, Cook et al. 1994). Recent studies performed in dogs, using implants coated with tricalcium phosphate (TCP) ceramic or a mixture of TCP and hydroxyapatite (HA), have demonstrated that recombinant human TGF-ß1 (rhTGF-ß1) enhances bone ongrowth and mechanical fixation (Sumner et al. 1995, Lind et al. 1996a,b). In the study using TCP-coated implants, 0.3 µg rhTGF-ß1 increased bone ongrowth twofold and mechanical strength threefold. These studies indicate possibilities for the use of growth factor-stimulated bone healing to endoprosthetic components.

TCP ceramic coatings are readily resorbed in a physiological environment but have osteoconductive properties (Winter et al. 1981, Klein et al. 1991). HA is resorbed much more slowly than TCP, and thus provides much stronger implant fixation than TCP due to formation of a more stable implant-ceramic and ceramic-bone interface (Klein et al. 1983, Tisdel et al. 1994). It would, therefore, be of interest to investigate whether rhTGF-ß1 could further enhance the effect of HA on bone ongrowth and mechanical fixation.

We investigated whether rhTGF-ß1 adsorbed onto pure HA-coated implants stimulated bone ongrowth and mechanical fixation of unloaded implants. This was done in a well-proven animal model where the implants were inserted into trabecular bone of mature dogs (Søballe et al. 1991).

Animals and methods

Design

10 mongrel and 10 Labrador dogs, all skeletally mature and weighing 22 (18–28) kg were used for the study. Cylindrical grit-blasted titanium implants coated with hydroxyapatite ceramic were inserted into the proximal humerus bilaterally (Figure 1). 10 animals had implants with 0.3 µg rhTGF-ß1 adsorbed onto the HA-coated surface inserted into one humerus and another implant without rhTGF-ß1 was inserted into the contralateral humerus as a control. In another 10 animals, a dose of 3.0 µg rhTGF-ß1 was tested using the identical design. An unloaded gap model was used and the observation period was 6 weeks (Søballe et al. 1991). The protocol and all animal handling procedures were approved by the Danish Board for Animal Research.

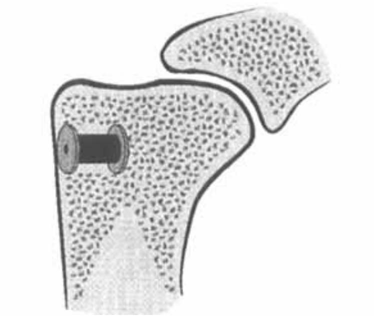


Figure 1. Schematic drawing illustrating the implant inserted orthogonally to the long axis of the humerus and centralized in a drilled canal in the proximal humerus surrounded by a two mm gap. The deep part of the implant is fixed by the footplate in press-fit. A titanium washer centralizes the implant superficially.

Surgical procedures

The implants were inserted under halothane anesthesia. Prophylactic ampicillin was administered in two 1-gram doses, the first dose 1 hour preoperatively, the second dose immediately postoperatively.

In the proximal humerus, the implantation sites were exposed through lateral extraarticular approaches immediately distal to the greater tubercle of the humerus. The periosteum was removed only from the area to be perforated by the drill. Initially, a 1.8 mm guide wire was inserted under fluoroscopic control. A 10 mm cannulated hand-drill was used to obtain the final diameter of the hole which ensured a 2 mm gap around the implants and a depth of 12 mm. Hand drilling was performed to avoid thermal trauma. The implantation site was cleared of debris with physiological saline. The implants were press-fitted into place, using the footplate and top washer (Figure 1).

All dogs were fully weight bearing within 2 days after surgery. All 20 dogs completed the observation period of 6 weeks and had an average weight gain of 0.35 kg over that period. No signs of infection or displacement of any of the implants were observed during preparation.

Implant characteristics

Cylindrical grit-blasted implants of Ti-6Al-4V alloy with a diameter of 6 mm and a length of 10 mm were used. The implant has been described in detail (Søballe et al. 1992). A footplate and a top washer made of titanium alloy with a diameter of 10 mm and a thickness of 1 mm ensures a 2 mm gap around the entire circumference of the implant and avoids connection to the periosteal surface. HA ceramic coatings were plasma-sprayed onto the implant surface.

The HA coating had a thickness of 50 mm, a purity of 95%, and a crystallinity of 68%. The grit-blasted titanium had an average roughness of 8.20 Ra (SD 0.62) before HA coating and an average roughness of 6.25 Ra (SD 0.47) after HA coating. The implants were sterilized by gamma irradiation.

Growth factor adsorption

Recombinant human TGF-ß type 1 was produced by a Chinese hamster ovary cell expression system and processed to 98% purity (Genentech Inc. South San Francisco, CA, USA). The loading of RhTGF-ß onto the implants was done by direct adsorption. To adsorb 3.0 mg rhTGF-ß1 to the ceramic surface, the implants were incubated for 2 hours with a 2.0 mL solution containing 12 µg/mL rhTGF-ß1 in 20 mM sodium acetate buffer, pH 5.0. The buffer contained 0.1% gelatin to avoid non-specific binding of the growth factor to plastic tubes during handling. For the 0.3 µg dosage, a rhTGF-ß1 concentration of 1.6 µg/mL was used. This corresponds to 1.6 and 0.16 µg rhTGF-ß1 pr cm^2, respectively. The incubation procedure was performed aseptically at ambient temperature. The implants were placed in sterile plastic containers immediately after the incubation procedure. The dose adsorbed was ensured by measuring rhTGF-ß1 content of the buffer before and after incubation. Release of rhTGF-ß1 from ceramic-coated implants into serum and saline was tested in vitro by performing the adsorption procedure using ^{125}iodine-labeled rhTGF-ß1. Total radioactivity adsorbed was then measured initially and the released rhTGF-ß1 was determined by measuring radioactivity in hourly-exchanged saline or serum. In this assay 90% of the rhTGF-ß1 was released to serum within 4 hours and virtually no release was seen to the physiological sodium chloride solution. The incubation and release analysis was performed by Genentech.

The rhTGF-ß1-loaded implants were implanted 8–12 days after the adsorption procedure. During that period they were transported by airmail from USA to Denmark and stored at 5 °C.

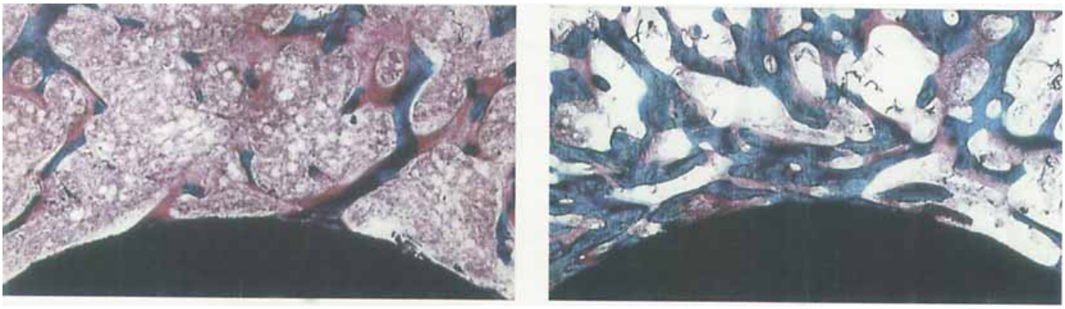
Specimen preparation

The proximal humeri were harvested and cleared of soft tissues and stored at –20 °C. Sections perpendicular to the long axis of the implants were cut on a water-cooled diamond band saw. The first cut was made 1 mm below the top washer. The first section, 4 mm thick, was used for mechanical testing and stored at –20°C until testing. The second section was 1 mm thick, from which a ground section was prepared for histomorphometry.

Mechanical testing

All implants were tested to failure by a push-out test.

Figure 2. Photomicrograph of hydroxyapatite-coated implants showing bone ongrowth to the implant and bone formation in the gap (Stain: basic fuchsin and light green; original magnification ×25)..



A control implant without rhTGF-ß1. Note the moderate bone ongrowth and sparse new bone formation consisting of thin lamellae of woven bone.

An implant loaded with 0.3 mg rhTGF-ß1. Note the increased bone in contact with the implant surface and the increased bone volume in the gap with thick bone lamellae .

Testing was performed on an Instron universal testing machine. The specimens were placed on a metal platform with a central circular opening supporting the bone within 500 mm of the bone-implant interface. A displacement rate of 5 mm/minute was used for all tests, and load displacement curves were obtained by a X-Y recorder.

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

Histological evaluation

Specimens were dehydrated in graded ethanol from 70% to 100% containing 0.4% basic fuchsin. The sections were then embedded in methylmethacrylate, mounted on acrylic slides, and ground to 50 µm using a micro grinding system and finally counterstained with 2% light green for 15 minutes. These sections were used for blinded histomorphometric evaluation of bone ongrowth and woven bone volume in the initial gap.

Quantitative histomorphometry was performed using an image-analysis system which is based on a user-specified grid counting on microscope view fields captured onto a computer screen. Bone ongrowth was determined by light-microscopy at 100× magnification, using the line intercept technique further magnified to ×230 on the screen. The number of intersections with bone in contact with the implant surface were counted in successive adjacent fields around the entire implant circumference (approximately 250 intersections). Bone gap healing was determined as percentage of bone in the initial gap by a point-counting technique. 20 random fields (approximately 1500 points) were evaluated within the gap area at a magnification of ×100.

Statistics

Data from the two rhTGF-ß1 groups are presented as percentage of intra-individual control and shown as mean values and 95% confidence limits. Median values and range for pooled absolute control values are also presented. All data sets were tested for normality by the Kolmogorov-Smirnov test, without any prior transformations. Control and stimulation groups in the humerus were tested by one-way analysis of variance (ANOVA) and Students-Newman-Keuls multiple range test to compare different doses of growth factor stimulation with controls. P-values of less than 0.05 were considered statistically significant.

Results

Bone histomorphometry

Bone ongrowth was significantly increased in the 0.3 µg rhTGF-ß1 group ($p = 0.01$; Table 1, Figure 2). Implants with 0.3 mg rhTGF-b1 demonstrated a 36% increase in bone coverage, whereas bone ongrowth in the 3.0 mg rhTGF-b1 group was equal to control levels. Gap healing was increased by 22% in the 0.3 µg rhTGF-ß1 group compared to the control group, but this difference was not statistically significant ($p = 0.2$) (Table 1).

Table 1. Absolute values (median and range) for pooled data from control implants (n 20) and data from histomorphometric analyses and push-out test (mean and 95% confidence limits in parentheses). The two rhTGF-ß1-stimulated groups are shown as mean percentage of intraindividual control, since each dose was tested in independent animal series (n 10).

Factor	Control Absolute values	0.3 mg rhTGF-ß1	3.0 mg rhTGF-ß1
Bone ongrowth	37.7 (12–55) %	136[a] (27)	98 (27)
Bone in gap	13.8 (7–29) %	122 (48)	95 (23)
Shear strength	2.37 (0.1–5.4) MPa	123 (33)	112 (72)
Shear stiffness	14.8 (2–23) MPa/mm	109 (30)	111 (68)
Energy absorp	433 (120–1045) J/m^2	141 (51)	139 (69)

[a] Statistically significant difference between intraindividual control and TGF-ß-stimulated implants (p = 0.01).

Mechanical tests

Bone-implant interface shear strength in the 0.3 mg rhTGF-ß1 group was increased by 23% compared to control implants, but this difference was not statistically significant ($p = 0.3$) (Table 1). The same trend was found for apparent shear stiffness and energy absorption. Shear stiffness was increased by 9% and energy absorption by 41% in the same lower dose group ($p = 0.2$ for energy absorption). For the 3.0 µg rhTGF-ß1 group, comparable changes in the mechanical parameters were observed (Table 1).

Discussion

We found that 0.16 mg/cm^2 rhTGF-ß1 can increase bone ongrowth to unloaded HA-coated implants in skeletally mature dogs. Mechanical fixation of the implants was not increased to a significant level after 6 weeks' observation for the two doses investigated.

Several different ceramic coatings have been developed to enhance bone ongrowth to implants: tricalcium phosphate, hydroxyapatite, and fluorapatite (Winter et al. 1981, Klein et al. 1991). These coatings have different physical and chemical properties which modify their biocompatibility in relation to bone (Klein et al. 1983, 1991). Plasma-sprayed HA is probably the coating which has the best-documented stimulatory effects on bone ongrowth and mechanical fixation (Geesink et al. 1988, Geesink 1990, Søballe et al. 1990, 1991, 1993). The mechanisms underlying these effects are not very clear, but it has been hypothesized that the ceramic liberates calcium ions that stimulate bone-healing activity in the surrounding bone (Søballe 1993). Moreover, the ceramic coating is thought to adsorb bioactive factors which initiate bone formation directly on the ceramic surface. This leads to bidirectional bone formation, with osteogenesis from the implant surface towards the surrounding bone and from the surrounding bone towards the implant surface (Søballe 1993). Such bidirectional bone formation could, in theory, be activated additionally by adding growth factors to the ceramic surface of an implant prior to insertion. Our study tested this concept, using the growth factor rhTGF-ß1 adsorbed onto the HA coating.

Growth factors, and especially members of the TGF-ß superfamily, have been shown to be powerful stimulators of bone healing in various models. RhTGF-ß1 stimulates bone healing of both calvarial defects and osteotomies in rabbits (Beck et al. 1993, Lind et al. 1993). Very few reports concern the use of growth factors as stimulators of implant fixation. Lynch et al. (1991) used a combination of platelet-derived growth factor and insulin-like growth factor to stimulate bone ongrowth into dental implants. However, these early results have not been reproduced. In 1995, we and others have independently found stimulatory effects of rhTGF-ß1 on bone ingrowth into TCP and TCP/HA-coated implants (Sumner et al. 1995, Lind et al. 1996a,b), but related studies have not so far been performed using pure HA coatings on implants. The use of growth factors as biochemical enhancers of bone healing is a new concept in cementless endoprosthetic surgery which could improve the clinical outcome in the future.

In the present study, bone ongrowth was increased when 0.3 µg rhTGF-ß1 was applied to the implants. This indicates that it is possible to enhance further the stimulatory effects of HA coating alone. However, an increased value for bone formation in the gap was found in the 0.3 µg rhTGF-ß1 group, but this increase was not significantly different from the control value. The same pattern was found for all mechanical parameters. Mechanical fixation of the implant with rhTGF-ß1 should be expected to be stronger when more bone ongrowth is present. However, we could not demonstrate such a correlation. This might be explained by the fact that the mechanical test is a more crude method than histomorphometry, and that the modest increase in bone ongrowth found in the present study might be too small to increase significantly the mechanical fixation. Histomorphometry and mechanical tests were performed on two adjacent but separate sections, and it is speculated that this could also explain the lack of correlation between the two tests. However, several previous studies using the same methods have shown a correlation between histomorphometry and mechanical parameters (Søballe et al. 1990, 1991, Lind et al. 1996a). In these previous studies, however, the relative increases demonstrated by the various stimulatory agents were higher than the 36% increase found in this study.

The correct dose of rhTGF-ß1 is essential, since a lack of effect at the higher dose of the growth factor was observed. Data from the present study provide no explanation of this observation, but previous in vitro studies have shown that TGF-ß can inhibit mineralization in high doses (Antosz et al. 1989, Bonewald and Dallas 1994). Such an effect could contribute to the lack of stimulatory effects of rhTGF-ß1 at the 3.0 µg dose. We selected the doses for this study from a similar study performed on tricalcium phosphate-coated implants (Lind et al. 1996a). In that study a 100% increase in bone ongrowth and a 200 % increase in mechanical strength was found as a result of the 0.3 µg rhTGF-ß1 dose.

Our findings are interesting for future use of rhTGF-ß1 as a stimulant of bony ingrowth in endoprosthetic surgery, since this growth factor was able to enhance bone ongrowth to HA-coated implants, which currently is the best known stimulator of bone ongrowth to implants. A combination of HA ceramic and rhTGF-ß1 could improve bone healing to cementless prosthetic components. Additional long-term clinical studies are needed to indicate whether this concept will lead to an improved clinical outcome for cementless prostheses.

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