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Improved early bone–implant integration in transgenic mice overexpressing bovine growth hormone

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Titanium implants were inserted in transgenic mice overexpressing bovine growth hormone. Four weeks after insertion the implants were cut out *en bloc* with the surrounding bone. The undecalcified specimens were cut and ground to a thickness of approximately 10 μm. Histomorphometry demonstrated significantly more direct bone to metal contact for the transgenic mice than for the non-transgenic littermates. The results indicate that endogenous high levels of bovine growth hormone result in improved early bone-implant integration. This study indicate that it may be possible to systemically administrate growth hormone in man, in the early healing in phase, to improve implant integration.

1. Introduction

During the past years several *in vitro* studies have demonstrated positive effects on osteoblast-like cell metabolism and proliferation under stimulation of growth hormone (GH) [1–3]. Positive effects on bone have also been demonstrated *in vivo* using GH-loaded polymethylmetacrylate (PMMA) bone cement and bioactive ceramics [4–6].

Improved fracture healing or bone formation has been demonstrated in animal experiments after administration of GH [7–9]. In contrast other studies have found no effect on fracture healing after systemic administration of GH [10] or an effect only for GH administered early in the fracture healing process [11]. It has been demonstrated that the effect of injected GH depends on when the effect is evaluated [12]. One explanation for the different results regarding experimental fracture healing in animals may be antibody formation against injected non-species-specific GH that has been demonstrated 8 days after administration [13]. In this study we investigate the effect of systemic high levels of GH on implant integration. To avoid the possible negative effects with antibody formation against non-species-specific GH we used transgenic animals with endogenous high levels of bovine GH.

2. Materials and methods

A transgenic mouse has a normal mouse DNA. However, due to genetic manipulation, an extra gene has been added in the DNA, in this case a gene expressing b-GH, resulting in high endogenous production of b-GH.

2.1. Animal groups

Seven transgenic mice and seven non-transgenic littermates were used in the study. The animals were 41 weeks old at the implant insertion.

2.2. Generation of transgenic animals

A BstEII-EcoRI fragment was isolated from the plasmid MTbGh 2016 (kindly provided by Dr Richard Palmiter) and used for pronuclear injection. This DNA fragment contains the mouse metallothionein I promotor linked to a genomic sequence encoding bovine growth hormone (bGH). The generation of transgenic mice was performed as described earlier [14]. Mice that integrated the bGH gene were identified with polymer chain reaction analysis of DNA from tail biopsy specimens obtained 3 weeks after birth of the animals. One primer hybridizing to the metallothionein promotor and another primer hybridizing to the bGH gene were used.

2.3. Measurements of bGH

The concentration of bGH in serum was determined by using a RIA with antisera kindly provided by AF Parlow, Pituitary hormones and antisera center, Torrance, CA, USA. Serum was collected from the animals tails at the time of implant insertion and analysed in triplicate samples. The assay was carried out in 200 μl PBS (pH 7.4) containing 0.05% bovine serum albumin (RIA grade, Sigma Chemicals Co, St Louis, MO, USA), 1.25 microlitres mouse serum anti bGH-antiserum (1/400.000) and ^{125}I-labelled bGH (5000 cpm). The bGH standard (0.1–50 ng/tube) was contained

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Figure 1 Undecalcified ground section with the implant *in situ* (total length of the implant 2.0 mm).

1.25 µl normal mouse serum. After overnight incubation (4 °C) the bound hormone was precipitated by adding 1.4 ml of a mixture of polyethylene glycol (16% w/v, final concentration), bovine gammaglobulin (2 mg/ml; Cohn fraction II, III Sigma) and triton X-100 (0.02%) in 0.05 M TRIS-HCl buffer (pH 8.5). The samples were further incubated for 30 min at + 4 °C, centrifugated, and the supernatants aspirated. The pellets were counted for gamma-radioactivity and the results were expressed as ng bGH/ml.

2.4. Implants and surgical technique

Threaded implants with an outer diameter of 1.4 mm and a length of 2 mm were manufactured from commercially pure (c.p.) titanium. A curved skin incision was made on the forehead of the mice. Gentle surgical technique and only well sharpened instruments were used. After removal of the soft tissue a 1.2 mm wide hole was drilled through the bone and the implant was screwed home in the bone. The bottom of the implant was with this procedure situated into the nose cavity while the top of the implant was one or two threads over the cortical level (Fig. 1).

Four weeks after implant insertion the animals were sacrificed and the implants were cut out *en bloc* with the surrounding bone.

2.5. Histological procedures

The specimens were dehydrated and embedded in methylmethacrylate plastic. Using the procedure of

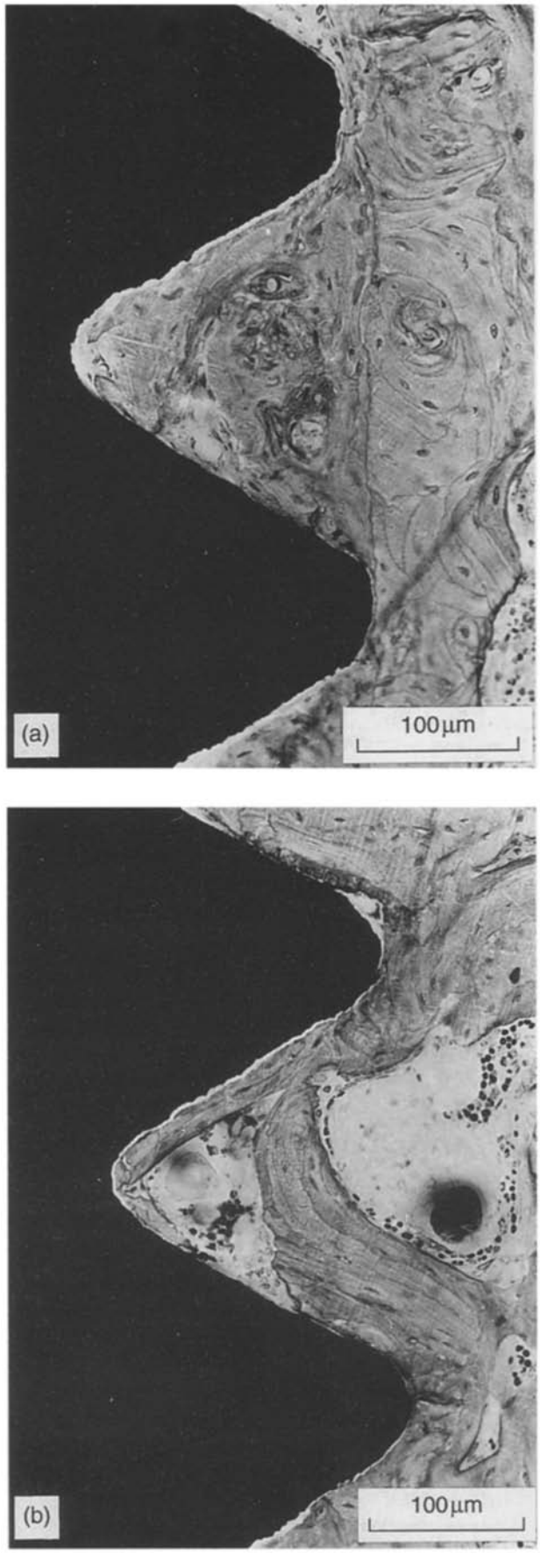

Figure 2 Undecalcified ground section of the implant (a) and (b) the undecalcified mice bone demonstrating direct bone-to-metal contact.

Donath [15], sections were made through the implants and the surrounding undecalcified bone. After grinding the sections to a thickness of approximately 10 µm they were stained in 1% toluidine blue in a 1%

TABLE I The GH-transgenic mice had significantly higher amount of bone to metal contact ($P < 0.01$). Area and cortical thickness measurements revealed a tendency to more bone inside the threads and thicker cortical bone in the transgenic mice even though these tendencies were not statistically significant

bGh-transgenic mice Sample number	BMC	BMC-all	Area	Area-all	CT	W	ngGH/ml
1	37	15.9	62	21.6	310	53/52	1579
2	36	14.1	56	24.4	392	51/48	896
3	49	20.7	58	38.0	500	47/45	752
4	50	43.5	60	48.5	300	52/50	726
5	71	34.0	65	35.0	375	45/45	801
6	42	21.5	59	26.0	43.5	61/61	225
7	34	14.7	72	43.6	325	63/64	1103
mean	45	23.5	61	33.9	377	53/52	869
Negative controls							
8	40	14.5	60	31.5	215	38/38	
9	0	0	57	24.4	315	52/51	
10	20	7.7	31	17.5	500	43/43	
11	0	0	22	13.0	230	38/36	
12	8	2.1	38	16.6	465	47/47	
13	25	12.8	45	30.3	240	38/38	
14	23	16.6	62	41.2	290	36/35	
mean	17	7.7	45	24.9	322	42/41	

BMC = percentage of direct bone metal contact for the best cortical thread, BMC-all = percentage of direct bone to metal contact for all threads, Area = percentage of bone inside the best cortical thread, Area-all = percentage of bone in all threads, CT = cortical thickness (μm) at site of implantation, W = weight (g) at implantation/at sacrifice, bGH = serum bGh levels at time of implantation

borax solution mixed in proportions 4 to 1 with 1% pyronin-G solution .

2.6. Histomorphometry

The amount of immature and mineralized bone apposed to the surface and the percentage of the total area inside the threads with immature and mineralized bone were calculated for the best cortical thread as well as for all the threads of the implants using a Leitz Aristoplan light microscope with objectives 1.6× to 50×, and a zoom of 2.5×. The light microscope was connected to a Leitz Microvid unit and coupled to a computer-based morphometric assessment.

2.7. Statistics

Statistical differences were calculated using a non-parametric test (Wilcoxon rank sum test): $p < 0.05$ was considered statistically significant.

3. Results

There were no macroscopical findings of adverse tissue reactions at sacrifice of the animals. Microscopic investigation revealed mostly immature bone in the threads and in contact with the implants. Endosteal bone regeneration was found in most of the sections. Numerous macrophages and fibroblasts were observed in areas without direct bone to metal contact. Giant cells were occasionally observed.

The levels of bGh in the transgenic mice were, on average, ten times higher than the normal peak levels of mouse-GH in normal mice. Normal littermates have no detectable levels of bGH.

The bGH-transgenic mice had a significantly higher percentage of direct bone-to-metal contact than the controls ($p < 0.01$). The transgenic animals demonstrated more bone in the threads than the control group, even through this difference was not statistically significant. The transgenic animal were larger with an average weight of 53 g versus 42 g and had a thicker cortical bone at the implantation site, mean 377 μm versus mean 322 μm for the non-transgenic control group. The difference in cortical thickness was not statistically significant.

The percentage BMC, the amounts of bone in the threads, cortical thickness, weight at implant insertion and at sacrifice and concentration of bGH for the animals are shown in Table I.

4. Discussion

This study has demonstrated that transgenic mice with endogenous high levels of b-GH have an improved early implant incorporation capacity compared to non-transgenic littermates. To our knowledge this is the first time it has been demonstrated that high systemic levels of b-GH may improve implant incorporation in bone. The transgenic animals had a tendency to thicker cortical bone at the implant site even though this difference was not statistically significant. The observed difference in cortical bone thickness may result in a better primary fixation of implants in the transgenic group. However, the difference in cortical thickness is quite small compared to the large difference in bone-to-metal contact for the transgenic versus the non-transgenic animals. We therefore conclude that the high levels of b-GH substantially contributes to the improved early implant

integration of the b-GH transgenic animals. These results are encouraging and indicate that systemically administrated GH may result in an improved implant/prosthesis incorporation and probably better long-term survival of implants. However, before such studies are conducted in man further animal studies are needed and we are therefore now investigating the incorporation capacity in b-GH transgenic mice after a longer follow up time.

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