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# Osteogenesis promoted by bone matrix combined with marrow

## Titanium implants studied in rats

We evaluated the bone-forming potential of isogeneic bone marrow combined with antigen-extracted, autolyzed allogeneic bone matrix (AAA bone a.m. Urist). The purpose of the experiment was to evaluate bone-inducing materials for application in orthopaedic devices designed for fixation by bone ingrowth into a porous surface. The bone-forming materials were packed into tubes of porous fiber titanium and placed in the back musculature of rats for 12 or 25 days. At 12 days the combination of bone marrow and AAA bone had produced more bone than marrow only. At 25 days, however, there was no difference. The bone-inducing materials produced substantial amounts of new bone, and may become an adjuvant for achieving fixation by bone ingrowth. In particular, a combination of AAA bone and marrow might enhance fixation at a very early postoperative stage.

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Numerous investigators have been able to show that living bone can invade porous implants and thereby fix them to the bone. Perfect immobilization of a biocompatible implant with interconnected pores in the range 100–400 μm, and placed in intimate contact with normal bone are essential for a successful outcome (Galante 1983). Occasionally the surgeon is compelled to accept a gap at the bone-implant interface. This situation calls for an aid to promote new bone formation that can fill the gap. The first alternative that presents itself is autografting. But recent research has developed materials that challenge the grafting techniques, in particular when availability and convenience are considered (Urist & Dawson 1981, Iwata et al. 1981, Oikarinen 1982).

In a previous experiment we found that Antigenextracted, Autolyzed, demineralized Allogeneic (AAA) bone (Urist 1980), combined with bone marrow, had an osteogenic capacity matching that of isogeneic bone transplants (Rønningen et al. 1985). But without bone marrow added, the AAA bone produced small amounts of new bone. Thus it seemed that the osteogenic capacity depended mainly on the bone marrow tissue.

As marrow without AAA bone was not among the bone-inducing materials tested in the previous experiment, we designed the present experiment to assess the osteogenic potential of bone marrow only, compared to the combination of marrow and AAA bone.

## Material and methods

*Fiber titanium implants.* Cylindrical tubes of porous fiber titanium 8.0 mm long by 6.5 mm OD, and with a 2.0-mm open central canal were used (Zimmer, USA). The implants had been manufactured from a 0.25-mm-thick wire of pure titanium, and compressed to a relative density of 50 per cent (Rostoker et al. 1974).

*Bone-inducing materials.* (1) *AAA bone* The femurs of 16 male rats (Outbred Wistar/Af/Mol/SPF) 288–316 g were used. An 8.5-mm segment of the mid-diaphysis was removed for preparation of the AAA bone (Urist 1980, Rønningen et al. 1985). Before freeze drying, the segments from the right femurs were cleaved longitudinally to make eight nails of AAA bone from each. The segments from the left femurs were stored to serve as reference samples.

(2) *Bone marrow* Bone marrow was obtained from both femurs of 32 inbred, female rats (Wistar Kyo/Nih/Mol/SPF) 250–300 g (Rønningen et al. 1985).

*Surgical procedure.* Sixteen inbred, male rats (Wistar Kyo/Nih/Mol/SPF) 277–322 g were used as recipient animals. The titanium implants were packed with bone-inducing materials and placed in the back musculature as described earlier (Rønningen et al. 1985). Each animal carried two implants: one packed with marrow and AAA bone, and one with marrow only.

Bone marrow from one donor femur was first installed in the central canal of the implant. For the implants scheduled to have AAA bone, as many AAA bone nails as possible were inserted into the canal. After the implant had been placed in the recipient animal, marrow from the other donor femur was placed in the pouch at the ends of the fiber metal cylinders. The AAA bone nails had been weighed before insertion, and surplus nails were weighed after, to obtain the weights of the implanted AAA bone. The reference AAA bone was weighed at the same time. Fluanisone (Hypnorm Vet®, LEO, Denmark) 5 mg/kg s.c. was used to anaesthetize the animals.

*Analyses.* Eight of the animals were killed 12 days after implantation and eight animals 25 days after. Twenty-four hours before sacrifice the animals had received $7.4\times10^5$ Bq/kg $^{47}$Ca (Amersham Int., Ltd., England) and $3.7\times10^7$ Bq/kg L-(5-$^3$H)-proline (New England Nuclear, USA) intraperitoneally for labelling of bone mineral and collagen. After sacrifice, the implants and right tibias were removed and counted for activity of $^{47}$Ca. The tissue within the implants was extracted in 6.0 M HCl, as described by Rønningen et al. (1984). The extracts, tibias and reference AAA bone were hydrolyzed and analysed for total content and radioactivity of hydroxyproline (Firschein 1969). Calcium in the hydrolysates was measured by atomic absorption spectrophotometry. The specific activities of the tibias were expressed as radioactivity per mol Ca and hydroxyproline, respectively. The rate of bone formation in the implants was computed by dividing the total radioactivities of the tissues from the implants by specific activities of tibias, denoted as collagen and mineral indexes (Rønningen et al. 1985).

The amounts of hydroxyproline in the AAA bone that had been put into the implants were assessed by combining their weights with weights and hydroxyproline contents of the reference AAA samples.

*Statistics.* Averages and dispersions were expressed as arithmetic means ± 1 SD. The data sets for mineral deposit and net bone formation had a linear relationship between means and variances which could be abolished by log transformation before the tests were applied. Two-way analysis of variance (ANOVA) was used to test differences between time intervals and implant types. Where differences were found, the Student-Newmann-Keuls' (SNK) test was used for contrasting means (Sokal & Rohlf 1969).

## Results

There were no postoperative complications. The animals in the 12-day group weighed 302–348 g and those in the 25-day group 330–366 g at sacrifice. The implanted AAA bone weighed 9.2 ± 1.3 mg and contained 8.0 ± 1.1 μmol of hydroxyproline at the time of implantation. The reference AAA bone did not contain any calcium. Thus the calcium content of tissue extracted from the implants was used as a valid measure of the net bone formation during the experiment.

Tables 1–3 present the bone formation data. Regarding the collagen indexes, there were no

*Table 1. Collagen synthesis rate in implants expressed as collagen indexes by division of total radioactivity of tritiated hydroxyproline in implants by specific activity of the right tibia (mean ± SD)*

| Graft | 12 days | 25 days |
|---|---|---|
| Isogeneic bone marrow w/AAA bone | 58.9±18.1 | 42.5± 7.2 |
| Isogeneic bone marrow | 49.2±25.0 | 58.5±31.1 |

*Table 2. Mineral deposit rate within implants expressed as mineral indexes by division of total radioactivity of $^{47}$Ca in implants by specific activity of the right tibia (mean ±SD)*

| Graft | 12 days | | 25 days |
|---|---|---|---|
| Isogeneic bone marrow w/AAA bone | 279±168 | | 484±288 |
| isogeneic bone marrow | 124±126 | ---*--- | 606±410 |

*Significant increase from 12 to 25 days, $p < 0.025$, two-way ANOVA. **$p < 0.05$, Student-Newmann-Keuls test. (The tests refer to log-transformed data.)

*Table 3. Net bone formed in implants expressed by contents of calcium (μmol, mean ± SD)*

| Graft | 12 days | | 25 days |
|---|---|---|---|
| Isogeneic bone marrow w/AAA bone | 33.6±14.8 | | 58.0±36.3 |
| Isogeneic bone marrow | 13.7± 6.9** | | 63.8±43.3 |

*Significant increase from 12 to 25 days, $p < 0.025$, two-way ANOVA. **Significantly lower than all other groups, $p < 0.05$, Student-Newmann-Keuls' test. (The tests refer to log-transformed data.)

differences between type of implant or between time intervals (Table 1). There was a general increase in the mineral indexes from 12 to 25 days, but no difference between the two types of bone-inducing material (Table 2). The mineral indexes for bone marrow at 12 and 25 days differed from each other also when contrasted by the SNK-test. The net amounts of new bone formed showed the same pattern as the mineral indexes with an increase from 12 to 25 days (Table 3). In particular, the 12-day implants with bone marrow only had produced less bone than any other group. Expressed as amount of calcium/g implanted AAA bone, AAA bone with marrow has produced 4.2 mmol/g at 12 days and 6.9 mmol/g at 25 days.

## Discussion

Bone marrow and bone marrow combined with bone matrix (AAA bone) produced substantial amounts of new bone within porous fiber titanium implants. Our results indicate that AAA bone contributes positively at the early interval (12 days), while the effect fades at the longer interval (25 days).

The net bone formation in our present study corresponds well to the bone formation by isografts and AAA bone/marrow in our previous experiment where similar amounts of bone-inducing materials were implanted (Rønningen et al. 1985). Bone formation by AAA bone with marrow expressed by *calcium content per gram implanted AAA bone* was in the same range as found by Firschein & Urist (1972), although it was somewhat higher due to the contribution from the marrow.

The osteogenic potential of bone marrow is confined to osteoprogenitor cells that are derived from the stroma cells, one of the two main cellular systems of bone marrow (Owen 1978). The term *bone induction* applies to the differentiation of osteoprogenitor cells into osteoblasts. The mineral indexes and net bone formations at 12 days demonstrate that AAA bone has bone-inducing potential. Thus it seems that AAA bone can play a more active role in bone formation than we found earlier (Rønningen et al. 1985). Other authors have maintained that the bone marrow is the predominant and active factor in bone induction (Nade 1977, Cummine et al. 1983). Craig Gray & Elves (1979) suggested that most of the osteogenic potential of bone marrow resides in endosteal lining cells which must have contaminated many batches of "gently removed bone marrow", and caused inconsistent results in various reports. Thus it may be that surgical removal of bone marrow will remove these cells close to the bone, while the aspiration technique applied by other investigators (Lindholm & Urist 1980, Lindholm et al. 1982) probably left them *in situ*.

Wittbjer et al. (1983) applied a bone-defect model in rabbits and found that bone marrow combined with matrix was more efficient than bone matrix 14 days after implantation, while there was no difference at 28 days. Our results also agree with those of other investigators who have found that a combination of bone marrow and bone matrix is a potent bone-forming material with a short lag time before bone production starts. (Newman & Boyne 1971, Lindholm & Urist 1980, Lindholm et al. 1982).

We conclude that a combination of specially prepared bone matrix and bone marrow induces rapid and abundant bone formation.

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