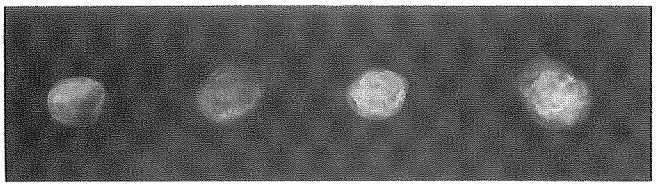

Fig. 2. Soft X-ray photographs of the ossicles at 3 weeks after implantation. A typical ossicle from each group is shown

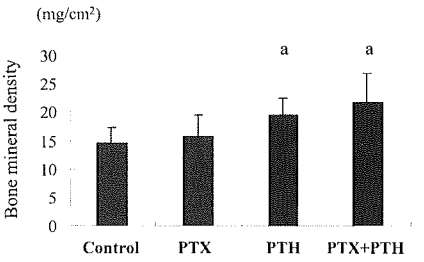

Fig. 3. The bone mineral density (BMD) values of the harvested tissues from the PTH-alone and PTX + PTH group were significantly higher than that in the control group (n = 10). Data values are means ± SD. [a] $P < 0.05$ vs control

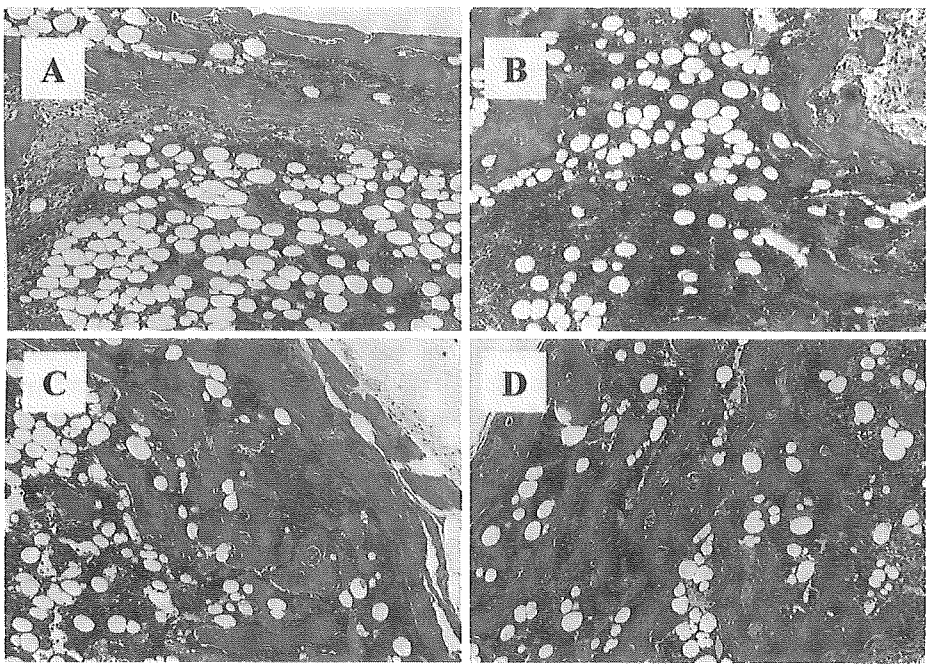

Fig. 4. Photomicrographs of the harvested tissues at 3 weeks after implantation. **A** Control; **B** PTX; **C** PTH; **D** PTX + PTH. New bone formation with hematopoietic bone marrow and trabeculae is visible in the tissue. In the PTH-alone treatment group and the PTX + PTH treatment group, there were visible increases in the number and thickness of bony trabeculae when compared to the PTX and control group. **A–D** H&E, ×100

control group was 55.6 ± 2.5 mm³; that of the PTX group was 70.9 ± 6.3 mm³; and that of the PTX + PTH group, 77.3 ± 6.5 mm³. The values for the PTX and PTX + PTH groups were significantly higher than that recorded for the control group ($P < 0.05$). However, there was no significant difference between the controls and the PTH group (62.1 ± 3.5 mm³).

Radiographic findings

The radiographic appearances of the harvested tissues 3 weeks after implantations are shown in Fig. 2. All of the pellets harvested 3 weeks after implantation showed radiographic evidence of calcification and calcified trabeculae.

Bone mineral density (BMD) of the ossicles

Figure 3 shows the mean BMD of ossicles from each group, measured with SXA. There was no significant difference in BMD between the PTX group (15.7 ± 3.8 mg/cm²) and the control group (14.5 ± 2.7 mg/cm²). However, the BMDs of the PTH group (19.4 ± 3.1 mg/cm²) and the PTX + PTH group (21.8 ± 5.2 mg/cm²) were significantly higher than those of the control group and the PTX group.

Histology

Ossicles from all of the four groups revealed normal bone histology, with hematopoietic marrow and bony trabeculae (Fig. 4). Small amounts of collagen carrier remnants were seen in the centers of all the ossicles.

Calcium content in the ossicles

The mean calcium content in ossicles harvested from each group is shown in Fig. 5. The calcium content in

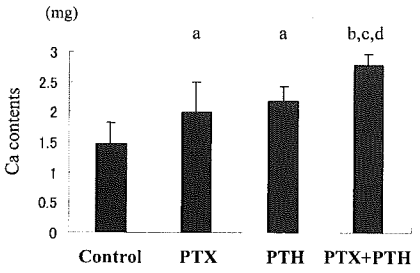

Fig. 5. Ca content of the harvested tissues at 3 weeks after implantation. Ca content was measured by OCPC method (Calcium C test kit; Wako) ($n = 7$). The Ca content of the ossicles from the PTX group and the PTH group was significantly higher than that in the control group. The Ca content of the ossicles from the PTX + PTH concurrent treatment group was significantly higher than that in the ossicles from the control, PTX-alone, and PTH-alone treatment groups. Data values are means ± SD. [a] $P < 0.05$ vs control; [b] $P < 0.01$ vs control; [c] $P < 0.05$ vs PTX group; [d] $P < 0.05$ vs PTH group

ossicles from the PTX (1.99 ± 0.51 mg), PTH (2.18 ± 0.24 mg), and PTX + PTH groups (2.78 ± 0.18 mg) was significantly higher than the value in the control group (1.46 ± 0.36 mg). The Mean calcium content in the ossicles of the PTX + PTH group was significantly higher than that in the PTH and PTX groups ($n = 7$).

Discussion

The results of the present experimental study revealed additive anabolic effects of the daily injection of active fragment of human PTH and a nonselective PDEi (PTX) on endochondral ossification elicited ectopically by rhBMP-2 in mice. The additive and complementary effects of both agents on endochondral bone formation were postulated from the results of our previous studies using the same model, indicating two aspects of the enhancement of bone formation, i.e., increased mass of the new bone and increased density of the new bone. Daily injection of PTX increased the mass of BMP-induced ectopic bone without changing the BMD [9], and PTH increased the BMD without changing the bone mass. In the initial phase of new ectopic bone formation in the BMP-retaining collagen pellets, the pellet is resorbed and replaced by a cartilage shell at the periphery of the pellet. This shell is then thought to be replaced by new bone through endochondral ossification, with the shell determining the final size of the BMP-induced ossicle [19]. In previous studies of ours, it was radiographically determined that the calcified rings in tissues harvested from mice treated for 1 week with PTX and other PDEi agents were larger than those observed in the control group [9,10]. Because the chondro-osseous differentiation of undifferentiated mesenchymal cells is initiated when these cells come into contact with BMP at the periphery of the BMP-retaining disk, this increase in the bone mass may indicate greater sensitivity and earlier initiation of the response to BMP-2 induced by PTX compared with the control. PTX therefore appears to have a stimulatory effect on the early stage of bone formation during BMP-induced osteogenesis. In an in vitro study, PTX has been shown to enhance BMP effects predominantly in less differentiated cells which have the potential for osteogenic or chondrogenic differentiation [20]. PTX may target undifferentiated mesenchymal cells that come into contact with BMP in the initial phase of ossification and may increase their responsiveness to BMP. This, is turn, may lead to the earlier initiation of chondrogenic differentiation, resulting in a larger cartilagenous anlage to be replaced later by new bone tissue, and leading to an increase in implant size. However, our study did not find an increase in the size of the harvested tissue as a result of PTH treatment. This indicates that PTH may not affect the cartilage formation phase in this model. The tissues harvested from mice treated with PTH for only the first week were not significantly different from the control, either in size or in BMD (data not shown). The BMD of the tissue harvested from the PTH-treatment group at 3 weeks after implantation was significantly higher than that of the tissue from the control group and the group treated with PTX alone. These findings suggest that PTH may affect a late rather than an early phase of this BMP-induced endochondral ossification process. The mechanisms of the anabolic effect of PTH remain unknown, however. Several studies have reported that the receptor for PTH is expressed in the cells of both chondrocytes and osteoblasts [21,22]. However, this claim does not seem to support the findings of the present study, in that the anabolic effects of PTH were observed at a later phase of the BMP-induced endochondral ossification process. Further studies to clarify this point therefore appear to be necessary.

Thus, PTX and PTH appear to act on distinct phases of the osteogenic process. Therefore, we hypothesized that the two agents would work additively when used concurrently. Our data seemed to confirm this hypothesis, with concurrent PTX + PTH treatment resulting in the induction of new ossicles of a larger size and with a higher BMD than the controls.

Several studies have reported that the cortical area of the tibia or femoral diaphysis increased significantly after injections of 50–200 μg/kg BW per day of PTH(1–34) [23–28]. However, other studies found that injection at doses of 1.5–10 μg/kg BW had an anabolic effect on bone formation [29–30]. We performed preliminary experiments in the model used in the present study. The injection of 4, 10, and 40 μg/kg BW per day of PTH (1–

34) resulted in a significant increase in the BMD of the harvested tissues compared with that produced by the vehicle alone. However, it should be noted that the increase in BMD generated by the 40 μg/kg-dose (18.5 ± 3.9 mg/cm²) was less than that resulting from the doses of 4 μg/kg (20.5 ± 3.3 mg/cm²) and 10 μg/kg (21.0 ± 3.5 mg/cm²). The dose of 10 μg/kg was therefore considered optimal, and a low dose of PTH(1–34) seemed to have an effect on this ectopic bone-formation model.

The mechanisms underlying the anabolic effects of PTH and PTX on bone formation are not fully understood. PTH appears to increase the bone-forming activity of osteoblasts, and it may increase the rate of maturation of pre-osteoblasts into osteoblasts, or it may increase the bone-forming activity of osteoblasts [31]. Bone mass can be increased by intermittent PTH administration, but the mechanism of this phenomenon is not known. In the study presented here, PTH treatment was found to increase the BMD and the calcium content of BMP-2 induced ectopic new bone, but it did not increase bone volume. These results indicate that intermittent administration of PTH is likely to have an anabolic effect on BMP-2-induced ectopic new bone formation. Further studies are needed, however, to clarify the mechanisms involved.

The exact mechanism by which a PDEi stimulates BMP-induced bone formation also awaits elucidation. Elevation of intracellular cAMP level by a PDEi, coupled with intracellular signaling through the PKA cascade by PTH, may stimulate bone formation [20]. For the future, it will be important to study the crosstalk between BMP, BMP receptors, Smads, Cbfa-1, and the PKA signaling cascade. There is considerable evidence in the literature to suggest that the anabolic effects of PTH are mediated by cAMP [1,2,32] and, by extrapolation, PDEs [11,20].

A recent study aimed at further understanding of the anabolic actions of PTX on bone formation has also implicated crosstalk between BMP signaling and PKC signaling cascades [12]. However, that report mentioned that PDEis, including PTX, could promote osteoblast differentiation by a mechanism independent of PKA activation. We speculate that this mechanism may be one of the reasons why PTH and PDEi have different effects on osteoblast differentiation. Future studies to investigate the molecules and signal pathways by which PTH and PDEis mediate osteoblast differentiation should contribute to an understanding of their anabolic effect on bone.

In conclusion, the present study has confirmed that daily injections of PTH and PTX enhance rhBMP-2 induced endochondral new bone formation in an additive and complementary manner in an animal model of bone induction. These agents may provide a new approach to enhancing the clinical efficacy of BMP-mediated new bone formation for the treatment of fracture and the correction of bone defects.

References

1. Partridge NC, Bloch SR, Pearman AT (1994) Signal transduction pathways mediating parathyroid hormone regulation of osteoblastic gene expression. J Cell Biochem 55:321–327
2. Ishizuya T, Yokose S, Hori M, Noda T, Suda T, Yoshiki S, Yamaguchi A (1997) Parathyroid hormone exerts disparate effects on osteoblast differentiation depending on exposure time in rat osteoblastic cells. J Clin Invest 99:2961–2970
3. Shen V, Dempster DW, Mellish RW, Birchman R, Horbert W, Lindsay R (1992) Effects of combined and separate intermittent administration of low-dose human parathyroid hormone fragment (1–34) and 17 beta-estradiol on bone histomorphometry in ovariectomized rats with established osteopenia. Calcif Tissue Int 50:214–220
4. Shen V, Dempster DW, Birchman R, Xu R, Lindsay R (1993) Loss of cancellous bone mass and connectivity in ovariectomized rats can be restored by combined treatment with parathyroid hormone and estradiol. J Clin Invest 90:2479–2487
5. Wronski TJ, Yen CF, Qi H, Dann LM (1993) Parathyroid hormone is more effective than estrogen or bisphosphonates for restoration of lost bone mass in ovariectomized rats. Endocrinology 132:823–831
6. Sogaard CH, Wronski TJ, McOsker JE, Mosekilde L (1994) The positive effect of parathyroid hormone on femoral neck bone strength in ovariectomized rats is more pronounced than that of estrogen or bisphosphonates. Endocrinology 134:650–657
7. Qi H, Li M, Wronski TJ (1995) A comparison of the anabolic effects of parathyroid hormone at skeletal sites with moderate and severe osteopenia in aged ovariectomized rats. J Bone Miner Res 10:948–955
8. Kinoshita T, Kobayashi S, Ebara S, Yoshimura Y, Horiuchi H, Tsutsumimoto T, Wakabayashi S, Takaoka K (2000) Phosphodiesterase inhibitors, pentoxifylline and rolipram, increase bone mass mainly by promoting bone formation in normal mice. Bone 27:811–817
9. Horiuchi H, Saito N, Kinoshita T, Wakabayashi S, Tsutsumimoto T, Takaoka K (2001) Enhancement of bone morphogenetic protein-2-induced new bone formation in mice by the phosphodiesterase inhibitor pentoxifylline. Bone 28:290–294
10. Horiuchi H, Saito N, Kinoshita T, Wakabayashi S, Yotsumoto N, Takaoka K (2002) Effect of phosphodiesterase inhibitor, Rolipram, on new bone formations by recombinant human bone morphogenetic protein-2. Bone 30:589–593
11. Wakabayashi S, Tsutsumimoto T, Kawasaki S, Kinoshita T, Horiuchi H, Takaoka K (2002) Involvement of phosphodiesterase isozymes in osteoblastic differentiation. J Bone Miner Res 17: 249–256
12. Rawadi G, Ferrer C, Spinella-Jaegle S, Roman-Roman S, Bouali Y, Baron R (2001) 1-(5-oxohexyl)-3,7-Dimethylxanthine, a phosphodiesterase inhibitor, activates MAPK cascades and promotes osteoblast differentiation by a mechanism independent of PKA activation (pentoxifylline promotes osteoblast differentiation). Endocrinology 142:4673–4682
13. Casperson GF, Bourne HR (1987) Biochemical and molecular genetic analysis of hormone-sensitive adenylyl cyclase. Annu Rev Pharmacol Toxicol 27:371–384
14. Conti M, Jin SL, Monaco L, Repaske DR, Swinnen JV (1991) Hormonal regulation of cyclic nucleotide phosphodiesterases. Endocr Rev 12:218–234
15. Hyacinthe M, Jaroszeski MJ, Dang VV, Coppola D, Karl RC, Gilbert RA, Heller R (1999) Electrically enhanced drug delivery for the treatment of soft tissue sarcoma. Cancer 15:409–417

16. Horiuchi A, Nikaido T, Mitsushita J, Toki T, Konishi I, Fujii S (2000) Enhancement of antitumor effect of bleomycin by low-voltage in vivo electroporation: a study of human uterine leiomyosarcomas in nude mice. Int J Cancer 15:640–644
17. Hashimoto J, Takaoka K, Yoshikawa H, Miyamoto S, Suzuki S, Ono K (1991) Preservation of ectopically induced bone in the mouse by estradiol. Bone 12:249–255
18. Yoshikawa H, Taniguchi SI, Yamamura H, Mori S, Sugimoto M, Miyado K, Nakamura K, Nakao K, Katsuki M, Shibata N, Takahashi K (1998) Mice lacking smooth muscle calponin display increased bone formation that is associated with enhancement of bone morphogenetic protein responses. Genes Cells 3:685–695
19. Takaoka K, Yoshikawa H (1996) Bone morphogenetic protein derived from osteosarcoma. In: Lindholm TS (ed) Bone Morphogenetic Proteins: Biology, Biochemistry, and Reconstructive Surgery. Landes Com, TX Georgetown, pp 83–103
20. Tsutsumimoto T, Wakabayashi S, Kinoshita T, Horiuchi H, Takaoka K (2002) A phosphodiesterase inhibitor, pentoxifylline, enhances the BMP-4-dependent differentiation of osteoprogenitor cells. Bone 31:396–401
21. Lee K, Deens JD, Chiba S, Un-no M, Bond AT, Segre GV (1994) Parathyroid hormone induces sequential c-fos expression in bone cells in vivo: in situ localization of its receptor and c-fos messenger ribonucleic acids. Endocrinology 134:441–450
22. Lee K, Deeds JD, Bond AT, Juppner H, Adou-Samra AB, Segre GV (1993) In situ localization of PTH/PTHrP receptor mRNA in the bone of fetal and young rats. Bone 14:341–345
23. Liu CC, Kalu DN (1990) Human parathyroid hormone-(1–34) prevents bone loss and augments bone formation in sexually mature ovariectomized rats. J Bone Miner Res 5:973–982
24. Ibbotson KJ, Orcutt CM, D'Souza SM, Paddock CL, Arthur JA, Jankowsky ML, Boyce RW (1992) Contrasting effects of parathyroid hormone and insulin-like growth factor I in an aged overiectomized rat model of postmenopausal osteoporosis. J Bone Miner Res 7:425–432
25. Wronski TJ, Yen CF (1994) Anabolic effects of parathyroid hormone on cortical bone in ovariectomized rats. Bone 15:51–58
26. Andreassen TT, Ejersted C, Oxlund H (1999) Intermittent parathyroid hormone (1–34) treatment increases callus formation and mechanical strength of healing rat fractures. J Bone Miner Res 14:960–968
27. Holzer G, Majeska RJ, Lundy MW, Hartke JR, Einhorn TA (1999) Parathyroid hormone enhances fracture healing. A preliminary report. Clin Orthop 366:258–263
28. Kimmel DB, Bozzato RP, Kronis KA, Coble T, Sindrey D, Kwong P, Recker RR (1993) The effect of recombinant human (1–84) or synthetic human (1–34) parathyroid hormone on the skeleton of adult osteopenic ovariectomized rats. Endocrinology 132:1577–1584
29. Okimoto K, Tsurukami H, Okazaki Y, Nishida S, Sakai A, Ohnishi H, Hori M, Yasukawa K, Nakamura T (1998) Effects of a weekly injection of human parathyroid hormone (1–34) and withdrawal on bone mass, strength, and turnover in mature ovariectomized rats. Bone 22:523–531
30. Nakajima A, Shimoji N, Shiomi K, Shimizu S, Moriya H, Einhorn TA, Yamazaki M (2002) Mechanisms for the enhancement of fracture healing in rats treated with intermittent low-dose human parathyroid hormone (1–34). J Bone Miner Res 17:2038–2047
31. Ejersted C, Andreassen TT, Hauge EM, Melsen F, Oxlund H (1995) Parathyroid hormone (1–34) increases vertebral bone mass, compressive strength, and quality in old rats. Bone 17:507–511
32. Boguslawski G, Hale LV, Yu XP, Miles RR, Onyia JE, Santerre RF, Chandrasekhar S (2000) Activation of osteocalcin transcription involves interaction of protein kinase A- and protein kinase C-dependent pathways. J Biol Chem 275:999–1006

A new bone-inducing biodegradable porous β-tricalcium phosphate

Naofumi Matsushita,[1] Hidetomi Terai,[1] Takao Okada,[2] Kazutoshi Nozaki,[3] Hikaru Inoue,[4] Shimpei Miyamoto,[1] Kunio Takaoka[1]

[1]*Department of Orthopaedic Surgery, Osaka City University School of Medicine, 1-4-3 Asahi-machi, Abeno-ku, Osaka 545-8585 Japan*

[2]*Research Dept., R&D Division, Taki Chemical Co., Ltd., 64-1 Nishiwaki, Befu-cho, Kakogawa-shi, Hyogo 675-0125, Japan*

[3]*Applied Pharmacology Laboratories, Institute for Drug Discovery Research, Yamanouchi Pharmaceutical Co., Ltd., 21 Miyukigaoka, Tsukuba, Ibaraki 305-8585, Japan*

[4]*Olympus Co., Ltd., 2-3 Kuboyamacho, Hachioji city, Tokyo 192-8512, Japan*

A new bone-inducing biodegradable porous β-tricalcium phosphate

Naofumi Matsushita,[1] Hidetomi Terai,[1] Takao Okada,[2] Kazutoshi Nozaki,[3] Hikaru Inoue,[4] Shimpei Miyamoto,[1] Kunio Takaoka[1]

[1]*Department of Orthopaedic Surgery, Osaka City University School of Medicine, 1-4-3 Asahi-machi, Abeno-ku, Osaka 545-8585 Japan*

[2]*Research Dept., R&D Division, Taki Chemical Co., Ltd., 64-1 Nishiwaki, Befu-cho, Kakogawa-shi, Hyogo 675-0125, Japan*

[3]*Applied Pharmacology Laboratories, Institute for Drug Discovery Research, Yamanouchi Pharmaceutical Co., Ltd., 21 Miyukigaoka, Tsukuba, Ibaraki 305-8585, Japan*

[4]*Olympus Co., Ltd., 2-3 Kuboyamacho, Hachioji city, Tokyo 192-8512, Japan*

Received 15 January 2004; revised 12 April 2004; accepted 6 May 2004
Published online 25 June 2004 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jbm.a.30102

Abstract: A new type of degradable biomaterial with bone-inducing capacity was made by combining porous β-tricalcium phosphate (β-TCP) with a delivery system for recombinant human bone morphogenetic protein-2 (rhBMP-2). The BMP delivery system consisted of a block copolymer composed of poly-D,L-lactic acid with random insertion of *p*-dioxanone and polyethylene glycol (PLA-DX-PEG), a known biocompatible and biodegradable material. The efficacy of this biomaterial in terms of its bone-inducing capacity was examined by ectopic bone formation in the dorsal muscles of the mouse. In the β-TCP implants coated with the PLA-DX-PEG polymer containing more than 0.0025% (w/w) of rhBMP-2, new ectopic bone tissues with marrow were consistently found on the surface of implants. The radiographic density of β-TCP was diminished in a time-dependent manner. On histological examination, numerous multinucleated osteoclasts with positive tartrate-resistant acid-phosphatase (TRAP) staining were noted on the surface of the β-TCP. These experimental results indicate that β-TCP implants coated with synthetic rhBMP-2 delivery system might provide effective artificial bone-graft substitutes with osteoinductive capacity and biodegradable properties. In addition, this type of biomaterial may require less rhBMP-2 to induce significant new bone mass. © 2004 Wiley Periodicals, Inc. J Biomed Mater Res 70A: 450–458, 2004

Key words: BMP; β-TCP; synthetic delivery system; osteoinductive; biodegradable

INTRODUCTION

Repair of bone fractures or defects is achieved by local new bone formation. However, the regenerative repair of bone is often impaired when the damage is severe as seen in comminuted open fractures or large bone defects associated with bone tumor resection. In these cases, autogenous bone grafting is routinely indicated to reactivate the regenerative potential and promote local bone formations because of its demonstrated efficacy.[1] The osteogenic potential of autogenous bone graft is due to the retention of osteogenic precursor cells with the ability to proliferate and differentiate to osteoblasts. Additionally, the grafted bone is resorbed and replaced by newly formed bone, thereby reestablishing a level of structural integrity at the grafted site. However, there are a couple of disadvantages associated with autogenous bone grafting. These include a limited source of donor bone coupled with donor site morbidities. To avoid these problems, new bone graft substitutes that exhibit bone-inducing capacity together with absorbability would be desirable. To date, there have been no absorbable materials with both osteoinductive and osteoconductive properties that have been proven as ideal substitutes for autogenous bone grafts, although a variety of biocompatible and osteoconductive materials have been reported.[2]

Porous beta-tricalcium phosphate (β-TCP) is well known as a biodegradable material with good osteoconductive capacity and demonstrated clinical efficiacy.[3] Some researchers have attempted to add bone-inducing capacity to β-TCP by combining this material with recombinant human bone morphoge-

Correspondence to: H. Terai; e-mail: hterai@med.osaka-cu.ac.jp

netic protein-2 (rhBMP-2) to accelerate bone healing.[4–7] Most of these studies have showed successful results by using β-TCP itself as a BMP carrier.

Meanwhile, considerable efforts have been focused on finding ways to reduce the minimum dose of rhBMP-2 that is essential for adequate bone regeneration. One of the difficulties when using rhBMP-2 clinically is the significant amount of this protein required for complete bone healing in humans. Therefore, the development of a carrier system that controls the release of rhBMP-2 is very important to reduce the dosage of rhBMP-2. We have already developed a biodegradable delivery system for rhBMP-2 that has been shown to enhance bone formation.[8–12]

In this study, we attempted to make a new biodegradable bone-inducing material by adding osteoinductive capacity of rhBMP-2 to porous β-TCP granules using a newly developed delivery system for rhBMP-2. Our goal was to test whether this approach could enhance bone formation using lower doses of rhBMP-2. The efficacy of this new bone graft substitute was examined in terms of its bone-inducing capacity and degradability in an experimental mouse model as a first step to further study in the clinic.

MATERIALS AND METHODS

Materials

As a rhBMP-2 delivery system, a block copolymer composed of poly-D,L-lactic acid with random insertion of *p*-dioxanone and polyethylene glycol (PLA-DX-PEG) was synthesized and provided by Taki Chemical (Kakogawa, Japan). The details of physicochemical properties of this polymer have been reported previously elsewhere.[10] RhBMP-2 was produced at Genetics Institute (Cambridge, MA) and donated to us through Yamanouchi Pharmaceutical Company (Ibaraki, Japan). Porous β-TCP (OSferion®, coarse granule, approximately 3 mm in particle diameter and 5 mg in weigh, from 100 to 400 μm in pore size, porosity of 75%, 1050° sintering temperature) was manufactured by Olympus (Tokyo, Japan) and donated to us for the purpose of these studies.[3,13]

Preparation of porous β-TCP granules combined with BMP delivery system

To prepare implants, 600 mg of β-TCP together with 200 mg of PLA-DX-PEG and various amounts of rhBMP-2 (0, 1.25, 5, 20, or 100 μg in 200 μL of 0.01 N HCl) were mixed in 3 mL of distilled acetone in glass vials. The resultant mixtures were then placed in a vacuum for a few seconds to replace air in the pores of the β-TCP with solvent. Acetone was then removed from the β-TCP granules by evaporation with a centrifuge evaporator. The glass vials were shaken several times during evaporation so that rhBMP-2 delivery material thoroughly impregnated the β-TCP granules. The resultant dried β-TCP granules coated with rhBMP-2 delivery system were stocked in a freezer at −30°C until use. A total of eight experimental groups including controls were prepared (Table I). The surface of porous β-TCP granule was observed by scanning electron microscopy (SEM; Hitachi 4700SI) to examine a structural characteristic of the PLA-DX-PEG coating.

TABLE I
Contents of rhBMP-2/PLA-DX-PEG in 600 mg of β-TCP and Bone Formation at 3 and 6 Weeks

	rhBMP-2 (μg)	PLA-DX-PEG (mg)	Concentration of rhBMP-2 (wt %)	Bone Formation	
				3 Weeks	6 Weeks
1	100	200	0.0125	+	++
2	20	200	0.0025	−	+
3	5	200	0.000625	−	−
4	1.25	200	0.000156	−	−
5	0	200	0	−	−
6	100	0	0.0167	+	+
7	20	0	0.0033	−	+
8	0	0	0	−	−

Materials from each group were divided into 20 implants (five to six coarse granules), respectively, and implanted into the back muscle pouch. Bone formation was rated in three grades by the pattern of newly formed bone induced around the implant. (++; uniformly covered by new bone +; partially covered by new bone −; no bone formation, assessed in soft X-ray radiographs)

Experimental protocol

One hundred sixty male ddY mice at 5 weeks of age, weighing 25–30 g, were used (20 per group) for this experiment in strict accordance with the institutional guidelines for the care and use of laboratory animals. The implants were aseptically placed into the left dorsal muscle pouch of mouse under anesthesia with diethyl ether. Approximately 30 mg of β-TCP granules including PLA-DX-PEG and rhBMP-2 (5–6 granules per animal) were implanted. Five animals from each group were sacrificed at 1, 2, 3, and 6 weeks after surgery, and the implants were harvested together with surrounding soft tissues. Harvested specimens were fixed in 10% neutral-buffered formalin solution and processed for radiological and histological examinations.

Radiographic and histological examination

The samples were radiographed with a soft X-ray apparatus (SOFRON®, Tokyo, Japan). For histological examination, samples were decalcified in 10% formic acid, dehydrated in a gradient ethanol series, mounted in paraffin, sectioned in 4 μm thickness and stained with hematoxylin-eosin. To detect osteoclasts, tartrate resistant acid phosphatase (TRAP) was stained by use of a histochemical method.

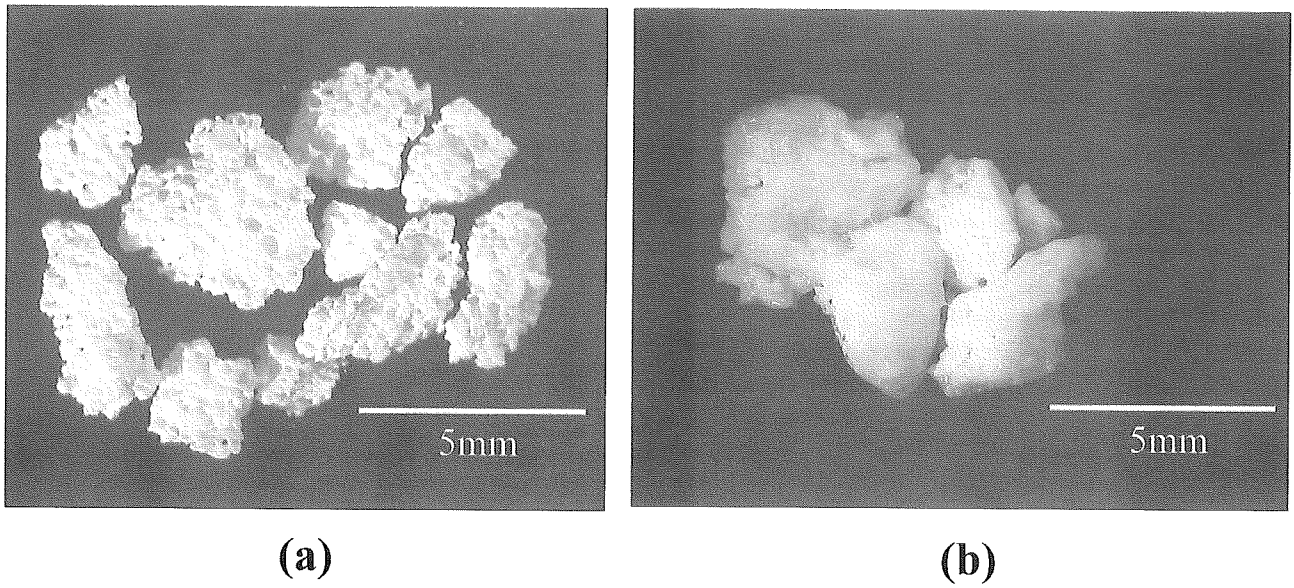

Figure 1. Macroscopic aspect of β-TCP coarse granules: (a) before coating, (b) after coating with PLA-DX-PEG. β-TCP granules exhibit adhesive-like properties at room temperature when coated with the polymer.

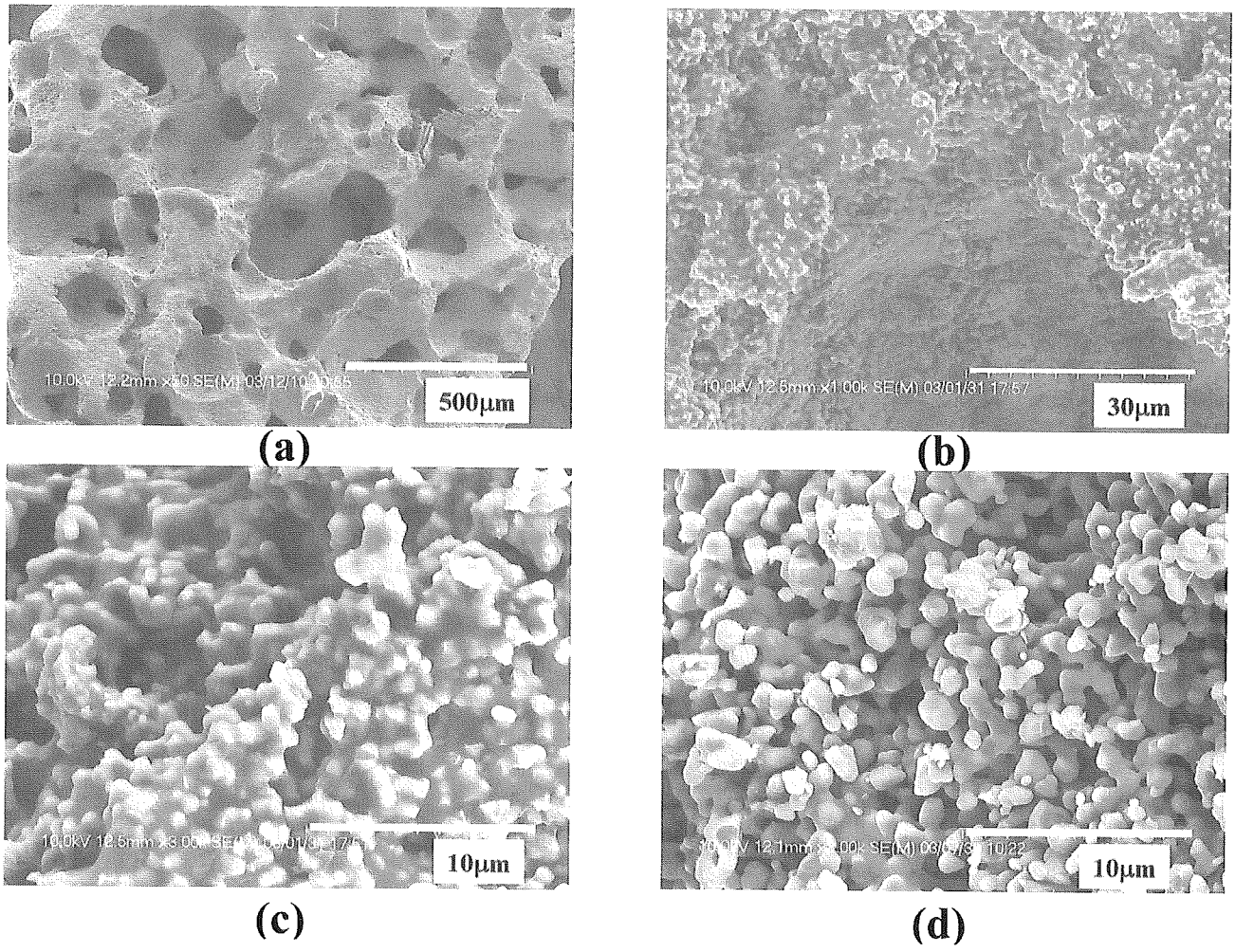

Figure 2. SEM micrographs of β-TCP granules coated with PLA-DX-PEG: (a) original magnification ×100, (b) original magnification ×1000, (c) original magnification ×3000 and noncoated β-TCP as a control; (d) ×3000. OSferion® is composed of 1 μm sintered β-TCP granules with 100~400 μm interconnected pores. The surface of β-TCP granules in the experimental group is uniformly covered by a thin polymer layer.

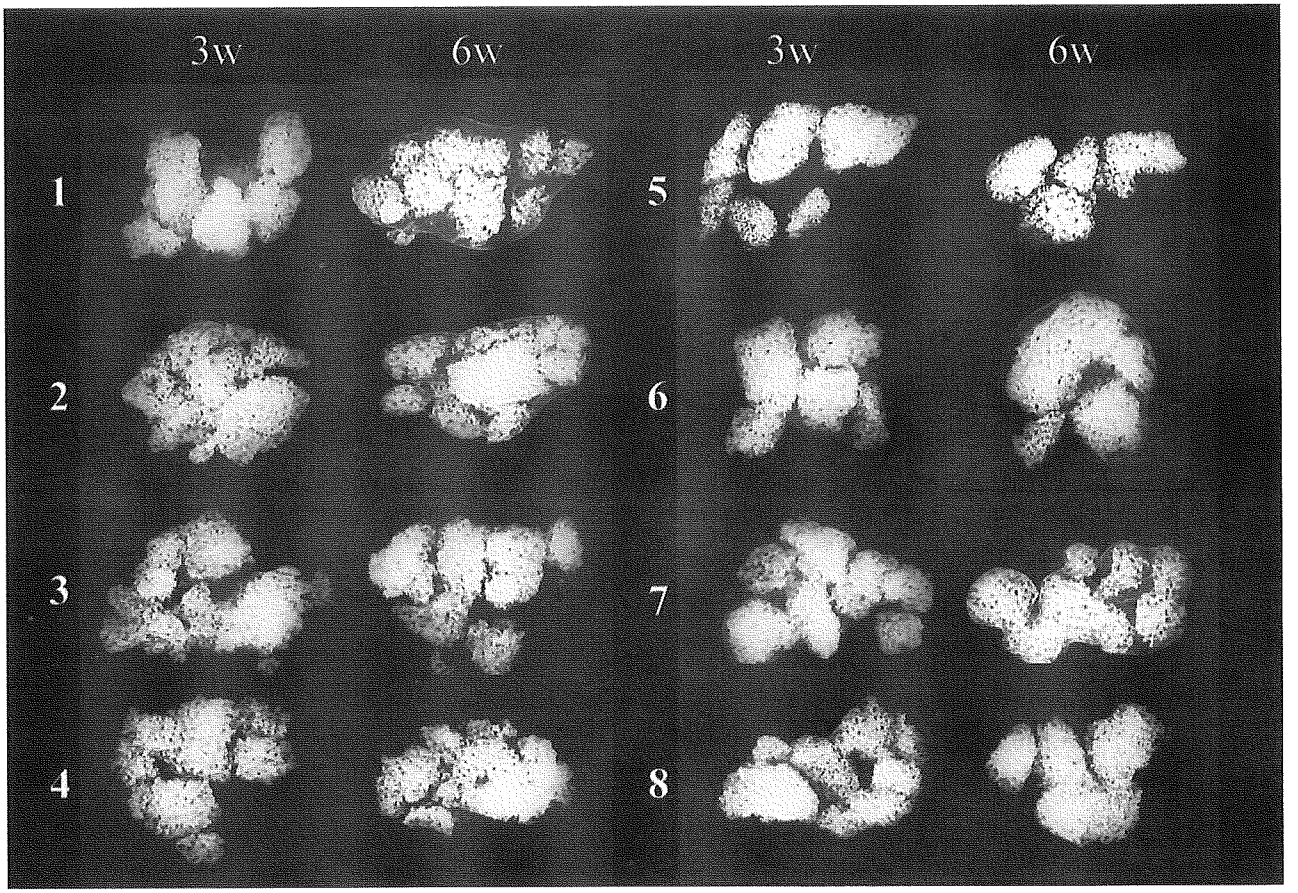

Figure 3. Typical soft X-ray photographs at 3 and 6 weeks after implantation are shown. The number on left side of each photograph indicates the number listed in Table I. Note that the new bone encasing the β-TCP granules is significant in group 1, 2, 6, and 7 compared to other experimental groups. The diminished density of β-TCP is recognized in the encased bone at 6 weeks in group 1.

Briefly, the deparaffinized sections were placed in the TRAP-staining solution consisting of acetate buffer (pH 5.0) 50 m*M* sodium tartrate, 25 mg/mL Naphthol-AS MX phosphate (Sigma Chemical Co., St. Louis, MO) and 0.5 mg/mL fast red violet salt (Sigma Chemical Co.). The specimens were incubated with the solution at 37°C for 120 min. After the solution was removed by washing, the specimens were counterstained with hematoxylin and observed under a light microscopy.

RESULTS

Surface structure of β-TCP granules

The porous granules of β-TCP are white in color and are easily crumbled by hand pressure. When combined with the polymer, the surface of the β-TCP granules became sticky to the touch at room temperature (Fig. 1). Scanning electron microscopy of the β-TCP granules revealed pores of 100–400 μm in diameter and the wall of the pores were comprised of tiny granules of approximately 1 μm in diameter. Electron micrographs (×3000) showed the uniform covering of each tiny β-TCP granule with the polymer (Fig. 2).

Ectopic bone formation on and in the implants

Soft X-ray examination

On soft X-ray radiographs, a shell-like radiopaque image encasing the β-TCP granules (indicating new bone formation) was noted in experimental groups 1 and 6 at 3 weeks after implantation. The same shell-like bone formation was recognized in groups 1, 2, 6, and 7 at 6 weeks after implantation. No bone formation on the X-ray radiographs was confirmed in other experimental groups at either 3 or 6 weeks after implantation. In the experimental group 1, the β-TCP granules had a more porous appearance and overall reduced density when compared with the original granules. At 6 weeks after implantation, the margins of the β-TCP granules became unclear as they were

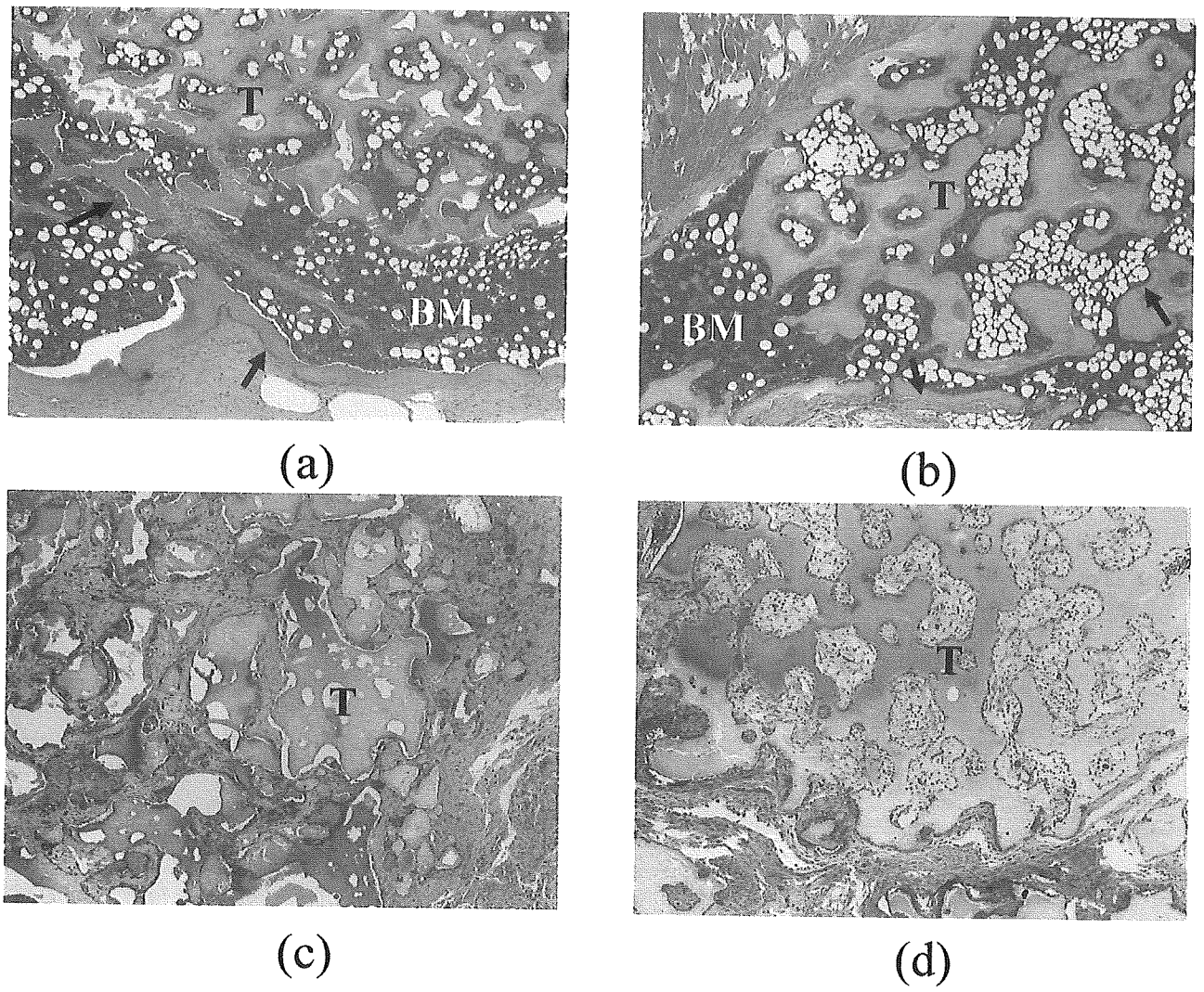

Figure 4. Typical histological sections at 6 weeks are shown: (a) group 1. (b) group 6, (c) group 5, and (d) group 8 (T, β-TCP; BM, bone marrow-like tissue; arrows, newly formed bone; Hematoxylin-eosin, decalcified sections; original magnification ×40).

progressively resorbed. The controls and composite implants from other groups did not show this appearance at the same interval after implantation (Table I and Fig. 3).

Histological examination

Histological examination of the composite implants revealed that both the outer and inner surface of the β-TCP was covered predominantly with new bone in the experimental groups 1, 2, 6, and 7 at 6 weeks. Hematopoietic and fatty marrows, like tissues, were also seen in the pore spaces. A significant difference in the amount of ectopically induced bone mass was noted between experimental groups 1 and 6. The implants used in these groups contained the same dose (5 μg per implant) of rhBMP-2 but used different amounts of carrier polymer (10 mg and 0 mg per implant, respectively). Harvested implants from group 1 were encased within the induced bone. However, in experimental group 6, a small amount of new bone mass unevenly covered the peripheral surface of the β-TCP granules. The implants lacking either rh-BMP-2 (group 5) or PLA-DX-PEG (group 8) failed to induce ectopic bone, and granulation tissues filled the β-TCP granules at 6 weeks (Fig. 4).

A typical pattern of ectopic bone formation induced by the material over time is shown. A large number of hypertrophic chondrocytes and mesenchymal cells were located between the β-TCP granules at 1 week. Trabecular bone encasing β-TCP granules and a few residual hypertrophic chondrocytes were seen at 2 weeks. The encasing bone had thickened and fatty marrow-like tissue occupied the space between the β-TCP granules at 3 weeks. At 6 weeks, prominent trabecular bone, and marrow-like tissue enveloped the

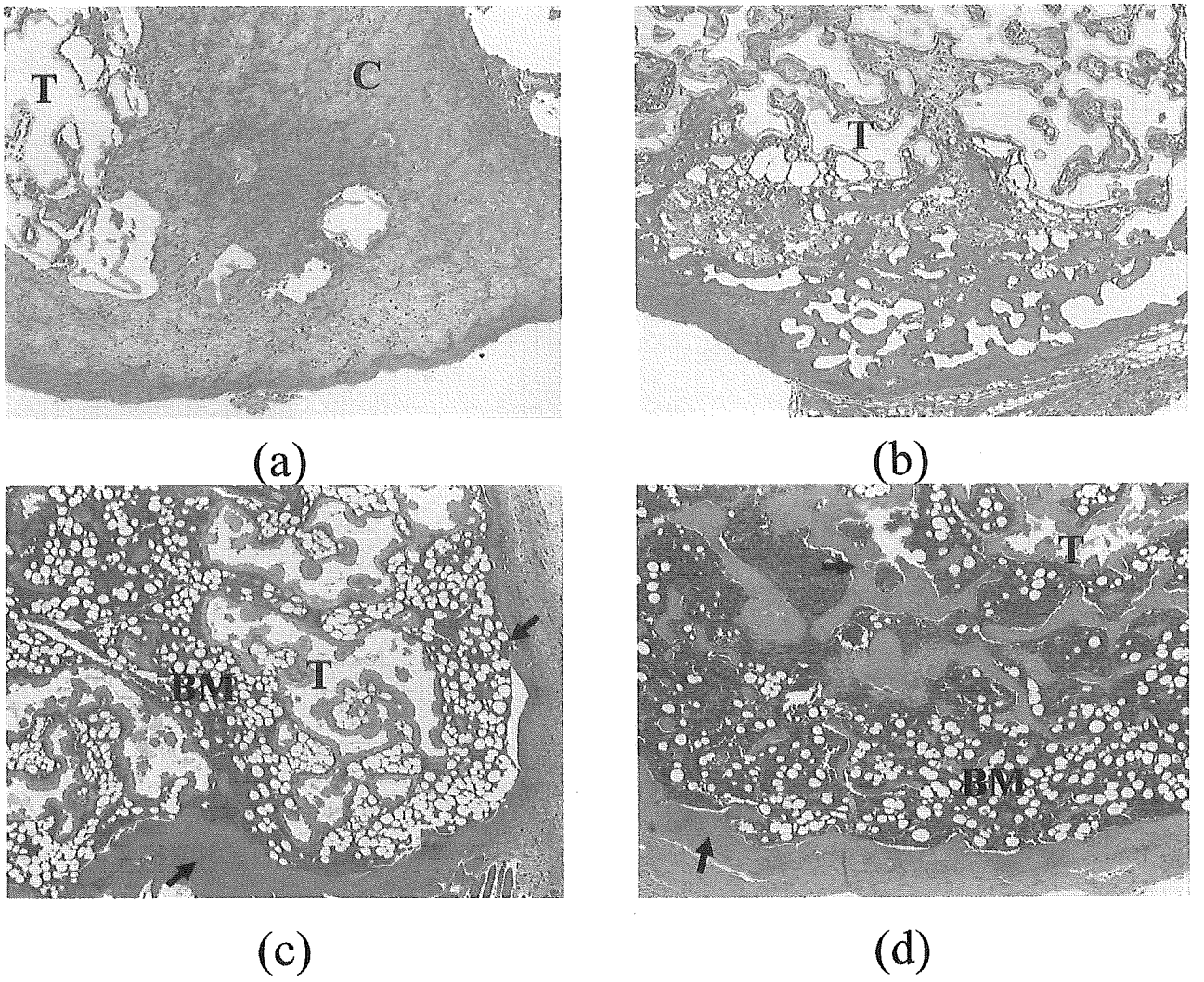

Figure 5. Time-dependent bone formation in group 1: (a) 1 week, (b) 2 weeks, (c) 3 weeks, and (d) 6 weeks after implantation (C, cartilage; T, β-TCP; BM, bone marrow-like tissue; arrows, newly formed bone; Hematoxylin-eosin, decalcified sections; original magnification ×40).

remnants of the β-TCP granules (Fig. 5). Dose-dependent ectopic bone formation was shown (Fig. 6). TRAP staining revealed that most of the surface of the BMP2/polymer-coated β-TCP granules was surrounded by numerous multinucleated TRAP positive osteoclasts. Few osteoclasts were seen on the surface of β-TCP in the group without polymer or rhBMP-2 for the duration of the experiment (Fig. 7).

DISCUSSION

To construct an artificial implant with bone-inducing capacity similar to that obtained with autogenous bone graft materials, porous β-TCP coarse granules were combined with a degradable polymer and rhBMP-2. The data from the present study indicates that this composite material retains bone-inductive and biodegradable properties. Because β-TCP itself is known to be osteoconductive, we examined the bone-inducing potential of the composite in tissues other than bone.[3,13] β-TCP granules with pore sizes of 100–400 μm in diameter were chosen in the present study to provide a sufficient local concentration of BMP2 via the polymer carrier material. The polymer carrier was critical for the induction of new bone in and on the β-TCP granules. In this study, we compared the ectopically induced bone among each implant at 3 and 6 weeks after implantation because the obvious bone formation was not detected by radiographs until 3 weeks. In control implants bearing rhBMP-2 in the absence of the carrier polymer, only a small amount of new bone was observed within the pores of the porous β-TCP. In a previous study, we had demonstrated that the carrier polymer enhanced new bone formation through its slow degradation and continuous release

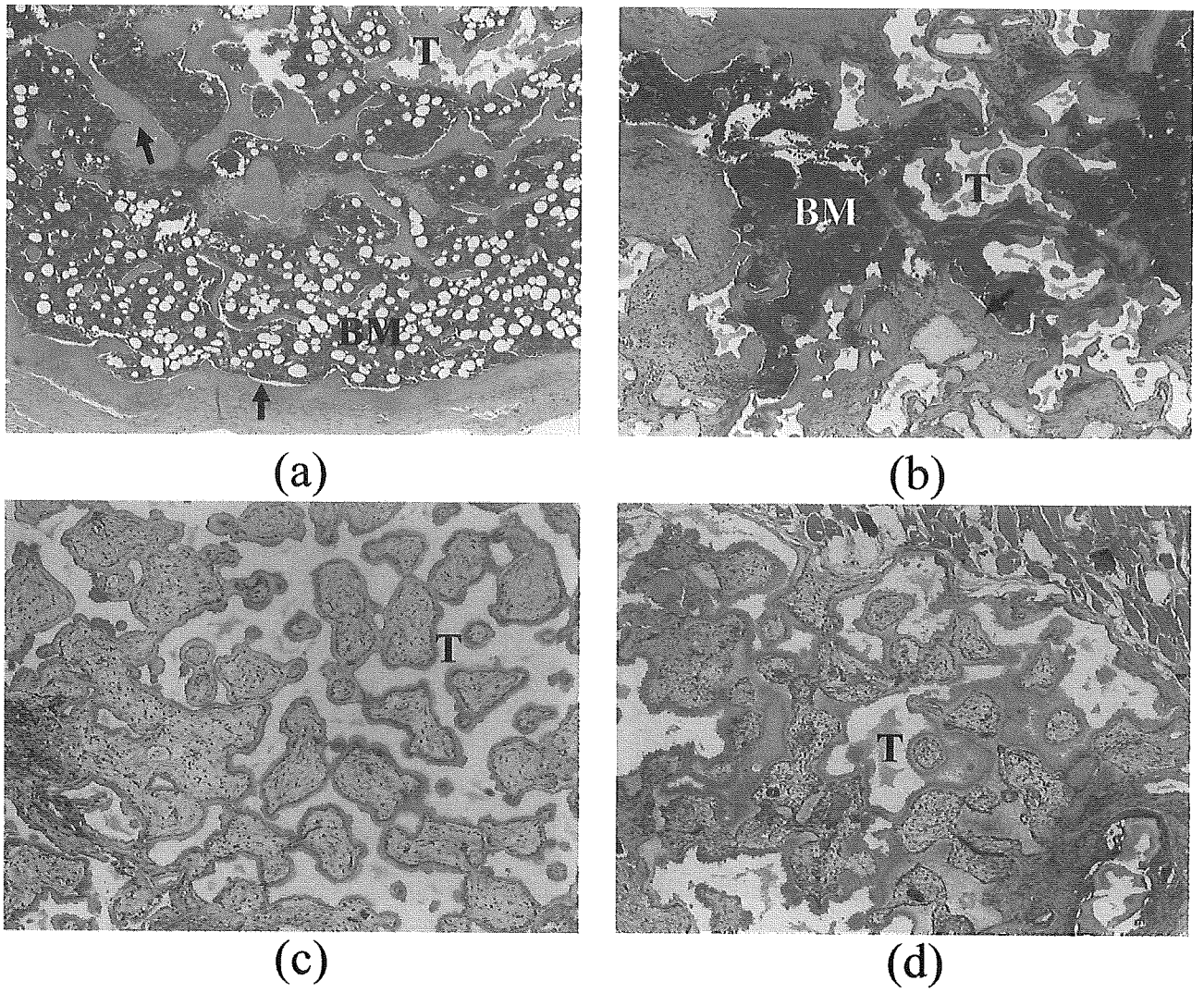

Figure 6. Dose-dependent bone formation at 6 weeks after implantation: (a) group 1 (b) group 6, (c) group 5, and (d) group 8 (T, β-TCP; BM, bone marrow-like tissue; arrows, newly formed bone; Hematoxylin-eosin, decalcified sections; original magnification ×40).

of rhBMP-2 *in vivo*. The dose of rhBMP-2 in β-TCP appeared to be the determinant of the bone mass in the present study.

The dose of rhBMP-2 required to elicit ectopic bone formation has been the focus of considerable experimental attention. It is known that the minimum dose of the rhBMP-2 required for bone formation is different depending on the animal species. The minimum dose required in primates or humans is approximately 1mg per 1 cm^3 carrier material (collagen sponge). This requirement makes this approach a very expensive clinical option. We have, therefore, pursued methods that would reduce the dose of rhBMP-2 required to repair injured bone in clinical practice. Another important consideration is the delivery system for rhBMP-2. Currently, collagen sponge, which has no mechanical strength, is used as the standard carrier material for the clinical use of rhBMP-2.[14–16] We postulated that one possible way to overcome these issues might be to coat the surface of biomaterials such as ceramics or metals with a thin layer of the rhBMP-2/polymer delivery material. The coating might enable a reduction in the efficacious dose ofrhBMP-2 required for bone induction. Additionally, the combination of the rhBMP-2 delivery system with the biomaterial would provide the implant with mechanical strength.[17]

In the present study, β-TCP granules with a porous structure and absorbable potential were used with expectation that they would enable bone formation by BMP and replacement by normal bone. This absorbable nature of β-TCP has been reported both in experimental animal models and in clinical cases.[3,13] In the murine model used in the present study, the β-TCP granules, as expected, appeared to be resorbed in the induced bone. Their reduced density and marginal line image became equivocal on radiography over the experimental period. The changes of the β-TCP im-

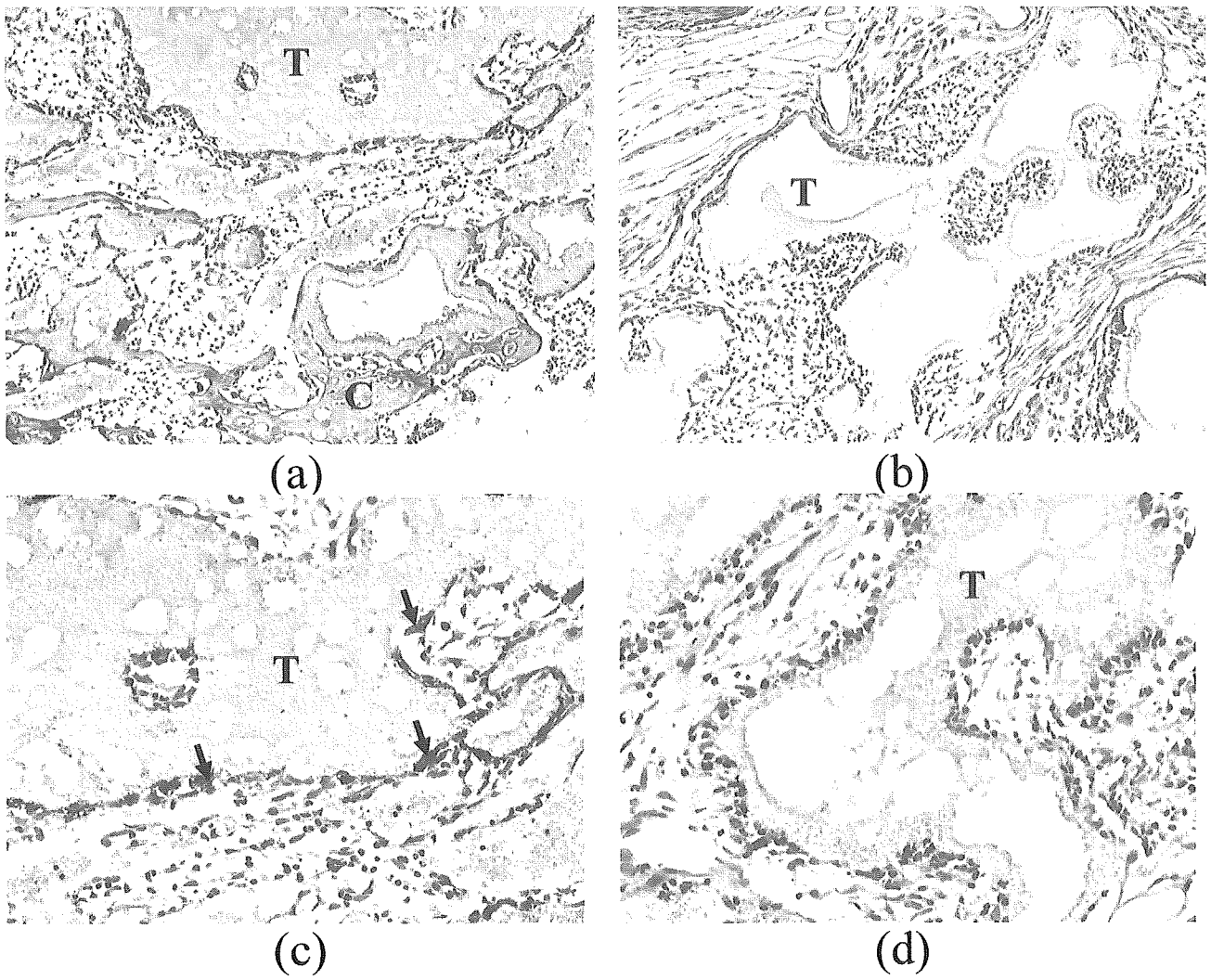

Figure 7. Tartrate resistant acid phosphatase (TRAP) stained histological sections of decalcified specimens harvested at 2 weeks: (a) original magnification ×40 and (c) original magnification ×200 from group 1, (b) original magnification ×40 and (d) original magnification ×200 from group 8. A wide range of β-TCP granules coated with PLA-DX-PEG and rhBMP-2 are surrounded by TRAP positive multinucleated osteoclasts (arrows, stained red). (T, β-TCP; OC, osteoclast; C, cartilage).

ages were seen consistently in samples with new bone and may have occurred through resorption of the β-TCP by osteoclasts within the bone. The reduction of β-TCP mass indicates enhanced degradation of β-TCP within bone because the densities of the β-TCP granules were not reduced in control samples in the absence of new bone formation over the experimental period. The time for complete disappearance of β-TCP granules in the ectopically induced bone was not determined due to the short length of the observation period.

In conclusion, an implant with properties similar to autogenous bone-graft material was constructed by combining porous β-TCP granules with a hydrogel polymer and rhBMP-2. The efficacy of the composite implants was determined by the degree of ectopic bone formation and mass reduction of β-TCP over a 6-week period. The composite implants retaining an adequate amount of the BMP delivery system consistently induced new bone on and in the porous β-TCP with a reduction in mass of β-TCP at 3 weeks after implantation. These findings suggest that it may be possible to develop a bone graft substitute through the combination of specific osteoinductive materials. This type of bone graft would obviate the need for autogenous bone and thereby eliminate the functional and cosmetic morbidities associated with surgical procurement of the bone grafts. However, further studies in large mammals or primates to check for safety and efficacy will be necessary before this material can be used in the clinic.

References

1. Einhorn TA. Enhancement of fracture-healing. J Bone Joint Surg Am 1995;77:940–956.

2. Bauer TW, Muschler GF. Bone graft materials. An overview of the basic science. Clin Orthop 2000;371:10–27.
3. Ogose A, Hotta T, Hatano H, Kawashima H, Tokunaga K, Endo N, Umezu H. Histological examination of beta-tricalcium phosphate graft in human femur. J Biomed Mater Res 2002;63:601–604.
4. Jingushi S, Urabe K, Okazaki K, Hirata G, Sakai A, Ikenoue T, Iwamoto Y. Intramuscular bone induction by human recombinant bone morphogenetic protein-2 with beta-tricalcium phosphate as a carrier: In vivo bone banking for muscle-pedicle autograft. J Orthop Sci 2002;7:490–494.
5. Alam I, Asahina I, Ohmamiuda K, Enomoto S. Comparative study of biphasic calcium phosphate ceramics impregnated with rhBMP-2 as bone substitutes. J Biomed Mater Res 2001; 54:129–138.
6. Laffargue P, Hildebrand HF, Rtaimate M, Frayssinet P, Amoureux JP, Marchandise X. Evaluation of human recombinant bone morphogenetic protein-2-loaded tricalcium phosphate implants in rabbits' bone defects. Bone 1999;25(Suppl):55S–58S.
7. Urist MR, Nilsson O, Rasmussen J, Hirota W, Lovell T, Schmalzreid T, Finerman GA. Bone regeneration under the influence of a bone morphogenetic protein (BMP) beta tricalcium phosphate (TCP) composite in skull trephine defects in dogs. Clin Orthop 1987;214:295–304.
8. Saito N, Okada T, Horiuchi H, Ota H, Takahashi J, Murakami N, Nawata M, Kojima S, Nozaki K, Takaoka K. Local bone formation by injection of recombinant human bone morphogenetic protein-2 contained in polymer carriers. Bone 2003;32: 381–386.
9. Murakami N, Saito N, Horiuchi H, Okada T, Nozaki K, Takaoka K. Repair of segmental defects in rabbit humeri with titanium fiber mesh cylinders containing recombinant human bone morphogenetic protein-2 (rhBMP-2) and a synthetic polymer. J Biomed Mater Res 2002;62:169–174.
10. Saito N, Okada T, Horiuchi H, Murakami N, Takahashi J, Nawata M, Ota H, Nozaki K, Takaoka K. A biodegradable polymer as a cytokine delivery system for inducing bone formation. Nat Biotechnol 2001;19:332–335.
11. Saito N, Okada T, Horiuchi H, Murakami N, Takahashi J, Nawata M, Ota H, Miyamoto S, Nozaki K, Takaoka K. Biodegradable poly-D,L-lactic acid-polyethylene glycol block copolymers as a BMP delivery system for inducing bone. J Bone Joint Surg Am 2001;83-A(Suppl 1-Pt 2):S92–S98.
12. Saito N, Okada T, Toba S, Miyamoto S, Takaoka K. New synthetic absorbable polymers as BMP carriers: Plastic properties of poly-D,L-lactic acid-polyethylene glycol block copolymers. J Biomed Mater Res 1999;47:104–110.
13. Saito M, Shimizu H, Beppu M, Takagi M. The role of beta-tricalcium phosphate in vascularized periosteum. J Orthop Sci 2000;5:275–282.
14. Boden SD, Kang J, Sandhu H, Heller JG. Use of recombinant human bone morphogenetic protein-2 to achieve posterolateral lumbar spine fusion in humans: A prospective, randomized clinical pilot trial: 2002 Volvo Award in clinical studies. Spine 2002;27:2662–2673.
15. Sandhu HS. Bone morphogenetic proteins and spinal surgery. Spine 2003;28:S64–S73.
16. Sandhu HS, Kanim LE, Kabo JM, Toth JM, Zeegen EN, Liu D, Delamarter RB, Dawson EG. Effective doses of recombinant human bone morphogenetic protein-2 in experimental spinal fusion. Spine 1996;21:2115–2122.
17. Murakami N, Saito N, Takahashi J, Ota H, Horiuchi H, Nawata M, Okada T, Nozaki K, Takaoka K. Repair of a proximal femoral bone defect in dogs using a porous surfaced prosthesis in combination with recombinant BMP-2 and a synthetic polymer carrier. Biomaterials 2003;24:2153–2159.

ELSEVIER

Bone 35 (2004) 375–382

BONE

www.elsevier.com/locate/bone

A meta-analysis of previous fracture and subsequent fracture risk

J.A. Kanis,[a,*] O. Johnell,[b] C. De Laet,[c] H. Johansson,[d] A. Oden,[d] P. Delmas,[e] J. Eisman,[f] S. Fujiwara,[g] P. Garnero,[e] H. Kroger,[h] E.V. McCloskey,[a] D. Mellstrom,[i] L.J. Melton,[j] H. Pols,[c] J. Reeve,[k] A. Silman,[l] and A. Tenenhouse[m]

[a] *WHO Collaborating Centre for Metabolic Bone Diseases, WHO Collaborating Centre, University of Sheffield Medical School, Sheffield, UK*
[b] *Department of Orthopaedics, Malmo General Hospital, Sweden*
[c] *Department of Public Health, Erasmus Medical Center, Rotterdam, The Netherlands*
[d] *Consulting Statistician, Gothenburg, Sweden*
[e] *INSERM Unite 403, Hopital Edouard Herriot, Lyon, France*
[f] *Bone and Mineral Research, Garvan Institute of Medical Research, NSW, Australia*
[g] *Radiation Effects Research Foundation, Hiroshima, Japan*
[h] *Department of Surgery, Kuopio University Hospital, Kuopio, Finland*
[i] *Department of Geriatric Medicine, University of Goteborg, Sweden*
[j] *Division of Epidemiology, Mayo Clinic, Rochester, MN, USA*
[k] *Strangeways Research Laboratories, Cambridge, UK*
[l] *ARC Epidemiology Research Unit, University of Manchester, Manchester, UK*
[m] *Division of Bone Metabolism, The Montreal General Hospital, Montreal, Canada*

Received 15 January 2004; revised 17 March 2004; accepted 24 March 2004

Abstract

Previous fracture is a well-documented risk factor for future fracture. The aim of this study was to quantify this risk on an international basis and to explore the relationship of this risk with age, sex, and bone mineral density (BMD). We studied 15259 men and 44902 women from 11 cohorts comprising EVOS/EPOS, OFELY, CaMos, Rochester, Sheffield, Rotterdam, Kuopio, DOES, Hiroshima, and two cohorts from Gothenburg. Cohorts were followed for a total of 250000 person-years. The effect of a prior history of fracture on the risk of any fracture, any osteoporotic fracture, and hip fracture alone was examined using a Poisson model for each sex from each cohort. Covariates examined were age, sex, and BMD. The results of the different studies were merged by using the weighted β-coefficients.

A previous fracture history was associated with a significantly increased risk of any fracture compared with individuals without a prior fracture (RR = 1.86; 95% CI = 1.75–1.98). The risk ratio was similar for the outcome of osteoporotic fracture or for hip fracture. There was no significant difference in risk ratio between men and women. Risk ratio (RR) was marginally downward adjusted when account was taken of BMD. Low BMD explained a minority of the risk for any fracture (8%) and for hip fracture (22%). The risk ratio was stable with age except in the case of hip fracture outcome where the risk ratio decreased significantly with age.

We conclude that previous history of fracture confers an increased risk of fracture of substantial importance beyond that explained by measurement of BMD. Its validation on an international basis permits the use of this risk factor in case finding strategies.

Keywords: Prior fracture; Meta-analysis; Hip fracture; Osteoporotic fracture

Introduction

It is well established from many cohort, case-control, and cross-sectional studies that a prior osteoporotic fracture increases the risk of future fractures [1–8]. A prior forearm fracture is associated with about a twofold increase in the subsequent risk of fracture [9–13]. More recently, significant increases in risk have been described for prior fractures at other sites characteristic of osteoporosis [6,7,14–21]. The risk of another vertebral fracture is particularly high after a spine fracture [7,22–24]. Similar observations are found in the setting of randomized clinical trials. In the placebo arm, the risk of vertebral deformities is approximately fivefold

* Corresponding author. WHO Collaborating Centre for Metabolic Bone Diseases, University of Sheffield Medical School, Beech Hill Road, Sheffield S10 2RX, UK. Fax: +44-114-285-1813.
E-mail address: w.j.Pontefract@shef.ac.uk (J.A. Kanis).

8756-3282/$ - see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.bone.2004.03.024

higher in patients with a prior vertebral deformity than in those without [4,25,26]. The interrelationships between the site of prior fracture and site of subsequent fracture have been summarized by meta-analysis [27] and a large case-control study, published more recently found broadly similar relationships [8].

Increased fracture risk may be in part due to the fact that patients with fracture have low bone mineral density (BMD). Studies that have adjusted for BMD suggest that the relative risk is only modestly downward adjusted [3,20,24,28–31].

The consistent association between a prior fracture and subsequent fracture risk has led to the inclusion of prior fracture as a risk factor to be used in assessment guidelines [32–35]. For example, in Europe, it is recommended that patients be identified on the basis of risk factors for subsequent assessment by BMD [33–35]. Patients are then considered for intervention on the finding of osteoporosis (i.e., a T score of ≤ -2.5 SD). This approach is conservative since it does not recognize the independent contribution of the risk factor from BMD. This has been recognized in some guidelines where the intervention threshold is less conservative in the presence of a risk factor such as a prior fragility fracture [32,36]. The consideration of several independent risk factors permits the more accurate categorization of risk [37], and attention has focussed recently on the assessment of fracture risk using multiple risk factors, rather than the use of BMD alone, to define intervention thresholds [38,39]. This demands knowledge of the interrelationships between these risk factors.

The aim of the present study was to quantify the risk associated with a history of prior fracture for future fracture in an international setting and to explore the dependence of this risk with age, sex, and BMD.

Methods

We studied 60 161 men and women of whom 26% had a prior fracture history taken from 11 prospectively studied cohorts. Brief details of the cohorts studied are given below and summarized in Table 1.

OFELY

The OFELY cohort comprises an age-stratified cohort of 1039 women aged 31–89 years randomly selected from the regional section of a large health insurance company (Mutuelle Generale d'Education Nationale, Lyon) [40]. Eighteen percent of women contacted participated in the study. Baseline characteristics were obtained using a standardized questionnaire, including the documentation of prior wrist, humeral, vertebral, and hip fracture that occurred after the age of 45 years. Only low trauma fractures (falls from a standing height or less) were recorded. BMD was measured at the lumbar spine, at the proximal femur, distal radius, and whole body by DXA using a Hologic QDR 2000. Women were reviewed annually and fractures registered. Peripheral fractures were confirmed by radiography. Vertebral fractures were identified from sequential X-rays of the thoracic and lumbar spine by morphometry in 80% of patients, but only clinical fractures were used for this analysis.

EVOS

The European Vertebral Osteoporosis Study (EVOS) comprised age- and sex-stratified random samples from 36 centers in 19 European countries [41–43]. Equal numbers of men and women were drawn in each center within six 5-year age bands (50–54 to 75–79 years). A baseline radiograph for vertebral fracture prevalence was undertaken in 15 570 men and women (response rate, 29%). BMD was measured in 3461 men and women from 13 centers by DXA at the femoral neck using pencil beam machines that were cross-calibrated using the European spine phantom. The sample provided the framework for the European Prospective Osteoporosis study (EPOS) where repeated assessment was undertaken in 29 of the centers. Information on clinical fractures were used for this report.

Table 1
Details of cohorts studied including individuals with information on follow-up time, prior fracture, and subsequent fracture

Cohort	Sample size	Person-years	Mean age (years)	Age range (years)	% Female	Fracture history (%)	Any fracture	Osteoporotic fracture	Hip fracture
EPOS	13366	40160	63.8	41–91	52	36	715	715	44
OFELY	426	2124	64.2	50–89	100	16	53	–	–
CaMos	9400	26653	62.1	25–103	69	44	586	316	42
Rochester	1001	6228	56.8	21–94	65	18	289	244	42
Sheffield	2147	6826	80.0	74–96	100	51	284	236	62
Rotterdam	7774	43606	70.3	55–106	61	14	992	768	284
Kuopio	11798	56602	52.3	47–57	100	17	1053	–	–
Gothenburg I	2375	16439	78.8	69–86	61	9	431	431	336
Gothenburg II	7098	29750	58.9	21–89	100	18	441	312	29
Dubbo	2163	16333	70.7	57–96	61	15	532	418	107
Hiroshima	2613	9861	65.1	47–95	70	26	187	90	32
Totals	60161	254582	62.9	21–106	75	26	5563	3530	978

CaMos

The Canadian Multicentre Osteoporosis Study (CaMos) is an ongoing prospective age-stratified cohort. The study is documenting the incidence of fractures and risk factors in a random sample of 9424 men and women aged 25 years or more selected by telephone listings. The sampling frame is from nine study centers in nine provinces [44]. Characterization of individuals was by interview. BMD was measured by DXA at the hip (Hologic QDR 1000) and lumbar spine, and an ultrasound scan taken at the heel in individuals aged 50 years or more.

Rochester

The Rochester cohort was recruited from two random population samples stratified by decade of age, one comprising women who were subsequently followed for up to 20 years [45] and another sample of women and men followed for 8 years [46]. The response rates were 49.8% in the women and 38.7% in the men. BMD of the right femoral neck was measured by dual photon absorptiometry in the first cohort (cross calibrated to DXA) and by DXA (Hologic QDR 2000) in the second group. Fractures were ascertained by periodic interview combined with review of the inpatient and outpatient medical records of all local care providers.

Sheffield

The Sheffield cohort comprised women aged 75 years or more selected randomly from the population of Sheffield, UK, and surrounding districts between 1993 and 1999. Approximately 35 000 women, identified from general practitioner listings, were contacted by letter and invited to attend for assessment of their skeletal status. Five thousand eight hundred and seventy-three women were willing to attend for the screening visit. Of these, 281 were excluded, and the remainder randomly allocated after informed consent to treatment with the bisphosphonate clodronate, or to an identical placebo. This study is still in progress, and the material used for the present paper comprised 2148 women allocated to treatment with placebo [47]. All women had baseline assessment of BMD undertaken at the femoral neck using the Hologic 4500. Outcomes were assessed by 6 monthly home visits.

Rotterdam

The Rotterdam study, begun in 1990, is a prospective cohort study that aimed to examine and follow-up all residents aged 55 years and older living in Ommoord, a district of Rotterdam [48]. By 1993, 7983 residents had been included (response rate, 78%). Bone mineral density was assessed at the femoral neck by DXA using a Lunar DPX-L [49]. Fracture follow-up was undertaken using an automated link with general practitioner computer systems and hospital admission data. Fracture data were collected and validated by two independent research physicians. For this analysis, validated fracture follow up was available for 7774 participants (3065 men) with an average follow up time of 6 years. Femoral neck BMD was measured in 5776 individuals (2432 men).

Kuopio

The Kuopio osteoporosis risk factor and prevention (OSTPRE) study in Finland comprised a postal inquiry sent to all 14 220 women aged 47–56 who were residents of Kuopio province in 1989. Thirteen thousand and one hundred women responded to the inquiry, of whom 1214 were excluded for incomplete information. This left a study population of 11 886 women. A random stratified sample of 3222 women underwent bone mineral densitometry by DXA using the Lunar DPX [50].

Gothenburg I

This study comprised four birth cohorts of 2375 randomly sampled men and women aged 70 years or more followed for up to 20 years at Gothenburg [51,52] after a baseline BMD measurement. The participation rate was 73%. The participants were drawn randomly from the population register in Gothenburg by the date of birth to provide cohorts aged 70, 76, 79, and 85 years at the time of investigation. Bone mineral density was measured at the right heel using dual photon absorptiometry.

Gothenburg II

The Gothenburg study comprised a randomly drawn population cohort of women aged 21–89 years followed up to 7.9 years (mean 4.2 years) [53]. Seventy percent of those invited (approximately 7000 women) participated in the study that examined risk factors for osteoporosis by use of a standardized questionnaire. BMD was assessed at baseline at the distal forearm using the Osteometer DTX 200.

DOES

The Dubbo Osteoporosis Epidemiology Study (DOES) is a population-based study with multiple assessments of skeletal status in men and women aged 60 years or more from Dubbo, Australia [54]. Participation in the study was 56% of the population. Baseline measurements included BMD at the femoral neck assessed using DXA (GE-Lunar, DPX). Fractures are identified through radiologists reports from the two centers servicing the region.

Hiroshima

The Adult Health Study in Hiroshima (AHS) documents the late health effects of radiation exposure among atomic

Table 2
Details of the construct of the questionnaire on fracture history in the cohorts studied

Cohort	Question
EVOS/EPOS	Have you ever suffered a fracture?
CaMos	Have you ever fractured any bones?
Rochester	Prior fracture with moderate trauma
Rotterdam	History of any fracture in the past 5 years
Gothenburg I	Evidence of any fracture (in hospital records)
OFELY	History of a fragility fracture from low trauma since age of 30 years
Sheffield	History of any fracture
Kuopio	History of any fracture from the age of 15 years
Gothenburg II	History of fracture after the age of 25 years
Dubbo	Self-reported
Hiroshima	Self-reported

bomb survivors in Hiroshima and Nagasaki. The original AHS cohort consisted of about 15 000 atomic bomb survivors and 5000 controls selected from residents in Hiroshima and Nagasaki using the 1950 national census supplementary schedules and the Atomic Bomb Survivors Survey. AHS subjects have been followed through biennial medical examinations since 1958 with a participation rate of approximately 80% throughout this period. BMD at the lumbar spine and proximal femur has been measured at each biennial health examination using DXA (Hologic QDR-2000) since December 1993. Trained nurses interviewed the subjects about baseline risk factors and measured height and weight at each biennial visit [55,56].

Baseline and outcome variables

The construct of the question to determine a prior fracture history differed between the cohorts studied (Table 2). Prospective fracture ascertainment was undertaken by self-report (Sheffield, Kuopio, EVOS/EPOS; Hiroshima) and/or verified from hospital central data bases (CaMos, Sheffield, EVOS/EPOS, Rochester, Rotterdam, Kuopio, Gothenburg I and II, CaMos, DOES). The EPOS and OFELY study also included sequential systematic radiography to define incident vertebral deformities, but were not used in this analysis. Information on all clinical fractures was used for this report. In addition, fractures considered to be due to osteoporosis were analyzed, and finally, hip fracture alone was considered separately. For Kuopio and OFELY, all fractures were recorded and no distinction made between fracture sites. In the case of the EPOS study and Gothenburg I, osteoporotic fractures only were recorded. In the other cohorts, an osteoporotic fracture was one considered to be due to osteoporosis either by the investigator or by the Coordinating Centre. For the EVOS study, osteoporotic fractures comprised hip, forearm, humeral, or limb fractures. For the CaMos study, they comprised fractures of the spine, pelvis, ribs, distal forearm, forearm, and hip. In the other cohorts (Sheffield, Rotterdam, Rochester, Gothenburg I, DOES, Hiroshima), fractures at sites considered to be characteristic for osteoporosis were [38] extracted from the data.

Statistical methods

The risk of fracture was estimated by Poisson regression applied to each cohort and each sex separately. Covariates included time since start of follow up, current age, prior history of fracture, and BMD. We additionally excluded BMD from the model. A further model included the interaction term prior fracture × time to determine whether the strength of the association of prior fracture and fracture risk waned with time. The beta value for each sex in each cohort is age-dependent, beta_{k+} beta_{k+1}·age. The estimated value of beta_{k+} beta_{k+1}·age was determined for each age from 50 to 85 years, together with the variance. Results of each cohort and the two sexes were weighted according to the variance and merged to determine the weighted means and standard deviations. The risk ratio (RR) of those with a prior fracture history versus those without a prior fracture history was equal to e^{mean}.

The component of the risk ratio explained by BMD was computed from a meta-analysis of BMD and fracture risk [57]. The risk of any fracture was assumed to increase 1.6-fold for each SD decrease in BMD. For hip fracture, the gradient of risk was assumed to be 2.6 per SD. The proportion of risk attributed to a low BMD was computed as

$$\frac{[\log RR_a/\log GR] - [\log RR_b/\log GR]}{[\log RR_a/\log GR]}$$

where RR_a is the unadjusted risk ratio, RR_b is the risk ratio adjusted for BMD, and GR is the gradient of risk.

Results

Of 60 161 men and women studied, 877 men and 4686 women were identified as having a subsequent fracture of any kind, of which 680 and 2850 were characterized as osteoporotic in men and women, respectively. Two hundred and eleven men and 767 women sustained a hip fracture. The total follow-up was 61 938 person years in men and 192 644 in women. BMD measurements were available in 62% of individuals.

Table 3
Prevalence of a prior fracture history in men and women by age

Age (years)	Probability of fracture history (%)		
	Men	Women	Combined
30	44	15	24
40	43	18	27
50	42	23	30
60	41	29	34
70	40	35	37
80	39	41	41
90	38	48	45

Table 4
Risk ratio (RR) and 95% confidence interval (CI) of fracture associated with a history of prior fracture in men and women, without and with adjustment for BMD

Outcome fracture	Men		Women		Combined	
	RR	95% CI	RR	95% CI	RR	95% CI
A. Without BMD						
Any	2.02	1.73–2.38	1.84	1.72–1.96	1.86	1.75–1.98
Osteoporotic	1.93	1.61–2.33	1.85	1.70–2.01	1.86	1.72–2.01
Hip	2.30	1.56–3.41	1.77	1.49–2.11	1.85	1.58–2.17
B. With BMD						
Any	2.04	1.67–2.48	1.73	1.59–1.88	1.77	1.64–1.91
Osteoporotic	1.91	1.50–2.43	1.74	1.57–1.92	1.76	1.60–1.93
Hip	1.97	1.12–3.48	1.56	1.23–1.98	1.62	1.30–2.01

Probability of fracture history rose almost linearly with age (Table 3). The probability of recording a history of a prior fracture was higher in men than in women (OR = 1.19; 95% CI = 1.14–1.25).

Risk of any fracture

Previous fracture was associated with a significantly increased risk of any subsequent fracture (Table 4). There was no difference in the risk ratio between men and women. In men and women combined, the risk ratio ranged from 1.83 to 2.03 depending upon age (Table 5). The risk ratio was marginally lower by approximately 10% when account was taken of BMD. If it is assumed that the risk of any fracture increases 1.60-fold for each SD deviation decrease in hip BMD, then the difference in risk between those with and without a prior fracture is equal to an expected difference in BMD of 1.32 SD [log(1.86)/log(1.60)]. In reality, the difference in BMD at all ages in men and women combined was approximately 0.11 SD ([log(1.86)/log(1.60)] − [log(1.77)/log(1.60)]). Thus, low BMD accounts for the minority (8%; 0.11/1.32) of the difference in risk between those with or without a prior fracture.

Table 5
Risk ratio (RR) for any fracture and 95% confidence intervals (CI) comparing men and women with and without a previous fracture by age, with or without adjustment for BMI

Age (years)	RR without BMD[a]		RR with BMD[a]	
	Mean	95% CI	Mean	95% CI
50	1.92	1.63–2.20	1.91	1.59–2.29
55	1.90	1.73–2.09	1.83	1.60–2.10
60	1.98	1.80–2.18	1.94	1.73–2.17
65	2.02	1.86–2.20	1.99	1.81–2.20
70	2.03	1.87–2.21	1.98	1.79–2.18
75	1.96	1.80–2.13	1.82	1.65–2.02
80	1.88	1.72–2.06	1.72	1.54–1.91
85	1.83	1.65–2.04	1.72	1.51–1.96
All ages	1.86	1.75–1.98	1.77	1.64–1.91

[a] Prior fracture versus no fracture.

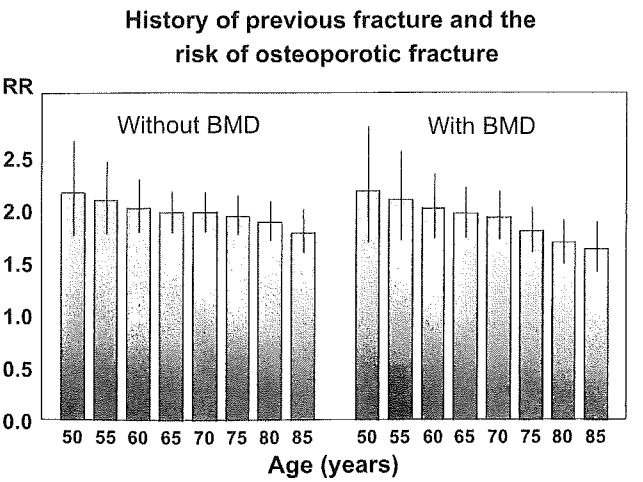

Fig. 1. Risk ratio for an osteoporotic fracture in men and women with a prior history of fracture with and without adjustment for BMD.

Risk of osteoporotic fracture

Previous fracture was also associated with a significantly increased risk of an osteoporotic fracture at all ages with and without adjustment for BMD (Table 4). The unadjusted risk ratios for an osteoporotic fracture were almost identical with the risks of a prior fracture for any fracture. For example, at the age of 80 years, the risk of any fracture was 1.88 (95% CI = 1.72–2.06) and for an osteoporotic fracture was 1.89 (95% CI = 1.72–2.09). There was no difference in risk ratio between men and women. Fracture risk decreased somewhat with age by about 10% per decade of age (Fig. 1), but the trend was short of conventional significance ($P = 0.089$).

Risk of hip fracture

A prior fracture history was a significant risk factor for hip fracture at all ages (Table 6). The risk ratio was highest at younger ages and decreased progressively with age ($P < 0.002$ for the interaction term). The risk decreased by 3%

Table 6
Risk ratio (RR) for hip fracture and 95% confidence intervals (CI) comparing men and women with and without a prior fracture by age, with and without BMD

Age (years)	RR without BMD[a]		RR with BMD[a]	
	Mean	95% CI	Mean	95% CI
50	5.04	2.66–9.56	3.88	1.79–8.43
55	4.20	2.46–7.15	3.98	2.08–7.62
60	3.40	2.21–5.24	3.16	1.88–5.32
65	2.60	1.85–3.64	2.28	1.52–3.41
70	2.31	1.76–3.02	1.90	1.37–2.65
75	2.14	1.71–2.68	1.64	1.24–2.17
80	1.90	1.58–2.28	1.41	1.12–1.78
85	1.66	1.39–1.98	1.32	1.04–1.68
All ages	1.85	1.58–2.17	1.62	1.30–2.01

[a] Prior fracture versus no fracture.

(95% CI = 1–5%) for each year of age. The RR was significantly increased at all ages, but at ages less than 60 years, the confidence estimates were wide (very few hip fractures). There was no difference in RR between men and women. Adjustment for BMD had an effect on the risk estimate for hip fracture that was quantitatively greater than for all fractures. The RR adjusted for BMD fell by approximately 30%. As in the case of all fractures, differences in BMD explained a minority of the increased risk ratio for hip fracture. In men and women combined, low BMD explained 22% of the increase in risk ratio and was constant by age (assuming a gradient of risk for hip fracture of 2.6/SD decrease in BMD).

The exclusion of data from Gothenburg (where BMD was assessed at the forearm or heel) had no material effect on these results (data not shown). There was no significant interaction of fracture history with time since baseline assessment.

Discussion

The present study confirms that a history of prior fracture is a significant risk factor for future fractures. In addition, the effect is over and above that which can be explained by variations in BMD. The risk of subsequent fractures is not as great as that identified in some studies [8], but as expected, falls within the confidence estimates of most estimates [27]. Discrepancies may be related to the duration of follow-up since the risk of subsequent fracture may not be linear over time [4,5]. Other possible reasons may relate to differences in the populations studied and the questionnaire used to identify prior fractures. A particular strength of the present study is that the estimate of risk is made in an international setting from randomly selected population cohorts. Calculations were based on the primary data, decreasing the risk of publication biases. The consistency of the association between cohorts (data not shown) additionally indicates the international validity of the importance of this risk factor. The risk of any subsequent fracture was comparable to the risk of a new osteoporotic fracture or a hip fracture. The large sample size permitted the quantification of risk by age. For all fractures and for osteoporotic fractures, the risk ratios were relatively constant with age. In the case of hip fracture, risk ratios decreased with age.

The present study also quantifies the independent contributions of low BMD and prior fracture. At all ages, low BMD explained a minority of the total risk, a proportion that decreased with age. The mechanism for the BMD-independent increase in risk could not be determined from this study but is likely due, in part, to coexisting morbidity that might increase the risk of falls or impair the protective responses to injury [6,21,29,58]. In addition, changes in the microarchitecture of cancellous and cortical bone with rapid bone loss after fracture or immobilization [59–61] may weaken the resistance to mechanical force out of proportion to any effect on BMD.

Irrespective of the underlying mechanism, these data indicate that the risk of fractures is substantially greater in individuals with a prior fragility fracture than in individuals of the same age, sex, and BMD without such a fracture. This has important implications for intervention thresholds. Health economic analysis suggests that intervention is cost-effective when treatment is targeted to women without a prior fracture with a T score of −2.5 SD at the femoral neck [39]. Since a prior fracture confers a risk over and above that provided by BMD, intervention thresholds for BMD can be less stringent (say at a T score of −1.5 SD) for those with a prior fracture, and still yield the same cost effectiveness. This approach has been incorporated into health economic analyses [32,62]. However, a large number of additional independent risk factors for fracture have been identified. These include smoking, corticosteroid exposure, a family history of fracture, secondary osteoporosis [39], and possibly the biochemical indices of bone turnover [63]. The interrelationships of all these risk factors will need to be determined before they can be easily used for assessing fracture risk in the general population.

The majority of reports have provided risk ratios for fracture in those with a prior fracture history compared to those without. For practical use, it is appropriate to express risk relative to the general population since risks expressed in this way can be more readily adjusted with other risk factors, such as the risk provided by BMD measurements [64]. For this purpose, knowledge of the prevalence of the risk factor is required. The adjustment decreases the risk ratios by a factor proportional to the prevalence of the risk factor. The relative risk is computed as $RR/[p.RR + (1 - p)]$ where RR is the risk ratio and p the prevalence of the risk factor [64]. Since the prevalence of fracture history is high (26%), the quantitative effect of adjustment is substantial.

The present study has some limitations that should be mentioned. As with nearly all randomly drawn populations, nonresponse biases may have occurred, which we were unable to document for all cohorts. The effect is likely to exclude sicker members of society, and may underestimate the absolute risk of fracture. Thus, the probability of a prior fracture may be underestimated from a societal perspective (Table 3), but this is unlikely to affect risk ratios. The greatest problem is the construct of the question concerning prior fractures and the methods of documenting and characterizing subsequent fracture events. These differed substantially between cohorts. The effect of this heterogeneity is likely, however, to weaken rather than to strengthen the associations that we found, that is, the association in reality may be stronger.

We conclude that prior fracture confers a substantial risk for future fractures and that this risk is largely independent of BMD. The consistency of the association in an international setting provides the rationale for the use of this risk factor in case of finding strategies. Moreover, patients