

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 ($18.5 \pm 3.9 \text{ mg/cm}^2$) was less than that resulting from the doses of 4 µg/kg ($20.5 \pm 3.3 \text{ mg/cm}^2$) and 10 µg/kg ($21.0 \pm 3.5 \text{ mg/cm}^2$). 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.

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A new bone-inducing biodegradable porous β -tricalcium phosphate

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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 radio-

graphic 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 efficacy.³ Some researchers have attempted to add bone-inducing capacity to β -TCP by combining this material with recombinant human bone morphoge-

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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 weight, from 100 to 400 μ m 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 μ g in 200 μ L 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

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 (μ g)	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)

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.

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 μ m thickness and stained with hematoxylin-eosin. To detect osteoclasts, tartrate resistant acid phosphatase (TRAP) was stained by use of a histochemical method.

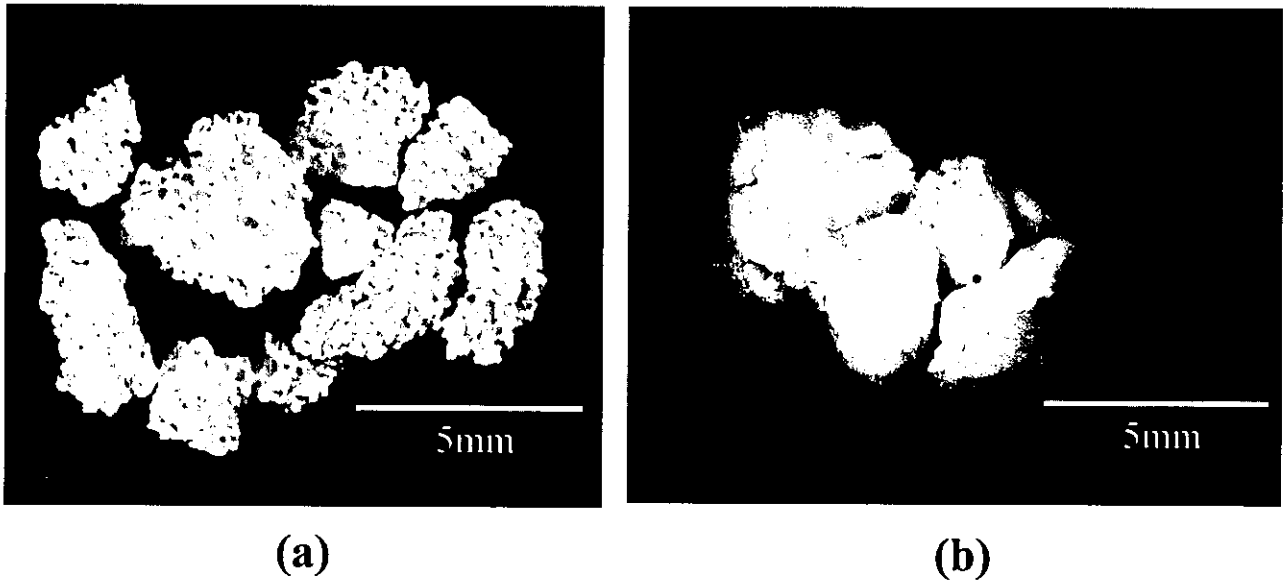


Figure 1. Macroscopic aspect of β -TCP coarse granules: (a) before coating, (b) after coating with PLA-DX-PEG. β -TCP granules exhibit adhesive-like properties at room temperature when coated with the polymer.

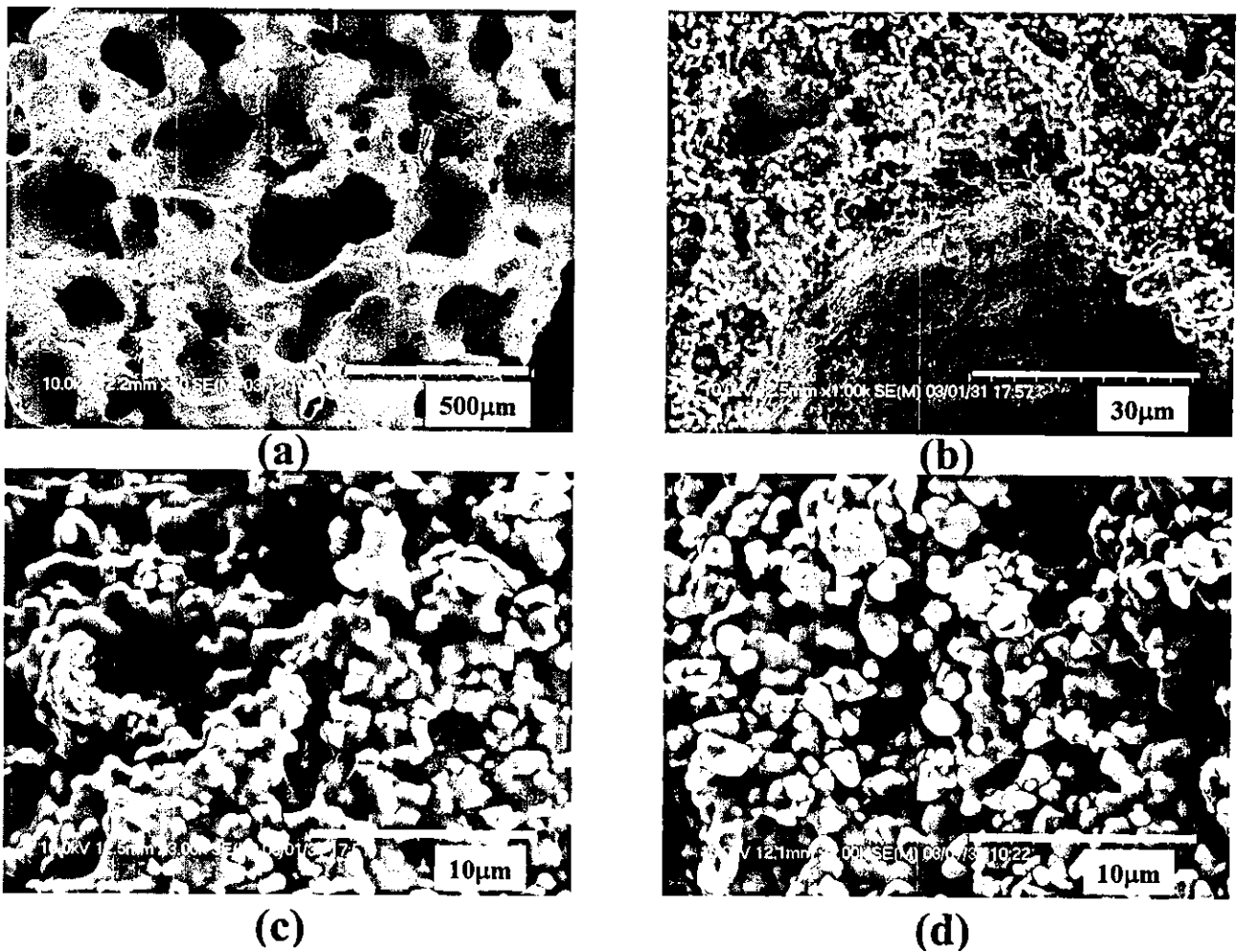


Figure 2. SEM micrographs of β -TCP granules coated with PLA-DX-PEG: (a) original magnification $\times 100$, (b) original magnification $\times 1000$, (c) original magnification $\times 3000$ and noncoated β -TCP as a control; (d) $\times 3000$. OSferion[®] is composed of $1\ \mu\text{m}$ sintered β -TCP granules with $100\sim 400\ \mu\text{m}$ interconnected pores. The surface of β -TCP granules in the experimental group is uniformly covered by a thin polymer layer.

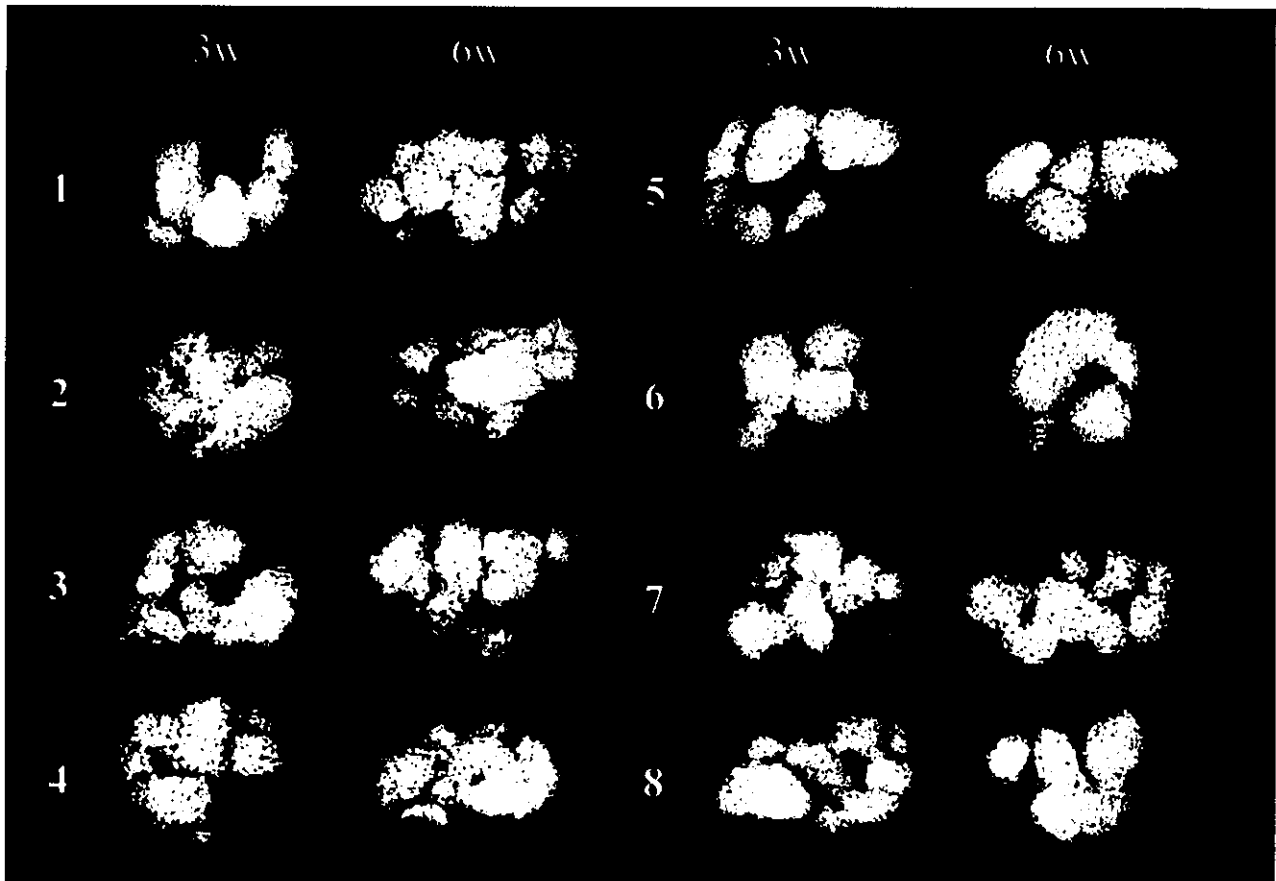


Figure 3. Typical soft X-ray photographs at 3 and 6 weeks after implantation are shown. The number on left side of each photograph indicates the number listed in Table I. Note that the new bone encasing the β -TCP granules is significant in group 1, 2, 6, and 7 compared to other experimental groups. 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 mM 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 μ m in diameter and the wall of the pores were comprised of tiny granules of approximately 1 μ m in diameter.

Electron micrographs ($\times 3000$) 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

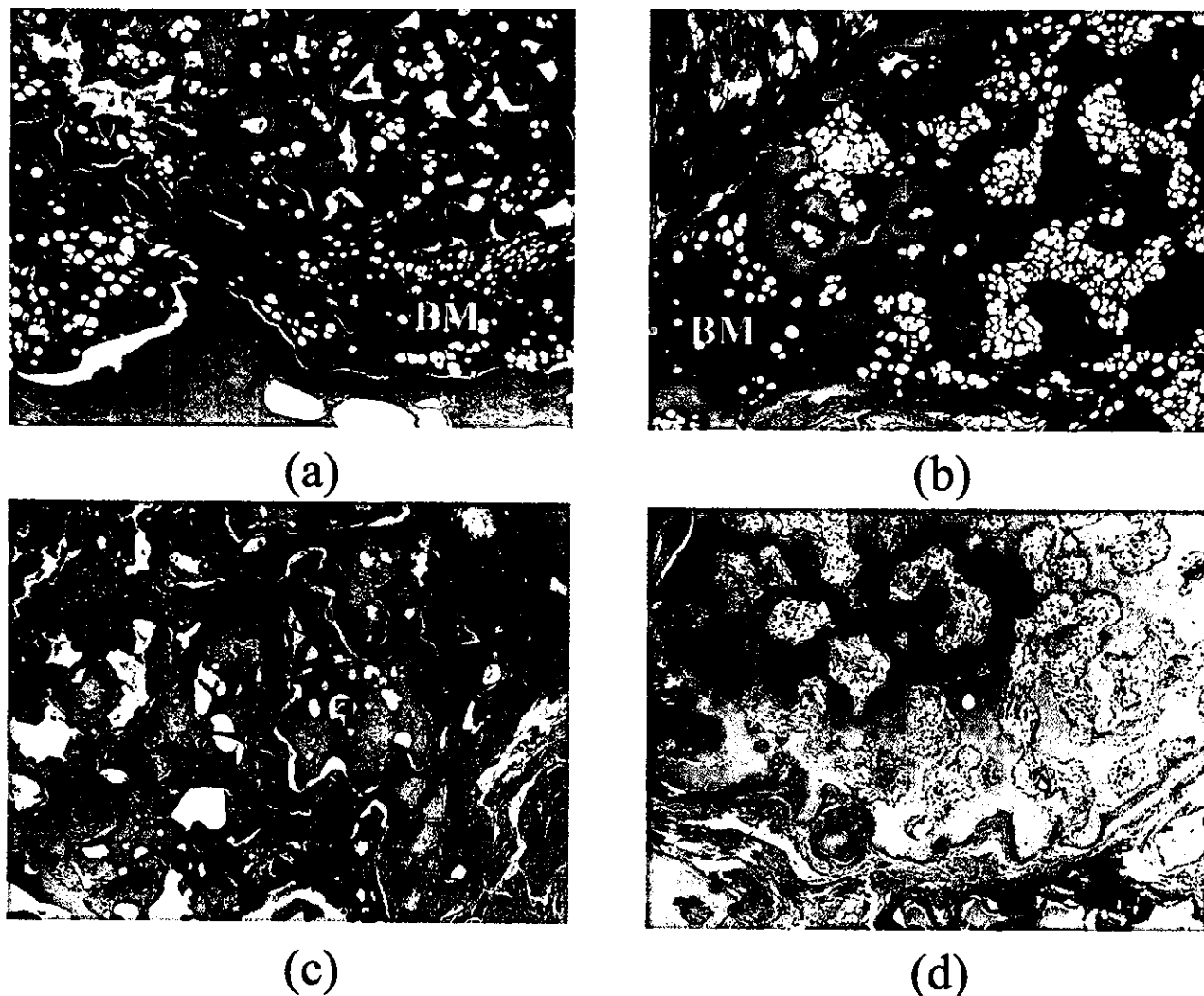


Figure 4. Typical histological sections at 6 weeks are shown: (a) group 1, (b) group 6, (c) group 5, and (d) group 8 (T, β -TCP; BM, bone marrow-like tissue; arrows, newly formed bone; Hematoxylin-eosin, decalcified sections; original magnification $\times 40$).

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 ($5 \mu\text{g}$ 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 rhBMP-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

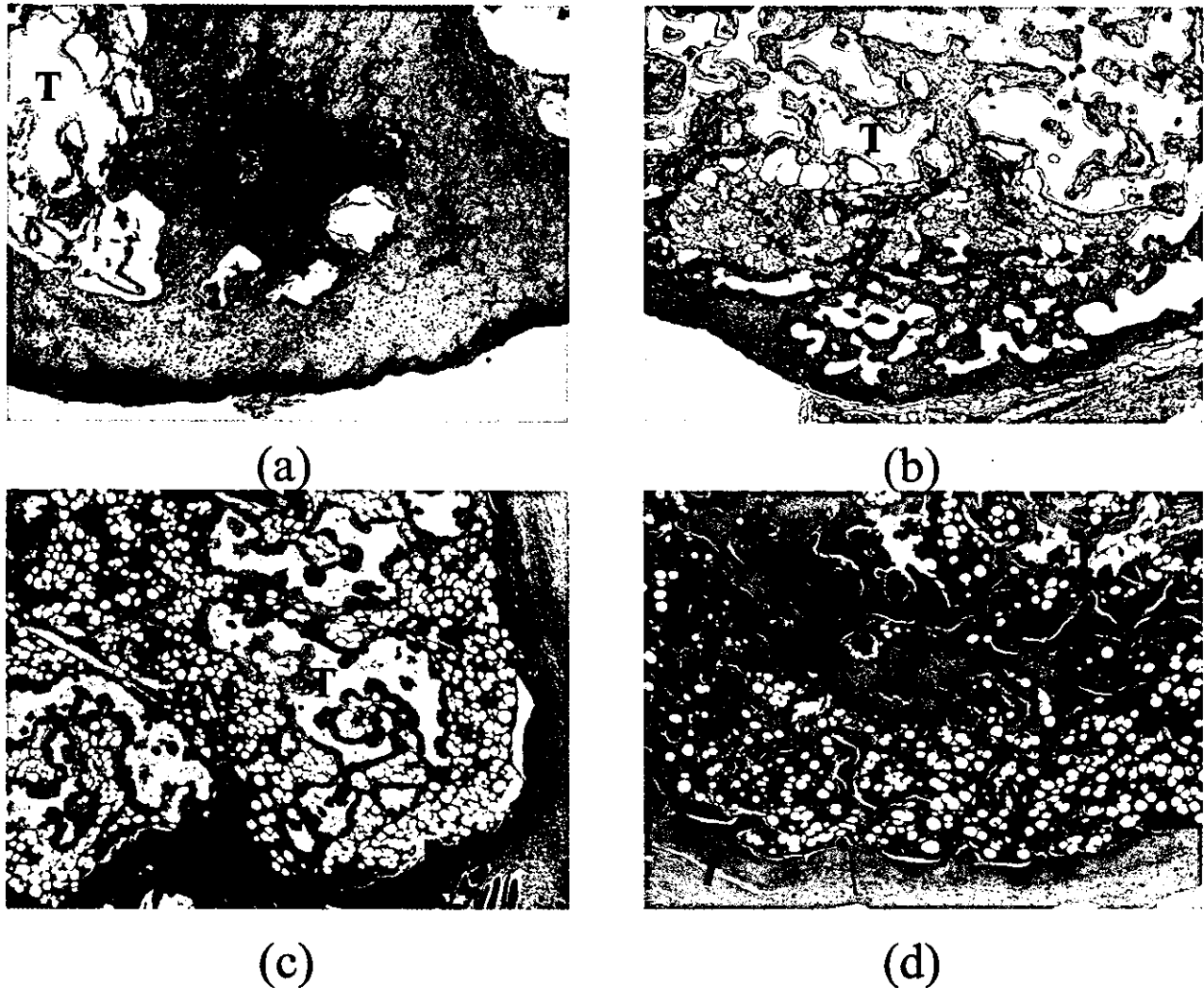


Figure 5. Time-dependent bone formation in group 1: (a) 1 week, (b) 2 weeks, (c) 3 weeks, and (d) 6 weeks after implantation (C, cartilage; T, β -TCP; BM, bone marrow-like tissue; arrows, newly formed bone; Hematoxylin-eosin, decalcified sections; original magnification $\times 40$).

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

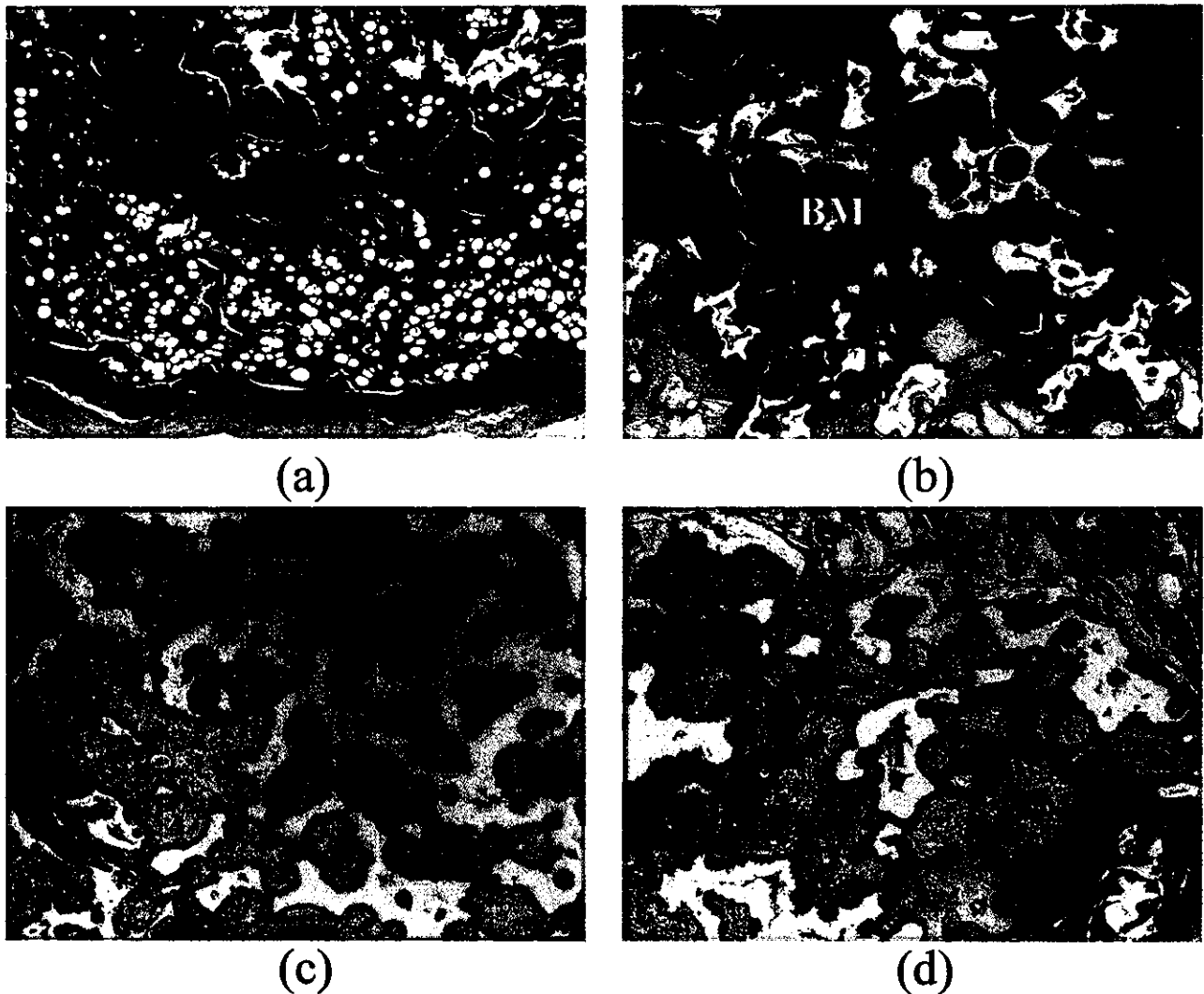


Figure 6. Dose-dependent bone formation at 6 weeks after implantation: (a) group 1 (b) group 6, (c) group 5, and (d) group 8 (T, β -TCP; BM, bone marrow-like tissue; arrows, newly formed bone; Hematoxylin-eosin, decalcified sections; original magnification $\times 40$).

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 cm³ 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 of rhBMP-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-

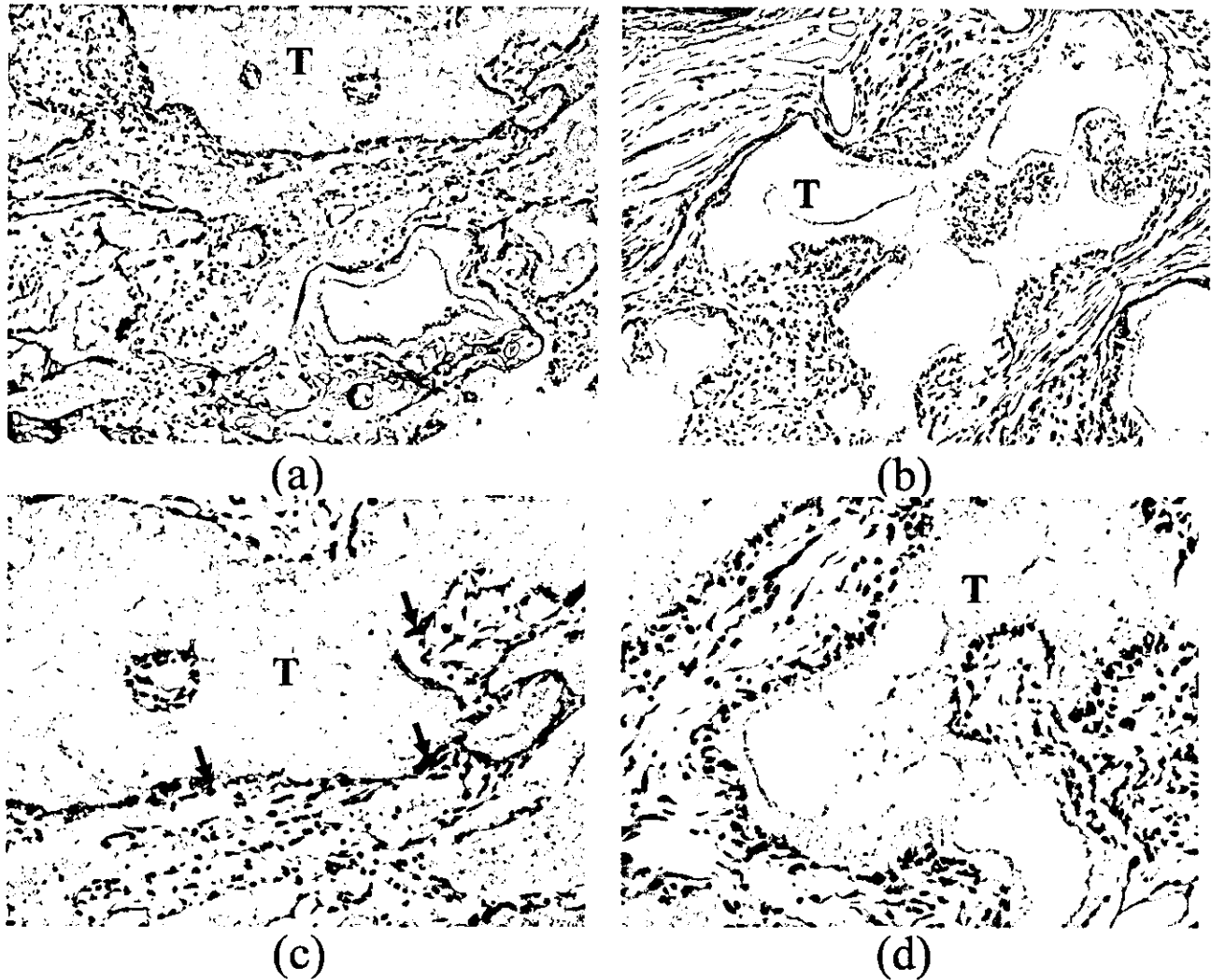


Figure 7. Tartrate resistant acid phosphatase (TRAP) stained histological sections of decalcified specimens harvested at 2 weeks: (a) original magnification $\times 40$ and (c) original magnification $\times 200$ from group 1, (b) original magnification $\times 40$ and (d) original magnification $\times 200$ 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.

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骨粗鬆症治療

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TNF- α

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はじめに

Tumor necrosis factor (TNF)が、はじめて論文に登場したのは今から30年ほど前のことで、名前が示すとおり、*in vivo*における腫瘍壊死誘導物質として同定された。その後、リンパ球が産生する細胞障害性因子として知られていたlymphotoxin (LT)と蛋白一次構造に高い類似性があることや共通のレセプターを介して作用発現すること、さらに染色体上で遺伝子が近接することから、発生学的に同一の遺伝子から進化したと考えられるに至った。そこで、TNFをTNF- α とし、LTをTNF- β とするように名称が統一された。TNF- α がマクロファージや繊維芽細胞から、TNF- β がリンパ球から産生されるという違いはあるが、その生物活性はほぼ同じである。

その後、これらのサイトカインと類似の構造と機能を有する物質がつぎつぎと同定され、現在ではTNFスーパーファミリーとして、19種類以上が知られている。発見の経緯から、TNF関連の臨床応用で最も期待されたことは、TNFの抗癌剤としての利用である。しかし、TNFの腫瘍細胞に対する障害性は、正常細胞に対しても認められ、生体に投与した際には副作用が前面に出てしまい、臨床

応用は困難であった。悪性腫瘍の末期状態である悪液質の原因物質がTNFであることから、この結果は容易に想像できる。現在では、同じくTNFスーパーファミリーの1つであり、腫瘍細胞にのみ障害性を示すTRAIL (TNF-related apoptosis-inducing ligand, 別名Apo2L)に抗癌剤としての期待がかかっている。

一方、TNFの作用を阻害することを目的とした抗TNF療法は、炎症性疾患の治療において劇的な効果を示し、関節リウマチ (RA) やクローン病 (CD) 治療の歴史に新しいページを開いた。

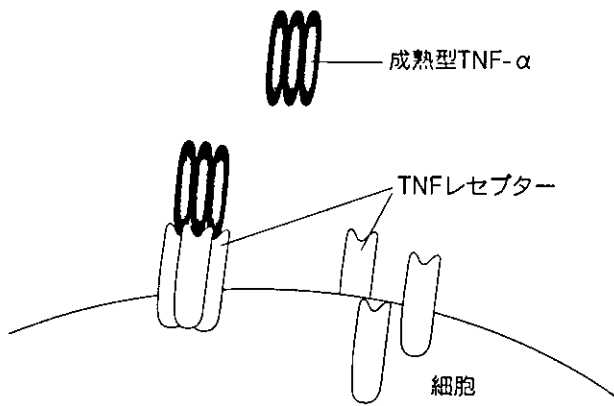


TNF-TNFR (receptor) 系

TNF- α はさまざまな細胞 (マクロファージ, 単球, T細胞, B細胞) で産生され、26 kDaの膜蛋白として存在する。これが炎症などの刺激を受け、メタロプロテアーゼにより細胞外ドメインが分離され、17 kDaの成熟型可溶性TNF- α として細胞外環境に放出される。成熟型TNF- α は常に三量体の形をとり、レセプター結合部位は隣接する二つの鎖のあいだに形成される溝として存在するため、1つのTNF- α 三量体は3カ所のレセプター結合部位を有する。1つのレセプターと結合してもシグナルは伝達されず、2つあるいは3つのレセプターと結合する必要がある (図①では3つのレセプターと結合している)。レセプターには、55 kDa (TNFR1) と75 kDa (TNFR2) の2種類があり、TNF- α もTNF- β もどちらにも結合する (図②)。レセプターの細胞外ドメインは相同性が高いが、

■ 関連語 ■

- ・ 炎症性サイトカイン
- ・ 関節リウマチ
- ・ 抗TNF療法



図① TNFとレセプター

成熟型可溶性TNF- α は、膜蛋白として生成されたTNF- α がプロテアーゼにより分離され、細胞外に放出される。細胞外では三量体として存在し、2~3個のレセプターと結合する。

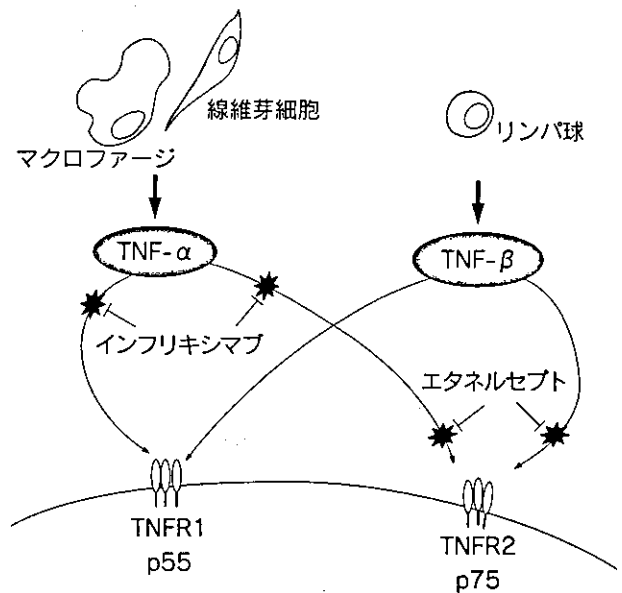
シグナル伝達を担う細胞内ドメインは両者で異なり、機能の差につながっている。

TNFがTNFR1に結合すると、図③に示すように、TNFレセプターファミリーに特徴的な「死のドメイン」を介してアポトーシスが誘導される。同様にTNFレセプターファミリーに共通のシグナル伝達因子であるTNF receptor associated factors (TRAFs)のうち、TRAF2を介するシグナルは分化誘導につながり、アポトーシスには抑制的に作用する。TNFスーパーファミリーの一員で、破骨細胞の分化活性因子として同定されたRANK (receptor activator of nuclear factor- κ B)-RANKL (RANK ligand)系では、TRAF2やTRAF6を含んだ複数のTRAFを介して、破骨細胞の分化および活性化が制御されている。破骨細胞の分化にはTRAF2が、活性化にはTRAF6が重要であるが、炎症性サイトカインのもう一方の雄であるインターロイキン-1 (IL-1)はTRAF6を介するシグナル伝達をおこなう。TNF- α がTRAF2を、IL-1がTRAF6を介するシグナル伝達をおこなうなら、両者が存在する炎症の場では破骨細胞の分化も活性化も促進可能であり、RAに伴う骨破壊を考えるうえで興味深い。



抗TNF- α 療法

生理的条件下では、TNFはマクロファージや好中球を



図② TNF- α 、- β とTNFR1、2の関係

TNF- α はさまざまな細胞で産生され、TNFの2種類のレセプターのいずれにも結合する。TNF- β はリンパ球より放出され、やはり両方のレセプターに結合しうる。抗TNF製剤であるインフリキシマブとエタネルセプト(図④参照)は、図の部位でTNFの作用をブロックする。

活性化することにより防御的炎症反応に関与している。また、各種接着因子の発現を高めることにより炎症担当細胞の血管外浸潤をもコントロールしている。RAは滑膜増生と関節破壊を特徴とする疾患であり、真の原因は今も不明であるが、その病勢の進展に各種サイトカインが関与していることに疑いはない。滑膜増生や軟骨破壊に最も大きな役割を果たしているのは、IL-1とTNF- α であるが、他のサイトカインの誘導を含めて、TNFがカスケードの最上流に位置していると考えられている。in vitroの実験系では、IL-1のほうが劇的な作用を示すのに対して、後述するように生体に抗IL-1や抗TNFを投与した際に、後者のほうがより効果的である理由は、このヒエラルキーにある。

現在、臨床で用いられている抗TNF療法としては、キメラ型(ヒトとマウス)抗TNF- α モノクローナル抗体を用いるものとヒトIgG1Fc部分とTNFR2の細胞外ドメインの融合蛋白を用いる方法とが実施されている(後者は、2004年春の時点でわが国では未承認)(図④)。前者が一般名インフリキシマブ(商品名レミケード®)で、TNF- α

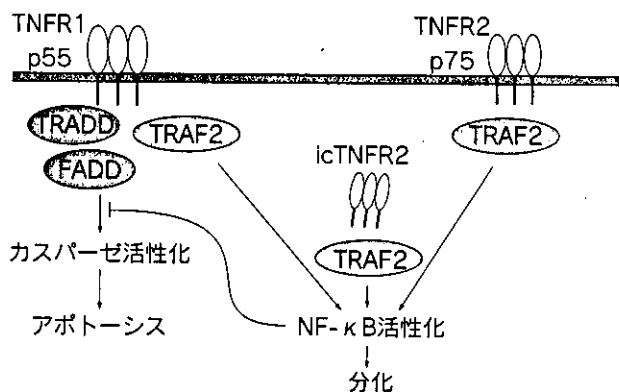


図3 TNFの細胞内シグナル伝達
 TNFR1にTNFが結合すると、TRADD(TNFR-associated death domain)やFADD(Fas-associated death domain)などの「死のドメイン」を介して、アポトーシスが誘導される。一方、TRAF2(TNFR-associated protein-2)を介するシグナルは分化誘導的に作用し、アポトーシスには抑制的にはたらく。また、細胞内にもTNFR2が存在する(intracellular TNFR2; icTNFR2)。

に直接作用し、その活性を中和する。マウスで作成された抗TNF- α 抗体のV領域を残し、他の部分をヒト免疫グロブリンに置き換えたもので、マウス領域が残っているために、くり返し投与によりキメラ抗体に対する中和抗体が出現し、効果が減弱する。メソトレキサートを併用することにより中和抗体出現を抑制しうることが示され、現在臨床では併用療法がおこなわれている。さらに、完全ヒト型抗TNF- α 抗体も開発されている。

一方、ヒトIgG1Fc部分とTNFR2の細胞外ドメインの融合蛋白は、一般名エタネルセプト(商品名Enbrel[®])とよばれ、TNFR2(p75)を2分子有し、Fc部分が存在するため生体内での安定性が期待される。TNF- α のみならずTNF- β のレセプターへの結合をも阻害し、細胞表面に存在するTNFに対しても結合する。

いずれの抗TNF製剤もTNF- α が関与することが想定される疾患(RA, CD, 敗血症, 心不全など)すべてに臨床応用が考えられる。しかし、RAやCDでは劇的な臨床成績が示されたが、敗血症患者では有用性は見出されなかった。また、これらの療法では本来のTNFが果たしている生理的作用もブロックしてしまうため、長期使用に

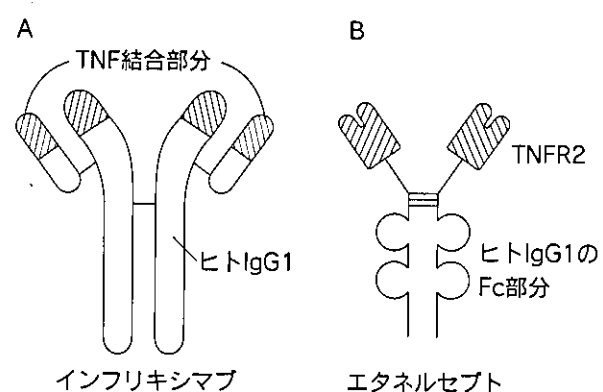


図4 インフリキシマブとエタネルセプト
 A インフリキシマブ
 ヒトTNF- α と高い親和性を有する可変領域(斜線部:25%)とヒトIgG1 κ 鎖からなるキメラ抗体。抗原結合部位を含むIg可変領域がマウス由来で、残り75%がヒトIgG定常領域。
 B エタネルセプト
 ヒトTNFR2(p75)の2分子をヒトIgG1のFc部分と結合させた融合蛋白。斜線部がレセプター部位。Fc部分を付けることで生体内での安定性をはかっている。

よる感染症や悪性腫瘍の発生が危惧されている。実際に、市販後調査で結核などの感染症の多発が指摘されており、専門医による厳重な管理下に使用すべき薬剤である。しかし、われわれは明らかに新しい武器を手に入れたことは間違いない。



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骨粗鬆症学

—基礎・臨床研究の新しいパラダイム—

X. 骨粗鬆症の治療法

運動療法

高齢女性に対する運動療法の長期効果

大川得太郎¹ 佐藤哲也² 小池達也³

X. 骨粗鬆症の治療法

運動療法

高齢女性に対する運動療法の長期効果

Effect of exercises on bone mineral density and physical strength in elderly women

大川得太郎¹ 佐藤哲也² 小池達也³

Key words : 骨粗鬆症, 体操療法, bone mineral density, osteoporosis, exercise

はじめに

近年, 高齢者人口の増加とともに骨粗鬆症による骨折が問題となってきている。骨粗鬆症による骨折には, 大腿骨頸部骨折や脊椎圧迫骨折などがあり, これらの骨折は寝たきり状態を引き起こし, 老後の生活の質(quality of life: QOL)を低下させる。骨粗鬆症の予防は老後のQOLを向上するために必須の事項となっている。

運動は骨粗鬆症に対して予防的に作用すると考えられているが, 実際にどのような運動が効果的であるのか, また高齢者にどの程度の効果が見込まれるのかを, 明確に示した報告はみられない。著者らはevidenceに基づく骨粗鬆症の運動療法を確立することを目標として, 約10年前より独自の骨粗鬆症体操教室を主催してきた。本稿では, 体操教室参加の高齢女性(体操教室群)と, 特に運動を行っていない高齢女性(非運動群)において, 開始時より6年間の骨密度(bone mineral density: BMD)の経年変化を報告する。また, 体操教室群においては, 運動能力の経年変化も報告する。

1. 著者らの骨粗鬆症体操の開発

諸家の縦断的研究によると¹⁻⁶, 歩行よりもインパクトを与えるような運動が更に骨塩量の維持増大効果があるといわれている。

藤井は⁷, 男子大学生でスポーツ種目による骨塩量の相違を検討した。その結果, 柔道, 野球, 短距離選手はすべての部位で高い骨塩量を示し, バレーボール, バasketボール選手は荷重部のみ高い骨塩量を示した。水泳選手は筋力が強いのに荷重付加が加わらないために, すべての部位で低骨塩量を示した。陸上選手では短距離選手の方が長距離選手よりもすべての部位で高骨塩量を示した。これより, 持久力を要する運動よりも瞬発力を要する荷重運動が骨塩量増大効果が高いと考えられた。

次に, 著者らは男子大学生を対象とし, 骨に衝撃力を与える運動(かしわ手, しこ踏みなど)を行ってもらい, 骨塩量を高めるのに有効であることを明らかにした⁸⁻¹⁰。

以上より, 骨に衝撃力を加える運動を中心に, 転倒予防効果もねらい, 筋力増強, バランス強化を加えた運動を著者らの骨粗鬆症体操(コッコ体操)とした。

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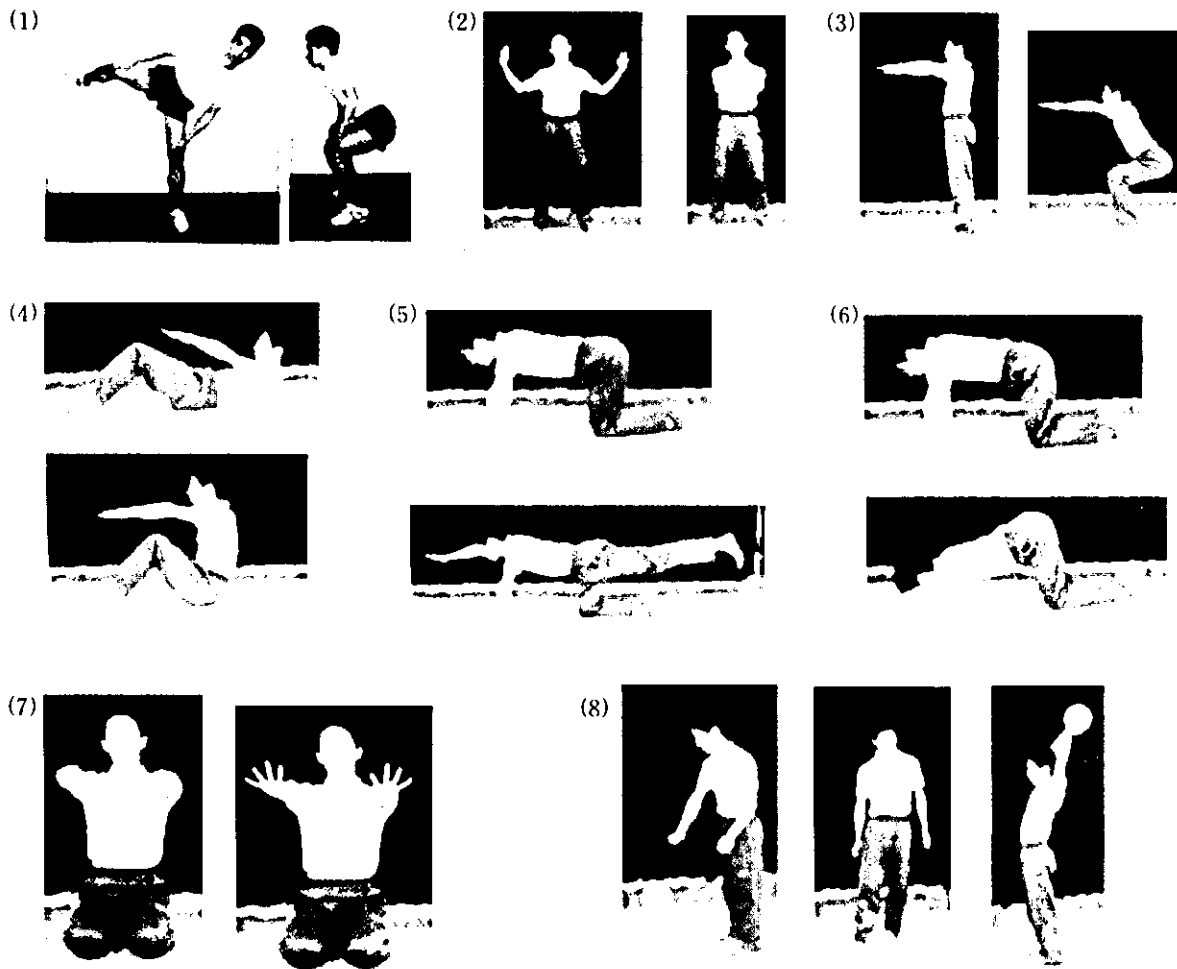


図1 コツコツ体操

(1) しこ踏み, (2) かしわ手, (3) ハーフスクワット, (4) 腹筋, (5) 膝立での背筋強化, (6) 膝立での腕立て伏せ, (7) グーパー運動, (8) ボール体操

2. コツコツ体操^{9,11-16)}

転倒時の骨折予防および体操時の準備運動として、ストレッチングを十分に行い、図1を中心とした体操を行っている。骨に衝撃力を与え、更に筋力強化をねらった(しこ踏み, かしわ手), 筋力強化として(ハーフスクワット, 腹筋, 膝立での背筋強化と腕立て伏せ), 更に体操教室ではグーパー運動, 踵上げ, ハイハイ運動およびバランス強化目的としたボール体操を追加している。体操は体力に合わせて7割くらいの強度で行い、徐々にアップするようにしている。体操は、週1回当院の体育館でトレーナーとともにを行い、他の日は自宅で行うよう指導している。

3. 高齢女性に対するコツコツ体操の長期効果

a. 対象および方法

対象は、体操教室群(平均63.2±3.8歳, n=17)と非運動群(平均61.2±6.1歳, n=17)である。すべて女性で、閉経後10年以上経過しており、また特に骨代謝に影響を与える疾患、手術そして投薬を受けていないものとした。コツコツ体操教室を週1回開催し、トレーナーとともに体操前後のストレッチングを加えて行った。また、自宅でもストレッチングと図1の項目を週1回以上行うよう指導している。BMDの測定は、体操開始前および開始後1年ごとに dual energy X-ray absorptiometry(DXA: QDR-1000,

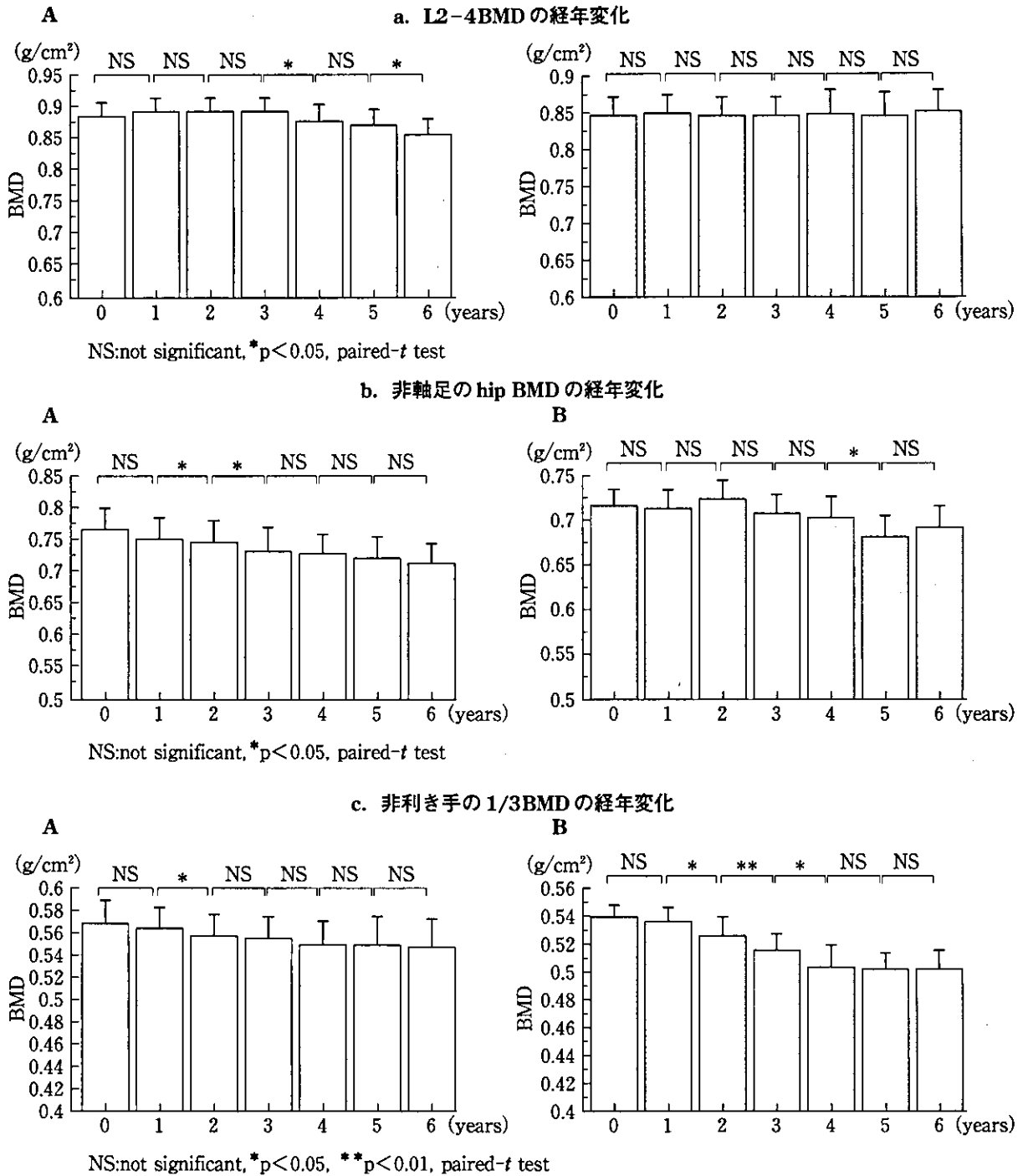


図2 各群でのBMDの経年変化(A: 非運動群, B: 体操教室群)

Hologic社)にて、第2-4腰椎平均BMD(L2-4 BMD)、非軸側大腿骨近位部BMD(hip BMD)、非利き手橈骨遠位1/3BMD(1/3BMD)を測定した。体操教室群の運動能力測定は、両手握力(kg)、ハイドロマシキュレーター(OG技研)を用いて測定した非軸足の大腿四頭筋筋力(WBI)(%)と非軸足片脚起立時間(秒)、長座体

前屈(cm)、上体起こし(回/分)、ハーフスクワット(回/分)、グラビコーダーGS-10(アニマ社)を用いて測定した15秒間の重心動揺総軌跡長(cm)を行った。経年変化はpaired-t検定にて解析した。

b. 結果

開始時に両群間において、年齢、身長、体重