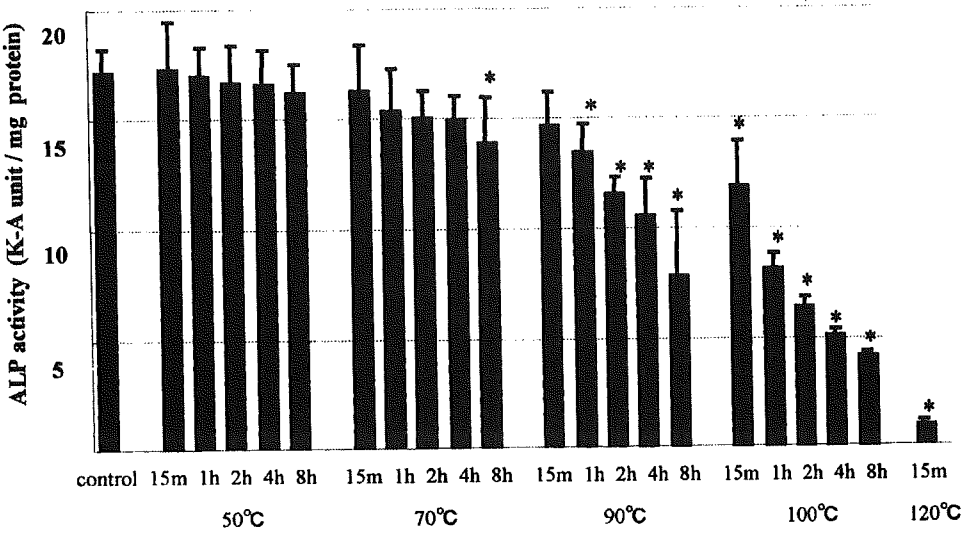

Fig. 1. The influence of heat treatment on rhBMP-2-induced alkaline phosphatase (*ALP*) activity of MC3T3-E1 cells. Data are means ± SD of three culture wells. A significant difference from the control is indicated as * ($P < 0.05$)

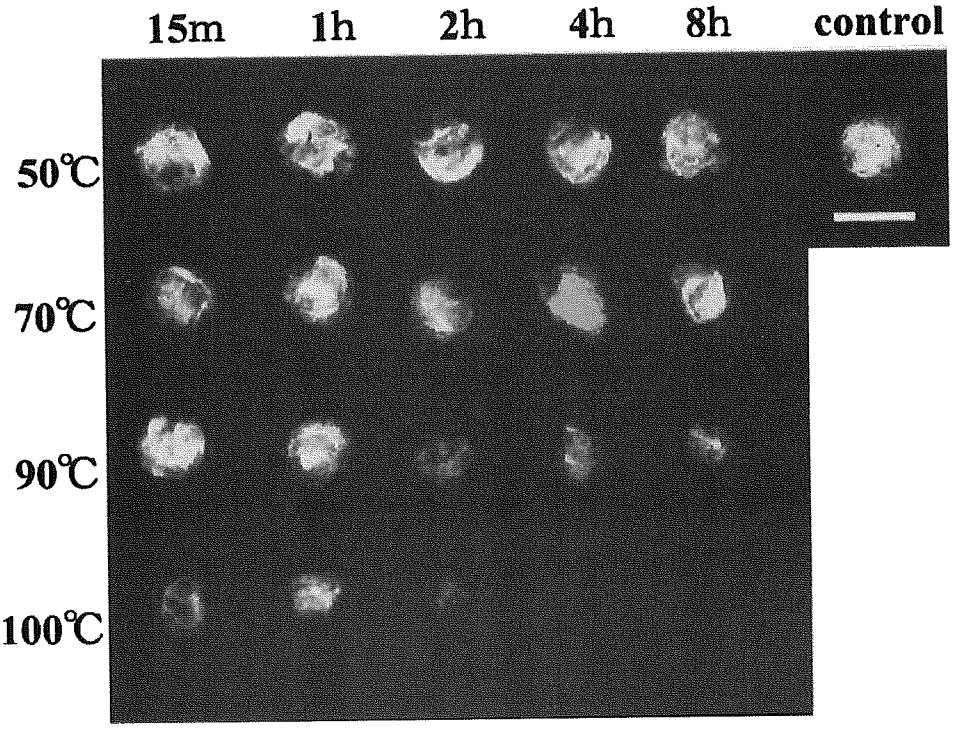

Fig. 2. Soft X-ray photograph of an ossicle formed at 3 weeks after implantation. A typical implant from each group is shown. *Scale bar* = 6 mm. There is no difference in the trabecular pattern within ossicles from each group. However, a reduction in size was observed over 90°C in a time-dependent manner. In the groups heated at 100°C for 4 h, at 100°C for 8 h, and at 120°C for 15 min, there was no firm mass around the implant location. Thus, we decided not to collect tissues from these areas for further analysis (softex and bone mineral content)

Results

Effects on ALP expression by heated rhBMP-2

Figure 1 shows the effects of the heat treatment on the ALP-inducing activity of rhBMP-2 in MC3T3-E1 cells. ALP-inducing activity was preserved at 50°C even after the rhBMP-2 was heated at this temperature for 8 h. A notable reduction in the ALP-inducing activity of rhBMP-2 was seen using protein samples heated at 70°C for 8 h. At 90°C, a significant reduction of activity was noted by heating for 1 h or more in a time dependent manner. By heating at 100°C, the time-dependent reduction was profound. The ALP-inducing activity was reduced to less than 1 unit/mg protein when the rhBMP-2 was heated at 120°C for 15 min.

Change in the bone-inducing activity of rhBMP-2 by heating

Figure 2 shows representative ossicles elicited in mice within a 3-week period by implanting collagen disks impregnated with 5 μg rhBMP-2 heated at 50, 70, 90, or 100°C for 15 min, or for 1, 2, 4, or 8 h. Ossicle size tended to become

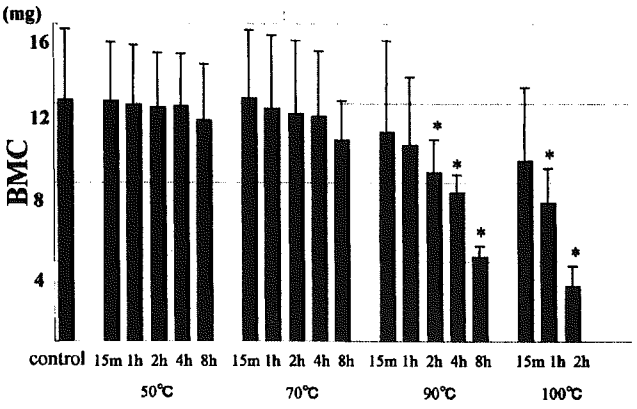

Fig. 3. The bone mineral content (*BMC*) of the tissues harvested at 3 weeks after surgery. Data are means ± SD of 8 samples. A significant difference from the control is indicated as * ($P < 0.05$). There was no significant difference between the BMC of the 50°C and 70°C groups and the control group. However, the mean BMCs of groups heated at 90°C for 2, 4, or 8 h and the one heated at 100°C for 1, or 2 h ($P < 0.01$) were significantly lower than that found in the control group. In the groups heated at 100°C for 4 h, at 100°C for 8 h, and at 120°C for 15 min, we found no evidence of new ectopic bone formation, as described in the legend to Fig. 2

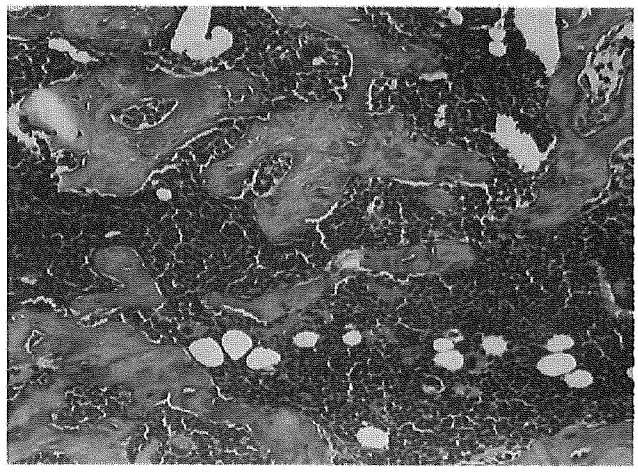

Fig. 4. Photomicrograph of the tissue harvested at 3 weeks after implantation (heated to 50°C for 8 h) (H&E, ×200). Normal bone histology with hematopoietic marrow and bony trabeculae was observed

small when the rhBMP-2 was treated at 90°C for 2 h or more. After treatment for more than 4 h at 100°C or for 15 min at 120°C, the bone-inducing activity of rhBMP-2 was lost.

Figure 3 shows the mean BMC values of the ossicles from each of the groups. The mean (±SD) BMC of the control group was 12.2 ± 3.56 mg. In the group treated at 50°C for 8 h, the mean BMC was 11.2 ± 2.81 mg, which was not significantly lower than that of the controls. In the group with protein heated at 70°C, the mean BMC in the group treated at 70°C for 15 min was 12.2 ± 3.45 mg. The BMC in the 70°C group showed a downward trend, but no statistically significant difference was observed when compared with the control group. Similarly, in the group using protein heated at 90°C, the BMC of the harvested ossicles decreased with an extension of the heating time. The BMC of ossicles from the groups treated at 90°C for 15 min or 90°C for 1 h were not significantly different from those of the control group. However, the mean BMC values of the groups treated at 90°C for 2 h (8.48 ± 1.68 mg), at 90°C for 4 h (6.98 ± 1.06 mg), or at 90°C for 8 h (4.26 ± 0.53 mg) were significantly reduced when compared with those of the control group ($P < 0.01$). Again, in the group treated at 100°C for 15 min, the BMC was less than that of the controls, although there was no significant difference between the values. The mean BMC of the groups treated at 100°C for 1 h (6.96 ± 1.71 mg) or at 100°C for 2 h (2.80 ± 0.99 mg) were significantly lower than those from the control group ($P < 0.01$).

Histological analysis of the ossicles from all groups revealed normal bone histology with hematopoietic marrow and bony trabeculae (Fig. 4).

Changes in the SDS-PAGE profile of rhBMP-2 by heating

The SDS-PAGE profiles of rhBMP-2 before and after heating at 90 or 120°C are shown in Fig. 5. The original rhBMP-2 showed three dark bands at around 30-k Daltons (kD) and two light bands below 20 kD without DTT (lane 1). With DTT, the 30-kD bands became lighter and the lower molecular-sized bands became darker (lane 4). By heating at 90°C for 2 h, in the absence of DTT the 30-kD bands became a little indistinct and two lower sized bands disappeared (lane 2), and with DTT the 30-kD bands became lighter and the lower bands became darker (lane 5). By heating at 120°C for 2 h, in the absence of DTT the 30-kD bands remained although the three bands became indistinct (lane 3), and with DTT both of the 30-kD bands and the lower bands were lost (lane 6).

Discussion

BMPs (BMP-2, BMP-7) with potent bone-inducing activity have been successfully produced and developed for clinical use through DNA recombinant technology. Examples of clinical applications include the repair of damaged bone, the reconstruction of bone defects resulting from trauma, and resection of bone tumors. In terms of a successful clinical outcome, the bone-inducing activity of rhBMP-2 must be able to withstand the conditions associated with transportation and storage. In addition, rhBMP has to survive sterilization and the high temperatures encountered when implants are used in close contact with curing bone cement. Previous studies have qualitatively described the stable character of natural BMP based on the ability of crude insoluble BMP-retaining decalcified bone matrix to induce ectopic new bone. Nakanishi et al. [12] reported that rabbit bone derived BMP extracts elicited ectopic bone after

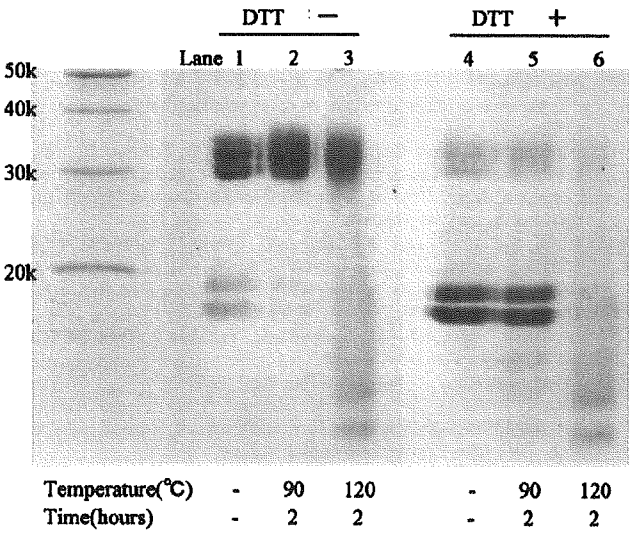

Fig. 5. SDS-PAGE profiles of rhBMP-2 before (*lane 1*) and after (*lane 2*) heating at 90°C and at 120°C (*lane 3*) for 2h. *Lanes 4, 5,* and *6* show the respective samples under reducing conditions in the presence of dithiothreitol (DTT, a disulfide-bond reducing agent). The original rhBMP-2 showed three dark bands at around 30k Daltons (kD) and two light bands below 20kD without DTT (*lane 1*). With DTT, bands at 30kD became lighter and the lower bands became darker (*lane 4*), which indicates that the dimers were reduced to monomers. By heating at 90°C for 2h in the absence of DTT, the major 30-kD bands became a little indistinct, and the two lower-sized bands disappeared (*lane 2*). With DTT, bands at 30kD became lighter and the lower bands became darker (*lane 5*), indicating that the bands at 30kD without DTT maintained a dimeric structure. By heating at 120°C for 2h, the original size of rhBMP-2 was maintained, although the three distinct bands were lost (*lane 3*). With DTT, both of the original 30-kD bands and those lower molecular-sized bands were lost (*lane 6*), indicating complete denaturation of the original configuration of the molecules

heating at 70°C for 10min. However, the bone-inducing activity was lost when the sample was heated at over 100°C for 20min. Some reports have also described similar results using crude BMP extracts as the bone-inducer in rodent models [13,14]. One report demonstrated that BMP activity was retained in a rabbit model after heating the matrix protein at 170°C for 10min or at 140°C for 30min [15].

In this study, the bone mineral content of the rhBMP-2-induced ossicles was used to evaluate the bone-inducing activity of the heat-treated rhBMP-2 in an in vivo system. In addition, the ability of rhBMP-2 to induce ALP activity in osteoblastic cells in culture was used as a model to confirm the results from the in vivo experiments. The ALP-inducing activity of the rhBMP-2 was little affected by heat treatment up to 70°C for 8h or up to 90°C for 1h, but then decreased gradually with increasing temperature and time. The ALP-inducing activity was eventually lost after heating the rhBMP-2 at 120°C, a treatment that denatured the molecular structure of the rhBMP-2 as shown on a SDS-PAGE profile. These results were in accordance with the in vivo results, and suggest that rhBMP-2 is largely stable in nature. The use of autopolymerising polymethylmethacrylate (PMMA)-based bone cement is the most common method chosen for prosthetic component fixation in total joint replacement surgery. During the polymerization of PMMA, high peak temperatures are reached. The temperature peak ranges from 48°C to 105°C at the bone–cement interface, and from 80°C to 124°C in the cement. The exposure time over 50°C is reported to be between 30 and 400s [19]. Thus, the data from the present study suggest that contact with curing bone cement would not affect the bone-inducing activity of rhBMP-2. Sterilizing by autoclave (120°C) would effectively inactivate the biological action of this protein, but sterilizing by ethylene oxide gas (60°C for 3h or 40°C for 6h) would not, as reported previously [20].

The heat-stable character of BMP may be derived from its molecular structure. It is known that the bioactive BMP-2 molecule consists of a homodimer which is essential for its biological activity. The homodimer consists of two 114 amino acid monomers with 7 cysteine residues that form three intramolecular disulfide bonds, with one intermolecular disulfide bond forming a cysteine knot [21]. The heat-resistant nature of the BMP might be explained by the cysteine-knot formation, which is known to contribute to the structural stability of the protein.

The biological activity of the rhBMP was reduced following heat denaturation at 90°C for 2h as evaluated in an in vivo assay system, and for 1h in an in vitro assay system. At these higher temperatures, the reduction in rhBMP-2 activity was temperature- and time-dependent. In order to visualize the heat-dependent changes in molecular structure, changes in the SDS-PAGE gel profile after heating were observed. As previously described, the rhBMP-2 molecule has a molecular size of around 16kD with one N-glycosylation site, and it forms a homodimer which has biological activity [6–8,22]. Therefore, the three bands at 30kD in the control lane on the SDS-PAGE with no disulfide bond reduction indicated dimeric BMP-2 molecules with 2, 1, or no sugar chains, respectively (see Fig. 5). Further, the two faint bands below 20kD were monomers with or without glycosylation because of their similarity to the molecular sizes of the DTT-reduced rhBMP-2 monomer. By heating at 90°C for 2h, in the absence of DTT the dimer bands became a little indistinct and the monomer bands were lost, which indicated complete deformation of the rhBMP-2 monomer. With DTT, the dimer bands became lighter and the monomer bands became darker. These changes most likely reflect partial deformation of the original three-dimensional configuration of the rhBMP-2 dimers, which corresponds with the partial loss of bone inducing activity. A higher temperature (120°C for 2h) made the dimer bands more indistinct and eventually degraded the structure of the monomers, as seen on DTT-reduced SDS-PAGE, with the resultant loss of biological activity.

In conclusion, the biological activity and molecular structure of rhBMP-2 were shown to be fairly resistant to heat treatment. In considering the potential clinical uses of rhBMP-2, the stable character of the protein suggests that it would not need to be cooled during transportation or storage. In addition, it was found that contact with heated material such as curing bone cement would not affect the

bone-inducing activity of the rhBMP-2. However, the autoclaving of rhBMP-2 causes this protein to lose its biological activity based on the results of the present study.

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ORIGINAL ARTICLE

Yukio Nakamura · Shigeyuki Wakitani · Naoto Saito
Kunio Takaoka

Expression profiles of BMP-related molecules induced by BMP-2 or -4 in muscle-derived primary culture cells

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Abstract The formation of ectopic bone in muscle following the implantation of decalcified bone matrix led to the search and eventual discovery of bone morphogenetic proteins (BMPs) in bone matrix. The precise sequence of molecular events that underpin the cellular transformation of undifferentiated mesenchymal cells into bone has not been established, and is the subject of this study. Northern and Western blot analyses were used to examine changes in gene expression of cells treated with BMP-2 or -4. The molecules, which included BMP receptors (BMPRs), Noggin (a BMP-specific antagonist), osteocalcin (OC), Smad-4, and MyoD, were examined at messenger RNA (mRNA) and protein levels. The changes in expression of these molecules were followed in mouse muscle-derived primary culture cells, and osteoblastic or nonosteoblastic embryonic cell lines. We show the early up-regulation of BMPR-1A, -2, Noggin, OC, and Smad-4 in muscle-derived primary culture cells in a dose-dependent manner in response to BMP-2 or -4. MyoD expression was not detected after BMP stimulation. The differential expression of these positive and negative regulators of BMP signaling points to a potential regulatory mechanism for bone induction in mesenchymal cells.

Key words BMP signaling · Feedback · Mesenchymal cells · Muscle · Up-regulation

Introduction

Bone morphogenetic proteins (BMPs) are multifunctional cytokines belonging to the transforming growth factor-β (TGF-β) superfamily. Among the BMP family, BMP-2, -4, and -7 (osteogenic protein-1) have been recognized as potent bone inducers [1–3], and BMP-2 and -4 also play critical roles in early embryogenesis and skeletal development [4]. Signaling by BMPs requires binding of the BMP (BMP-2, -4, and -7) molecules to the BMP receptors (BMPRs), which consist of two different types of serine-threonine kinase receptors, known as BMP type I receptors (1A and 1B) and BMP type 2 receptor [5]. These receptors then phosphorylate intracellular proteins such as the Smad-1 or -5 to effect intracellular signaling and physiological responses [6–9]. Therefore, BMPR expression is a prerequisite for the biological action of the BMPs [1–5]. BMP action may also be modulated by a group of BMP-binding proteins outside the responding cells. Noggin is a representative molecule with such a function, and is assumed to contribute to the negative regulation of BMP action or bone formation under physiological conditions [6–9].

Changes in expression of those molecules involved in BMP signaling are critical to understanding the mechanism of BMP-induced osteogenic differentiation and feedback mechanisms following treatment with BMPs. We previously examined an ectopic bone-forming model in mice [10], and found that BMPR-1A, -2, and Noggin were induced by BMP-2 in muscle tissues during the early phase of the reaction. To confirm this mechanism in in vitro systems, we used muscle-derived primary culture cells, and osteoblastic or non-osteoblastic embryonic cell lines were used as controls.

Materials and methods

Recombinant human BMP-2 (rhBMP-2) and mouse BMP-4 (mBMP-4)

rhBMP-2 was produced by the Genetics Institute (Cambridge, MA, USA) using DNA recombination techniques, and donated to us through Yamanouchi Pharmaceutical (Tokyo, Japan), as described elsewhere [11].

Y. Nakamura (✉) · S. Wakitani · N. Saito
Department of Orthopaedic Surgery, Shinshu University School of Medicine, 3-1-1 Asahi, Matsumoto 390-8621, Japan
Tel. +81-263-37-2659; Fax +81-263-35-8844

K. Takaoka
Department of Orthopaedic Surgery, Osaka City University Hospital, Osaka, Japan

Conditioned media of mBMP-4-transfected Chinese hamster ovary (CHO) cells (BMP-CHO) were the source of mBMP-4. Details of the BMP-CHO cells have been described previously [12,13]. In mBMP-4-conditioned media, the alkaline phosphatase activity of the 10% conditioned media corresponds to approximately 70ng/ml rhBMP-2 [13]. The BMP-CHO cells transfected with mBMP-4 cDNA or mock vector (for a control) were propagated at a density of 1×10^6 cells/100-mm plastic dish (Falcon no. 3003; Becton Dickinson Labware, Tokyo, Japan), and were then cultured in 10ml Dulbecco's modified Eagle's medium (DMEM; Gibco-BRL, Grand Island, NY, USA) with 10% fetal calf serum (FCS; Sigma Chemical, St. Louis, MO, USA) at 37°C for 5 days. The conditioned media were collected after 5 days and stored at 4°C.

Cell culture

Muscle-derived primary culture cells were prepared from the thigh muscles of newborn ddy mice (Nippon SLC, Shizuoka, Japan), as described previously [14], and cultured on a 100-mm plastic dish in DMEM containing 10% (vol/vol:v/v) heat-inactivated FCS and penicillin–streptomycin (PSM) antibiotic mixture (Invitrogen). A murine osteoblastic cell line, MC3T3-E1, and murine embryonic fibroblast-like cell line, NIH3T3, were obtained from the RIKEN Cell Bank (Tsukuba, Japan) and cultured on a 100-mm plastic dish in α-minimal essential medium (Gibco-BRL) and DMEM, respectively, containing 10% (v/v) heat-inactivated FCS.

Experimental protocols

To examine the effects of rhBMP-2 and mBMP-4 on the expression of BMPRs, Noggin, OC, Smad-4, and MyoD in muscle-derived primary culture cells, and MC3T3-E1 and NIH3T3 cells, the culture media were replaced with fresh media containing rhBMP-2 or mBMP-4 at various concentrations (0, 10, 100, 500, 1000, or 1500ng/ml for rhBMP-2, and 0%, 10%, 20%, 40%, 60%, or 80% conditioned media for mBMP-4). The cells were cultured at 37°C in a humidified 5% CO_2 incubator for a period of 6 days, with a change of media on day 3. Cells cultivated for 0, 12, 24, 48, 72, 96, 120, or 144h with each medium containing rhBMP-2 (0, 10, 100, 500, 1000, or 1500ng/ml) or mBMP-4 (0%, 10%, 20%, 40%, 60%, or 80%) were collected and processed for Northern blot analysis. Cells cultivated for 24, 48, 72, or 96h with each medium containing rhBMP-2 (1000 or 1500ng/ml) or mBMP-4 (60% or 80%) were collected and processed for Western blot analysis, as described below.

RNA preparation and reverse transcriptase-polymerase chain reaction (RT-PCR)

Total RNA from primary culture cells derived from embryonic mouse thigh muscle and MC3T3-E1 and NIH3T3 cells was extracted using Isogen (Nippon Gene, Tokyo, Japan) according to the manufacturer's instructions. After treating with RNase-free deoxyribonucleases II (Gibco-BRL), complementary DNA (cDNA) was synthesized using an RNA polymerase chain reaction (PCR) kit (Takara Shuzo, Ohtsu, Japan) according to the manufacturer's instructions. The reaction time was 30min at 42°C. Aliquots of the cDNA pool obtained were subjected to PCR and amplified in a 20μl reaction mixture using *Taq* polymerase (Takara Shuzo). Amplifications were performed in a Program Temp Control System (PC800; ASTEC, Fukuoka, Japan) for 30 cycles after an initial denaturation step at 94°C for 3min, denaturation at 94°C for 30s, annealing for 30s at 60°C, and extension at 72°C for 90s, with a final extension at 72°C for 10min. Reaction products were electrophoresed in a 1.5% agarose gel, and the amplified DNA fragments were visualized by ethidium bromide staining under UV light. PCR products were subcloned and sequenced using a DNA sequencing kit (Applied Biosystems, Warrington, UK). The primers of Noggin, Smad-4, OC, and MyoD for PCR were set as described previously [10,13,15]. The primers of BMPRs for PCR were set as follows: BMPR-1A, 5′-CTCATGTTCAAGGGCAG-3′ (5′ sense) and 5′-CCCCTGCTTGAGATACTC-3′ (3′ antisense; 346–362 and 850–833, respectively); BMPR-1B, 5′-ATGTGGG CACCAAGAAG-3′ and 5′-CTGCTCCAGCCCAATGC T-3′ (215–231 and 681–664, respectively); BMPR-2, 5′-GTGCCCTGGCTGCTATGG-3′ and 5′-TGCCGCCTC CATCATGTT-3′ (47–64 and 592–575, respectively). Nucleotide sequences of the cDNA fragments were checked and found to be identical to mouse BMPRs (BMPR-1A, NM009758; BMPR-1B, NM007560; BMPR-2, NM007561). The specificity of these cDNAs was confirmed by sequencing using an autosequence analyzer (ABI Prism 310 Genetic Analyzer; Perkin-Elmer Japan, Tokyo, Japan).

Northern blot analysis

Twenty micrograms of total RNA were separated by electrophoresis on a 1.0% agarose–formaldehyde gel and blotted onto Hybond-N^+ membrane (Amersham Intl., Piscataway, NJ, USA) for Northern blotting. Filters were hybridized overnight with random-primed [^{32}P]-labeled mouse BMPRs, Noggin, OC, Smad-4, and MyoD cDNA fragment probes at 65°C for 3h in hybridization buffer (50mM Tris-HCl (pH 7.5), 1mg/ml denatured salmon sperm DNA, 1% SDS, 1M NaCl, 10mM EDTA, 0.2% Ficol 400, 0.2% polyvinylpyrolidone, and 0.2% bovine serum albumin) and washed three times with 0.1 × SSC and $NaDodSO_4$ for 1h at 68°C. The signals were detected by a BioImaging Analyzer BAS-1500 (Fuji Photo Film, Tokyo, Japan). For reprobing, each hybridized probe was removed by boiling the membrane in 0.5% SDS, and then sequentially hybridized with the respective target probes.

Western blotting

Muscle-derived primary culture cells, MC3T3-E1 cells, NIH3T3 cells, and mouse skeletal muscle tissue (as a posi-

tive control) were homogenized and dissolved in 0.5ml sample buffer (0.05M Tris-HCl (pH 6.8), 2% SDS, 6% β-mercaptoethanol, and 10% glycerol) and centrifuged at 12000*g* for 5min at 4°C. The supernatant was used as the sample, and the protein content of each sample was measured by UV assay at an OD of 280nm. Anti-mouse Noggin antibody (R&D Systems, Minneapolis, MN, USA) was used at 1μg/ml. Polyclonal goat antibodies for BMPRs (Santa Cruz, San Diego, CA, USA) were also used at a dilution of 1μg/ml. Aliquots of protein solution (5μl) were adjusted to 1μg/μl, mixed with 1% BPB (1μl), and then boiled for 2min and loaded onto each lane of SDS (10%–20%) acrylamide gradient gels (35mA, low voltage, 90min). After running the gels, BMPR-1A, -1B, -2, and Noggin proteins in mouse embryo muscle-derived cells, and MC3T3-E1 and NIH3T3 cells, and mouse skeletal muscle tissue were stained with Coomassie brilliant blue (Sigma Chemical). The protein bands were then transferred to polyvinylidene difluoride membrane (Immunobilon-P Transfermembrane, Millipore, Bedford, MA, USA) according to the manufacturer's instructions. After treatment with Blocking Reagent (Nippon Roche, Tokyo, Japan) for 1h at room temperature, the membranes were washed with PBS for 5min, and then incubated for 1h with primary antibody (BMPRs, 1:200; Noggin, 1:100). After two 5-min washes with PBS, the membranes were incubated with peroxidase-conjugated rabbit anti-goat antibody (1:50; Histofine, Nichirei, Tokyo, Japan) for 1h. After two further 5-min washes with PBS, the immunoblot was developed using an ImmunoStar Kit for Rabbit (Wako Pure Chemical Industries, Tokyo, Japan) to detect biotin and chemiluminescence.

Results

Expression level of messenger RNA (mRNA)

The increase in transcription of BMPR-1A, -2, Noggin, OC, and Smad-4 appeared to be dose-dependent. The expression pattern of these molecules in muscle-derived primary culture cells after 24h stimulation by rh-BMP-2 or mBMP-4 is shown in Fig. 1. When we performed Northern blotting on all cell sources using 0, 10, 100, 500, 1000, and 1500ng/ml doses of rhBMP-2, or 0%, 10%, 20%, 40%, 60%, and 80% doses of mBMP-4, the gene expression levels of these molecules were the similar at the following doses: rhBMP-2 (1000ng/ml), rhBMP-2 (1500ng/ml), or mBMP-4 (60%) and mBMP-4 (80%). Therefore, the expression of BMPR-1A, -2, Noggin, OC, and Smad-4 appeared to reach a plateau at the 1000ng/ml dose of rhBMP-2 and 60% mBMP-4. The mRNA expression of these molecules was readily detected at the 10ng/ml dose of rhBMP-2 and 10% mBMP-4 (Figs. 1 and 2).

The expression levels of BMPR-2 and Noggin mRNA were sharply elevated on day 1, and then decreased gradually in the muscle-derived primary culture cells at all concentrations. Representative expression patterns using rhBMP-2 (100ng/ml) or mBMP-4 (20%) are shown in Figs. 3A and 4A. BMPR-1A transcription was also elevated on day 1, but at lower levels when compared with BMPR-2 at all concentrations (Figs. 3 and 4).

Similar patterns for BMPR-1A, -2, and Noggin were observed in MC3T3-E1 and NIH3T3 cell lines, but to a much lower degree than that seen in the muscle-derived primary culture cells at all concentrations. The typical expression figures using rhBMP-2 (1000ng/ml) or mBMP-4 (60%) in MC3T3-E1 and NIH3T3 cell lines are shown in Figs. 5–8. BMPR-1B expression was not detected by Northern blotting before or after BMP stimulation in any cell sources examined, or in OC later on day 4. The Smad-4 mRNA level gradually increased, and reached a plateau from day 2 (Figs. 5–8).

MyoD mRNA expression was detected without BMP-2 or -4 exposure throughout the experimental period. A typical figure in which muscle-derived primary culture cells were stimulated by BMP-2 or -4 after 24h is shown in Fig. 9A or 9B.

Protein expression levels

Western blotting on the muscle-derived primary culture cells using either 1000 or 1500ng/ml doses of rhBMP-2, or 60% or 80% of mBMP-4, revealed that the protein expression levels of BMPR-1A, -2, and Noggin were the same at the 1000ng/ml and 1500ng/ml doses of rhBMP-2, and at 60% or 80% concentrations of mBMP-4. Therefore, rhBMP-2 (1000ng/ml) or mBMP-4 (60%) was used for all subsequent studies of protein expression levels in the muscle-derived primary culture cells (Fig. 10). We did not perform Western blotting on MC3T3-E1 and NIH3T3 cell lines because of the weak expression revealed by Northern blotting.

The translational expression levels of BMPR-1A, -2, and Noggin were enhanced on day 2 and then decreased gradually in the muscle-derived primary culture cells. BMPR-1B expression was not detectable by Western blotting before or after BMP stimulation in the muscle-derived primary culture cell (Fig. 10).

Discussion

This study showed increased transcription and translation of BMPR-1A, -2, and Noggin and increased transcription of OC and Smad-4 in response to rhBMP-2 or mBMP-4 in muscle-derived primary culture cells. Clearly, the muscle-derived primary culture cells are capable of responding to changes in the external concentrations of the bone growth factors. Induction of BMPR-1A and -2 following exposure to BMPs points to the activation of a receptor-mediated pathway to effect intracellular signaling by these molecules. Although the reason for the predominant induction of BMPR-2 among BMPRs is unknown at present, it is possible that an increased number of BMPR-2 molecules with a high affinity for BMP might allow greater capture of this

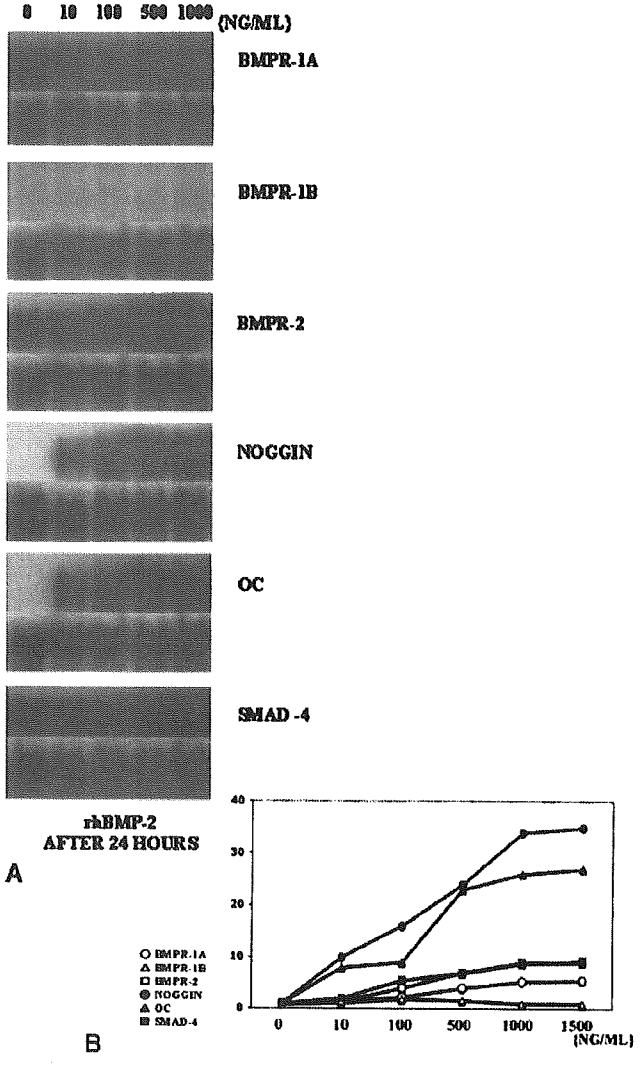

Fig. 1. Gene expression level of BMPR-1A, -1B, -2, Noggin, OC, and Smad-4 after 24-h stimulation of rhBMP-2 (0, 10, 100, 500, 1000 ng/ml) in muscle-derived primary culture cells by Northern blot analysis (**A**) and quantitation of the data of Northern blot analysis by Densitometry (**B**) G3PDH mRNA levels (*the bottoms of all lanes* are G3PDH) obtained by Northern blotting were used for normalization (**A**). The score on hour 0 (just after BMP stimulation) was used as a standard (**B**) BMPR-1A, -2, Noggin, OC, and Smad-4 were up-regulated dose-dependently. No increase in BMPR-1B expression was observed during the course of the study. The expression levels of these molecules were almost the same using 1000 ng/ml and 1500 ng/ml rhBMP-2 (1500 ng/ml rhBMP-2 data not shown in Fig. 1A)

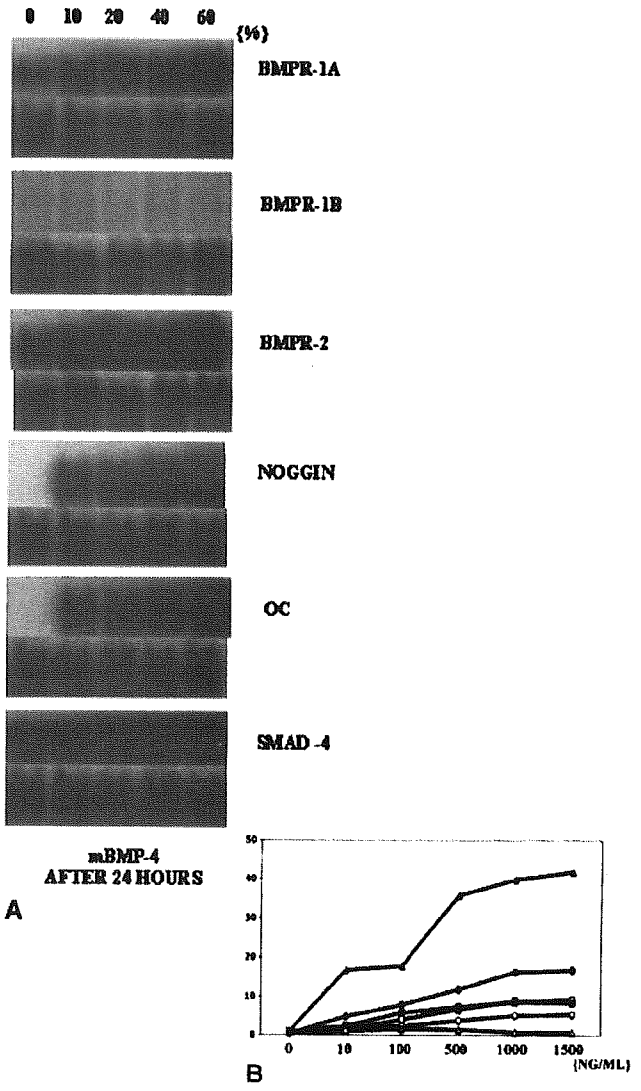

Fig. 2. Gene expression level of BMPR-1A, -1B, -2, Noggin, OC, and Smad-4 after 24-h stimulation of mBMP-4 [0%, 10%, 20%, 40%, and 60% (v/v) conditioned media] in muscle-derived primary culture cells by Northern blot analysis (**A**) and quantitation of the data of Northern blot analysis by Densitometry (**B**). G3PDH mRNA levels (*the bottoms of all lanes* are G3PDH) obtained by Northern blotting were used for normalization (**A**). The score on hour 0 (just after BMP stimulation) was used as a standard (**B**) The gene expression pattern of the molecules after stimulation of mBMP-4 was similar to that observed after stimulation of rhBMP-2. The expression levels of these molecules were almost the same using 60% and 80% mBMP-4. (80% mBMP-4 data not shown in Fig. 2A)

ligand and subsequent activation of BMPR-1A for enhanced transduction of the BMP signal into cells.

We have observed that expression of BMPR-1A and -2 is significantly increased during the early phase of ectopic bone formation following the implantation of rhBMP-2 into the back muscles of adult mice [10]. Based on these data from in vivo and in vitro studies, the activation of BMPR-1A after BMPR-2 might be a key event following BMP stimulation of muscle tissue. BMPR-1A, -2, and Noggin were induced in MC3T3-E1 and NIH3T3 cell lines, but to a much lesser degree than that seen in the muscle-derived primary culture cells used in this study.

A specific role of BMPR-1B in skeletal development has been proposed based on the abnormal interphalangeal joint formation in an animal with a null mutation in this receptor. However, the expression of BMPR-1B appeared to be limited in the muscle-derived primary culture cells and the osteoblastic or nonosteoblastic embryonic cell lines, even after exposure to BMPs [16–19]. The lack of expression of BMPR-1B was in accordance with results in a previous

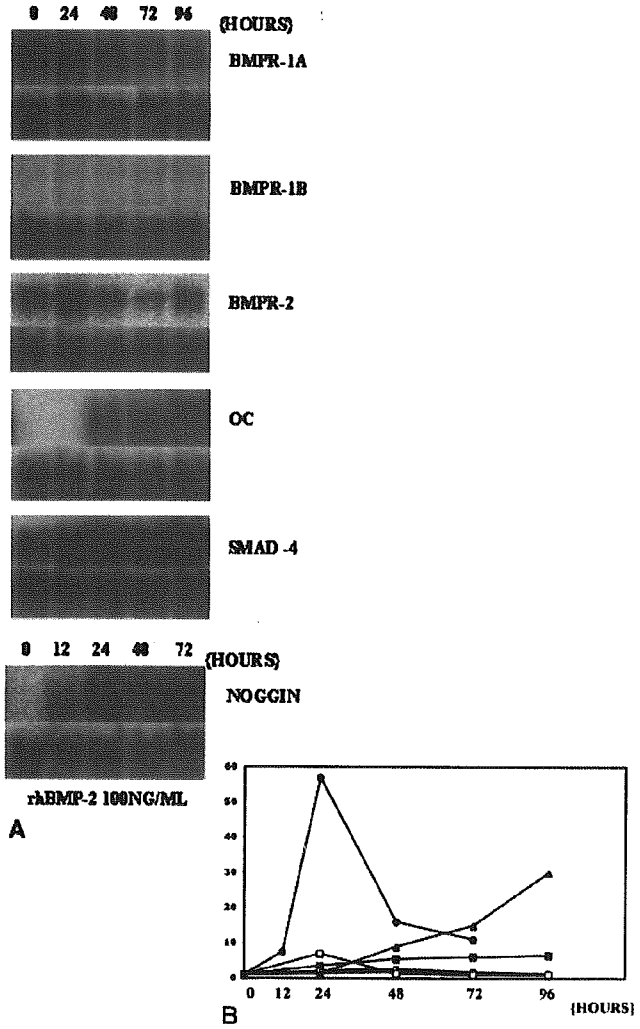

Fig. 3. Gene expression of BMPR-1A, -1B, -2, OC, and Smad-4 for 0, 24, 48, 72, and 96 h and Noggin for 0, 12, 24, 48, and 72 h after 100 ng/ml rhBMP-2 stimulation in muscle-derived primary culture cells by Northern blot analysis (**A**) and quantitation of the data of Northern blot analysis by Densitometry (**B**). G3PDH mRNA levels (*the bottoms of all lanes* are G3PDH) obtained by Northern blotting were used for normalization (**A**). The score on hour 0 (just after BMP stimulation) was used as a standard (**B**). After rhBMP-2 stimulation, OC was up-regulated time-dependently. Noggin level peaked at 24 h. Expression of BMPR-1A and -2 was increased moderately after 24 h, then gradually decreased thereafter. Smad-4 was gradually and weakly up-regulated after stimulation. BMPR-1B was not increased during the experimental period

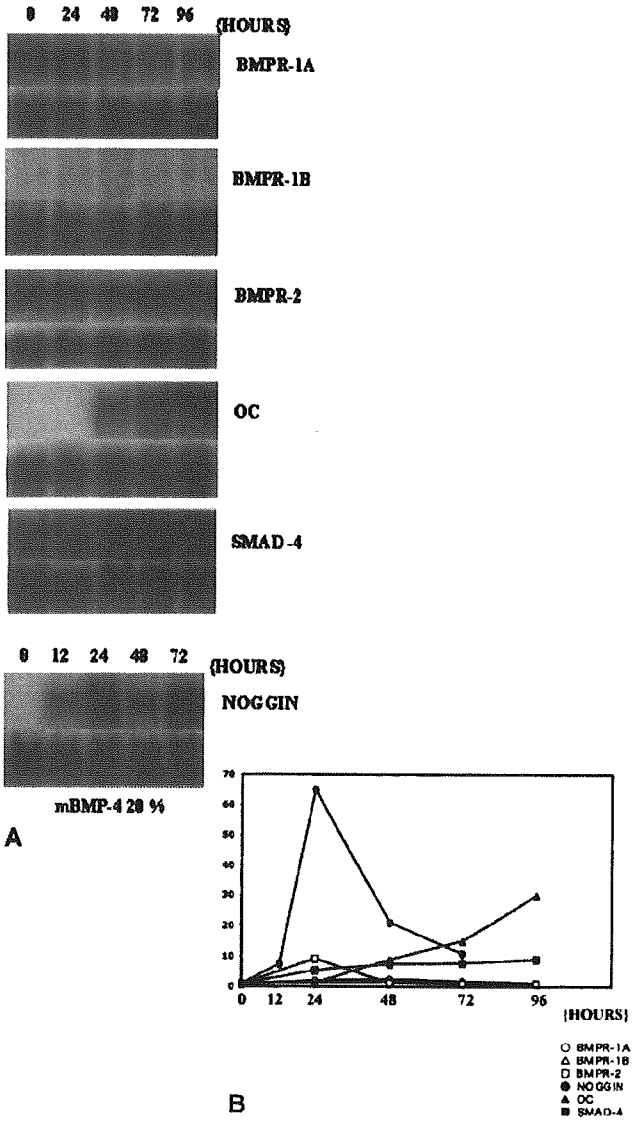

Fig. 4. Gene expression of BMPR-1A, -1B, -2, OC, and Smad-4 for 0, 24, 48, 72, and 96 h and Noggin for 0, 12, 24, 48, and 72 h after 20% mBMP-4 stimulation in muscle-derived primary culture cells by Northern blot analysis (**A**) and quantitation of the data of Northern blot analysis by Densitometry (**B**). G3PDH mRNA levels (*the bottoms of all lanes* are G3PDH) obtained by Northern blotting were used for normalization (**A**). The score on hour 0 (just after BMP stimulation) was used as a standard (**B**). The gene expression pattern of the molecules after stimulation of mBMP-4 (20%) was similar to that seen after stimulation of 100 ng/ml rhBMP-2

report using the pluripotent C2C12 cell line, and another study that revealed predominant expression of BMPR-1B in brain and not skeleton [20].

The induction of Noggin gene expression in cells of the osteoblastic lineage following exposure to rhBMP-2, and in fetal rat limb explants by BMP-7, has been reported [21,22]. In this study, Noggin gene expression was also confirmed in muscle-derived primary culture cells, an osteoblastic cell line (MC3T3-E1), and a nonosteoblastic, embryonic fibroblast-like cell line (NIH3T3) [16,23,24]. As Noggin is a representative antagonist of BMP action, the expression of Noggin might act as a negative regulator of the BMP-induced cellular reactions, and consequently reduce the susceptibility of the cells to BMPs.

Three classes of Smads, termed receptor-activated Smads (R-Smads), common Smads (Co-Smads), and inhibitory Smads (I-Smads), have been identified in mammals. Smads1, 5, and 8 are R-Smads that primarily mediate BMP signaling from the receptors to the nucleus [16,25]. Therefore, the up-regulation of Smad-4, which is a representative BMP signaling Co-Smad, in a time- or dose-

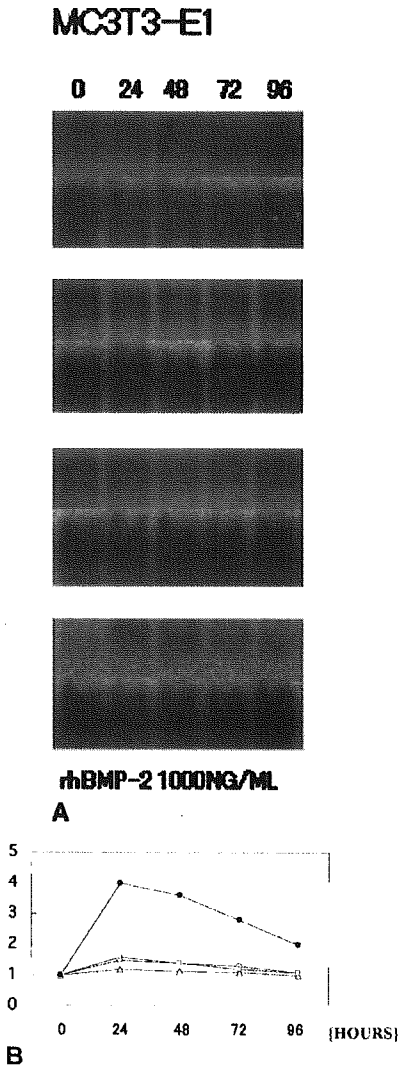

Fig. 5. Gene expression of BMPR-1A, -1B, -2, and Noggin for 0, 24, 48, 72, and 96 h after 1000 ng/ml rhBMP-2 stimulation in MC3T3-E1 cell line by Northern blot analysis (**A**) and quantitation of the data of Northern blot analysis by Densitometry (**B**). G3PDH mRNA levels (*the bottoms of all lanes* are G3PDH) obtained by Northern blotting were used for normalization (**A**). The score on hour 0 (just after BMP stimulation) was used as a standard (**B**). BMPR-1A and -2 were weakly induced after rhBMP-2 stimulation, peaked at 24 h, then decreased gradually. Noggin was also moderately induced after stimulation showed maximal expression at 24 h, then decreased thereafter. BMPR-1B was not induced during the course of the reaction

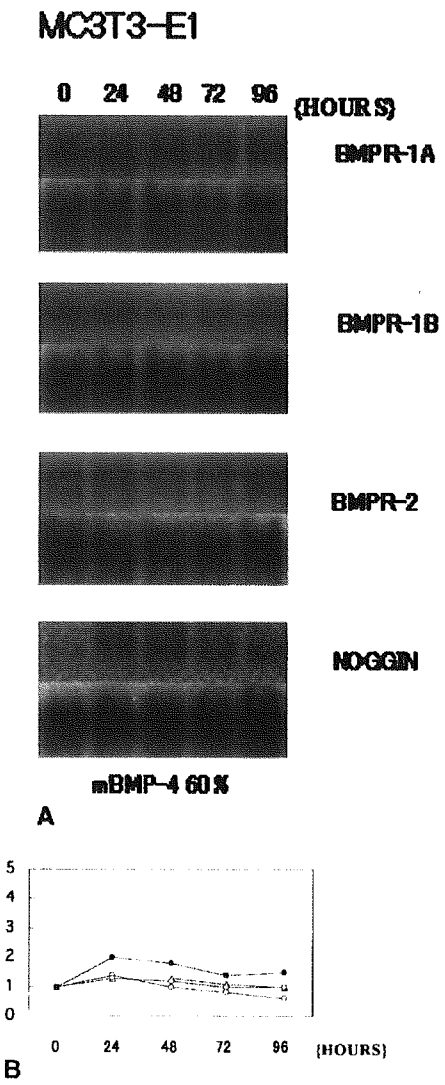

Fig. 6. Gene expression of BMPR-1A, -1B, -2, and Noggin for 0, 24, 48, 72, and 96 h after mBMP-4 (20%) stimulation in MC3T3-E1 cell line by Northern blot analysis (**A**) and quantitation of the data of Northern blot analysis by Densitometry (**B**). G3PDH mRNA levels (*the bottoms of all lanes* are G3PDH) obtained by Northern blotting were used for normalization (**A**). The score on hour 0 (just after BMP stimulation) was used as a standard (**B**). The gene expression pattern of the molecules after stimulation of mBMP-4 (20%) was similar to that seen after stimulation of 1000 ng/ml rhBMP-2, but the expression levels with mBMP-4 (20%) were smaller than those with 1000 ng/ml rhBMP-2

dependent manner suggests that BMP signaling in muscle tissue is regulated in a coordinated manner. OC is a well-characterized osteoblast differentiation marker, and MyoD is also a good marker for myoblastic differentiation [26]. Although the expression of MyoD was not detected in this study, the expression of OC was enhanced on day 2 after BMP-2 or -4 stimulation. These results indicate that BMP-induced osteogenic differentiation in muscle tissue might occur through a BMP/Smad signaling pathway, and muscle-derived primary culture cells might lose the muscle phenotype after BMP exposure.

The expression profiles were much more prominent for primary undifferentiated mesenchymal cells derived from muscle than for MC3T3-E1 or NIH3T3 cells in this study. Muscle-derived primary culture cells include a large population of undifferentiated mesenchymal cells, as described elsewhere [14]. Clearly, undifferentiated mesenchymal cells in muscle tissue are highly responsive to BMPs, based on

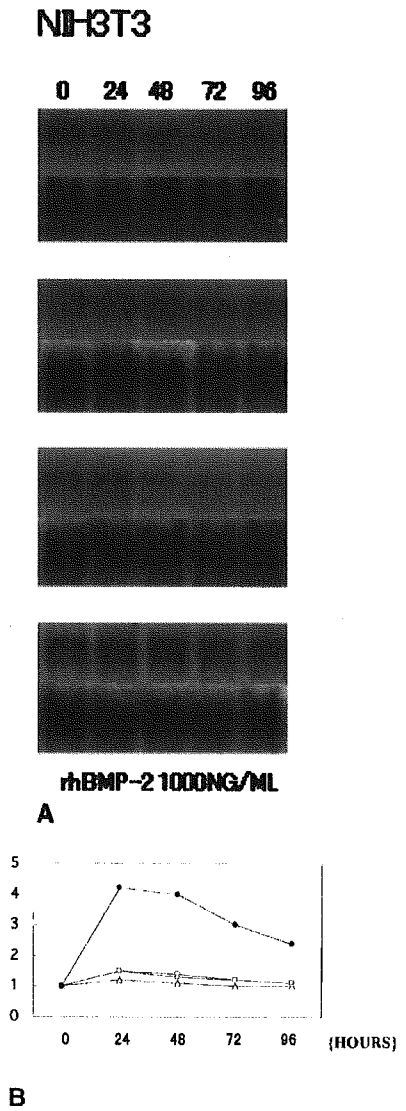

Fig. 7. Gene expression of BMPR-1A, -1B, -2, and Noggin for 0, 24, 48, 72, and 96 h after 1000 ng/ml rhBMP-2 stimulation in NIH3T3 cell line by Northern blot analysis (**A**) and quantitation of the data of Northern blot analysis by Densitometry (**B**). G3PDH mRNA levels (*the bottoms of all lanes* are G3PDH) obtained by Northern blotting were used for normalization (**A**). The score on hour 0 (just after BMP stimulation) was used as a standard (**B**). BMPR-1A and -2 were weakly induced after rhBMP-2 stimulation, peaked at 24 h, then decreased gradually. Noggin was moderately induced after stimulation showed maximal expression at 24 h, then decreased thereafter

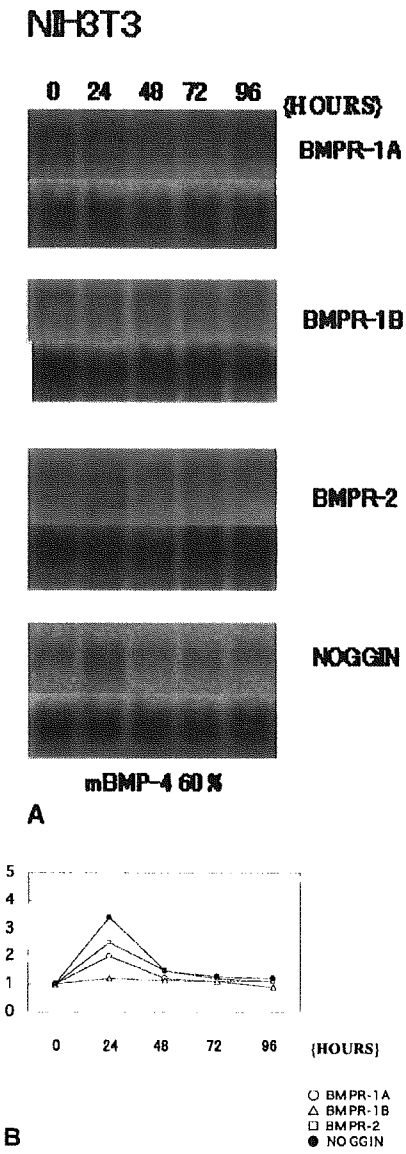

Fig. 8. Gene expression of BMPR-1A, -1B, -2, and Noggin for 0, 24, 48, 72, and 96 h after mBMP-4 (20%) stimulation in NIH3T3 cell line by Northern blot analysis (**A**) and quantitation of the data of Northern blot analysis by Densitometry (**B**). G3PDH mRNA levels (*the bottoms of all lanes* are G3PDH) obtained by Northern blotting were used for normalization (**A**). The score on hour 0 (just after BMP stimulation) was used as a standard (**B**). BMPR-1A and -2 were weakly induced after rhBMP-2 stimulation, peaked at 24 h, then decreased gradually. Noggin was moderately induced after stimulation showed maximal expression at 24 h, then decreased thereafter. BMPR-1B was not induced in all experimental stages. In NIH3T3 cells, the expression pattern was similar to that observed in the MC3T3-E1 culture experiments. Expression levels were greater in NIH3T3 cells than in MC3T3-E1 cells

the changes in gene and protein expression levels observed in this study. The proliferation and differentiation of osteoblasts from osteoprogenitor cells in murine bone marrow cultures induced by BMP-2 or -4 have been reported [27,28]. However, there have been few reports using muscle-derived primary culture cells with BMPs. In this study, the expression of BMP-related molecules was examined using undifferentiated mesenchymal cells derived from mouse muscle tissue.

The majority of undifferentiated mesenchymal cells in muscle-derived primary culture cells showed a fibroblastic appearance. These cells are considered to be heterogenous, and contain some kinds of precursor cells such as bone, cartilage, and muscle. They differentiate into each phenotype when they are placed in each differentiation condition.

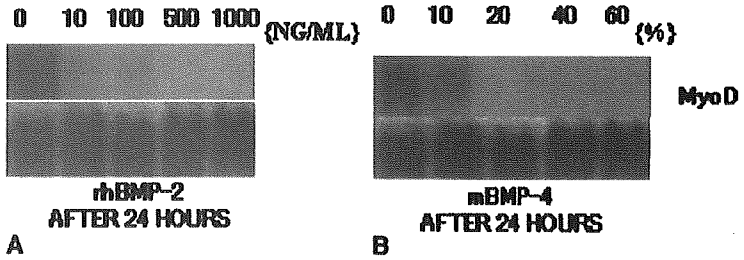

Fig. 9. The expression of MyoD in muscle-derived primary culture cells by Northern blot analyses. G3PDH mRNA levels obtained by Northern blotting were used for normalization. The expression of MyoD mRNA was not detected after BMP-2 or -4 exposure, and the expression was detected only at 0 and 24h, and not after 24h BMP stimulation

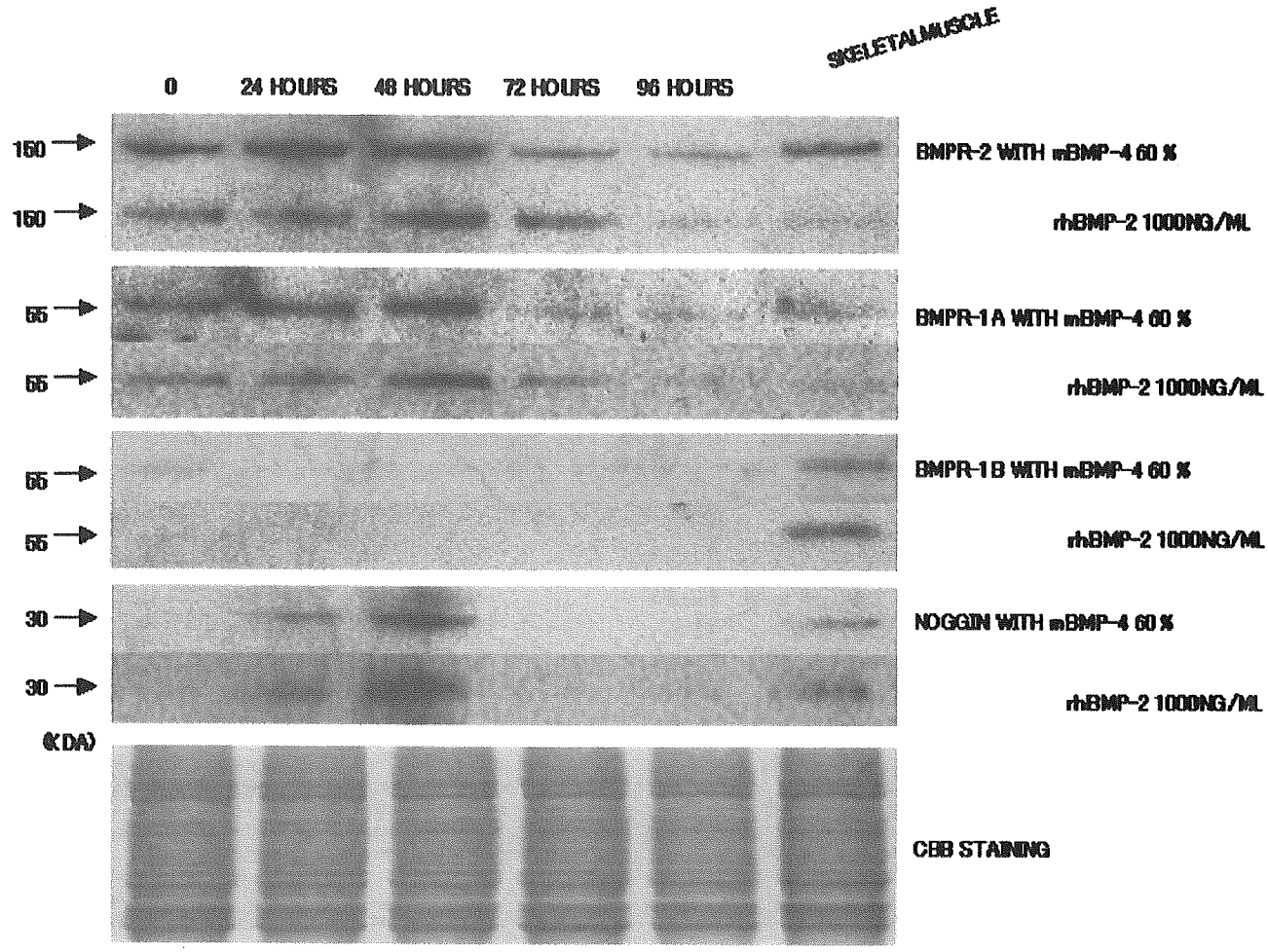

Fig. 10. Western blot analysis of BMPR-1A, -1B, -2, and Noggin after 60% mBMP-4 or 1000ng/ml rhBMP-2 stimulation in muscle-derived primary culture cells. Equivalent loading and integrity of protein were confirmed by Coomassie brilliant blue staining on the gel (*lower panel*). Mouse skeletal muscle proteins were used as positive controls. BMPR-1A and -2 were detected at 0h, induced at 24h, peaked at 48h, and then gradually decreased in both 60% mBMP-4 and 1000ng/ml rhBMP-2 stimulation groups. Expression was greater for BMPR-2 than for BMPR-1A. BMPR-1B was not detectable during any stages in either treatment group. Noggin was not detected at 0h, was up-regulated at 24h, peaked at 48h, and decreased thereafter

In our study, BMPs stimulated them to upregulate the expressions of a bone marker (OC) and cartilage markers (type II collagen and aggrecan, data not shown), but not the muscle marker examined previously. However, it is unclear whether bone and cartilage phenotypes were induced by BMPs in separate cells or in a single cell.

To further understand the potential autoregulatory mechanism in response to BMP, further gene expression

studies will be necessary. Ultimately, this knowledge may provide new approaches to the regulation of local and systemic bone formation.

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運動についての評価

小池 達也*

骨粗鬆症および関連する骨折は，高齢化が進行する社会において，重要な問題になっている。骨量が減少した状態である骨粗鬆症においては，目に見えない形で骨の微細構造が破綻しており，骨強度が減少し軽微な外傷により骨折を引き起こす。これまで運動は，骨量を増加維持する有効な手段であると考えられてきた。スポーツ選手を対象とした横断分析では，運動と骨量の間に有意な相関が認められてきたが，もともと体格の大きい人がスポーツ活動を行う傾向があるというバイアスが入り込んでいる可能性がある。一般高齢女性において，身体活動性が高い群に大腿骨頸部骨折が少ないという報告もあるが，骨を増加させるような激しい運動によって得られる骨量増加効果はわずかであり，ビスホスホネート製剤を投与した時よりも劣る。骨粗鬆症の予防法として運動を評価する場合には，転倒防止や骨折予防に焦点を当てるべきである。

Evaluation of Exercise

Rheumatosurgery, Osaka City University Medical School

Tatsuya Koike

Osteoporosis and osteoporotic fractures have become an epidemic in the industrialized world. Osteoporosis, low bone mass, is a silent condition with microarchitectural deterioration of the bone structure leading to decreased bone strength and osteoporotic fractures. Physical activity has been advocated as offering a potential means to increase and maintain bone mineral density. Previous cross-sectional studies showed that there is a strong association between exercise and bone mineral density, especially in athletic individuals. However, there might be a self-selection bias ; i.e. individuals with larger muscles and bones are more likely to choose an athletic lifestyle. Although there is a report that physical activity is associated with a reduced risk for hip fracture among older community-dwelling women, the effects of vigorous exercises building bone mass is modest and considerably less than bisphosphonates. The proper evaluation of exercise as a preventative therapy for osteoporosis should focus on prevention of falls or osteoporotic fractures.

*大阪市立大学大学院医学研究科リウマチ外科学・助教授（こいけ・たつや）

はじめに

骨粗鬆症は，文明化が進み高齢者人口が増加している国々において，非常に重要な問題になりつつある。最も大きな問題は大腿骨頸部骨折や脊椎変形であり，これらのイベントが発生することにより，高齢者の日常生活動作は極度に制限され，非介護人口の増加に結びつくため，医療経済学的に見ても解決策の確立が急務である。

骨粗鬆症には，他の退行期疾患同様に危険因子が存在し，逆に危険因子に対する予防措置をとることで予防や治療に結びつく可能性がある。その一つが身体活動性である。これまで多くの研究が行われ，横断分析において身体活動性と骨量の正の相関が報告され，骨折との関係も論じられてきた。しかし，骨量増加を目的とした縦断分析においては，緩やかな効果しか報告されておらず，目標設定が誤っているという指摘[1]もある。

本稿では，これまでの運動と骨粗鬆症の関係を論じた研究の変遷をたどってみたい。

骨量に及ぼす運動の影響

不動性は骨量低下の重要な因子であり，運動により骨量が増える程度に比して，非動化による骨量減少はより顕著である。健常人に強制的にベッドレストを行わせても骨量低下は観察されるし[2]，脳卒中や脊髄損傷により片麻痺や対麻痺が生じた場合にも，運動機能を失った四肢には著明な骨量減少が生じる。極端な例では，宇宙空間で無重力状態での生活を行うと，バネやゴムを用いて強力な運動を行っても骨量低下を完全に防止することはできない[3]。

逆に，骨に負荷をかけることによって，骨密度は増加する。多くの横断分析は，荷重負荷運動により，peak bone massが高くなると報告している。20歳前後の男子大学生を対象に我々が行った研究においても，短距離走・野球・バレーボール・柔道などの衝撃力あるいは荷重負荷がかかる運動を日常的に行っている群が，運動を行っていない群よりも高い骨塩量を示した（図1）。

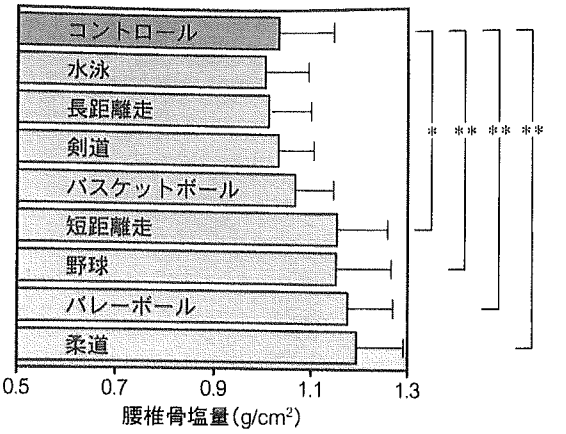

図1　運動種目別の腰椎骨塩量

男子大学生180人(平均年齢20歳)の腰椎骨塩量を種目別に比較。体重補正済み。
*：$p < 0.05$，**：$p < 0.01$，共分散分析。
（筆者作成）

運動効果と年齢の関係

骨粗鬆症は高齢女性において最も大きな問題となるが，低骨塩量は成人前から存在する可能性がある。高いpeak bone massを有する人たちは，その後の骨量減少に耐えうると考えるのは妥当な推論であろう。従って，骨量を高めるような運動をいつから開始すべきであるかという疑問が生じる。初潮前の少女に対して衝撃性の強い運動を10カ月間処方することにより，筋力も骨量も増加し得たとする報告[4]や，思春期前の少年に対する8カ月間にわたる中程度の運動負荷による骨量増加[5]の報告から考えて，成長期の骨は運動負荷に対して感受性が高いと考えられる。しかも，これらの運動効果は成人後も維持されていると考えられる[6]ので，成長期に運動を行うことは，将来の骨粗鬆症を予防する意味で非常に重要であろう。

では，すでに骨粗鬆症の危険年齢域にある閉経前後の女性に対する運動効果は存在するのだろう

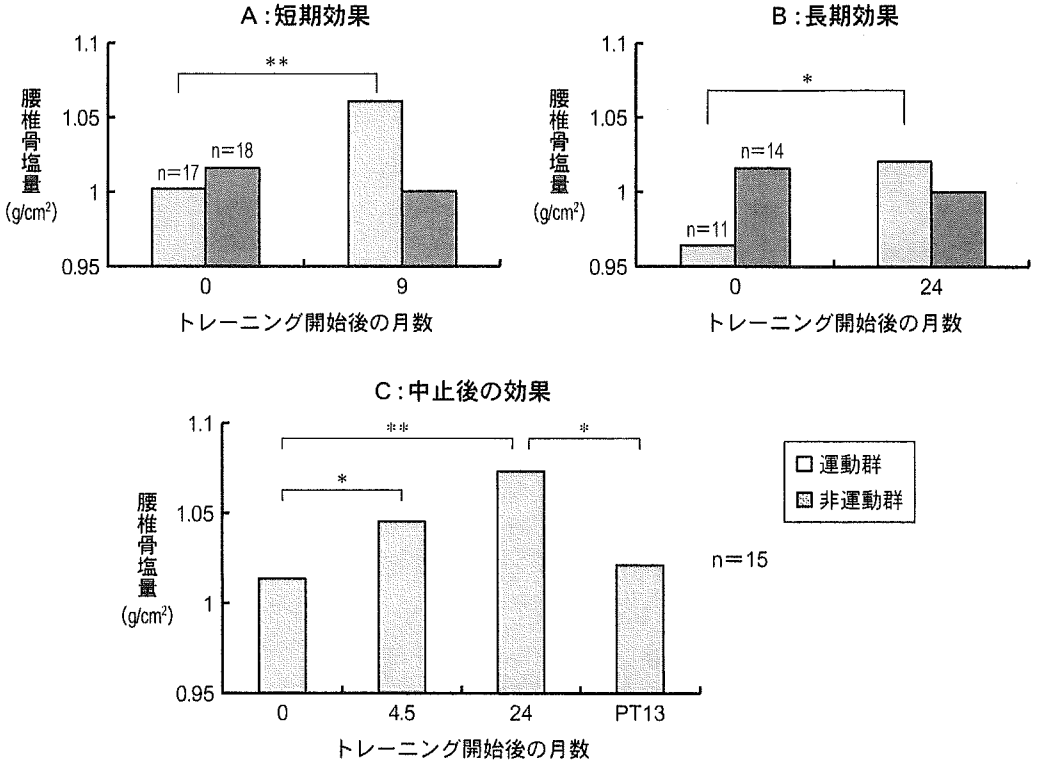

図2　運動効果が腰椎骨塩量に与える影響

55～70歳の特に運動をしていない女性を対象に，最大酸素摂取量の70～90%の負荷となるように，週に3回1時間の荷重運動（歩行，ジョギング，階段昇降）を行わせた。

A：短期効果。運動群は9カ月後に有意に腰椎骨塩量が増加したが，非運動群では有意ではないものの骨塩量の低下を認めた。

B：長期効果。24カ月でも同様の効果を認めた。

C：中止後の効果。運動負荷群のみの経過を見ると，運動負荷後24カ月までは有意に腰椎骨塩量は増加したが，運動を中止すると，その効果は消失した。

PT13：運動負荷終了後13カ月。

*：$p < 0.05$，**：$p < 0.01$

（文献8より筆者作成）

か？Wallaceらによるメタアナリシスの結果[7]では，最も多く研究対象となっていた閉経後女性の腰椎骨塩量に対しては，衝撃性の強い運動（エアロビクスなど）でも，衝撃性のない運動（ウエートトレーニングなど）でも，骨量増加作用が認められた。一方，大腿骨骨塩量に対しては，衝撃性のある運動では骨量減少防止効果が認められたが，衝撃性の少ない運動では，研究の数が少ないこともあって明確な結論は得られていない。

Dalskyら[8]は，人数は少ないが縦断分析で閉経後の女性に対して運動負荷を行い，運動終了後の影響も観察している。彼らの結果を図2に示す。短期であっても長期であっても，荷重負荷運動は腰椎骨塩量を増加させうるが，運動を終了するとその効果は消失するようである。少なくとも，閉経後に始めた骨量維持運動には継続が必須である。

ところで，骨粗鬆症の治療目標は時代とともに

変化しており，疼痛の除去から骨量増加へ，そして骨折予防へと変遷してきた。実際に，近年開発されてきたビスホスホネート製剤などは，はっきりとした骨折抑制効果が証明されている。では，運動にはそのような効果が存在するのだろうか？

骨粗鬆症に伴う骨折のリスクファクターとしての運動の影響

日常生活レベルでの活動性が，脊椎変形に及ぼす影響を観察したヨーロッパでの横断分析の報告[9]がある（図3）。50歳以上の男女14,261人を対象にした研究で，過去の活動レベルを年代ごとに聴取し，脊椎変形との関係を論じている。結果は，男性では激しい活動性を続けた場合に脊椎変形が強く認められたが，女性ではそのような影響は認められなかった。彼らは同時に，現在の屋外での活動性を調査し，女性において屋外で良く歩く女性に脊椎変形が少なかったとも報告している。この結果は，女性と男性において，脊椎変形の機序が異なる可能性を示しており，骨粗鬆症治療の戦略においても男女別の方法を考える必要性があるかもしれない。

身体活動性が，脊椎以外の骨折発生にも影響しているかを調べた研究も存在する。ノルウェーで行われた16,676人（平均年齢男性47.3歳，女性45.1歳）を対象とした大規模調査で，調査期間中に1,435件の非脊椎骨折が生じ，女性ではどの部位の骨折も年齢に伴い増加したが，男性ではそのような傾向は認められなかった。45歳以上の対象者のうち，活動性が最も高い群において荷重骨の骨折が有意に少なかった（相対危険率0.6）が，非荷重骨では活動性と骨折の間に有意な関係は認められなかった。

より高齢の女性（65歳以上）を対象としたコホート研究では，活動性の高い女性において，年齢・栄養・転倒・機能健康状態を補正した上でも，大腿骨頸部骨折の発生頻度が低いことが報告[10]されている（図4）。身体活動性を5分割し，上位2群に限れば，活動性の一番低い群に比して，実に36%の大腿骨頸部骨折発生率の低下を認めている。この低下は，ビスホスホネートの大規模試験の結果[11]に匹敵する。しかし，これらの結果は，脊椎骨折や前腕骨骨折には当てはまらなかった。

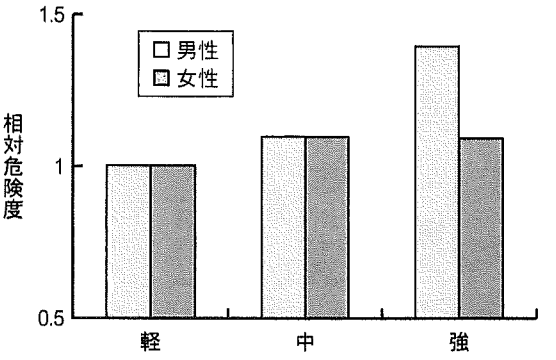

図3　脊椎変形に及ぼす過去の身体活動性の影響

50～79歳の男女14,261人にアンケート調査と脊椎レントゲン撮影を行い，過去の活動性をアンケートから評価し，脊椎変形との関係を調査。活動性は，軽・中・強の三段階に分けている。

（文献9より筆者作成）

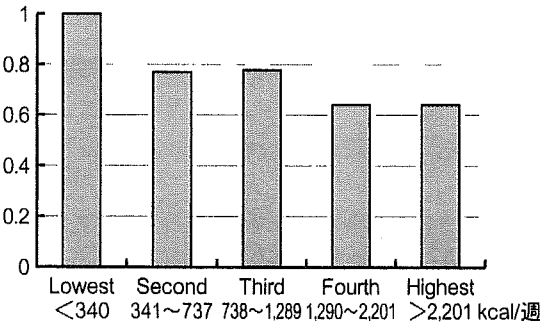

図4　身体活動性別に見た大腿骨頸部骨折発生相対危険度

9,704人の65歳以上の白人女性を対象に行ったコホート研究。週毎の消費カロリーを基準にして活動性を5段階に分けて，各群における大腿骨骨折発生頻度を比較。年齢・栄養・転倒・機能健康状態を補正。

（文献10より筆者作成）

骨粗鬆症に伴う骨折予防に対する運動の効果

高齢者における運動の効果が骨折予防に結びつくとすれば，転倒予防を介しての効果であると考えられる。転倒危険因子に関しても多くの研究があり，それから得られた結果をもとにして，多くの転倒予防プログラムが考案されている。多くの研究は，介入法として運動プログラムを用いているが，その効果は完全には証明されていない。Robertson らによるメタアナリシス[12]では，筋力強化とバランス改善プログラムにより，転倒は35%減少したが，重度外傷発生に関しては効果がなかったと結論している。

骨折を生じる転倒は，全転倒の10%程度にすぎないこともあり，骨折抑制を目標とした介入試験で有意な結果は出ていない。

おわりに

非運動性が骨量減少や骨折の危険因子として存在することは間違いない。しかし，これまでの研究は，運動処方により骨量が増えること，あるいは維持できることを証明することに焦点を当てすぎてきたきらいがある。骨粗鬆症の薬物治療の目標が，骨量増加から骨折予防に変化してきたように，骨粗鬆症の治療予防戦略の一環として運動プログラムを考えるならば，骨折予防を視野に入れた研究がなされるべきである。その際には，転倒予防がキーワードになるであろう。

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Ⅲ．栄養と運動は骨粗鬆症予防に役立つか

最も有効な骨粗鬆症の運動療法

小池　達也*

骨粗鬆症および関連する骨折は，高齢化が進行する社会において，重要な問題になっている。骨量が減少した状態である骨粗鬆症においては，目に見えない形で骨の微細構造が破綻しており，骨強度が減少し軽微な外傷により骨折を引き起こす。これまで，運動は骨量を増加維持する有効な手段であると考えられてきた。スポーツ選手を対象とした横断分析では，運動と骨量の間に有意な相関が認められてきたが，もともと体格の大きい人がスポーツ活動を行う傾向があるというバイアスが入り込んでいる可能性がある。一般高齢女性において，身体活動性が高い群に大腿骨頸部骨折が少ないという報告もあるが，骨を増加させるような激しい運動によって得られる骨量増加効果はわずかであり，ビスホスホネート製剤よりも劣る。骨粗鬆症の予防法として運動を評価する場合には，転倒防止や骨折予防に焦点を当てるべきである。

The best physical therapy for osteoporosis

Rheumatosurgery, Osaka City University Medical School

Koike Tatsuya

Osteoporosis and osteoporotic fractures have become an epidemic in the industrialized world. Osteoporosis, low bone mass, is a silent condition with microarchitectural deterioration of the bone structure leading to decreased bone strength and osteoporotic fractures. Physical activity has been advocated as offering a potential means to increase and maintain bone mineral density. Previous cross-sectional studies showed that there is a strong association between exercise and bone mineral density, especially in athletic individuals. However, there might be a self-selection bias; i.e. individuals with larger muscles and bones are more likely to choose an athletic lifestyle. Although there is a report that physical activity is associated with a reduced risk for hip fracture among older community-dwelling women, the effects of vigorous exercises building bone mass is modest and considerably less than bisphosphonates. The proper evaluation of exercise as a preventative therapy for osteoporosis should focus on prevention of falls or osteoporotic fractures.

*大阪市立大学大学院医学研究科リウマチ外科学・助教授（こいけ・たつや）

はじめに

骨粗鬆症は，文明化が進み高齢者人口が増加している国において，認知症と並び非常に重要な問題である。骨粗鬆症によって引き起こされる大腿骨頸部骨折や脊椎変形が問題であり，これらのイベントが発生することにより高齢者の日常生活動作は極度に制限され，被介護人口の増大につながる。

骨粗鬆症には他の退行期疾患同様に危険因子が存在し，それらの危険因子を除去することによって，予防や治療に結びつく可能性がある。危険因子には，遺伝・ライフスタイル・疾患・外傷などがあるが，介入できるとすれば身体活動性のコントロールであろう。

これまで多くの研究が行われ，横断分析において身体活動性と骨量の正の相関が報告され[1)~3)]，骨折との関係も論じられてきた[4)]。しかし，骨量増加を目的とした縦断分析においては緩やかな効果しか報告されておらず[5)]，目標設定が誤っているという指摘もある[6)]。では，現時点で最も有効な骨粗鬆症の運動療法は何で，その目的はどこにあるのだろうか。

不動性と骨代謝

不動性は骨量低下の重要な因子であり，運動により骨量が増える程度に比して，不動化による骨量減少はより顕著である。健常人に強制的にベッドレストを行わせても骨量低下は観察されるし[7)]，脳卒中や脊髄損傷により片麻痺や対麻痺が生じた場合にも，運動機能を失った四肢には著明な骨量減少が生じる。極端な例では，宇宙空間で無重力状態での生活を行うと，バネやゴムを用いて強力な運動を行っても骨量低下を完全に防止することはできない。従って，極端な不動性は骨代謝にとって不利であることは間違いないが，運動を行うことが骨代謝にとってどの程度有利であるのかははっきりしない。

運動種目と骨塩量

運動種目と骨塩量の関係を調査した数多くの研究は，ハイレベルスポーツ[8)]であってもアマチュアレベルスポーツ[9)]であっても，荷重負荷がかかる運動（重量挙げなど）の方が非荷重運動（水泳など）よりも高い骨塩量を獲得できるという結果で一致している。

しかし，これらの研究には大きなバイアスが含まれている。体格が大きいために重量挙げを選択し，身長が高いためにバスケットボールに参加したかも知れないからである。

同一人物の身体各部位の骨塩量を比較することで，この問題の答えを得ようとした研究がある。Huddlestonら[10)]は，比較的高齢まで長期にわたってテニスを続けてきた35名のテニスプレーヤーの利き手側前腕骨骨量が，反対側より有意に高いことを示した。また，Morelら[9)]は全身骨塩量に対する身体各部位の骨塩量の比率を種目別に検討し，サッカー・長距離では足の比率が高く，登山・水泳では腕の比率が高いことを報告している。

これらの事実は，運動による骨量増加作用が身体各部位に特異的に及んでいることを示しており，特定の部位に非日常的負荷がかかることが重要であることが分かる。

運動を始める時期

骨粗鬆症は高齢女性において最も大きな問題となるが，低骨塩量は成人前から存在する可能性がある。高いpeak bone massを有する人たちは，その後の骨量減少に耐え得ると考えるのは妥当な推論であろう。従って，骨量を高めるような運動をいつから開始すべきであるかという疑問が生じる。

初潮前の少女に対して衝撃性の強い運動を10カ月間処方することにより，筋力も骨量も増加し得たとする報告[11)]や，成長期の活動性が骨塩量増