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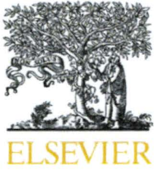

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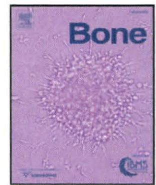

Epinephrine accelerates osteoblastic differentiation by enhancing bone morphogenetic protein signaling through a cAMP/protein kinase A signaling pathway

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ABSTRACT

Topical effects of a catecholamine on bone morphogenetic protein (BMP)-induced ectopic bone formation were investigated in both *in vivo* and *in vitro* experimental systems. Epinephrine enhanced bone induction by BMP-2. Thus, the mass of ossicles ectopically induced by BMP-2 (5 μg) was increased by the addition of a low dose (10, 20, 40, or 80 μg) of epinephrine into a biodegradable BMP-2 carrier, in a dose-dependent manner. To investigate the mechanism by which epinephrine enhances BMP activity, *in vitro* experiments were carried out using osteogenic cells. The expression level of alkaline phosphatase (ALP) in cells, a marker of osteoblastic differentiation, was consistently elevated by BMP-2 (50 ng/ml) and was further elevated by the addition of epinephrine (10^{-8} M). The epinephrine-enhanced ALP elevation was specifically abolished by an antagonist to β2-adrenergic receptors (Butoxamine) and by a protein kinase A inhibitor (H89). Furthermore, BMP-induced mRNA expression of ALP and osteocalcin (marker proteins of osteoblastic differentiation) and of Osterix (a transcription factor essential for terminal differentiation to osteoblasts) in ST2 cells was significantly enhanced by the addition of epinephrine (10^{-8} M). In luciferase expression assays using the promoter sequence of the Id1 gene (an immediate early response gene to BMP), luciferase activity was elevated by BMP-2 treatment (50 ng/ml) and this activity was further enhanced by the addition of epinephrine (10^{-8} M). Epinephrine-enhanced luciferase activity was abolished by mutation of the cAMP-response element (CRE) sequence in the Id1 promoter, indicating that CRE-binding transcription proteins induced by epinephrine addition may act as enhancers of Smad-mediated BMP signaling.

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Introduction

Recent reports indicate the involvement of catecholamines in the regulation of bone metabolism through activation of Gs-coupled adrenergic receptors [1–5], located on both osteoblasts and osteoclasts [6–9], and through elevation of intracellular cyclic adenosine 3′, 5′-monophosphate (cAMP) levels [10–14].

Our previous study indicated that intracellular cAMP accumulation consistently enhances bone morphogenetic protein (BMP)-mediated osteoblastic differentiation of mesenchymal cells and that this effect is regulated by enhancement of Smad-mediated transcriptional activity through the protein kinase A (PKA)/cAMP-response element-binding protein (CREB) signaling pathway [15–20]. In this context, we hypothesized that catecholamines might also have a potential role in enhancement of BMP-induced osteoblastic differentiation and subsequent bone formation. The present study was designed to clarify the effects of epinephrine on BMP-induced ectopic bone formation in an *in vivo* experimental system and to gain an insight into cross talk between intracellular BMP-induced signaling and catecholamine-signaling pathways using an *in vitro* system.

Materials and methods

Animal care and protocols for in vivo experiments

Male ICR mice (5 weeks old) were purchased from Japan SLC, Inc. (Hamamatsu, Japan) and were housed in an air-conditioned room with free access to food and water. After acclimation for 1 week, experiments were carried out in strict accordance with the Institutional Guidelines for the Care and Use of Laboratory Animals of Osaka City University.

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Preparation of pellet implants for ectopic bone induction by BMP-2

A biodegradable polymer, poly-D,L-lactic acid-p-dioxanone-polyethylene glycol (PLA-DX-PEG) block copolymer (MW: 9800, PLA/DX/PEG molar ratio: PLA/DX/PEG = 43/14/43) (Taki Chemicals Co., Kakogawa, Japan) was used for local delivery of BMP-2. The physicochemical characteristics and the efficacy of this biodegradable polymer as a carrier material for BMP have been previously reported [15,16,21–24].

To prepare BMP-2-containing pellet implants, 30 mg of the PLA-DX-PEG polymer was mixed with an aliquot of either the BMP-2 (5 μg/pellet) solution alone or with various doses of epinephrine (10, 20, 40, 80 or 160 μg/pellet) with or without BMP-2 (5 μg/pellet) and were then fashioned into discs for implantation. Various doses of fenoterol, a β2-adrenergic receptor selective agonist, (10, 20, 40 or 80 μg/pellet) were also mixed with 30 mg of the PLA-DX-PEG polymer, with or without BMP-2 (5 μg/pellet), using the same methods. All procedures were carried out under sterile conditions. The pellet implants were stored at −80 °C in a freezer until surgical implantation.

Protocol of in vivo experiment

The mice were anesthetized by diethyl-ether gas inhalation and the PLA-DX-PEG polymer discs, prepared as described above, were surgically implanted into the left dorsal muscle pouches (one pellet per animal) of the mice. Three weeks after surgery, the mice were sacrificed. The ossicles induced at the sites of implantation were explored and were then harvested and processed for radiological examination.

Radiological and histological analyses

All harvested bony tissues were radiographed using a soft X-ray apparatus (Soft Co., Ltd., Tokyo, Japan). The bone mineral content (BMC) in each ossicle was measured by dual-energy X-ray absorptiometry (DXA) using a bone mineral analyzer (DCS-600EX, Aloka Co., Tokyo, Japan). The ossicles were then fixed in neutralized 10% formalin and embedded in methyl methacrylate (MMA, Wako, Japan). Ossicle sections (7 μm thick) were cut and stained with hematoxylin–eosin.

Reagents for in vitro experiments

Recombinant human BMP-2 (BMP-2) was produced and kindly provided by Osteopharma, Inc. (Osaka, Japan) [24]. Epinephrine and fenoterol, were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Epinephrine was dissolved in 0.5 M hydrochloric acid for storage [25]. The following specific antagonists were used: an antagonist to α1-adrenergic receptor subtypes Prazosin (Sigma Chemical Co.), an antagonist to β2-adrenergic receptor subtypes Butoxamine (Sigma Chemical Co.), the PKA inhibitor H89 (Sigma Chemical Co.), the PKC inhibitor Gö6976 (Calbiochem Co.), the p38 mitogen-activated protein kinase (MAPK) inhibitor SB203580 (Sigma Chemical Co.), the MEK/ERK-kinase inhibitor PD98059 (Calbiochem Co.) and the c-Jun NH2-terminal kinase (JNK) inhibitor SP600125 (Calbiochem Co.).

Cell cultures

BMP-2-responsive pluripotent murine bone marrow-derived stromal cells (ST2) and pluripotent myoblastic cells (C2C12) were obtained from the Riken Cell Bank (Ibaragi, Japan). Primary calvarial osteoblasts were isolated from a newborn ICR mouse as described previously [26]. Cells were cultured in α-minimal essential medium (α-MEM; Sigma Chemical Co.) supplemented with 10% heat-inactivated fetal bovine serum (FBS; Gibco, Grand Island, NY, USA) or 2.5% FBS and antibiotics/antimycotic (100 U/ml penicillin; 100 μg/ml streptomycin; and 0.25 μg/ml amphotericin B; Sigma Chemical Co.) at 37 °C in 5% CO_2-humidified air. Upon reaching confluence, the cells were used in the following experiments.

Detection of adrenergic receptor subtypes

Total RNA (3 μg) was isolated from the respective cell lines and primary osteoblasts using NucleoSpin RNA II (Macherey-Nagel, Duren, Germany) according to the manufacturer's instructions and was reverse-transcribed into first-strand cDNA using an oligo-dT primer and Superscript II reverse transcriptase (Invitrogen, Carlsbad, CA, USA). PCR amplification was performed using gene-specific PCR primers and rTaq (Takara Bio, Otsu, Japan). A thermal cycle reaction was carried out at 95 °C for 4 min, followed by 35 cycles at 94 °C for 30 s, 60 °C for 30 s, 72 °C for 1 min, and 72 °C for 5 min. The sequences of the PCR primers used were as follows; α1b-adrenergic receptor (forward, 5'-GTGACATCTGGGCAGCGGTTGATG-3'; reverse, 5'-ATGACCGCCAGTGGGATGTAGAAG-3'; amplicon size 304 bp) [9], α1d-adrenergic receptor (forward, 5'-CGCTGTGGTGGGAACCGGCAG-3'; reverse, 5'-ACAGCTGCACTCAGTAGCAGGTCA-3'; amplicon size 282 bp) [9], and β2-adrenergic receptor (forward, 5'-GGTTATCGTCCTGGCCATCGTGTTTG-3'; reverse, 5'-TGGTTCGTGAAGT-CACAGCAAGTCTC-3'; amplicon size 468 bp) [27]. The PCR products were separated by electrophoresis on a 1% agarose gel and were visualized by staining with ethidium bromide.

Assay of intracellular cAMP

When cultured cells in 12-well plates ($n = 3$) reached confluence, the medium was replaced with fresh medium without FBS and the cells were pre-incubated for 1 h. Epinephrine (10^{-8} M) was then added once to the culture (single treatment), or epinephrine (10^{-9} M) was added 10 times at 3-min time intervals (cyclic treatments) resulting in a final cumulative concentration of epinephrine of 10^{-8} M. After further incubation for 1 min, 2.5 min, 5 min, 10 min, 15 min, 30 min, 1 h, 2 h and 3 h, the medium was removed and the cAMP level in the cultured cells was determined using a cAMP enzyme immunoassay system (GE Healthcare, Piscataway, NJ, USA) in accordance with the manufacturer's instructions. All experiments were performed independently in triplicate.

Assay of alkaline phosphatase (ALP) activity

Cells were seeded at a density of 2×10^4 cells per well in 24-well plates ($n = 4$). When the cells reached confluence, the medium was replaced with fresh treatment medium containing 2.5% FBS and BMP-2 (50 ng/ml). Cells (ST2, C2C12 or primary osteoblasts) were additionally stimulated by epinephrine using cyclic treatments (with a final cumulative concentration of epinephrine of 10^{-8} M) as described above, and were then cultured for an additional 3, 6 or 3 days, respectively. ST2 cells were also stimulated by cyclic fenoterol treatments (10 cycles of 10^{-8} M at 3-min time intervals, resulting in a final cumulative concentration of 10^{-7} M) with or without BMP-2 (50 ng/ml) and were then cultured for an additional 3 days. ALP levels were measured using p-nitrophenylphosphate as the ALP substrate and the obtained data were normalized to cellular protein levels (Bio-Rad Laboratories, Hercules, CA, USA). All experiments were performed independently in triplicate.

Effects of protein kinase inhibitors

To identify the adrenergic receptor subtype that mediates epinephrine signaling in order to modulate BMP signaling, the effect of antagonists specific to the α1-adrenergic receptor subtype (Prazosin; 10^{-7} M) or the β2-adrenergic receptor subtype

(Butoxamine; 10^{-6} M) on epinephrine activity was assayed. To identify protein kinases involved in the intracellular signaling induced by epinephrine, the effect of specific inhibitors of PKA (H89; 2 μM), PKC (Gö6976; 10^{-8} M), the p38 MAPK (SB203580; 10^{-8} M), MEK/ERK-kinase (PD98059; 10^{-8} M) or JNK (SP600125; 10^{-8} M) on epinephrine enhancement of BMP-induced ALP activity was assayed. ST2 cells were pre-treated with medium containing 2.5% FBS and the respective inhibitor for 2 h. BMP-2 (50 ng/ml) and/or epinephrine (10 cycles of 10^{-9} M) were then added to the media containing the inhibitors, and incubation was continued in this inhibitor-containing media for another 3 days. The mean level of ALP activity in the cells was determined from triplicate samples.

Quantitative real-time reverse transcription polymerase chain reaction

The medium of confluent ST2 cells in 6-well plates ($n = 3$) was replaced with fresh medium containing 2.5% FBS and BMP-2 (50 ng/ml), and the cells were treated with or without epinephrine (10 cycles of 10^{-9} M). At each time point, total RNA was isolated from the cells using NucleoSpin RNA II (Macherey-Nagel, Duren, Germany) according to the manufacturer's instructions. Total RNA (5 μg) was reverse-transcribed into first-strand cDNA with an oligo-dT primer using Superscript II reverse transcriptase (Invitrogen). Real-time reverse transcription polymerase chain reaction (RT-PCR) was performed according to the manufacturer's instructions (Applied Biosystems, Foster City, CA, USA) using an iCycler apparatus (Bio-Rad Laboratories) and iCycler Optical System Interface software (version 3.0; Bio-Rad Laboratories). TaqMan fluorogenic probes for Osterix, ALP, osteocalcin and GAPDH (an internal control) were purchased from Applied Biosystems. Real-time RT-PCR was performed using Absolute QPCR low rox Mixs (Applied Biosystems). To correct variability in RNA recovery and efficiency of reverse transcription, GAPDH cDNA was amplified and quantified for each cDNA preparation. Steps of normalization and calculation were

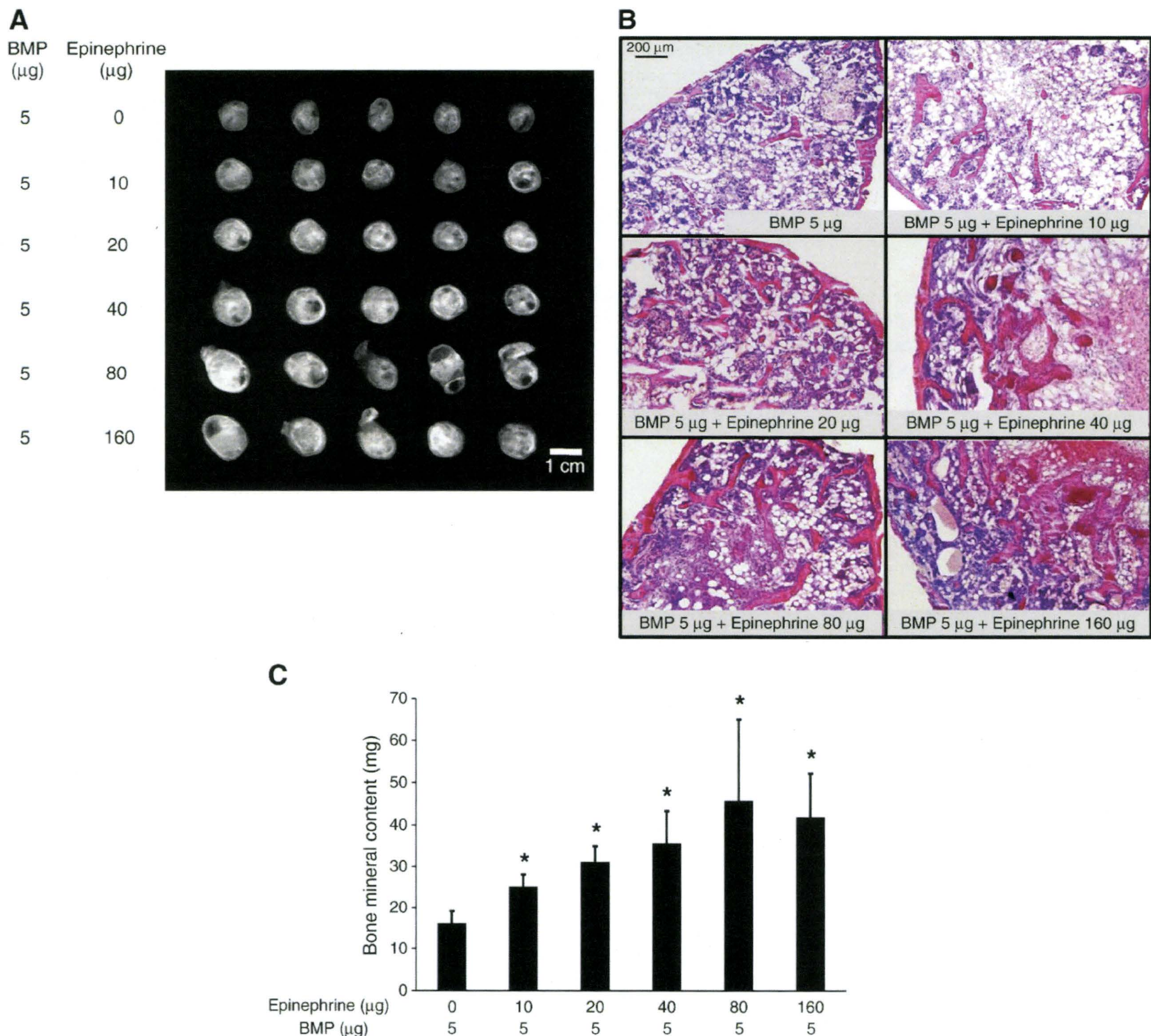

Fig. 1. Effect of epinephrine on BMP-2-induced ossicles. Mice were implanted with implants containing 5 μg BMP-2 and the indicated concentration of epinephrine. Three weeks after implantation newly formed ossicles were examined (A) by soft X-ray photography, (B) by hematoxylin and eosin staining and (C) for bone mineral content. In (A) both the radio-opaque areas and the radiological densities of the ossicles were larger in the groups stimulated by epinephrine than in the BMP-2 alone group (control). In (B), epinephrine addition induced visible increases in the number and thickness of bony trabeculae compared to those in the ossicles of the control group. In (C), the BMC of the ossicles peaked at 80 μg of added epinephrine. Bars and lines represent mean ± SD for 5 samples. $^{*}p < 0.05$.

performed as described by Pfaffl [28]. All experiments were performed independently in triplicate.

Id1 gene promoter-linked luciferase gene expression

A luciferase reporter plasmid driven by the Id1 promoter (Id985WT-luc) was kindly provided by Dr. Katagiri (Saitama Medical School, Saitama, Japan) [29]. Id1 is known to be an early response gene to BMP-2, to contain both a BMP-responsive element (BRE) and a cAMP-response element (CRE) [19], and to commit young mesenchymal cells to differentiate into osteoblasts. To investigate the effects of epinephrine on BMP-induced transcriptional activity in ST2 cells, we used wild-type luciferase reporter plasmids and a CRE-mutated Id1 promoter as described previously [19,20].

For the reporter assay, ST2 cells were plated at a density of 4×10^3 cells per well in 96-well plates ($n = 4$) and were then cultured until almost confluent before transfection of the luciferase gene. Cells were transfected with reporter plasmid constructs containing a BRE and a wild-type or a mutated CRE, using Lipofectamine 2000 according to the manufacturer's instructions (Invitrogen). At 5 h after transfection, the medium was replaced with fresh medium, and the cells were then treated with BMP-2 (50 ng/ml), and/or epinephrine (10 cycles of 10^{-9} M) for a further 24 h. Cells were then harvested, and luciferase activity in the cell extracts was determined using a Dual-Glo Luciferase assay system (Promega, Madison, WI, USA). All experiments were performed independently in triplicate.

Statistical analysis

Data are expressed as the mean ± standard deviation (SD) for each group. Differences between treatment groups were analyzed using Fisher's Protected Least Significant Difference (PLSD) test. Values of $p < 0.05$ were considered statistically significant.

Results

Effect of epinephrine on ectopic bone formation by BMP-2

The ectopic ossicles that were induced by PLA-DX-PEG polymer discs containing BMP-2 (5 μg) without or with epinephrine (10, 20, 40, 80 and 160 μg) are shown in Fig. 1. No evidence of ossicle formation was found in groups implanted with polymer discs with epinephrine alone without BMP-2 (data not shown). Ossicles induced by BMP-2 in conjunction with epinephrine were of a significantly larger size on soft X-ray radiograms (Fig. 1A) and showed significantly higher bone mineral content (BMC) on DXA, than those of ossicles induced by BMP-2 alone without epinephrine (control) (Fig. 1C). The mean BMC value of ossicles in the epinephrine (80 μg) + BMP-2 (5 μg)-treated group was approximately 3 times higher than that of the BMP-2 group without epinephrine. Histological sections of all ossicles showed normal bone characteristics with trabeculae and bone marrow (Fig. 1B). In the BMP-2 + epinephrine-treated groups, the number and thickness of bony trabeculae were visibly increased compared to the ossicles of the control group (BMP-2 alone).

Elevation of intracellular cAMP levels by epinephrine

We next determined the best conditions for induction of intracellular cAMP by epinephrine. Elevation of intracellular cAMP concentration was consistently noted in all cultured cells following epinephrine treatment. The profile of cAMP elevation by epinephrine stimulation was similar in the ST2, C2C12 and primary osteoblastic cells (data not shown). In ST2 cells, the epinephrine-elevated cAMP level was maintained for a longer time if epinephrine was added by 10 cyclic additions at a low dose (10^{-9} M), rather than by addition using a single high dose (10^{-8} M) (Fig. 2). Based on these results, epinephrine stimulation was carried out throughout the study by cyclic additions of the low dose (10 cycles of 10^{-9} M at 3-min time intervals, resulting in a final cumulative concentration of 10^{-8} M).

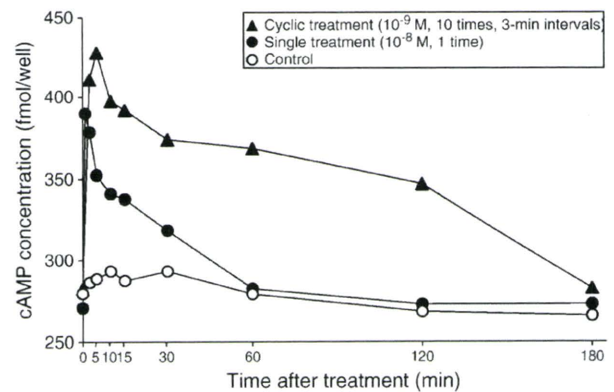

Fig. 2. Elevation of Intracellular cAMP levels by epinephrine. Cultured ST2 cells were stimulated with 10^{-8} M epinephrine, which was administered as a single high dose (10^{-8} M) or administered 10 times at 3-min intervals using a low dose (10^{-9} M) of epinephrine each time. The intracellular cAMP level was then measured using an ELISA. Intracellular cAMP remained elevated for a longer time following cyclic stimulation with low doses of epinephrine.

Expression of adrenergic receptor subtypes in osteogenic cells

Prior to analysis of the adrenergic receptors through which epinephrine might modulate BMP functions, we first confirmed the previously reported expression [7,14,30,31] of α1b-, α1d- and β2-adrenergic receptor subtypes genes in ST2, C2C12 and primary osteoblast cells by RT-PCR (Fig. 3). Of the two receptor subtypes, the β2-adrenergic receptor but not the α1d-receptor, is known to mediate epinephrine-induced intracellular cAMP accumulation [12,32–34]. To examine the potential involvement of the β2-adrenergic receptor in the anabolic action of epinephrine in BMP-2-induced bone formation, we tested the effect of addition of fenoterol, a β2-adrenergic receptor specific agonist, in place of epinephrine, in both the *in vivo* and *in vitro* systems, and analyzed its effect on BMP-2-induced ectopic bone formation. The effect of fenoterol was equivalent to that of epinephrine (Fig. 4), suggesting that the β2-

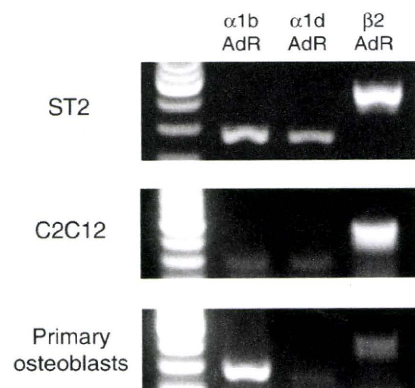

Fig. 3. The mRNA expression of adrenergic receptor subtypes in osteogenic cells. Total RNA, extracted from ST2 and C2C12 cells and from primary calvarial osteoblasts, was analyzed by RT-PCR using primers specific for each adrenergic receptor subtype. PCR products were analyzed by agarose gel electrophoresis. Molecular weight markers are at left. The mRNA expression of α1b-, α1d- and β2-adrenergic receptor subtypes was detectable in all of the cells tested. α1bAdR: α1b-adrenergic receptor, α1dAdR: α1d-adrenergic receptor, β2AdR: β2-adrenergic receptor.

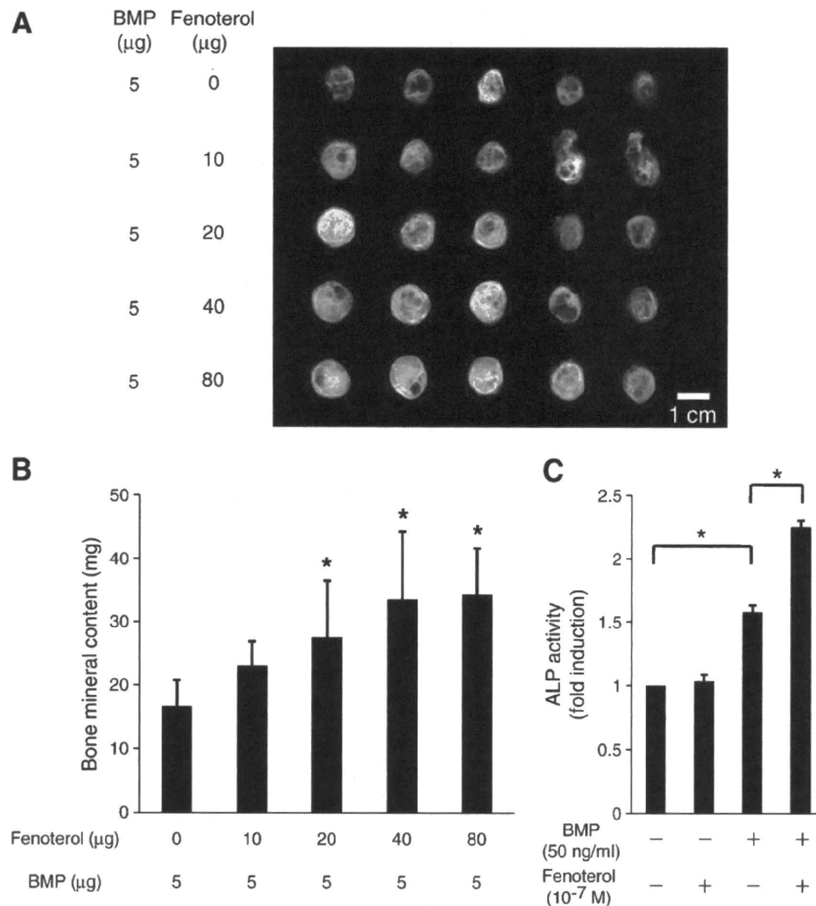

Fig. 4. Effect of fenoterol on BMP-2-induced ossicles and on ALP induction by BMP-2 in ST2 cells. The ectopic ossicles in mice that were induced by PLA-DX-PEG polymer discs containing BMP-2 (5 μg) without or with fenoterol (10, 20, 40 and 80 μg) are shown. Ossicles induced by BMP-2 in conjunction with fenoterol were of a significantly larger size on soft X-ray radiograms in (A) and showed significantly higher bone mineral content (BMC) on DXA in (B), than those of ossicles induced by BMP-2 alone without fenoterol (control). ST2 cells were stimulated by cyclic fenoterol treatment (10 cycles of 10^{-8} M at 3-min time intervals, resulting in a final cumulative concentration of 10^{-7} M) with or without BMP-2 (50 ng/ml) and were then cultured for an additional 3 days. (C) The ALP levels of ST2 cells were elevated in response to BMP-2 (50 ng/ml), and the BMP-2-induced ALP levels were further enhanced by the addition of fenoterol. ALP activity was normalized to that of the control group which was assigned a value of 1. The bars and lines represent the mean ± SD of 4 wells. BMP: BMP-2 (50 ng/ml), fenoterol (10^{-7} M): fenoterol (10^{-8} M) was added 10 times at 3-min intervals, *$p<0.05$.

adrenergic receptor is involved in mediation of the epinephrine-induced enhancement of BMP actions.

ALP induction by BMP-2 and/or epinephrine

The ALP levels of ST2, C2C12 and osteoblast cells were elevated in response to BMP-2 (50 ng/ml), and these BMP-2-induced ALP levels were further enhanced by the addition of epinephrine (Fig. 5). To investigate the intracellular signaling pathway by which epinephrine enhances BMP-2 signaling, we first assayed the effects of antagonists of adrenergic receptor subtypes in ST2 cells. A specific antagonist to β2-adrenergic receptor subtypes (Butoxamine) abolished epinephrine enhancement of BMP-2 signaling, whereas a specific antagonist to α1-adrenergic receptor subtypes (Prazosin) did not inhibit epinephrine-induced enhancement of BMP-2-induced ALP activity (Fig. 6A). To identify protein kinases involved in the intracellular signaling induced by epinephrine, the effect of specific inhibitors of PKA (H89), PKC (Gö6976), the p38 MAPK (SB203580), MEK/ERK-kinase (PD98059) and JNK (SP600125) on epinephrine enhancement of BMP-induction of ALP was assayed. H89 significantly abolished this epinephrine-induced effect (Fig. 6B). However, neither Gö6976 (Fig. 5B), nor SB203580, PD98059 or SP600125 (Fig. 6C) inhibited this epinephrine activity. Based on these results, it was concluded that epinephrine enhanced BMP-induced-osteoblastic differentiation, mainly through the PKA signaling pathway, via β2-adrenergic receptors.

Enhancement of BMP-2-induced mRNA expression of ALP, osteocalcin and Osterix by epinephrine

Induction of osteoblastic differentiation by BMP-2 involved enhancement of the mRNA expression of both the osteoblastic differentiation markers ALP and osteocalcin, as well as that of Osterix, which is a key transcriptional regulator for osteoblastic differentiation. In ST2 cells, epinephrine addition also enhanced BMP-2 upregulation of these mRNAs as assayed on days 3, 6 and 3, respectively, following epinephrine addition (Fig. 7A–C). These results indicate that epinephrine potentially enhanced BMP-2-induced mRNA expression of osteoblastic differentiation markers.

Id1 promoter-driven luciferase expression by BMP-2 and/or epinephrine

Upregulation of osteoblastic differentiation-specific cellular mRNA by BMP-2 suggested that BMP-2 may act by modulating transcriptional events. Indeed we have shown that epinephrine modulates

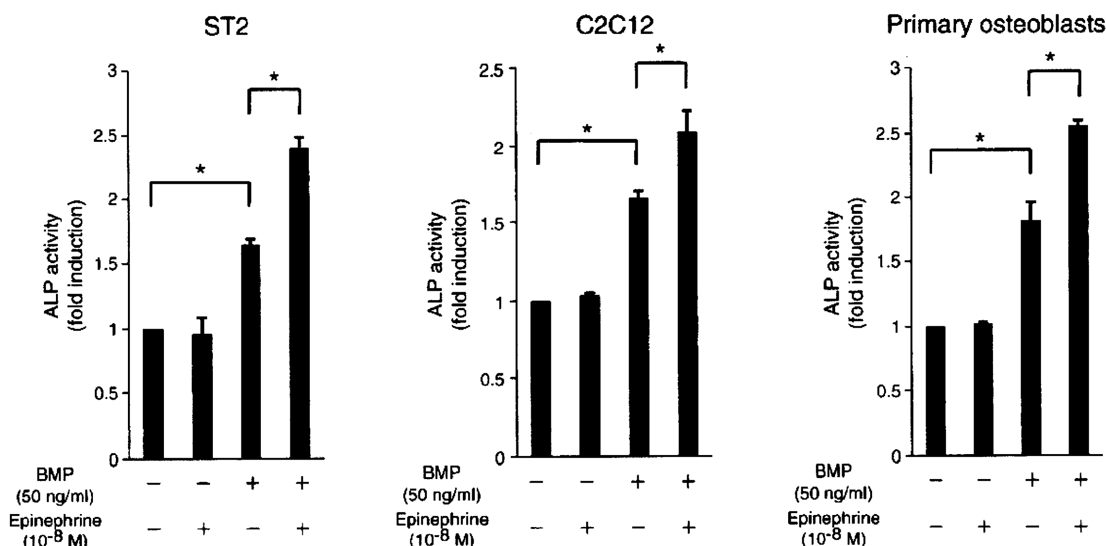

Fig. 5. Effect of epinephrine on ALP induction by BMP-2 in osteogenic cells. ST2 cells, C2C12 cells, and primary calvarial osteoblasts, were treated with BMP-2 (50 ng/ml) and/or epinephrine (10^{-8} M), or with buffer control, and ALP activity was assayed in ST2 cells and primary osteoblasts after 3 days and in C2C12 cells after 6 days. At these times, BMP-2 activated ALP in all cells and this activity was further enhanced by epinephrine. ALP activity was normalized to that of the control group which was assigned a value of 1. The bars and lines represent the mean ± SD of 4 wells. BMP: BMP-2 (50 ng/ml), Epinephrine (10^{-8} M): epinephrine (10^{-9} M) was added 10 times at 3-min intervals, * $p<0.05$.

Smad-mediated transcription resulting in the generation of specific mRNAs. To determine if epinephrine enhances BMP-2-modulated transcription by upregulation of cAMP and subsequent CRE activation by binding of activated transcription factor(s), we assayed the effect of epinephrine addition on the activity of a luciferase reporter with an Id1 promoter that contains both BRE and CRE. Elevation of luciferase activity in ST2 cells in response to BMP-2 treatment was further up-regulated by the addition of epinephrine (Fig. 8A). This epinephrine-induced upregulation was not observed in cells transfected with a luciferase gene in which the CRE sequence in the Id1 promoter was mutated (Fig. 8B), indicating that epinephrine accelerates BMP signaling through CRE-mediated transcriptional regulation.

Discussion

A catabolic action of catecholamines in bone metabolism has been suggested based on *in vivo* experimental systems [1–5]. In contrast, the present study showed an anabolic effect of epinephrine on BMP-2-induced ectopic new bone formation under *in vivo* conditions that manifested as an increase in size and in the BMC of BMP-2-induced ectopic ossicles. One possible explanation of the discrepancy between these effects of epinephrine on bone metabolism under *in vivo* conditions may be due to the concentrations and/or method of delivery of epinephrine in the different studies. Enhanced osteoclastogenesis, resulting in systemic bone loss, is known to occur during physiological systemic bone remodeling, which is regulated by various endocrine systems. Thus, repeated systemic administration of significant doses of epinephrine may have a catabolic effect on bone metabolism whereas continuous local release of lower doses of epinephrine from the biodegradable carrier may result in anabolic effects. A second possible explanation of this discrepancy may be that the anabolic effect of epinephrine on bone formation is confined to BMP-induced ectopic bone, which is exposed to the low dose of epinephrine that is continuously released from the pellet implants concomitant with BMP-2. This extremely low dose epinephrine is unlikely to strongly modulate systemic bone metabolism. Further study of the effects of continuously released, low dose epinephrine from implants on systemic bone metabolism is required to clarify this matter.

We also investigated the mechanism by which epinephrine enhances BMP-2 action using an *in vitro* system. As expected from previous classical reports, epinephrine addition to osteogenic cells instantly elevated the intra-cellular cAMP level [10–14]. This reaction of osteogenic cells to epinephrine appears to be mediated through β-adrenergic receptors based on the adrenergic receptor expression profile of the osteogenic cells and on the inhibition of this effect of epinephrine by a specific β-adrenergic receptor antagonist [10,12,33]. The expression profiles of adrenergic receptors that were observed by RT-PCR in the osteogenic cells, were in accordance with those of previous reports [7,14,30,31]. Moreover, the epinephrine-induced up-regulation of cAMP via β2-adrenergic receptors is consistent with the known activity of these receptors, which activate adenylylcyclase via G protein [35] to produce cAMP.

In order to maintain an elevated level of cAMP in the cells, cyclic (ten times) stimulation using a low dose (10^{-9} M) of epinephrine at each cycle, was a more effective way to administer epinephrine than using a single equivalent dose (10^{-8} M). Although the mechanism of this phenomenon remains unclear, as well as the our results (Fig. 2) are consistent with a previous report previous report that high levels of intracellular cAMP were maintained with cyclic stimulation of PTH [20]. We speculate that mechanisms that regulate intracellular cAMP-degrading phosphodiesterases may be involved in generation of the different intracellular cAMP retention profiles.

The level of enzymatic activity of ALP, a marker of osteoblastic differentiation for undifferentiated mesenchymal cells, was utilized as an index of BMP activity in the experiments on the effects of epinephrine on BMP-2-induced osteoblastic differentiation. Epinephrine enhanced BMP-induced ALP levels in all of the cells tested: ST2, C2C12 and primary osteoblastic cells (Fig. 5). Therefore, ST2 cells were used as representative cells for the rest of the study.

Although PKA and/or PKC have been reported to be activated by elevated levels of cAMP, epinephrine enhancement of BMP action was inhibited exclusively by a PKA specific inhibitor (H89) and not by a PKC inhibitor (Fig. 6). This finding that PKA plays a more important role than PKC in modulation of BMP-2 signaling is similar to the result

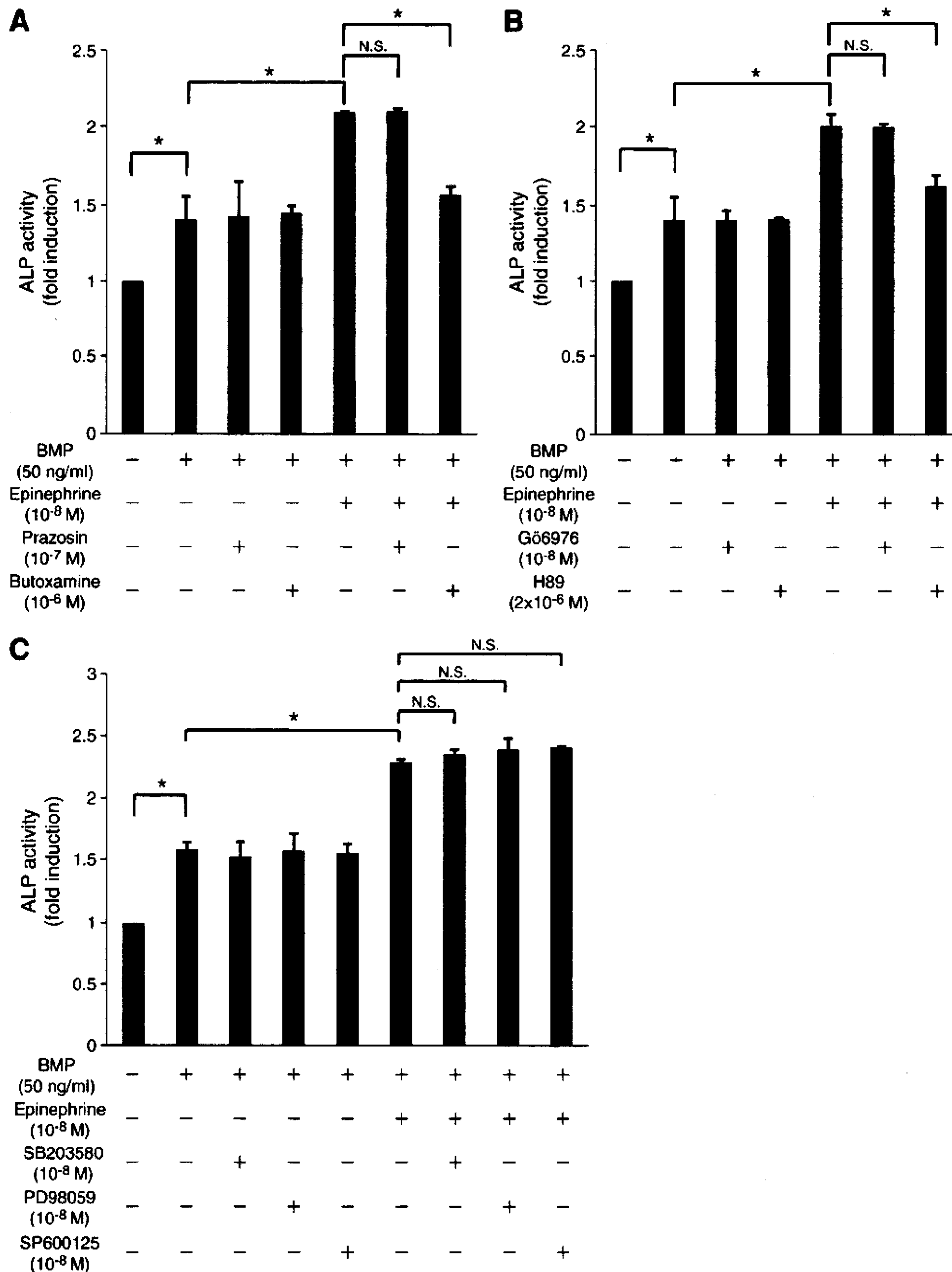

Fig. 6. Effect of adrenergic receptor subtype antagonists and protein kinase inhibitors on epinephrine-enhancement of BMP-2-induced ALP activity. ST2 cells were pre-incubated for 2 h without or with (A) a specific antagonist to α1-adrenergic (Prazosin; 10^{-7} M), or to β2-adrenergic (Butoxamine; 10^{-6} M) receptor subtypes, (B) a specific inhibitor of PKC (Gö6976; 10^{-8} M) or PKA (H89; 2 μM) or (C) a specific inhibitor of the p38 MAPK (SB203580; 10^{-8} M), MEK/ERK-kinase (PD98059; 10^{-8} M), or JNK (SP600125; 10^{-8} M). The cells were then incubated in the continued presence of the antagonists or inhibitors for an additional 3 days, with BMP-2 (50 ng/ml) in the presence or absence of epinephrine, or with buffer control. The epinephrine enhancement of BMP-induced ALP was abolished only by the specific antagonist to the β2-adrenergic receptor subtype and the specific inhibitor of PKA (H89) but not by the other inhibitors tested. The ALP value of the control group was assigned a value of 1. The bars and lines represent the mean ± SD of 4 wells. BMP: BMP-2 (50 ng/ml). Epinephrine (10^{-8} M): epinephrine (10^{-9} M) added 10 times at 3-min intervals. N.S.: not significant, *$p<0.05$.

of our previous study in which PKA played a critical role in PTH (teriparatide) enhancement of BMP-2 activity [20]. It has been reported that stimulation of β2-adrenergic receptor activates the p38 MAPK [36,37], MEK/ERK-kinase [38,39] and JNK [40,41] in addition to the cAMP/PKA signaling pathway. Cross-talk between the cAMP/PKA pathway and the p38 MAPK pathway in β2-adrenergic receptor signaling has also been demonstrated previously [36]. However, in the present study, enhancement of BMP activity by epinephrine was not abolished by inhibitors of p38 MAPK, MEK/ERK-kinase or JNK suggesting that the cAMP/PKA pathway is the dominant signaling pathway by which epinephrine enhances BMP activity.

The results of our real-time RT-PCR assay indicating that epinephrine enhanced BMP-2-induced upregulation of the mRNA expression of the osteogenic differentiation markers (ALP, osteocalcin

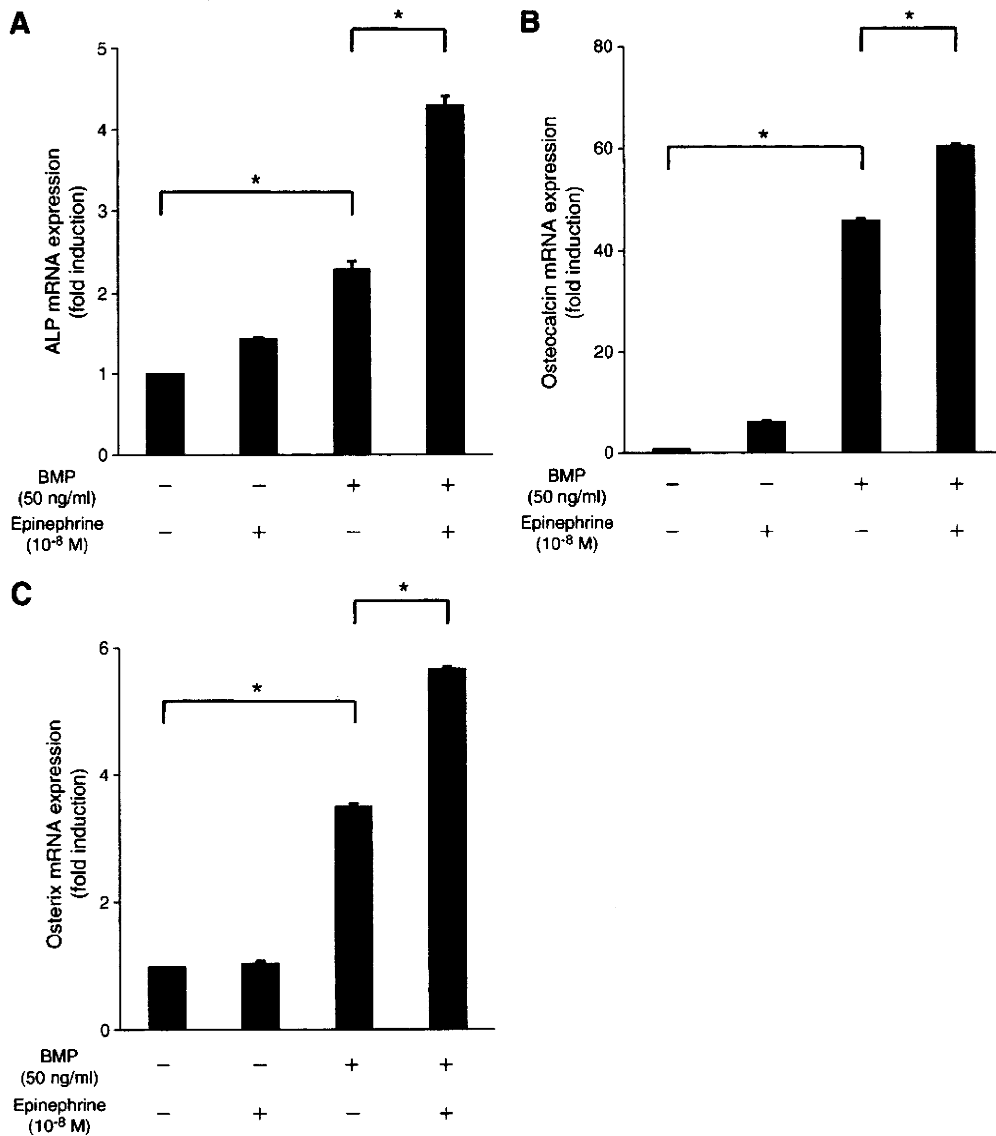

Fig. 7. Effect of epinephrine on BMP-2 induction of the mRNA of ALP, osteocalcin and Osterix. ST2 cells were treated with the indicated concentrations of BMP-2 and/or epinephrine, or with buffer control, following which the mRNA expression of (A) ALP, (B) Osteocalcin or (C) Osterix was assayed on days 3, 6 and 3, respectively, using quantitative RT-PCR. BMP increased the mRNA expression of all three genes and the mRNA expression was further enhanced by epinephrine addition in all cases. Relative mRNA expression was normalized using amplified GAPDH expression values. The bars and lines represent the mean ± SD of 3 wells. BMP: BMP-2 (50 ng/ml), Epinephrine (10^{-8} M); epinephrine (10^{-9} M) added 10 times at 3-min intervals, N.S.: not significant, $^*p<0.05$.

and Osterix) suggested that epinephrine might enhance BMP-induced transcriptional activity. This possibility was further suggested by the fact that epinephrine enhanced BMP-induced transcription from a luciferase reporter linked to the Id1 promoter sequence, which is known to be an early response gene in BMP-Smad signaling. Id1 was reported to suppress MyoD (a bHLH type transcriptional factor) function and to shift the differentiation commitment from a myogenic to an osteogenic commitment in response to BMP-2 [42,43]. Therefore, Id1 is an essential gene for BMP-2 induced osteoblastic differentiation. However, the role of Id1 in downstream transcriptional modulation that is required to achieve terminal differentiation into a mature osteoblast has not yet been clarified.

Furthermore, the inability of epinephrine to enhance BMP-2 activation of luciferase expression in experiments using an Id1 promoter with a non-functional mutation in the CRE sequence, suggested that epinephrine enhances BMP-2-evoked transcriptional regulation via CRE. Thus, transcriptional regulator(s) such as CREB or ATF4, that are activated in the cAMP/PKA pathway, and that bind to CRE, might act as enhancers of BRE-mediated transcriptional activity.

Our previous studies indicated that agents that cause elevation of intracellular cAMP, including phosphodiesterase inhibitors (pentoxifylline and rolipram), cell permeable analogues of cAMP (dibutyryl cAMP), a prostaglandin E_2 EP4 agonist (ONO-4819)) and parathyroid hormone (PTH), consistently enhance BMP-2 or BMP-4 action to induce osteoblastic differentiation of mesenchymal cells under *in vivo* and/or *in vitro* conditions [15–20,44–47]. The present study also indicated that epinephrine and other catecholamines are included in this group of agents that are able to promote BMP-induced bone formation through enhancement of the transcriptional activity of BMP-induced intracellular signaling via a common cAMP/PKA/CRE signaling pathway.

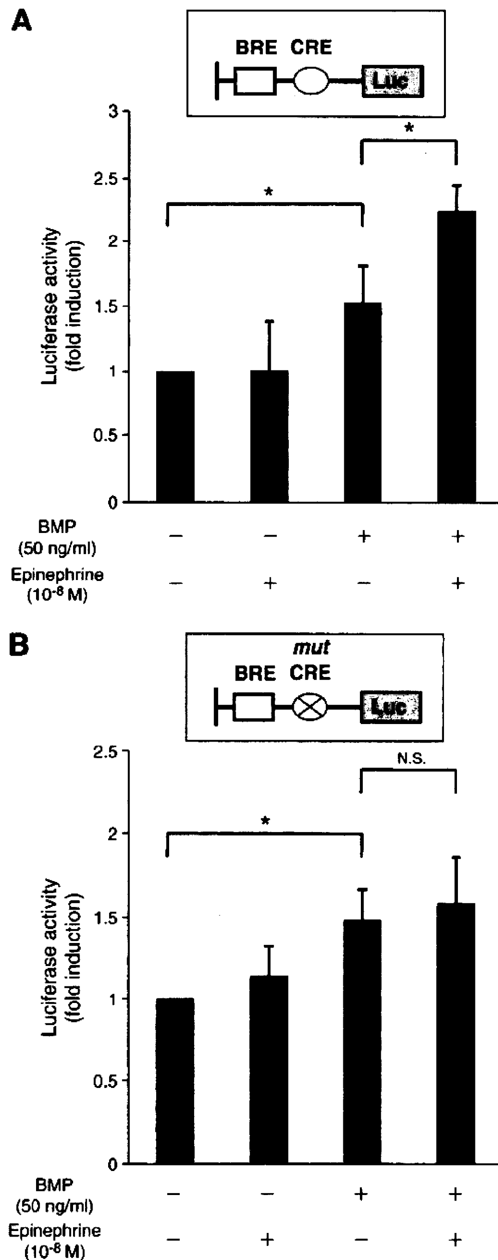

Fig. 8. Effect of epinephrine on BMP-2-induced luciferase activity driven by an Id1 promoter. ST2 cells were transfected with luciferase reporter plasmids driven by the Id1 promoter sequence containing (A) the BRE and CRE elements or (B) containing the BRE and a functionally mutated CRE element. The cells were then treated with the indicated concentrations of BMP-2 and/or epinephrine, or with buffer control, and luciferase activity was assayed after 24 h. BMP-2 enhanced the luciferase activity and this activity was further enhanced by the concurrent addition of epinephrine. The enhancement of BMP-2-induced luciferase activity by epinephrine was abolished when CRE was mutated. The luciferase activity of the control group was assigned a value of 1. The bars and lines represent the mean ± SD of 4 wells. BMP: BMP-2 (50 ng/ml), Epinephrine (10^{-8} M): epinephrine (10^{-9} M) added 10 times at intervals of 3 min, N.S.: not significant, $^{*}p<0.05$. BRE: BMP-responsive element, CRE: cAMP-response element.

Acknowledgments

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References

[1] Takeda S, Elefteriou F, Levasseur R, Liu X, Zhao L, Parker K, et al. Leptin regulates bone formation via the sympathetic nervous system. Cell 2002;111:305–17.

[2] Elefteriou F, Ahn J, Takeda S, Starbuck M, Yang X, Liu X, et al. Leptin regulation of bone resorption by the sympathetic nervous system and CART. Nature 2005;434:514–20.

[3] Elmquist J, Strewler G. Physiology: do neural signals remodel bone? Nature 2005;434:447–8.

[4] Sato S, Hanada R, Kimura A, Abe T, Matsumoto T, Iwasaki M, et al. Central control of bone remodeling by neuromedin U. Nat Med 2007;13:1234–40.

[5] Bouxsein M, Devlin M, Glatt V, Dhillon H, Pierroz D, Ferrari S. Mice lacking beta-adrenergic receptors have increased bone mass but are not protected from deleterious skeletal effects of ovariectomy. Endocrinology 2009;150:144–52.

[6] Takeuchi T, Tsuboi T, Arai M, Togari A. Adrenergic stimulation of osteoclastogenesis mediated by expression of osteoclast differentiation factor in MC3T3-E1 osteoblast-like cells. Biochem Pharmacol 2001;61:579–86.

[7] Togari A. Adrenergic regulation of bone metabolism: possible involvement of sympathetic innervation of osteoblastic and osteoclastic cells. Microsc Res Tech 2002;58:77–84.

[8] Arai M, Nagasawa T, Koshihara Y, Yamamoto S, Togari A. Effects of beta-adrenergic agonists on bone-resorbing activity in human osteoclast-like cells. Biochim Biophys Acta 2003;1640:137–42.

[9] Nishiura T, Abe K. Alpha1-adrenergic receptor stimulation induces the expression of receptor activator of nuclear factor kappaB ligand gene via protein kinase C and extracellular signal-regulated kinase pathways in MC3T3-E1 osteoblast-like cells. Arch Oral Biol 2007;52:778–85.

[10] Lipski S. Effects of beta-adrenergic stimulation on bone-marrow function in normal and sublethally irradiated mice. I. The effect of isoproterenol on cAMP content in bone-marrow cells in vivo and in vitro. Int J Radiat Biol Relat Stud Phys Chem Med 1976;29:359–66.

[11] Shultz P, Sedor J, Abboud H. Dopaminergic stimulation of cAMP accumulation in cultured rat mesangial cells. Am J Physiol 1987;253:H358–64.

[12] Kusaka M, Oshima T, Yokota K, Yamamoto S, Kumegawa M. Possible induction of fatty acid cyclooxygenase in mouse osteoblastic cells (MC3T3-E1) by cAMP. Biochim Biophys Acta 1988;972:339–46.

[13] Bjurholm A, Kreicbergs A, Schultzberg M, Lerner U. Neuroendocrine regulation of cyclic AMP formation in osteoblastic cell lines (UMR-106-01, ROS 17/2.8, MC3T3-E1, and Saos-2) and primary bone cells. J Bone Miner Res 1992;7:1011–9.

[14] Moore R, Smith Cn, Bailey C, Voelkel E, Tashjian AJ. Characterization of beta-adrenergic receptors on rat and human osteoblast-like cells and demonstration that beta-receptor agonists can stimulate bone resorption in organ culture. Bone Miner 1993;23:301–15.

[15] Sasaoka R, Terai H, Toyoda H, Imai Y, Sugama R, Takaoka K. A prostanoid receptor EP4 agonist enhances ectopic bone formation induced by recombinant human bone morphogenetic protein-2. Biochem Biophys Res Commun 2004;318:704–9.

[16] Toyoda H, Terai H, Sasaoka R, Oda K, Takaoka K. Augmentation of bone morphogenetic protein-induced bone mass by local delivery of a prostaglandin E EP4 receptor agonist. Bone 2005;37:555–62.

[17] Sugama R, Koike T, Imai Y, Nomura-Furuwatari C, Takaoka K. Bone morphogenetic protein activities are enhanced by 3′, 5′-cyclic adenosine monophosphate through suppression of Smad6 expression in osteoprogenitor cells. Bone 2006;38:206–14.

[18] Nakagawa K, Imai Y, Ohta Y, Takaoka K. Prostaglandin E2 EP4 agonist (ONO-4819) accelerates BMP-induced osteoblastic differentiation. Bone 2007;41:543–8.

[19] Ohta Y, Nakagawa K, Imai Y, Katagiri T, Koike T, Takaoka K. Cyclic AMP enhances Smad-mediated BMP signaling through PKA-CREB pathway. J Bone Miner Metab 2008;26:478–84.

[20] Nakao Y, Koike T, Ohta Y, Manaka T, Imai Y, Takaoka K. Parathyroid hormone enhances bone morphogenetic protein activity by increasing intracellular 3′, 5′-cyclic adenosine monophosphate accumulation in osteoblastic MC3T3-E1 cells. Bone 2009;44:872–7.

[21] Saito N, Okada T, Horiuchi H, Murakami N, Takahashi J, Nawata M, et al. 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):S92–8.

[22] Kato M, Toyoda H, Namikawa T, Hoshino M, Terai H, Miyamoto S, et al. Optimized use of a biodegradable polymer as a carrier material for the local delivery of recombinant human bone morphogenetic protein-2 (rhBMP-2). Biomaterials 2006;27:2035–41.

[23] Suzuki A, Terai H, Toyoda H, Namikawa T, Yokota Y, Tsunoda T, et al. A biodegradable delivery system for antibiotics and recombinant human bone morphogenetic protein-2: a potential treatment for infected bone defects. J Orthop Res 2006;24:327–32.

[24] Yano K, Hoshino M, Ohta Y, Manaka T, Naka Y, Imai Y, et al. Osteoinductive capacity and heat stability of recombinant human bone morphogenetic protein-2 produced by *Escherichia coli* and dimerized by biochemical processing. J Bone Miner Metab 2009;27:355–63.

[25] Suzuki A, Palmer G, Bonjour J, Caverzasio J. Catecholamines stimulate the proliferation and alkaline phosphatase activity of MC3T3-E1 osteoblast-like cells. Bone 1998;23:197–203.

[26] Nomura-Furuwatari C, Wakitani S, Hashimoto Y, Imai Y, Ohta Y, Nakagawa K, et al. Expression profiles of phosphodiesterase 4D splicing variants in osteoblastic cells. J Bone Miner Metab 2008;26:152–8.

[27] Lai L, Mitchell J. Beta2-adrenergic receptors expressed on murine chondrocytes stimulate cellular growth and inhibit the expression of Indian hedgehog and collagen type X. J Cell Biochem 2008;104:545–53.

[28] Pfaffl M. A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res 2001;29:e45.
[29] Katagiri T, Imada M, Yanai T, Suda T, Takahashi N, Kamijo R. Identification of a BMP-responsive element in Id1, the gene for inhibition of myogenesis. Genes Cells 2002;7:949–60.
[30] Togari A, Arai M, Mizutani S, Koshihara Y, Nagatsu T. Expression of mRNAs for neuropeptide receptors and beta-adrenergic receptors in human osteoblasts and human osteogenic sarcoma cells. Neurosci Lett 1997;233:125–8.
[31] Kellenberger S, Muller K, Richener H, Bilbe G. Formoterol and isoproterenol induce c-fos gene expression in osteoblast-like cells by activating beta2-adrenergic receptors. Bone 1998;22:471–8.
[32] Summers R, McMartin L. Adrenoceptors and their second messenger systems. J Neurochem 1993;60:10–23.
[33] Takahata Y, Takarada T, Iemata M, Yamamoto T, Nakamura Y, Kodama A, et al. Functional expression of beta2 adrenergic receptors responsible for protection against oxidative stress through promotion of glutathione synthesis after Nrf2 upregulation in undifferentiated mesenchymal C3H10T1/2 stem cells. J Cell Physiol 2009;218:268–75.
[34] Benovic J, Bouvier M, Caron M, Lefkowitz R. Regulation of adenylyl cyclase-coupled beta-adrenergic receptors. Annu Rev Cell Biol 1988;4:405–28.
[35] Bylund D, Eikenberg D, Hieble J, Langer S, Lefkowitz R, Minneman K, et al. International Union of Pharmacology nomenclature of adrenoceptors. Pharmacol Rev 1994;46:121–36.
[36] Zheng M, Zhang S, Zhu W, Ziman B, Kobilka B, Xiao R. beta 2-adrenergic receptor-induced p38 MAPK activation is mediated by protein kinase A rather than by Gi or gbeta gamma in adult mouse cardiomyocytes. J Biol Chem 2000;275:40635–40.
[37] Gong K, Li Z, Xu M, Du J, Lv Z, Zhang Y. A novel protein kinase A-independent, beta-arrestin-1-dependent signaling pathway for p38 mitogen-activated protein kinase activation by beta2-adrenergic receptors. J Biol Chem 2008;283:29028–36.
[38] Suzuki A, Palmer G, Bonjour J, Caverzasio J. Regulation of alkaline phosphatase activity by p38 MAP kinase in response to activation of Gi protein-coupled receptors by epinephrine in osteoblast-like cells. Endocrinology 1999;140:3177–82.
[39] Magne S, Couchie D, Pecker F, Pavoine C. Beta(2)-adrenergic receptor agonists increase intracellular free Ca(2+) concentration cycling in ventricular cardiomyocytes through p38 and p42/44 MAPK-mediated cytosolic phospholipase A(2) activation. J Biol Chem 2001;276:39539–48.
[40] Frost R, Nystrom G, Lang C. Epinephrine stimulates IL-6 expression in skeletal muscle and C2C12 myoblasts: role of c-Jun NH2-terminal kinase and histone deacetylase activity. Am J Physiol Endocrinol Metab 2004;286:E809–17.
[41] Yamauchi J, Hirasawa A, Miyamoto Y, Itoh H, Tsujimoto G. Beta2-adrenergic receptor/cyclic adenosine monophosphate (cAMP) leads to JNK activation through Rho family small GTPases. Biochem Biophys Res Commun 2001;284: 1199–203.
[42] Katagiri T, Akiyama S, Namiki M, Komaki M, Yamaguchi A, Rosen V, et al. Bone morphogenetic protein-2 inhibits terminal differentiation of myogenic cells by suppressing the transcriptional activity of MyoD and myogenin. Exp Cell Res 1997;230:342–51.
[43] López-Rovira T, Chalaux E, Massagué J, Rosa J, Ventura F. Direct binding of Smad1 and Smad4 to two distinct motifs mediates bone morphogenetic protein-specific transcriptional activation of Id1 gene. J Biol Chem 2002;277:3176–85.
[44] Kinoshita T, Kobayashi S, Ebara S, Yoshimura Y, Horiuchi H, Tsutsumimoto T, et al. Phosphodiesterase inhibitors, pentoxifylline and rolipram, increase bone mass mainly by promoting bone formation in normal mice. Bone 2000;27: 811–7.
[45] Horiuchi H, Saito N, Kinoshita T, Wakabayashi S, Yotsumoto N, Takaoka K. Effect of phosphodiesterase inhibitor-4, rolipram, on new bone formations by recombinant human bone morphogenetic protein-2. Bone 2002;30:589–93.
[46] Tsutsumimoto T, Wakabayashi S, Kinoshita T, Horiuchi H, Takaoka K. A phosphodiesterase inhibitor, pentoxifylline, enhances the bone morphogenetic protein-4 (BMP-4)-dependent differentiation of osteoprogenitor cells. Bone 2002;31:396–401.
[47] Wakabayashi S, Tsutsumimoto T, Kawasaki S, Kinoshita T, Horiuchi H, Takaoka K. Involvement of phosphodiesterase isozymes in osteoblastic differentiation. J Bone Miner Res 2002;17:249–56.

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ORIGINAL ARTICLE

Clinical outcomes of microscopic decompression for degenerative lumbar foraminal stenosis: a comparison between patients with and without degenerative lumbar scoliosis

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Abstract We performed microscopic lumbar foraminotomy in all the patients diagnosed with degenerative lumbar foraminal stenosis (DLFS) and retrospectively reviewed the clinical outcomes and the factors influencing them. The preoperative Japanese Orthopaedic Association (JOA) score of 13.8 significantly improved to 21.9 postoperatively. Although leg pain reduced in 44 patients (95.7%) immediately after surgery, it recurred in 9 patients (19.6%). The recurrence frequency was significantly higher and the JOA score improvement ratios significantly lower in patients with degenerative lumbar scoliosis (DLS) than in those without DLS. Even among patients with DLS, those with <3° Cobb angle difference between the supine and standing positions showed satisfactory results, with no recurrence. In conclusion, microscopic lumbar foraminotomy for DLFS produced satisfactory clinical outcomes even in patients with DLS. However, the outcomes were poor in patients with unstable DLS.

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Keywords Degenerative lumbar scoliosis · Foraminal stenosis · Microsurgical decompression · Less-invasive surgery

Introduction

Degenerative lumbar foraminal stenosis (DLFS) is a relatively common cause of lumbar radiculopathy, with a reported incidence rate of 8–11% [9, 10, 14, 17]. It is characterized by the narrowing of the canal space for the exiting nerve root, caused by osseous and ligamentous hypertrophy [8]. In addition, the L5 nerve root may be compressed by the L5 transverse process, sacral ala, or lumbosacral ligament in the extraforaminal zone [11, 18]. There are two surgical treatment options for DLFS: decompression without fusion and decompression with spinal fusion [1, 3, 6, 8–10, 12, 14]. With development in spinal instrumentation, fusion surgery is preferred, especially in patients with degenerative lumbar scoliosis (DLS) due to malalignment and instability. However, fusion surgery poses problems, such as adjacent segment disease and pseudoarthrosis [2, 4, 19]. Because patients with DLS are often elderly, surgical invasion and instrumentation failure due to osteoporosis also create problems in fusion surgery. Decompression without fusion is considered less invasive and capable of avoiding spinal fusion-associated sequelae, but recurrent radiculopathy may become a problem, especially in patients with deformity and/or instability [3, 5, 10, 16]. Many clinical studies have shown satisfactory clinical outcomes of foraminal decompression without fusion for lumbar foraminal disc herniation, but there have been only a few studies on DLFS [1, 5, 6, 12, 14] and no detailed analysis of the influence of DLS on the outcomes.

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We developed a less-invasive microscopic lumbar foraminotomy procedure for decompressing the compressed nerve root and minimizing invasion into the spinal posterior structure. We, then performed microscopic lumbar foraminotomy in all the patients diagnosed with DLFS, including even those with DLS. In the present study, we investigated the clinical outcomes of this surgical procedure and frequency of recurrent leg pain and evaluated the influence of DLS on the outcomes. Preoperative and postoperative radiographic features of leg pain recurrence in patients with DLS were also investigated.

Materials and methods

Patients and methods

Between 2005 and 2008, 51 patients diagnosed with DLFS were treated with microscopic lumbar foraminotomy at our institution. DLFS was diagnosed on the basis of the symptoms, neurological examination results, selective nerve root block, and presence of foraminal stenosis on computed tomography (CT) and magnetic resonance (MR) images. Surgical treatment was indicated only in patients who showed severe muscle weakness or intolerable leg pain even after conservative treatment, including nonsteroidal antiinflammatory medication, physical therapy, and selective nerve root block. Selective nerve root block was performed under fluoroscopic guidance. All patients who underwent surgeries for leg pain showed a good response to nerve block, but complained of leg pain again after a certain period. Patients with obvious disc herniation, isthmic spondylolisthesis, and congenital stenosis were excluded. Four patients were lost to follow-up, and one had an incomplete preoperative radiographic assessment. Thus, 46 patients (29 men and 17 women) who were followed up for >1 year were included in this study. The age at the time of surgery was 39–87 years (mean, 67.3 years). The follow-up period was 12–41 months (mean, 21.9 months).

Operative technique

All operations were performed or supervised by one senior author (H.M.). Under fluoroscopic guidance, a 3-cm skin incision was made, centered at the intervertebral foramen to be decompressed. After splitting, the erector spinae muscles, a self-retaining retractor and surgical microscope were set up.

The superolateral part of the superior articular process of the lower vertebrae and the lower border of the upper transverse process were resected using a high-speed drill. Tilting the surgical microscope to the medial side, the lateral part of the pars interarticularis and the superomedial part of the superior articular process were resected. By resecting the ligamentum flavum and the medial part of the intertransverse ligament, the foramen was unroofed. To confirm adequate decompression, probing with a Watson-Cheyne dissector, from the medial side of the foramen to the lateral extraforaminal zone, was carefully performed to confirm the movability of the decompressed nerve root (Fig. 1a).

On the basis of the preoperative or intraoperative findings, partial pediculectomy or discectomy were additionally performed in patients with stenosis in the craniocaudal direction. In partial pediculectomy, the inferior part of the pedicle (total length, 4–5 mm) was resected using the intrapedicular approach, similar to the method adopted by Kunogi and Hasue [10] or Sheehan et al. [15].

If patients had extraforaminal stenosis at the L5–S1 intervertebral level, additional extraforaminal decompression was performed by tilting the surgical microscope to the lateral side: the lower part of the L5 transverse process, superomedial part of the sacral ala, and lumbosacral ligament were resected (Fig. 1b).

Further, if patients showed intracanal stenosis on preoperative CT or MR images, an additional medial facetectomy, which decompressed the lateral recess to the medial aspect of the pedicle, was performed via the midline approach.

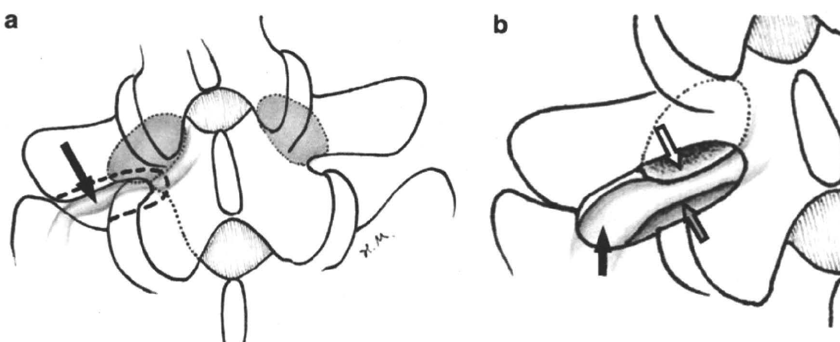

Fig. 1 Schematic representation of our decompression procedure. **a** The arrow indicates the area of unroofing at the foramen. **b** Additional decompression was performed depending on the stenotic condition: the white, gray, and black arrows indicate the areas of partial pediculectomy, discectomy, and extraforaminal decompression, respectively

Clinical and radiological evaluation

Patient data, including demographic information, duration of symptoms, level of operation, additional procedures, surgical complications, and revision surgery, were obtained from their clinical records. Revision surgeries were defined as the surgical procedures performed at the same decompressed level.

Clinical outcomes were evaluated using the Japanese Orthopaedic Association (JOA) scores obtained preoperatively and at the latest follow-up. When revision surgery for recurrent leg pain was performed, the score at the latest follow-up was obtained before the surgery. Ratios of JOA score improvement from preoperative evaluation to the latest follow-up were calculated using the formula proposed by Hirabayashi [7]. Clinical outcomes were classified into 4 categories according to the JOA score improvement ratios: (1) poor, <25%; (2) fair, 25–50%; (3) good, 50–75%; (4) excellent, ≥75%. Intensity of leg pain was evaluated according to the JOA score: 0 = frequent or continuous severe leg pain, 1 = continuous slight or occasional severe leg pain, 2 = occasional mild leg pain, and 3 = no pain. Recurrent leg pain was defined as leg pain similar to preoperative leg pain that had worsened to the 0 or 1 level at the latest follow-up, after postoperative improvement.

Radiological evaluation was performed by obtaining preoperative and postoperative plain radiograms. On the preoperative anteroposterior lumbar radiograms, Cobb angle was measured both in the supine and standing positions. The patients were classified into two groups according to the coronal standing Cobb angle: Group 1 (with DLS) comprised patients with ≥10° Cobb angle, and Group 2 (without DLS) comprised patients with <10° Cobb angle. Leg pain recurrence and clinical outcomes in the two groups were compared. Further, to clarify the factors associated with the recurrent leg pain in Group 1, other radiological parameters were investigated. Lateral rotatory olisthesis was considered present when >3 mm of lateral vertebral displacement was observed on anteroposterior radiograms. Sagittal olisthesis was considered present when >3 mm of vertebral displacement was observed on lateral neutral-position plain radiograms. Wedging angles were measured on the standing radiograms of the affected intervertebral level. Disc heights were measured as the distance between the posterior edges of the vertebral bodies on lateral radiograms. Range of motion (ROM) in flexion–extension and lateral bending was measured on dynamic lumbar radiograms. Cobb angle progression was evaluated on the latest postoperative standing lumbar radiograms. Curve progression was defined as positive when >5° progression was observed. Data are expressed as average ± standard deviation (SD). Statistical analysis was performed using StatView 5.0 (Abacus Concepts, Berkeley, CA) and the unpaired nonparametric Mann–Whitney test at a 95% confidence level or Chi-square test. A p value of <0.05 was considered statistically significant.

Results

The affected nerve roots and the procedures combined with the usual lateral lumbar foraminotomy are shown in Table 1. The most common nerve root decompressed was the L5 nerve root (79.2%), followed by the L4 nerve root (18.9%) and the L3 nerve root (1.9%). The preoperative JOA scores of 13.8 ± 4.4 significantly improved to 21.9 ± 5.5 postoperatively. The average JOA score improvement ratio was 54%. Good or excellent outcomes were shown by 28 patients (60.9%). Although leg pain reduced in 44 patients (95.7%) immediately after surgery, it recurred in 9 patients (19.6%) during the follow-up period.

Postoperative hematoma occurred in one patient, which was removed because of intractable leg pain. No other complications occurred in this series.

Revision surgeries for residual leg pain due to insufficient initial decompression were performed in two patients (2 and 12 weeks after the initial surgery), after which the symptoms decreased immediately. Revision surgeries for recurrent leg pain were performed in three patients. One of them underwent posterior lumbar interbody fusion 17 months after the first surgery. The other two patients underwent additional decompression surgery of the lateral intervertebral foramen (17 and 20 months after the initial surgery). The leg pain immediately disappeared after the revision surgery.

Comparison between patients with and without DLS

Depending on the Cobb angle, 26 and 20 patients were classified in Group 1 (with DLS) and Group 2 (without DLS), respectively. There were no significant differences in the other parameters, including follow-up period, between the two groups (Table 2). In Group 1, 27 of the 30

Table 1 Surgical procedures performed in Groups 1 and 2

	Group 1	Group 2
No. of nerve roots targeted for decompression	30	23
L3	1	0
L4	8	2
L5	21	21
No. of additional procedures		
Partial pediculectomy	11	12
Discectomy	10	5
Extraforaminal decompression	3	3
Medial facetectomy	6	9

Table 2 Demographic data of patients in Groups 1 and 2

	Group 1	Group 2	*P* value
No. of patients	26	20	
Preoperative Cobb angle (°)	15.0 ± 4.2	5.8 ± 2.7	**<0.0001**
Preoperative JOA score (points)	14.3 ± 4.8	13.1 ± 3.8	0.351
Duration of symptoms (mo)	11.9 ± 12.6	10.8 ± 13.2	0.973
Follow-up period (mo)	23.0 ± 8.9	20.6 ± 7.1	0.387

Bold value is statistically significant

JOA score Japanese Orthopaedic Association score for low-back pain assessment

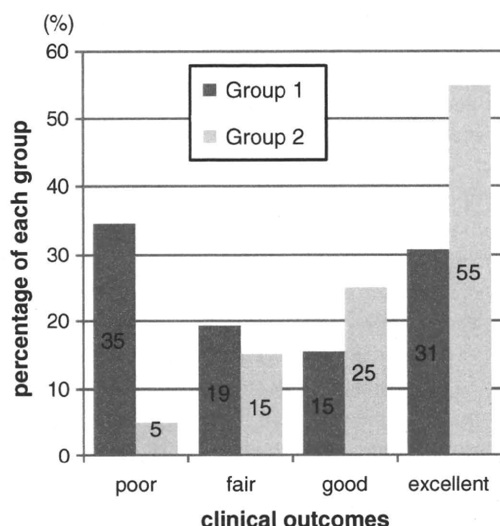

Fig. 2 Patient distribution with regard to clinical outcomes classified into four categories according to the JOA score. In Groups 1 and 2, 46 and 80% patients showed good or excellent results, respectively

nerve roots involved were at the concave side of their segmental curves. The JOA score improvement ratio in Group 2 was significantly higher than that in Group 1 ($p = 0.027$). The categorized outcomes are shown in Fig. 2.

Recurrent leg pain was observed in eight patients (30.8%) in Group 1 and in one patient (5%) in Group 2. The incidence was significantly higher in Group 1 ($p = 0.029$). Revision surgeries for recurrent leg pain were performed for two patients in Group 1 and for one patient in Group 2.

Overall, the postoperative clinical outcomes were better in patients without DLS than in those with DLS. Additionally, after the patients were divided into two groups on the basis of the follow-up period (lesser or greater than 24 months), same trends were observed in the recurrence ratio and improvement of JOA score between the DLS and non-DLS groups.

Radiographic features of patients with recurrent leg pain in Group 1

Table 3 shows the summarized data of patients with recurrent leg pain in Group 1.

Preoperative radiograms showed that the Cobb angles and angle differences between the standing and supine positions were significantly greater in patients with recurrent radiculopathy than in those without recurrent radiculopathy ($p = 0.007$ and $p = 0.006$, respectively). These two significantly correlated with each other ($R = 0.44$, $p = 0.023$). ROM in lateral bending was larger in patients with recurrence, although this difference was not statistically significant ($p = 0.052$). Postoperative curve progression was observed in four patients, but it was not related to recurrent radiculopathy (Table 4).

Patients in Group 1 were further divided into two groups on the basis of the presence of ≥3° and <3° Cobb angle difference between the standing and supine positions. No patient with <3° difference had recurrent radiculopathy during the follow-up, and 70% patients showed good or

Table 3 Summary of cases of leg pain recurrence in Group 1

Case no.	Age (year)/sex	Cobb angle (°)	Target nerve root	Additional procedure	Time to recurrence (mo)	JOA score		Treatment
						Latest leg pain (points)	Improvement ratio (%)	
1	67/M	25	L5	PP	9	1	41.2	Conservative
2	71/M	20	L5	PP	3	0	0.0	Conservative
3	73/M	18	L5	PP, D	21	1	−12.5	Conservative
4	78/F	16	L5	None	1	1	0.0	Surgical (PLIF)
5	74/F	16	L5	None	5	1	31.6	Conservative
6	60/M	16	L4	D	3	0	26.1	Surgical (decompression)
7	69/F	15	L5	PP, D	9	0	39.1	Conservative
8	79/F	15	L5	None	6	0	5.0	Conservative

PP partial pediculectomy, *D* discectomy, *PLIF* posterior lumbar interbody fusion, *JOA* Japanese Orthopaedic Association score for low-back pain assessment

Table 4 Radiographic evaluation of patients with and without recurrence in Group 1

	With recurrence	Without recurrence	*P* value
Cobb angle (°)	17.8 ± 3.2	13.8 ± 4.1	**0.007**
Cobb angle difference between supine and standing positions (°)	4.4 ± 1.3	2.4 ± 1.5	**0.006**
No. of patients with lateral rotatory olisthesis	2	5	0.671
No. of patients with sagittal olisthesis	Anterior: 1	Anterior: 2 Posterior: 5	0.335
Coronal wedging angle	6.4 ± 3.6	4.6 ± 2.7	0.159
Disc height (mm)	3.7 ± 1.7	2.9 ± 1.1	0.250
ROM in flexion–extension (°)	9.9 ± 5.1	6.7 ± 3.1	0.146
ROM in lateral bending (°)	5.8 ± 4.0	3.1 ± 2.3	0.052
No. of patients with postoperative curve progression (≥5°)	2	2	0.365

Bold values are statistically significant

ROM range of motion

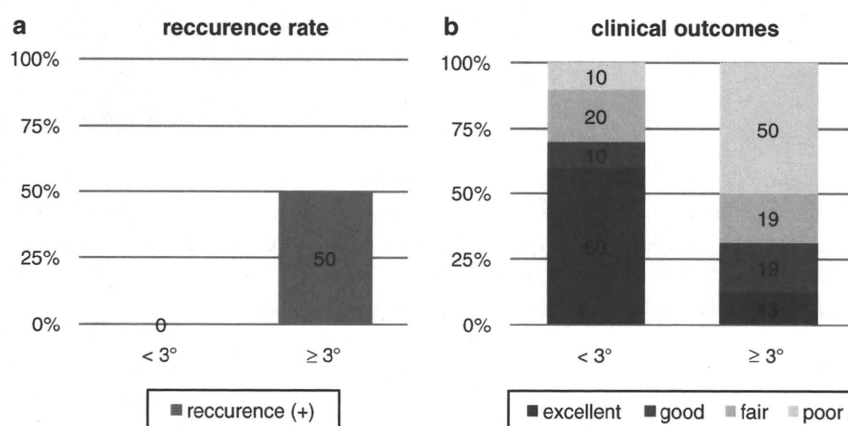

Fig. 3 Recurrence rate (**a**) and clinical outcomes (**b**) of patients with ≥3° and <3° Cobb angle difference between the standing and supine positions in Group 1. Patients with <3° difference showed a better clinical outcome than those with ≥3° difference

excellent results according to the JOA score improvement ratios. On the other hand, 50% patients with ≥3° difference had recurrent radiculopathy, and only 32% showed good or excellent results (Fig. 3).

A representative case

A 76-year-old woman had been experiencing right leg pain for 3 years. Her preoperative radiogram showed DLS with a 15° Cobb angle and L5 sacralization. Her preoperative CT and MR images showed foraminal stenosis at the right L4–L5 and intracanal stenosis at L3–L4. A microscopic lumbar foraminotomy at the right L4–L5 was followed by a partial pediculectomy at the left L4 and a medial facetectomy at L3–L4. After surgery, the leg pain completely disappeared. At 12 months after surgery, the patient had only mild low-back pain, and the JOA score improvement ratio was 75% (Fig. 4).

Discussion

In this series, we not only performed unroofing at the foramen but also partial resection of the pedicle, disc, transverse process, and/or sacral ala according to each patient's pathology using a microscope. This combination technique can sufficiently decompress the affected nerve root and preserve the facet joint and pars interarticularis. Although postoperative residual leg pain was observed in two patients, it disappeared after additional decompression surgery. Thus, all the patients with DLFS were successfully treated with decompression surgery. The short-term clinical outcomes were good or excellent in 60.9% patients. Hallett et al. [6] performed a randomized clinical trial of patients with foraminal stenosis without instability due to degenerative disc disease. They reported no differences in the clinical outcomes of decompression and additional fusion. Ozeki et al. [12] reported that short-term clinical results after microscopic decompression via the intrapedicular approach were satisfactory with an average JOA score recovery rate of 62.1%. Other studies on foraminal stenosis without instability have also suggested that sufficient decompression can produce satisfactory clinical outcomes even without spinal fusion [1, 10, 12, 13]. The present study, even including patients with DLS, showed similar satisfactory clinical outcomes after decompression surgery alone.

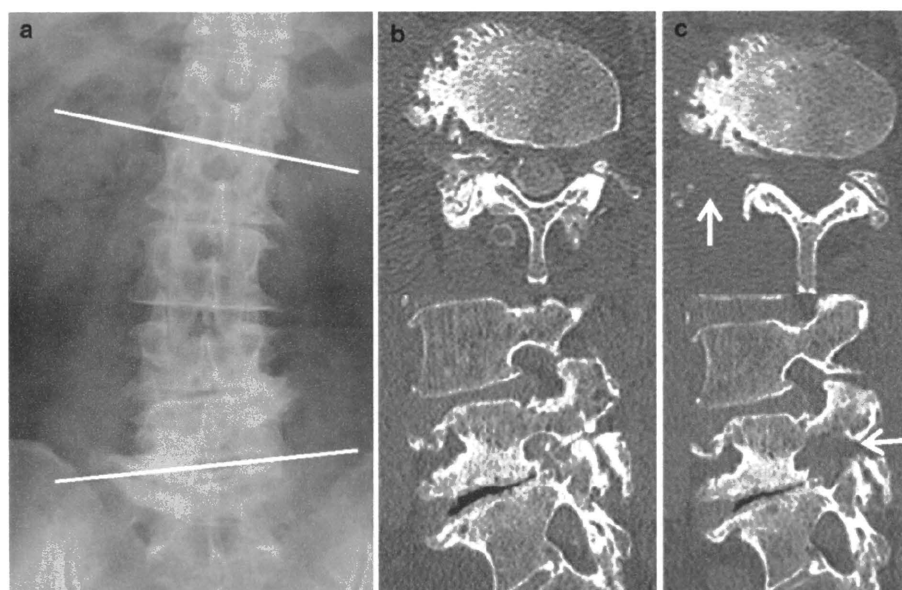

Fig. 4 Case representation. A 76-year-old woman with right L4–L5 foraminal stenosis underwent microscopic lumbar foraminotomy and additional partial pediculectomy at the left L4 and medial facetectomy at L3–L4. **a** Preoperative radiogram shows a 15° Cobb angle. **b** Preoperative axial (*above*) and sagittal (*below*) CT scan shows the right L4–L5 foraminal stenosis. **c** Postoperative axial (*above*) and sagittal (*below*) CT scan shows minimal resection at the intervertebral foramen and expanded intervertebral foramen by partial pediculectomy (*arrows*)

Recurrent radiculopathy is a serious problem following symptom improvement after decompression surgery. Chang et al. [3] reported that the incidence of residual or recurrent leg pain after decompression surgery was 21.7% in patients with foraminal stenosis, including foraminal disc herniation. In this study, the recurrence rate was 19.6%. Thus, the recurrence rates were similar despite different study populations. In our study, 89% patients with recurrent radiculopathy were those who had DLS. Among the patients with DLS, the Cobb angle difference between the standing and supine positions was significantly greater in the patients with recurrent radiculopathy than in those without it. The ROM of the affected level in lateral bending also tended to be higher in patients with recurrent radiculopathy than in those without it, whereas sagittal ROM did not differ between the two groups. These results suggest that the instability of the coronal curve affects the clinical outcomes of the surgical procedure. Hypermobility of the affected level, combined with scar tissue, may irritate the nerve root and cause recurrence. To prevent recurrent radiculopathy, sufficient decompression should be performed considering the dynamic instability, especially in patients with both DLS and its coronal instability.

Although the present study showed that the average outcomes of patients with DLS treated with decompression surgery were unsatisfactory, we believe that our foraminotomy technique can be applied to some patients with DLS because of the following reasons. First, it has been reported that fusion surgery for DLS has a relatively high complication rate than for other degenerative diseases such as spondylolisthesis [2, 4, 19]. Therefore, for patients who are poor candidates for massive spinal reconstruction surgery because of their general condition, our decompression technique is a treatment option. Second, DLS with a rigid curve may also be an indication for our decompression technique. The present study results showed that the patients with <3° Cobb angle difference between the supine and standing positions had no recurrence, and 70% patients achieved good or excellent outcomes. DLS with coronal instability may be an indication for fusion surgery, but it cannot be exactly determined from the present results. This study only showed the short-term outcomes and postoperative radiologic evaluation was performed only on the basis of the anteroposterior lumbar radiograms, with no total spine or pelvic radiograms. Further studies focusing on long-term follow-up, spinal balance, and influence of the pelvis will further clarify the conditions indicating decompression surgery.

Conclusion

Less-invasive microscopic lumbar foraminotomy produced satisfactory clinical outcomes in 60.9% patients with DLFS, including even those with DLS. However, the outcomes of some patients with DLS were not equivalent to those of the patients without DLS. Radiological evaluation indicated that coronal instability might affect the clinical outcomes.

Conflict of interest No funds or grants were received in support of this work.

References

1. Baba H, Uchida K, Maezawa Y, Furusawa N, Okumura Y, Imura S (1996) Microsurgical nerve root canal widening without fusion for lumbosacral intervertebral foraminal stenosis: technical notes and early results. Spinal Cord 34:644–650

2. Bono CM, Lee CK (2005) The influence of subdiagnosis on radiographic and clinical outcomes after lumbar fusion for degenerative disc disorders: an analysis of the literature from two decades. Spine (Phila Pa 1976) 30:227–234
3. Chang SB, Lee SH, Ahn Y, Kim JM (2006) Risk factor for unsatisfactory outcome after lumbar foraminal and far lateral microdecompression. Spine (Phila Pa 1976) 31:1163–1167
4. Cho KJ, Suk SI, Park SR, Kim JH, Kim SS, Choi WK, Lee KY, Lee SR (2007) Complications in posterior fusion and instrumentation for degenerative lumbar scoliosis. Spine (Phila Pa 1976) 32:2232–2237
5. Epstein NE (2002) Foraminal and far lateral lumbar disc herniations: surgical alternatives and outcome measures. Spinal Cord 40:491–500
6. Hallett A, Huntley JS, Gibson JN (2007) Foraminal stenosis and single-level degenerative disc disease: a randomized controlled trial comparing decompression with decompression and instrumented fusion. Spine (Phila Pa 1976) 32:1375–1380
7. Hirabayashi K, Watanabe K, Wakano K, Suzuki N, Satomi K, Ishii Y (1983) Expansive open-door laminoplasty for cervical spinal stenotic myelopathy. Spine (Phila Pa 1976) 8:693–699
8. Jenis LG, An HS (2000) Spine update: lumbar foraminal stenosis. Spine (Phila Pa 1976) 25:389–394
9. Jenis LG, An HS, Gordin R (2001) Foraminal stenosis of the lumbar spine: a review of 65 surgical cases. Am J Orthop 30:205–211
10. Kunogi J, Hasue M (1991) Diagnosis and operative treatment of intraforaminal and extraforaminal nerve root compression. Spine (Phila Pa 1976) 16:1312–1320
11. Nathan H, Weizenbluth M, Halperin N (1982) The lumbosacral ligament (LSL), with special emphasis on the "lumbosacral tunnel" and the entrapment of the 5th lumbar nerve. Int Orthop 6:197–202
12. Ozeki N, Aota Y, Uesugi M, Kaneko K, Mihara H, Niimura T, Saito T (2008) Clinical results of intrapedicular partial pediculectomy for lumbar foraminal stenosis. J Spinal Disord Tech 21:324–327
13. Park YK, Kim JH, Chung HS, Suh JK (2003) Microsurgical midline approach for the decompression of extraforaminal stenosis in L5–S1. J Neurosurg 98:264–270
14. Porter RW, Hibbert C, Evans C (1984) The natural history of root entrapment syndrome. Spine (Phila Pa 1976) 9:418–421
15. Sheehan JM, Helm GA, Kallmes DF, Bogaev CA, Jane JA (1997) Partial pediculectomy in the treatment of lumbar spinal stenosis: technical note. Neurosurgery 41:308–310
16. Weidenbaum M (2006) Considerations for focused surgical intervention in the presence of adult spinal deformity. Spine (Phila Pa 1976) 31:S139–S143
17. Weiner BK, Fraser RD (1997) Foraminal injection for lateral lumbar disc herniation. J Bone Joint Surg Br 79:804–807
18. Wiltse LL, Guyer RD, Spencer CW, Glenn WV, Porter IS (1984) Alar transverse process impingement of the L5 spinal nerve: the far-out syndrome. Spine (Phila Pa 1976) 9:31–41
19. Wu CH, Wong CB, Chen LH, Niu CC, Tsai TT, Chen WJ (2008) Instrumented posterior lumbar interbody fusion for patients with degenerative lumbar scoliosis. J Spinal Disord Tech 21:310–315

Risk Factor Analysis for Motor Deficit and Delayed Recovery Associated With L4/5 Lumbar Disc Herniation

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Study Design: Retrospective study of multivariable analysis for the risk factors of motor deficit associated with lumbar disc herniation (LDH).

Objectives: To identify the risk factors for motor deficit and delayed recovery after surgery in patients with LDH.

Summary of Background Data: LDH can cause motor deficit as well as pain and sensory disturbance. Even though motor deficit can lead to disabilities and affect treatment plans, few studies have described motor deficit and its risk factors in LDH patients.

Methods: Seventy-six consecutive patients who underwent microsurgical or microendoscopic discectomy for LDH at the L4/5 level were retrospectively reviewed. Motor deficit was defined as tibialis anterior muscle strength of lower than grade 4 by the manual muscle test, and delayed recovery was defined as cases requiring longer than 3 months to achieve complete recovery. The possible risk factors including sex, age, symptom duration, preoperative radiographic parameters, and type of herniation were evaluated by multivariate logistic regression analysis.

Results: Forty-three patients (56.6%) suffered from motor deficit before surgery. Forty cases (93%) completely recovered within a mean duration of 4 months. Multivariate logistic regression analysis revealed that noncontained-type ($P = 0.012$, odds ratio = 13.7) and migrated herniated nucleus pulposus ($P = 0.033$, odds ratio = 9.8) were important risk factors for motor deficit. Furthermore, severe motor deficit (preoperative manual muscle test ≤ 3; $P = 0.019$, odds ratio = 19.6) and noncontained type ($P = 0.049$, odds ratio = 5.17) were identified as important risk factors for delayed recovery.

Conclusions: Noncontained-type or migrated herniated nucleus pulposus seem to be the most important risk factors for motor deficit in LDH, whereas severe motor deficit and noncontained type seem to be associated with delayed recovery. The treatment options for patients with these factors at first visit should be carefully chosen during the follow-up period.

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Key Words: lumbar disc herniation, motor deficit, risk factor analysis, noncontained type, migration

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Lumbar disc herniation (LDH) is the most common cause of low back pain and leg pain. The natural history of LDH is favorable, wherein leg pain in most patients is reduced within 8 weeks from onset. Therefore, medical doctors regularly perform some kind of conservative therapy on patients with symptomatic LDH. There is, however, no consensus on how long should the conservative therapy be tried before surgical intervention.

Recently, some comparative studies have indicated that early surgical intervention can affect earlier pain relief, but there is no significant advantage in 1-year outcomes.[1–3] LDH can cause motor deficit as well as pain and sensory disturbance. Some patients, such as those with bowel/bladder dysfunction and/or progressive motor deficit, have definite indications for surgery. Motor deficit can lead to disabilities, especially in severe cases such as foot drop, and some studies have reported that 24% to 47% of such patients do not recover completely even after surgery.[4,5] Although the patients with motor deficit need early surgical intervention, only a few studies have described the characteristics or types of LDH associated with motor weakness. If the risk factors for motor deficit and its prognosis can be clarified, the treatment plan can be arranged at an early stage of treatment. The aims of this study were to identify the significant risk factors for motor weakness caused by LDH and for delayed recovery after surgery.

MATERIALS AND METHODS

Patients

Seventy-six consecutive patients (46 men and 30 women) who underwent microsurgical (n = 24) or microendoscopic (n = 52) discectomy for LDH at L4/5 level between January 2000 and October 2006 were retrospectively reviewed. The mean age was 41.1 years (range, 17 to 79 y) and the mean follow-up period was 16.6 months (range, 6 to 67 mo). Cases of LDH recurrence or more than 2-level lesions of LDH, were excluded from this study. Demographic data on the patients were

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retrieved from their medical charts. The Japanese Orthopaedic Association (JOA) score for the assessment of low back pain was evaluated before surgery and at final follow-up. The JOA score comprises 9 points assigned for subjective symptoms, 6 points for clinical signs, and 14 points for the restriction of activity of daily living; the total score being 29 points (Table 1).[6,7] Muscle strength was evaluated twice by 2 spinal surgeons using the manual muscle test (MMT) according to the classification of the Medical Research Council, which grades muscle strength on a scale of 0 (paralysis) to 5 (normal strength). In this study, an average MMT was used for evaluation, and motor deficit was defined as tibialis anterior (TA) muscle strength of lower than grade 4 by MMT, and delayed recovery was defined as cases requiring longer than 3 months to achieve complete recovery (recovery to MMT = 5). Postoperative motor recovery was examined at 3 days, 2 weeks, 4 to 8 weeks, 3 months, 6 months, 1 year after surgery, and then after every 6 months.

Risk Factor Analysis for Motor Weakness and Delayed Recovery

The possible risk factors for motor weakness including age, sex, duration of symptom, type of herniated nucleus pulposus (HNP), HNP occupancy percentage, and craniocaudal migration of HNP were evaluated by multivariate logistic regression analysis. Duration of symptom was defined as the interval from the onset of neurologic symptom (leg pain, numbness, and/or muscle weakness) to surgery. The types of HNP were classified by intraoperative findings according to the Barton classification (contained type or noncontained type).[8] HNP occupancy percentage was calculated as the cross-sectional area of HNP per cross-sectional area of the spinal canal (Fig. 1A). Each area was measured on the axial view of a T1-weighted magnetic resonance image by using Scion Image software (Scion Co, Frederick, MD). Craniocaudal migration of HNP was evaluated by whether the base of the extruded disc extended beyond the disc height on a sagittal T1-weighted magnetic resonance image, (Fig. 1B). For the risk factor analysis of delayed recovery, the preoperative muscle strength of TA (MMT > 4 or ≤ 3) was added to these factors for motor deficit.

Statistical Analysis

The clinical outcomes were evaluated by the Mann-Whitney U test. The difference was considered significant when the P value was < 0.05. To identify risk factors for motor deficit and delayed recovery, the relationships

TABLE 1. Japanese Orthopaedic Association Scoring System for Treatment of Low Back Pain (JOA Score)

Item			Score
Subjective symptoms	Low back pain (3 points)	None	3
		Occasional mild pain	2
		Frequent mild or occasional severe pain	1
		Continuous severe pain	0
	Leg pain and/or tingling (3 points)	None	3
		Occasional mild symptoms	2
		Frequent mild or occasional severe symptoms	1
		Continuous severe symptoms	0
	Gait (3 points)	Normal	3
		Able to walk farther than 500 m although results in pain, tingling, and/or muscle weakness	2
		Unable to walk farther than 500 m, results in pain, tingling, and/or muscle weakness	1
		Unable to walk farther than 100 m, results in pain, tingling, and/or muscle weakness	0
Objective symptoms	Straight leg raising test (2 points)	Normal	2
		30-70 degrees	1
		Less than 30 degrees	0
	Sensory abnormality (2 points)	Normal	2
		Mild disturbance (not subjective)	1
		Marked disturbance	0
	Motor disturbance (MMT) (2 points)	Normal (Grade 5)	2
		Slight weakness (Grade 4)	1
		Marked weakness (Grade 3-0)	0
Restriction of ADL (14 points)	Turning over while lying		
	Standing		
	Washing	No restriction	2
	Leaning forward	Moderate restriction	1
	Sitting (about 1 h)	Severe restriction	0
	Lifting or holding heavy objects		
	Walking		
Urinary bladder		Normal	0
Function (−6 points)		Mild dysuria	−3
		Severe dysuria	−6
Total score			29

ADL indicates activity of daily living; MMT, manual muscle test.

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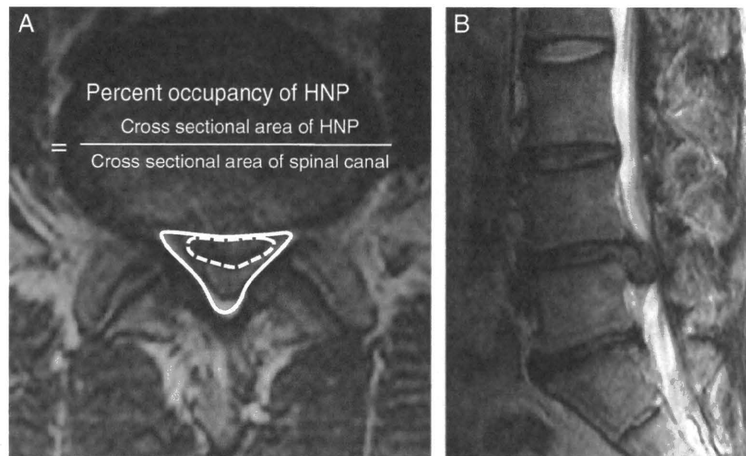

FIGURE 1. A, HNP occupancy percentage is calculated as the percentage of cross-sectional area of HNP (inner area of dotted line) divided by cross-sectional area of spinal canal (inner area of white line). B, Representative case of migration of HNP. When base of prolapsed disc extends beyond contour of intervertebral disc cranially or caudally, it is defined as migration of HNP. HNP indicates herniated nucleus pulposus.

between the categorical variables were analyzed by the χ^2 test. The odds ratios for significant variables and the 95% confidence intervals were calculated by multivariate logistic regression analysis. SAS software (version 9; SAS Institute Inc, Cary, NC) was used for all statistical analyses.

RESULTS

Clinical Outcomes

Forty-three patients suffered from motor deficit before surgery. Motor deficit was found in 29 patients with MMT4 (67.4%), 6 patients with MMT3 (14.0%), and 8 patients with MMT$\leq$2 (18.6%). Motor deficit recovered fully in 40 cases (93%), and the duration from surgery to complete recovery was an average of 4 months. The mean duration of each group was 2.9 months for MMT4, 6.9 months for MMT3, and 11.6 months in MMT < 3. Residual motor deficit at the final follow-up (> 18 mo after surgery) was observed in 3 patients (7%) who had severe muscle deficit before surgery (MMT3, 1 case; MMT2, 1 case; MMT1, 1 case).

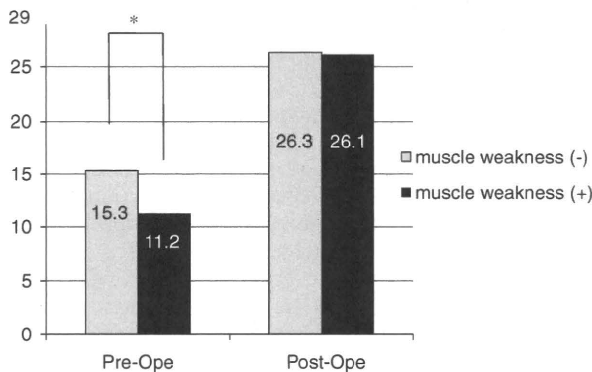

FIGURE 2. Preoperative and postoperative JOA scores for the assessment of low back pain in patients with or without motor deficit before surgery. Even in the cases with preoperative muscle weakness, postoperative score is equivalent as the cases without motor weakness despite lower preoperative score.

The mean preoperative JOA score of 13.2 (range, 3-23) points improved to 26.1 (range, 21 to 29) points at final follow-up. The JOA score was significantly lower in patients with motor weakness before surgery, but there was no difference at the final follow-up (Fig. 2).

Risk Factor Analysis for Motor Weakness and Delayed Recovery

Multivariate logistic regression analysis revealed that noncontained type of HNP ($P = 0.003$, odds ratio = 9.1) and migration of HNP ($P = 0.0002$, odds ratio = 12.3) were important risk factors for motor deficit (Table 2). Severe muscle deficit (MMT$\leq$3; $P = 0.019$, odds ratio = 19.6) and the noncontained type of HNP ($P = 0.049$, odds ratio = 5.17) were identified as the most significant risk factors for delayed recovery among 7 explanatory variables (Table 3).

DISCUSSION

Recently, some papers have revealed the efficacy of surgery in patients with LDH compared with conservative therapy.[1–3] Clinically, however, not all patients with LDH

TABLE 2. Multivariate Analysis for Motor Weakness

Factors	*P*	Odds Ratio (95% CI)
Age	0.996	1.00 (0.96-1.04)
Sex	0.429	0.69 (0.14-3.35)
Duration of symptom	0.943	1.02 (0.98-1.06)
Type of HNP	0.003	9.10 (1.54-34.3)
Percent occupancy of HNP	0.217	31.7 (0.98-1.09)
Migration of HNP	0.0002	12.3 (3.25-46.0)

CI indicates confidence interval; HNP, herniated nucleus pulposus.

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