

Osteogenesis versus chondrogenesis by BMP-2 and BMP-7 in adipose stem cells [☆]

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Abstract

Bone morphogenetic proteins (BMPs) initiate, promote, and maintain chondrogenesis and osteogenesis. We hypothesize that BMP-2 induces an osteogenic, and BMP-7 a chondrogenic phenotype in adipose tissue-derived mesenchymal stem cells (AT-MSCs). We compared the effects of a short 15 min BMP-2 or BMP-7 (10 ng/ml) treatment on osteogenic and chondrogenic differentiation of AT-MSCs. Gene expression was studied 4 and 14 days after BMP-treatment. At day 4 BMP-2, but not BMP-7, stimulated *runx-2* and *osteopontin* gene expression, and at day 14 BMP-7 down-regulated expression of these genes. At day 4 BMP-2 and BMP-7 stimulated biglycan gene expression, which was down-regulated by BMP-7 at day 14. BMP-7 stimulated aggrecan gene expression at day 14. Our data indicate that BMP-2 treatment for 15 min induces osteogenic differentiation, whereas BMP-7 stimulates a chondrogenic phenotype of AT-MSCs. Therefore, AT-MSCs triggered for only 15 min with BMP-2 or BMP-7 provide a feasible tool for bone and cartilage tissue engineering. © 2006 Elsevier Inc. All rights reserved.

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Nowadays, bone morphogenetic proteins (BMPs) in combination with cells and/or scaffolds gain more and more significance for their use in bone tissue engineering [1]. BMPs belong to the transforming growth factor- β (TGF- β) superfamily of polypeptides including TGF- β , activins/inhibins, and BMPs. They were discovered in extracts of demineralized bone matrix and were shown to induce ectopic bone formation in subcutaneous and intramuscular pockets of rodents [2–4]. BMPs induce differentiation of multipotential mesenchymal cells into both osteochondrogenic and osteoblast precursor cells [5]. Several BMPs have been described and can be classified into subgroups upon their degree of sequence homology, e.g.,

BMP-2 and BMP-4 belong to the BMP-2/-4 subgroup, BMP-5, -6, -7, and -8 belong to the osteogenic protein-1 (OP-1) group, and most of the members of these two groups induce formation of bone and cartilage in vivo [6,7]. Members of the BMP family bind to type I and type II serine/threonine kinase receptors, which in turn can signal by phosphorylating the cytoplasmatic proteins mother against decapentaplegic (*smad*) that are relocated into the nucleus [1,7,8].

Especially, BMP-2 and BMP-7, the latter also known as OP-1, are under evaluation for tissue engineering purposes and available as clinically approved recombinant human proteins [9–12]. BMP-7 has been demonstrated to have strong anabolic activity in young and adult cartilage [13,14]. BMP-7 promotes chondrogenic differentiation of human as well as goat perichondrium cells in vitro, and it is counteracting retinoic acid-induced dedifferentiation of bovine chondrocytes, suggesting that BMP-7 is involved

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in chondrogenic differentiation [12,15]. In vivo, BMP-7 leads to the formation of cartilage at the defect site [14]. BMP-2 on the other hand has been shown to be crucially involved in osteogenic differentiation [16,17].

Autologous adult mesenchymal stem cells provide new and innovative tools in tissue engineering. These cells may be used to restore or replace tissues and organs. Bone marrow is a common source for mesenchymal stem cells, but only available in limited amounts [18]. However, recently adipose tissue has been described as an alternative source for adult stem cells, which show mesenchymal stem cell markers [19–21]. Zuk et al. [20,22] demonstrated that adipose tissue-derived mesenchymal stem cells (AT-MSCs) can differentiate toward the adipogenic, chondrogenic, myogenic, neurogenic, and osteogenic lineage. BMP-2 has been shown to induce osteogenic differentiation in bone marrow-derived mesenchymal stem cells (BM-MSCs) and AT-MSCs in vitro and in vivo [17,23–25]. BMP-2-transfected stem cells derived from adipose tissue are able to induce bone formation in an in vivo SCID mouse assay and it stimulates healing of critical size bone defects in rats [17,26].

Gene expression of runt-related factor-2 (*runx-2*), also referred to as core-binding factor α -1 (*cbfa-1*), and osteopontin (OPN) can be used as markers for differentiation of stem cells toward the osteogenic lineage. *Runx-2* is the earliest transcription factor expressed during osteogenic differentiation and can be induced by BMP-2 and BMP-7 [27,28]. BMP-2 affects *runx-2* gene expression via the smad signaling pathway [7,29,30]. In addition, *runx-2* can increase OPN gene expression, indicating its important role during osteogenic differentiation [27,29]. OPN is one of the abundant non-collagenous proteins in bone extracellular matrix and is produced by osteoblasts involved in endochondral ossification during development [31,32]. OPN is considered to play a role in both bone formation and bone resorption [32,33].

Biglycan is a small leucine-rich proteoglycan present in bone and cartilage extracellular matrix and is involved in BMP-4 mediated osteogenic differentiation of osteoblast precursor cells [34,35]. Aggrecan is the predominant large chondroitin sulfate proteoglycan, and serves a direct, primary role providing the osmotic resistance necessary for cartilage to resist compressive loads [35]. In addition, increased aggrecan synthesis is associated with matrix assembly and cartilage formation [36]. Its gene expression can be stimulated by BMP-7 and BMP-2 in several cell types [36–38]. Aggrecan can be used as a marker for chondrogenic differentiation, and it is expressed by AT-MSCs after chondrogenic treatment with TGF- β 1 [20,22].

Short treatment with growth factors could be of clinical importance especially if freshly isolated stem cells could be stimulated with a growth factor during one surgical procedure and directly implanted into the patient. However, a direct comparison of the effects of BMP-2 or BMP-7 added for only 15 min in AT-MSCs has not been performed so far. Therefore, we investigated the effects of 15 min of

BMP-2 or BMP-7 treatment on osteogenic and/or chondrogenic differentiation of AT-MSCs. *Runx-2* and OPN gene expression, as well as ALP activity, were studied as markers for osteogenic differentiation, and aggrecan and biglycan gene expression was studied as markers for chondrogenic differentiation. We hypothesize that a short treatment with BMP-2 induces an osteogenic phenotype and a short treatment with BMP-7 a chondrogenic phenotype in AT-MSCs upon prolonged culture.

Materials and methods

AT-MSC isolation and culture. Goat adipose tissue was resected from the renal region of seven adult female goats (age \geq 3.5 years). The animal care and use committee of the Vrije Universiteit Amsterdam approved the use of goats in these experiments. The obtained tissue was washed with phosphate-buffered saline (PBS) to remove red blood cells, chopped into small pieces of about 50 mm³, and the extracellular matrix was digested for 90 min at 37 °C with 0.05% collagenase (type 1, Sigma, St. Louis, MO, USA) in PBS. A single cell suspension was obtained by filtering the digested material through a 100 μ m mesh filter (Stokvis & Smith B.V., IJmuiden, The Netherlands) to remove tissue debris. The AT-MSC-containing cell suspension was centrifuged at 600 g, and the pellet was resuspended in culture medium, which was composed of Dulbecco's modified Eagle's medium (D-MEM, Gibco, Paisley, UK) supplemented with 500 μ g/ml streptomycin sulfate (Sigma), 600 μ g/ml penicillin (Sigma), 50 μ g/ml gentamycin (Gibco), 2.5 μ g/ml fungizone (Gibco), and 10% fetal bovine serum (FBS, Hyclone, Logan, UT, USA). The resuspended cells were incubated for 15 min at 37 °C with 160 mM NH₄Cl (Merck, Darmstadt, Germany) to destroy remaining erythrocytes. AT-MSCs were then washed three times with culture medium, and immediately used for experiments. Growth factors (BMP-2 and BMP-7) were added, and cells were post-incubated as described below. Freshly isolated AT-MSCs were characterized as previously described [39]. Briefly, the isolated AT-MSCs were analyzed by fluorescence-activated cell sorting (FACS) for the expression of the mesenchymal stem cell markers CD166/ALCAM and CD105/endoglin, using either 0.1 mg/ml of monoclonal antibodies phycoerythrin (PE)-conjugated anti-CD166/ALCAM (BD Biosciences, Pharmingen, San Diego, CA, USA), or 0.1 mg/ml anti-CD105/endoglin (Abcam, Cambridge, UK) [19]. The latter antibody was visualized with 0.1 mg/ml of a fluorescein isothiocyanine (FITC)-conjugated secondary antibody (Biotrend, Cologne, Germany). Expression of both CD 166/ALCAM and CD105/endoglin was assessed with a FACSScan (BD Biosciences, Pharmingen).

Approximately 35% of the freshly isolated AT-MSCs was positive for the mesenchymal stem cell marker CD105/endoglin, and approximately 30% of the cells was positive for the mesenchymal stem cell marker CD166/ALCAM [39].

BMP-2, BMP-7. Recombinant human BMP-2 (Peprotech EC LTD, London, UK) was reconstituted at a concentration of 100 μ g/ml in PBS containing 0.1% BSA, and recombinant human BMP-7 (kindly provided by Stryker Biotech, Hopkinton, MA, USA) was dissolved in α -MEM at a concentration of 40 μ g/ml, aliquoted, and stored at -80 °C until further use. AT-MSCs were incubated for 15 min in culture medium alone (control), or in culture medium containing 0.1 mg/ml ascorbic acid (Merck), 10 mM β -glycerophosphate (Sigma), with or without 10 ng/ml BMP-2 or 10 ng/ml BMP-7 to induce osteogenic and/or chondrogenic differentiation. Thereafter, cells were seeded in 6-well culture dishes (Corning Incorporated, Corning, NY, USA) at 18×10^4 cells per well, and cultured without growth factor for 4 or 14 days in culture medium alone, or in medium containing 0.1 mg/ml ascorbic acid and 10 mM β -glycerophosphate. After 4 and 14 days, AT-MSCs were harvested using 0.25% trypsin and 0.1% EDTA in PBS, counted, and dissolved in Trizol[®] for RNA-isolation, cDNA synthesis, and real-time PCR analysis as described below.

To study the effect of 4 days of treatment with 10 ng/ml BMP-2 or BMP-7, cells were cultured in culture medium containing 10 ng/ml BMP-2

or BMP-7 in the presence of 10 mM β -glycerophosphate and 0.1 mg/ml ascorbic acid, and harvested for RNA isolation as described above. To determine aggrecan gene expression, AT-MSCs were incubated in spermine-supplemented medium for 15 min before the addition of growth factors for another 15 min of incubation to enhance the growth factor effect.

Alkaline phosphatase and protein. To assess the osteoblastic phenotype of AT-MSCs after 15 min of growth factor treatment, alkaline phosphatase (ALP) activity was measured after 7 and 14 days of the post-incubation period. Cells were seeded at 3.6×10^4 cells per well in 24-well culture dishes (Greiner Bio-One, Kremsmuenster, Austria), cultured for 7 and 14 days as described above, and ALP activity and protein content were determined in the cell lysate. As substrate *p*-nitrophenyl phosphate (Merck) at pH 10.3 was used to determine ALP activity, according to the method described by Lowry [40]. The absorbance was read at 410 nm. ALP activity data were expressed as nanomole per microgram of protein in the cell layer. The amount of protein was determined using a BCA Protein Assay Reagent kit (Pierce, Rockford, IL, USA), and the absorbance was read at 570 nm with a microplate reader (BioRad Laboratories Inc., Hercules, CA, USA).

Analysis of gene expression. Total RNA was isolated according to the manufacturer's instructions (Invitrogen, Carlsbad, CA, USA). cDNA synthesis was performed using 0.5–1 μ g total RNA in a 20 μ l reaction mix consisting of 5 U Transcriptor Reverse Transcriptase (Roche Diagnostics, Mannheim, Germany), 0.08 A_{260} units random primers (Roche Diagnostics), 1 mM of each dNTP (Invitrogen), and 1 \times concentrated Transcriptor RT reaction buffer. Real-time PCRs were performed using the SYBR-Green reaction kit according to the manufacturer's instructions (Roche Diagnostics) in a LightCycler (Roche Diagnostics), and relative housekeeping gene expression (18S) and relative target gene expression (runx-2, OPN, biglycan) were determined. Aggrecan PCR products were subjected to electrophoresis on a 1.5% agarose gel containing 0.5 μ g/ml ethidium bromide. Primers (Invitrogen) used for real-time PCR are listed in Table 1. Values of target gene expression were normalized for 18S housekeeping gene expression.

Statistical analysis. Data were obtained from 3 to 7 separate experiments. For statistical analysis treatment-over-control-ratios (T/C-ratios) of real-time PCR data were calculated. Differences between groups were tested with the one-tailed *t* test for unpaired samples and differences of T/C-ratios were tested with a one-tailed *t* test for single group mean and compared to 1. Differences were considered significant if $p < 0.05$.

Results

Fifteen minutes of BMP-2 treatment as well as 15 min of BMP-7 treatment significantly increased the number of AT-MSCs 14 days after treatment compared to control cultures (BMP-2 treated: $64.9 \times 10^4 \pm 7.8 \times 10^4$ cells, BMP-7 treated: $64.4 \times 10^4 \pm 8.2 \times 10^4$ cells, control: 36.2×10^4

$\pm 5.7 \times 10^4$ cells; mean \pm SEM) in the presence of β -glycerophosphate and ascorbic acid (Fig. 1A). The number of AT-MSCs treated with BMP-2 and BMP-7 was similar. Fifteen minutes of BMP-2 and BMP-7 treatment increased the protein content of the cell cultures in the presence of β -glycerophosphate and ascorbic acid 14 days after growth factor treatment compared to control (BMP-2 treated: $31 \pm 8.1 \mu$ g/well, BMP-7 treated: $35.8 \pm 6.7 \mu$ g/well, control: $18.4 \pm 4.3 \mu$ g/well; mean \pm SEM) (Fig. 1B). The protein content of the cell cultures treated with BMP-2 and BMP-7 was similar. ALP activity was measured in AT-MSCs 7 and 14 days after 15 min of treatment with

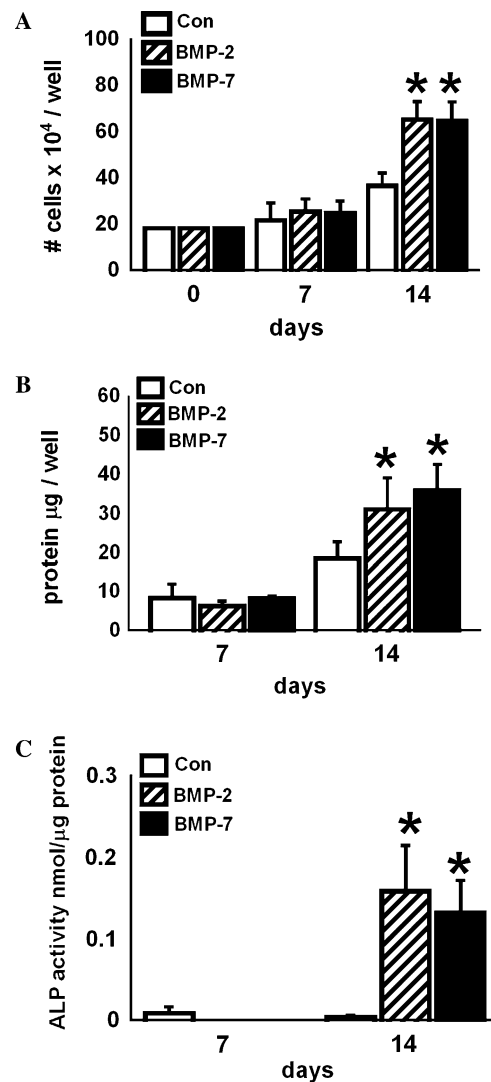


Fig. 1. Effect of 10 ng/ml BMP-2 and BMP-7 on the number of cells, protein content, and alkaline phosphatase activity by AT-MSCs. Freshly isolated AT-MSCs were treated for 15 min with 10 ng/ml BMP-2 or BMP-7 followed by a post-incubation period of 4, 7, or 14 days without growth factor. (A) BMP-2 and BMP-7 significantly increased the number of AT-MSCs at day 14. (B) BMP-7 increased protein content of AT-MSCs at day 14. (C) BMP-2 and BMP-7 up-regulated ALP activity by AT-MSCs at day 14. Values are means \pm SEM of 3–5 cultures. BMP, bone morphogenetic protein; AT-MSCs, adipose tissue-derived mesenchymal stem cells; ALP, alkaline phosphatase. * Significant effect of growth factor, $p < 0.05$.

BMP-2 or BMP-7 (Fig. 1C). Short incubation with BMP-2 or with BMP-7 significantly up-regulated ALP activity by 40- and 32-fold, respectively (BMP-2 treated: 0.16 ± 0.06 nmol/ μ g protein, BMP-7 treated: 0.13 ± 0.05 nmol/ μ g protein, control: 0.004 ± 0.003 nmol/ μ g protein; mean \pm SEM) in AT-MSCs (Fig. 1C).

The growth factors BMP-2 and BMP-7 modulated relative runx-2 gene expression (Fig. 2). Incubation of AT-MSCs for 15 min with 10 ng/ml BMP-2 significantly increased runx-2 gene expression by 1.8-fold, whereas the short treatment with 10 ng/ml BMP-7 did not affect relative runx-2 gene expression at day 4 of culture after growth factor treatment (Fig. 2A). Fourteen days after the short incubation with BMP-2, there was no effect on relative runx-2 gene expression, while 15 min of BMP-7-treatment significantly down-regulated relative runx-2 gene expression in AT-MSCs by 1.7-fold at day 14 (Fig. 2A). Four days of continuous treatment with either 10 ng/ml BMP-2 or BMP-7 did not affect runx-2 gene expression (Fig. 2B).

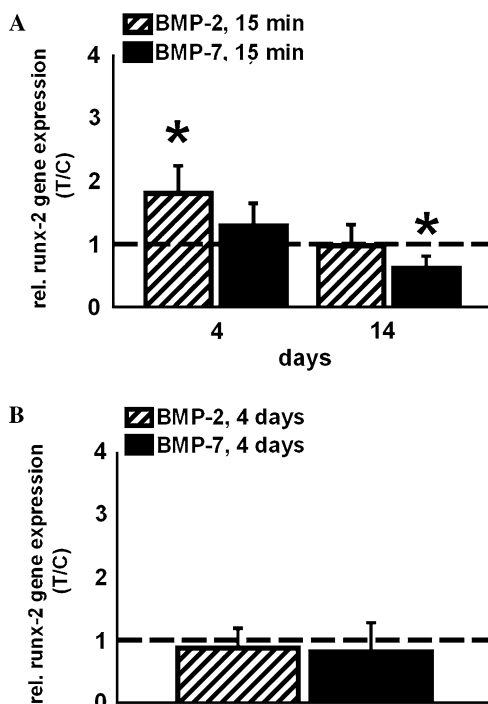


Fig. 2. Effect of 10 ng/ml BMP-2 and BMP-7 on relative runx-2 gene expression by AT-MSCs. Freshly isolated AT-MSCs were treated for 15 min with 10 ng/ml BMP-2 or BMP-7 followed by a post-incubation period of 4 or 14 days without growth factor, or AT-MSCs were treated with 10 ng/ml BMP-2 or BMP-7 for 4 days. (A) BMP-2 treatment for 15 min significantly increased relative runx-2 gene expression in AT-MSCs by 1.8-fold at day 4, while BMP-7 had no effect. BMP-7 treatment for 15 min decreased relative runx-2 gene expression in AT-MSCs by 1.7-fold at day 14, while BMP-2 had no effect. (B) Four days of treatment with BMP-2 or BMP-7 had no effect on runx-2 expression in AT-MSCs at day 4. Values are means \pm SEM of growth factor-treated-over-control ratios (T/C, $n = 3-8$), dashed line, T/C = 1 (no effect), real-time PCR data were normalized for 18S gene expression. Runx-2, runt-related transcription factor-2; BMP, bone morphogenetic protein; AT-MSCs, adipose tissue-derived mesenchymal stem cells. * Significant effect of growth factor, $p < 0.05$.

Incubation of AT-MSCs for 15 min with BMP-2 significantly increased relative OPN gene expression by 2.3-fold, whereas BMP-7 did not affect relative OPN gene expression 4 days after growth factor treatment (Fig. 3A). Short incubation with BMP-2 did not affect relative OPN gene expression after 14 days, while BMP-7 for 15 min significantly down-regulated OPN gene expression by 5.2-fold in AT-MSCs (Fig. 3A). Four days of continuous treatment with either 10 ng/ml BMP-2 or BMP-7 did not affect OPN gene expression (Fig. 3B).

BMP-2 and BMP-7 modulated relative biglycan gene expression by AT-MSCs (Fig. 4). Four days after the 15 min of BMP-2 or BMP-7 treatment, relative biglycan gene expression was significantly increased by 1.8- and 1.5-fold, respectively, in AT-MSCs (Fig. 4A). Fifteen minutes of incubation with BMP-2 did not affect biglycan gene expression after 14 days, while a short incubation with BMP-7 significantly decreased relative biglycan gene expression by 1.8-fold in AT-MSCs (Fig. 4A). Four days of continuous BMP-2 and BMP-7 treatment did not affect biglycan gene expression (Fig. 4B).

Aggrecan gene expression by AT-MSCs incubated with 10 ng/ml BMP-2 or BMP-7 for 15 min and under control conditions was below the detection limit of the Light Cycler, and was therefore visualized using agarose gel electrophoresis (Fig. 5). BMP-7 increased aggrecan and decreased OPN gene expression 14 days after 15 min of BMP-7 treatment in the presence of spermine, while BMP-2 had no effect (Fig. 5).

Discussion

In this study, we have compared the effect of the growth factors BMP-2 or BMP-7 on the differentiation of AT-MSCs along the osteogenic and/or chondrogenic pathway. We hypothesized that BMP-2 stimulates an osteogenic phenotype, while BMP-7 stimulates a more chondrogenic phenotype in AT-MSCs. Differences were found in gene expression of the early and essential transcription factor runx-2, the bone extracellular matrix protein OPN, the small leucine-rich extracellular matrix proteoglycan biglycan, and the cartilage extracellular matrix proteoglycan aggrecan in response to a short (15 min) BMP-2 or BMP-7 treatment. BMP-2 increased the expression of runx-2, OPN, as well as biglycan in AT-MSCs 4 days after 15 min of treatment, but not 14 days after. BMP-2 did not affect aggrecan gene expression. These findings indicate that a short incubation time of 15 min with BMP-2 is sufficient to stimulate osteogenic differentiation of AT-MSCs. Short treatment with BMP-7 stimulated biglycan gene expression, but did not affect runx-2 and OPN gene expression after 4 days. BMP-7 inhibited runx-2, OPN, and biglycan gene expression, and stimulated aggrecan gene expression 14 days after BMP-7 treatment, indicating that a short 15 min treatment with BMP-7 may stimulate a chondrogenic phenotype in AT-MSCs.

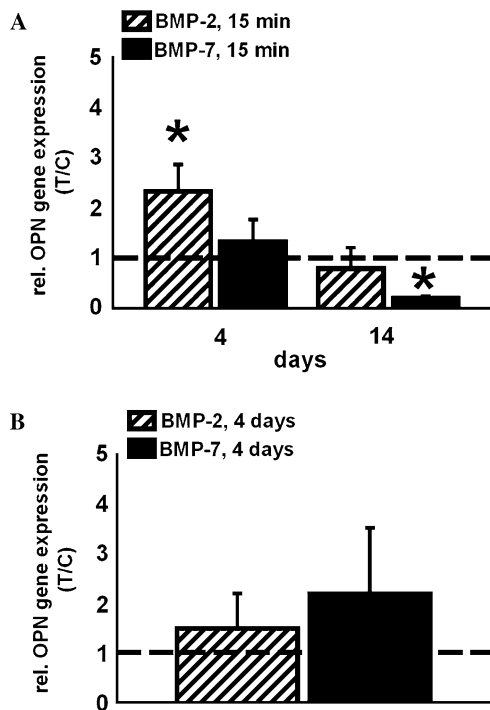


Fig. 3. Effect of 10 ng/ml BMP-2 and BMP-7 on relative osteopontin gene expression by AT-MSCs. Freshly isolated AT-MSCs were treated for 15 min with 10 ng/ml BMP-2 or BMP-7 followed by a post-incubation period of 4 or 14 days without growth factor, or AT-MSCs were treated with 10 ng/ml BMP-2 or BMP-7 for 4 days. (A) BMP-2 treatment for 15 min increased relative OPN gene expression in AT-MSCs by 2.3-fold at day 4, while BMP-7 had no significant effect. BMP-7 treatment for 15 min decreased relative OPN gene expression in AT-MSCs by 5.2-fold at day 14, while BMP-2 had no effect. (B) Four days of treatment with BMP-2 or BMP-7 had no effect on OPN gene expression in AT-MSCs at day 4. Values are means \pm SEM of growth factor-treated-over-control ratios (T/C, $n = 3-8$), dashed line, T/C = 1 (no effect), real-time PCR data were normalized for 18S gene expression. OPN, osteopontin; BMP, bone morphogenetic protein; AT-MSCs, adipose tissue-derived mesenchymal stem cells. * Significant effect of growth factor, $p < 0.05$.

Since runx-2 is an early and essential transcription factor during osteogenic differentiation, its gene expression by AT-MSCs in response to BMP-2 and BMP-7 was studied [27,30]. BMP-2 stimulated runx-2 gene expression, whereas BMP-7 had an inhibiting effect. Runx-2 can be stimulated by BMP-2 and BMP-7 in C2C12 mesenchymal precursor cells [28,41]. BMP-7 can increase runx-2 gene expression via an OSE2 element present in the gene's promoter, and BMP-2-stimulated runx-2 gene expression involves smad signaling pathways [29,42]. Runx-2 itself can interact with smad proteins to regulate its own gene expression and the expression of target genes, such as OPN, collagen 1 α I, and osteocalcin associated with the osteoblastic lineage [7,29,30]. The increase in OPN gene expression in AT-MSCs after BMP-2 treatment might therefore be due to a runx-2-dependent stimulation of OPN gene expression and a smad-transmitted repressor release mechanism [41,43]. The inhibition of runx-2 gene expression in AT-MSCs by BMP-7 might be due to the low concentration of 10 ng/ml used in this study, and a

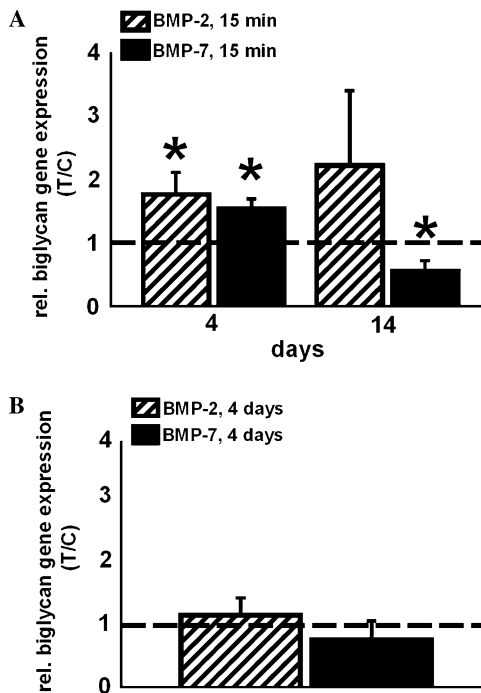


Fig. 4. Effect of 10 ng/ml BMP-2 and BMP-7 on relative biglycan gene expression by AT-MSCs. Freshly isolated AT-MSCs were treated for 15 min with 10 ng/ml BMP-2 or BMP-7 followed by a post-incubation period of 4 or 14 days without growth factor, or AT-MSCs were treated with 10 ng/ml BMP-2 or BMP-7 for 4 days. (A) BMP-2 and BMP-7 treatment for 15 min increased relative biglycan gene expression in AT-MSCs by 1.8- and 1.5-fold, respectively, at day 4. BMP-7 treatment for 15 min decreased relative biglycan gene expression in AT-MSCs by 1.8-fold at day 14, while BMP-2 had no effect. (B) Four days of treatment with BMP-2 or BMP-7 had no effect on biglycan gene expression at day 4. Values are means \pm SEM of growth factor-treated-over-control ratios (T/C, $n = 3-5$), dashed line, T/C = 1 (no effect), real-time PCR data were normalized for 18S gene expression. BMP, bone morphogenetic protein; AT-MSCs, adipose tissue-derived mesenchymal stem cells. * Significant effect of growth factor, $p < 0.05$.

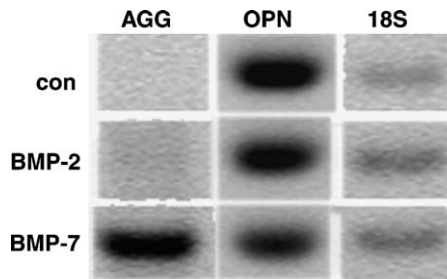


Fig. 5. Effect of 10 ng/ml BMP-2 and BMP-7 on aggrecan and OPN gene expression by AT-MSCs. Cells were treated with and without 10 μ M spermine for 15 min, followed by 15 min spermine with and without 10 ng/ml BMP-2 and BMP-7, real-time PCR experiments were performed after a post-incubation period of 14 days. BMP-7 increased aggrecan gene expression, whereas it decreased OPN gene expression in AT-MSCs in combination with spermine, whereas BMP-2 does not. BMP, bone morphogenetic protein; AGG, aggrecan; OPN, osteopontin; AT-MSCs, adipose tissue-derived mesenchymal stem cells.

short incubation period of only 15 min, since it has been shown that 200 ng/ml BMP-7 induces runx-2 gene expression in C2C12 precursor cells [28]. However, BMP-7 at these high doses also induces markers of chondrogenic differentiation, e.g., sox-9, indicating that BMP-7 is involved in chondrogenic differentiation [28]. This is in line with ex vivo gene therapy studies, where BMP-7 induces endochondral-like bone formation in vivo in rats [28,44].

The OPN promoter has a runx-2 binding site and can therefore be induced by directly binding of runx-2 protein [27,45]. BMP-7 is inhibiting runx-2 gene expression in AT-MSCs, indicating that this stimulator of OPN gene expression is lacking, subsequently leading to a decreased OPN gene expression in these cells. The inhibition of Runx-2 and OPN also suggests that there could be a promotion of a chondrogenic phenotype in AT-MSCs by BMP-7. Furthermore, BMP-7 treatment of AT-MSCs increased the gene expression of the important cartilage extracellular matrix proteoglycan aggrecan, but BMP-2 had no effect. BMP-7 up-regulated the small leucine-rich proteoglycan biglycan that is present in cartilage extracellular matrix. Rutherford et al. [46] have shown that fibroblasts secreting BMP-7 in a 3-dimensional environment acquire some chondroblastic traits, e.g., accumulation of cartilage proteoglycan. AT-MSCs treated for 15 min with BMP-7 placed in an environment that supports chondrogenic differentiation could therefore be beneficial for cartilage tissue engineering.

In this study, incubation with recombinant BMP-2 or BMP-7 at a low concentration (10 ng/ml) for a short period of only fifteen minutes affects osteogenic or chondrogenic differentiation of AT-MSCs in vitro. Current clinical studies, i.e., on spinal fusion, using recombinant BMPs on carrier materials in humans required high doses of the growth factors to be effective (0.9–2 mg/ml carrier), which raises concerns for bone overgrowth and subsequent potential neural compression or potential restenosis at the site of application [26,47,48]. An ex vivo incubation with growth factors at a million-fold lower concentration for only 15 min could easily be fitted within such a spinal fusion surgery, where autologous stem cells could be isolated, triggered with growth factors, and subsequently used for bone or cartilage regeneration in the patient.

For tissue engineering the delivery method of BMPs is of utmost importance [26]. To date, the use of recombinant human BMP-2 and BMP-7 on osteoinductive carriers, or ex vivo adenoviral transfection of stem cells, bone marrow-derived as well as adipose tissue-derived stem cells, with BMPs are under evaluation [16,17,26,47,48]. However, there are certain safety concerns surrounding adenoviral vectors, e.g., the unpredictable kinetics of protein production in vivo, or the unknown fate of the BMP-transfected cells [16].

We conclude that BMP-2 treatment for only 15 min is capable of inducing osteogenic differentiation in AT-MSCs, whereas BMP-7 may stimulate AT-MSCs to differentiate towards a more chondrogenic phenotype. Therefore, AT-

MSCs triggered with BMP-2 or BMP-7 at a concentration as low as 10 ng/ml for only 15 min may provide a feasible tool for both bone and cartilage tissue engineering and may be a clinically interesting alternative for gene therapy approaches.

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