

THE EFFECTS OF rhBMP-2 ON HUMAN OSTEOSARCOMA CELLS AND HUMAN GINGIVAL FIBROBLASTS *IN VITRO*

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Certain cells of the periodontium are necessary for the regeneration of tissues that are destroyed as a result of periodontal disease. There has been debate regarding which cells are the primary participants in periodontal regeneration. It is a well-known fact that osteoblasts are essential in new bone formation, but controversy surrounds the role that gingival fibroblasts may play in the regeneration of the hard tissues of the periodontium. If gingival fibroblasts could contribute to the regeneration of these tissues, they might provide an additional source of progenitor cells. The bone morphogenetic proteins are potent stimulators of cell differentiation and have been shown to induce new bone formation in many experimental models. This project investigated the ability of recombinant human bone morphogenetic protein-2 (rhBMP-2) to (1) enhance the production of markers of osteoblastlike cells (osteocalcin and mineralization in culture) in human osteosarcoma cells (MG63) and to (2) induce the expression of an osteoblast phenotype in cultured human gingival fibroblasts (HGFs). MG63 cells and pooled HGFs were exposed to varying concentrations of rhBMP-2 for 24, 48, and 72 hours after 9 days in culture, and osteocalcin levels were measured by enzyme immunosorbent assay in the cell supernatants. In addition, the cells were exposed to rhBMP-2 for 72 hours after 18 days in culture, and mineralization was determined by the Von Kossa stain. The rhBMP-2 had an inhibitory effect on both osteocalcin production and mineralization ($p < 0.05$) in MG63 cells compared with untreated controls. In addition, increasing doses of rhBMP-2 inhibited both osteocalcin and mineralization in HGF cells. These results suggest that HGFs can express an osteoblastic phenotype when exposed to rhBMP-2; however, rhBMP-2 has inhibitory effects at higher rhBMP-2 doses in both cell types and may, in fact, be inhibitory to MG63 cells.

INTRODUCTION

The regeneration of the periodontium that has been lost because of periodontal disease is a central goal of periodontal treatment. This goal requires deposition of new bone, formation of new cementum, and insertion and functional orientation of new connective fibers into both the new alveolar bone and cementum. Several methods have been utilized in an attempt to foster regeneration and, although these procedures work to some extent, they act only as a scaffold for vascular ingrowth and subsequent bone growth.¹

Because new bone formation is critical to the process of regeneration, the application of growth factors, morphogenetic proteins, or both has been necessary to successfully achieve regeneration of the periodontal attachment apparatus. Although the periodontal ligament and exposed alveolar bone contain mesenchymal cells that have the potential to differentiate into osteoblasts, cementoblasts, or fibroblasts when stimulated by growth factors and bone morphogenetic proteins (BMPs),² there is controversy regarding the presence of various human gingival fibroblast (HGF) phenotypes having the ability to express the osteoblastic phenotype. Cells isolated from bone synthesize cementumlike and bonelike tissue *in vitro*.^{3,4} In addition, periodontal ligament cells express the osteoblastic phenotype.⁴ Stimulation with BMP-2 increased alkaline phosphatase production in these cells *in vivo*.⁴ These findings suggest that similar effects may be found with the various cell types within the periodontium after exposure to factors that affect proliferation, differentiation, or both. To support this suggestion, it has been shown that certain phenotypes of HGFs have the ability to synthesize osteocalcin after stimulation with 1,25(OH)₂D₃ alone.⁵ It has been shown that BMP-2 is mitogenic and enhances the expression of the differentiated phenotypic

markers of both chondroblasts and osteoblasts.

BMPs, particularly BMP-2, induce the formation of both cartilage and bone *in vivo* and *in vitro*. Because of this action, BMP-2 may have tremendous therapeutic potential in regenerative procedures. The BMPs have received widespread attention because of their unique properties, particularly as they relate to bone induction. Marshall Urist is acknowledged as the pioneer in the isolation of BMP⁶ with his classic paper in 1965 that first described bone formation by autoinduction. The BMPs have major effects on the direction of cell differentiation, as well as acting as positional signals,⁷⁻⁹ and therefore are categorized as morphogens. Currently, 15 BMPs have been identified, and BMPs 2 through 8 are members of the TGF- β superfamily.¹⁰⁻¹² The BMPs, such as TGF- β , are expressed throughout embryonic development and into adulthood but are the only known molecules capable of forming bone at an ectopic site. In addition, BMPs 2 through 8 are also able to induce cartilage formation.^{13,14} It appears that the BMPs are responsible for the initial step in the differentiation of mesenchymal progenitor cells into chondrocytes and that bone-derived growth factors and other processes are responsible for promoting and maintaining the subsequent osteogenic cascade.^{15,16} In experimental models, it has been shown that increasing the amount of BMP-2 will affect the timing of bone deposition.¹⁷ Histologic studies have demonstrated new attachment formation in humans after treatment with decalcified freeze-dried bone.^{18,19} In addition, animal studies have demonstrated that recombinant human BMP-2 (rhBMP-2) has been used successfully to stimulate periodontal regeneration.²⁰ However, it remains unclear whether there is a particular sequence of exposure to the BMPs and whether there is a specific sequence or temporal pattern of exposure that is critical to wound healing and periodontal regeneration.

Osteoblastic and fibroblastic pheno-

types differ in their ability to synthesize osteocalcin and mineralize *in vitro*. Osteoblasts produce osteocalcin and mineralize in culture, whereas HGFs ordinarily do not. These phenotypic characteristics can be used to differentiate between osteoblastlike cells and gingival fibroblasts. Therefore, the purpose of this study was to evaluate two osteoblastic cell markers in order to determine the effects of rhBMP-2 on the production of these markers in a human osteosarcoma cell line (MG63 cells) and to determine whether rhBMP-2 could induce the differentiation of HGFs into an osteoblastic phenotype. In addition, the temporal sequence of rhBMP-2 exposure to these cells was evaluated.

MATERIALS AND METHODS

Cell culture

Human Gingival Fibroblasts

These cells were explanted from gingival tissue obtained from surgical sites involving healthy gingival tissue (4 patients, average age 51 years; 3 men and 1 woman). The cells were placed in 35 \times 10-mm tissue culture dishes, allowed to attach, and incubated with growth medium consisting of Eagle's minimum essential medium (EMEM; GIBCO BRL, Great Neck, NY) supplemented with 10% fetal bovine serum (FBS; Atlanta Biologicals, Norcross, Ga) and antibiotics (penicillin G, potassium, 100 units/mL; streptomycin sulfate, 100 μ g/mL). The cells were fed every 3 days until they reached confluency. Cultures were then trypsinized with 0.05% trypsin-0.02% EDTA and transferred to 25-cm² flasks (Corning Costar Corp, Cambridge, Mass). Once the cells reached confluency in these flasks, they were trypsinized, as above, and transferred to 75-cm² flasks. After reaching confluency, the cells were trypsinized, prepared for cryostorage, and defined as passage 3. All experimental cultures were established from pooled populations and utilized at no greater than passage 5.

Human Osteosarcoma Cells

MG63 cells were obtained through the American Type Culture Collection (Rockville, Md) and are derived from an osteosarcoma from a 14-year-old boy. This cell line has been characterized for alkaline phosphatase, osteocalcin, TGF- β , and osteogenic specific protein-1. The cell line was defined as passage 86 and contained approximately 3.7×10^6 cells per ampule. These cells were handled similarly to the HGF cells, except that they were initially seeded onto 75-cm² flasks. Once they were detached from the 75-cm² flasks, they were defined as passage 89. All experimental cultures were established from pooled cells and utilized at no greater than passage 91.

rhBMP-2 solutions

The rhBMP-2 was kindly donated by Dr Wozney of the Genetics Institute (Cambridge, Mass). Experimental rhBMP-2 solutions were prepared according to the Genetics Institute's recommendations in 1% FBS EMEM supplemented with 1,25 (OH)₂ vitamin D₃ at a concentration of 10^{-8} M (AGROS Organics; Fisher Scientific, Pittsburgh, Pa). A log scale of experimental solutions ranging from 0.1 to 100 ng/mL was used in all experiments.

Cell treatment for osteocalcin

Pooled MG63 cells and pooled HGF cells were seeded, in duplicate, onto six-well plates (Corning Costar Corp, Cambridge, Ma) at a density of 2×10^4 per well and cultured for 8 days in 3.0-mL culture medium. The cells were re-fed with fresh culture medium every third day. On the eighth day, the culture medium was removed, and the cells were rinsed three times with sterile phosphate-buffered saline (PBS) and then placed in 3.0 mL 1% FBS EMEM supplemented with 1,25 (OH)₂ vitamin D₃ for 24 hours. On the ninth day in culture, the cells were exposed to varying concentrations of rhBMP-2 (0.1, 1.0, 10, 50, or 100 ng/mL rhBMP-

2; experimental) in a 1% FBS EMEM supplemented with 1,25 (OH)₂ vitamin D₃ or, in the control, a 1% FBS EMEM supplemented with 1,25 (OH)₂ vitamin D₃ alone for 24, 48, and 72 hours. At each time point, an aliquot of the supernatant was removed and stored frozen until ready for assay.

Cell treatment for mineralization

Pooled MG63 cells and pooled HGF cells were seeded, in duplicate, onto four-well slides (Nunc Inc, Naperville, Ill) at a density of 1×10^4 cells per well and cultured for 18 days in 1.0 mL culture medium. The cells were re-fed with fresh medium every third day. On the 18th day, the culture medium was removed, and the cells were rinsed three times with sterile PBS and then placed in 1.0 mL 1% FBS EMEM supplemented with 1,25 (OH)₂ vitamin D₃ for 24 hours. On the 19th day in culture, the cells were rinsed three times with sterile PBS and exposed to the varying concentrations of rhBMP-2 (experimental) or to 1% FBS EMEM supplemented with 1,25 (OH)₂ vitamin D₃ alone (control) for 72 hours. After 72 hours, the cells were rinsed three times with sterile PBS and immediately fixed in neutral buffered formalin.

Measurement of osteocalcin

The amount of osteocalcin secreted into the culture supernatant was measured with an enzyme immunosorbent assay kit for intact human osteocalcin (Biomedical Technologies Inc, Stoughton, Mass). The osteocalcin assay was performed on duplicate samples from duplicate cultures.

Measurement of mineralization

The presence of mineralized extracellular matrix was determined by von Kossa staining of quadruplicate wells. Mineral deposition was quantitated with a computerized image analysis package (Scion Image, Frederick, Md).

Data analysis

The osteocalcin concentration for each sample was extrapolated from stan-

dard curves. By cells seeding cells at a density of 2×10^4 per well, cultures at confluency had similar cell numbers per well at time of sampling. The osteocalcin data were normalized per culture volume. Results were reported as ng/mL osteocalcin. Mineralization was quantitated by averaging the number of pixels per density slice via the Scion Image program. Data from treated (experimental) cells versus non-treated (control) cells were compared by analysis of variance (ANOVA) and a *post hoc* Tukey analysis. Differences between treated and control MG63 cells and HGFs for both osteocalcin production and mineralization were compared by ANOVA, and a statistically significant difference was established at $p < 0.05$.

RESULTS

Osteocalcin production

Experiments were designed to investigate the effects of exposing MG63 cells and HGF cells to varying concentrations of rhBMP-2 over three predetermined time points *in vitro*. Nonstimulated, control MG63 cells synthesized osteocalcin *de novo* by day 9 in culture, with a peak value observed at 72 hours (Fig 1). This value was statistically significantly greater ($p < 0.05$) compared with the 24- or 48-hour values for control MG63 cells (Fig 1) and to all values observed in rhBMP-2-exposed cells. Exposure to rhBMP-2 resulted in a steady decline in osteocalcin production that was statistically significant ($p < 0.05$) over all time points, and values never approached those observed in control cells (Fig 1). The decline in osteocalcin production observed in the experimental cells was dose dependent, and duration of exposure did have a significant effect. No detectable levels of osteocalcin were noted when these cells were stimulated with the highest doses of rhBMP-2 (50–100 ng/mL), and this was uniform over the three experimental time points (Fig 1).

The nonexposed, control HGF cells synthesized very small amounts of os-

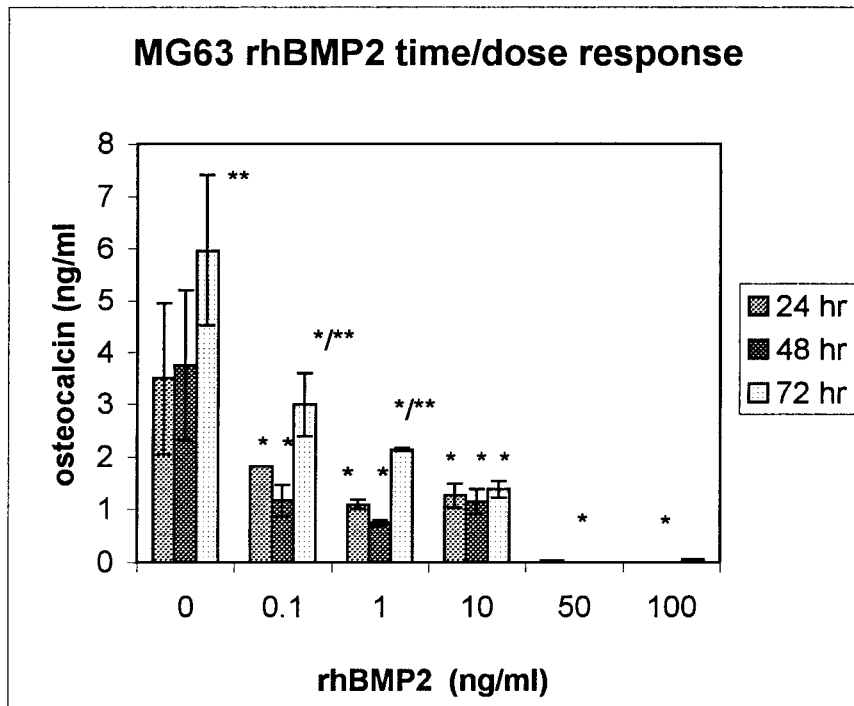


FIGURE 1. MG63 recombinant human bone morphogenetic protein-2 (rhBMP-2) time/dose response. Time-dependent effects of rhBMP-2 on osteocalcin production in MG63 cells. Cells were treated with various concentrations of rhBMP-2 over three experimental time points (24, 48, and 72 hours). Values are the means \pm SD of two separate experiments, each conducted in duplicate. * $p < 0.05$ versus untreated control; ** $p < 0.05$, significant difference among time points at each dose.

teocalcin *de novo*, with a peak value observed at 48 hours (Fig 2). The rhBMP-2-exposed cells synthesized statistically significantly ($p < 0.05$) greater, albeit small, levels of osteocalcin compared with the nonstimulated cells at all but the highest rhBMP-2 doses (Fig 2). The greatest osteocalcin level was noted at 24 hours, when HGFs were stimulated with 0.1 ng/mL rhBMP-2 (Fig 2). This value was statistically significantly ($p < 0.05$) greater than that seen with any other dose of rhBMP-2 and was approximately eightfold when compared with the control values (Fig 2). The duration of exposure had some statistically significant effects, but this was not seen uniformly (Fig 2). As with the MG63 cells, stimulation with the highest doses of rhBMP-2 resulted in negligible, if any, osteocalcin production (Fig 2).

Comparison of the two cell types revealed that the nonexposed control MG63 cells synthesized statistically sig-

nificantly ($p < 0.05$) greater amounts of osteocalcin compared with their counterpart control HGF cells at all time points (Fig 3). Although there were differences between cells among the various rhBMP-2 doses and among the three time points, no clear-cut pattern was observed. Comparison of the highest doses of rhBMP-2 at all time points revealed similar findings in both cell types (Fig 3).

Mineralization in culture

Mineralization was statistically significantly ($p < 0.05$) greater in the nonexposed control MG63 cells and those cells exposed to 0.1 ng/mL rhBMP-2, although these two were not significantly different from each other. Mineralization declined significantly ($p < 0.05$) with increasing rhBMP-2 dose (Fig 4). However, mineralization was significantly ($p < 0.05$) enhanced in the HGF cells, with the greatest degree of mineralization seen at the lowest rh-

BMP-2 dose. As with the MG63 cells, mineralization declined with increasing rhBMP-2 dose (Fig 4).

DISCUSSION

The BMPs, particularly BMP-2, have been shown to act as potent stimulators of osteoblast differentiation.²¹⁻²³ They also have been shown to induce more pluripotent stem cells to differentiate into various progenitor cells associated with bone formation²⁴⁻²⁶ and other periodontal functions.^{20,27}

To evaluate the potential use of the BMPs in periodontal regeneration, we investigated the *in vitro* effects of rhBMP-2 on osteocalcin and mineralization, which are phenotypic markers of osteoblastic differentiation in pooled HGF and MG63 cells. MG63 cells were chosen because they represent a more mature osteoblast cell line and synthesize these markers of the osteoblast phenotype. HGFs were included to test the hypothesis that rhBMP-2 could stimulate an osteoblastlike phenotypic expression in a nonosteoblast cell. HGFs do not exhibit the osteoblastic phenotype; however, Carnes *et al*⁵ demonstrated that cells obtained from human attached gingiva could express markers of the osteoblast phenotype when stimulated with 1,25(OH)₂D₃. These markers included alkaline phosphatase activity, osteocalcin production, and mineralization in culture.

The use of a cell line has limitations because transformed cells are dissimilar from primary cells in several respects. These cell lines typically contain cells with an aneuploid chromosomal number. They are prone to chromosomal aberrations and usually contain a range of genotypes. They have a capacity for genetic variation not usually seen in many primary cell lines. However, they are normally well characterized and reproducible phenotypically, are available in large quantities, and are easy to grow. Cell lines generally have reduced growth requirements and are less stringent in their requirements for attachment and spreading. These qualities are vital for

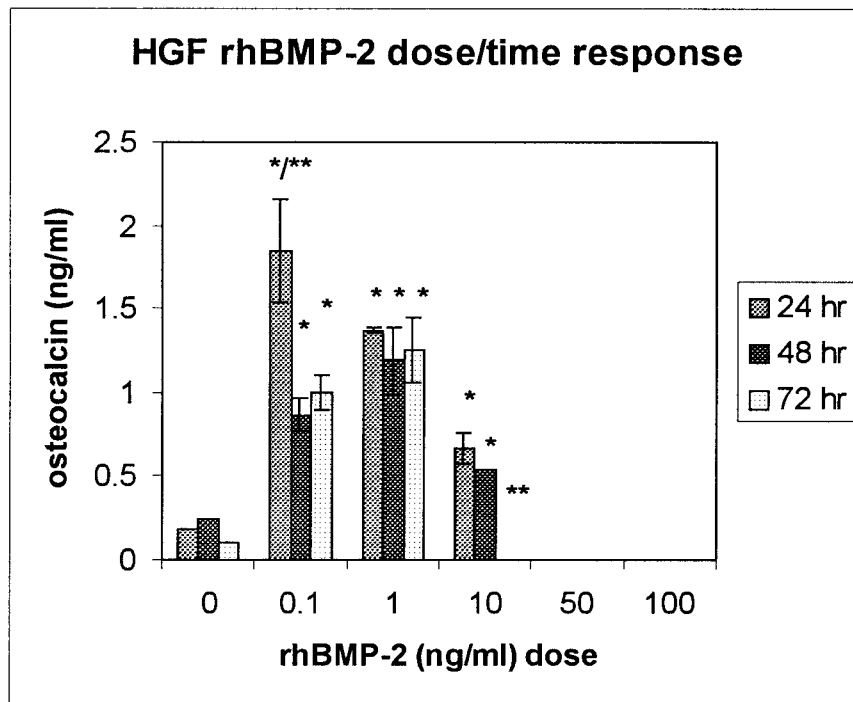


FIGURE 2. Human gingival fibroblast (HGF) recombinant human bone morphogenetic protein-2 (rhBMP-2) time/dose response. Time-dependent effects of rhBMP-2 on osteocalcin production in HGFs. Cells were treated with various concentrations of rhBMP-2 over three experimental time points (24, 48, and 72 hours). Values are the means \pm SD of two separate experiments, each conducted in duplicate. * $p < 0.05$ versus untreated control; ** $p < 0.05$ significant difference among time points at each dose.

proliferation. Thus, conclusions and interpretations based on findings from transformed cells need to be drawn with caution. However, primary cell cultures of human origin are diploid in chromosomal number and are more representative of the human organism. These primary cells are initially characterized by phenotypic drift, which continues until senescence ensues. One of the drawbacks associated with primary cells is that they are usually more heterogeneous than transformed cell lines and thus contain a variety of cell types in various stages of differentiation. Another drawback is difficulty in maintaining sufficient quantity and their increased growth requirements.

We hypothesized that rhBMP-2 would stimulate osteoblastic differentiation, as evidenced by increased osteocalcin production and mineralization in MG63 cells when compared with nonstimulated cells. We found that rhBMP-2 suppressed osteocalcin

production at all doses in these cells, with complete inhibition at 50 and 100 ng/mL rhBMP-2 over all experimental time periods.

In agreement with our findings, Takiguchi *et al*²³ found that rhBMP-2 inhibited 1,25(OH)₂ D₃-induced osteocalcin production in human bone cells in a dose-dependent manner at concentrations over 5 ng/mL, whereas Kobayashi *et al*¹² reported similar findings in human periodontal ligament cells. However, Lecande *et al*²⁸ reported that rhBMP-2 (100 ng/mL for 7 days) increased osteocalcin expression in both human bone marrow stromal cells and human osteoblasts. The bone marrow stromal cells produced approximately twice the amount of osteocalcin as human bone cells. Several investigators have shown similar results when comparing a less differentiated population with a more differentiated one.^{21,23}

In our study, osteocalcin levels were significantly greater in cells treated for

72 hours when compared with those treated for either 24 or 48 hours at the lower doses of rhBMP-2 (0.1 and 1.0 ng/mL). One explanation for this increase over 72 hours could be that there were cells still responsive to the differentiating effects of rhBMP-2, with a resultant increase in osteocalcin production. However, this explanation is confounded by the fact that nonstimulated cells always had higher levels of osteocalcin. Thus, it could be argued that rhBMP-2 had an inhibitory effect on osteocalcin production. This is supported by the finding that maximum levels of BMP-2, BMP-4, and BMP-6 parallel the formation of mineralized bone nodules²⁹ and that osteocalcin gene expression was downregulated in nearly mineralized cultures.³⁰ This is also supported by Wada *et al*²⁹ in their study that identified three different BMP receptors that were preferentially expressed in osteoblasts localized within bone nodules. BMP-2 had the capability of binding to all three of them. Another possible explanation for the inhibiting effects of rhBMP-2 could be that rhBMP-2 receptors were becoming saturated over time, thereby rendering the cells less responsive to the effects of rhBMP-2.

It has been shown that osteoblasts synthesize and secrete a variety of proteinases that are capable of participating in proteolysis and extracellular matrix remodeling.³⁰ It is not unwarranted to speculate that the overall decline in osteocalcin levels in rhBMP-2 cells may be caused by some interaction between rhBMP-2 and these proteinases (*ie*, upregulation of proteinase gene expression). In this study, we evaluated only intact osteocalcin; thus, fragments resulting from proteolysis were not measured. It would be interesting to measure both intact and fragmented osteocalcin in the cell supernatants to see if a relationship exists between the two.

We also hypothesized that rhBMP-2 would induce expression of the osteoblastic phenotype in HGFs. As stated previously, HGFs have been shown to

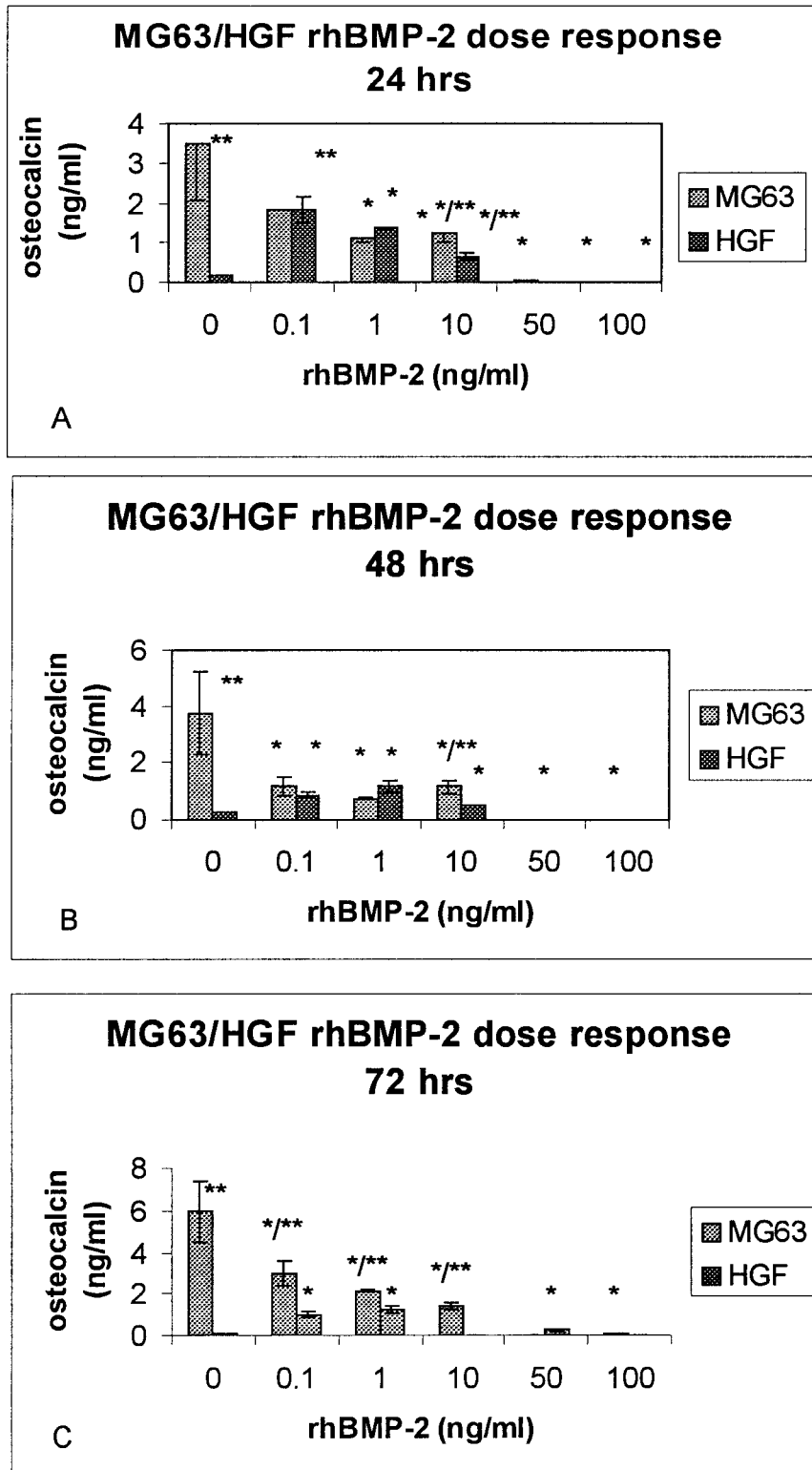


FIGURE 3. MG63/human gingival fibroblast (HGF) recombinant human bone morphogenetic protein-2 (rhBMP-2) dose response at 24, 48, and 72 hrs. Comparison of osteocalcin production between MG63 cells and HGFs after (A) 24, (B) 48, and (C) 72 hours of exposure to various concentrations of rhBMP-2. Values are the means \pm SD of two separate experiments, each conducted in duplicate. * $p < 0.05$, treatment versus untreated control; ** $p < 0.05$, MG63 versus HGFs.

express phenotypic markers of the osteoblast in response to vitamin D₃ stimulation.⁵

Our findings support the concept that HGFs can express phenotypic characteristics of osteoblasts. In this study, growth media and rhBMP-2 dosing media contained 10⁻⁸ M vitamin D₃. One could argue that the rhBMP-2 had no effect and that the results observed were caused by the action of the vitamin D₃ in the growth or dosing media. Although no studies have looked at the effects of rhBMP-2 on HGFs, it is not unreasonable to expect some response by these cells in light of the response of Carnes *et al*⁵ to vitamin D₃ stimulation of HGFs. In an earlier study, we were unable to induce osteocalcin production in these cells with vitamin D₃. Therefore, we feel that it is valid to conclude that the results were caused by the effects of rhBMP-2.

The HGFs treated with rhBMP-2 demonstrated significantly greater amounts of osteocalcin compared with the untreated controls, although there were low, basal levels of osteocalcin in nonstimulated control cells. This increase was dose and time dependent. There was an inverse relationship between osteocalcin concentration and dosing time. There was a direct, positive relationship between rhBMP-2 dose and osteocalcin concentration at the lower doses; however, there was almost complete inhibition of osteocalcin at doses greater than 50 ng/mL rhBMP-2. Clearly, this cell culture contained cells that were capable of synthesizing osteocalcin *de novo*. This suggests that the culture was (1) populated with cells that were osteoblastlike, (2) contained cells that, although not genotypically similar to osteoblasts, were capable of expressing the osteoblastic phenotype, or (3) a combination of both. It is most likely that this HGF pooled cell line contained immature mesenchymal cells that differentiated along the osteoblast phenotype in culture. Further support for this conclusion was advanced by Melcher *et al*³ in a study suggesting that it was not like-

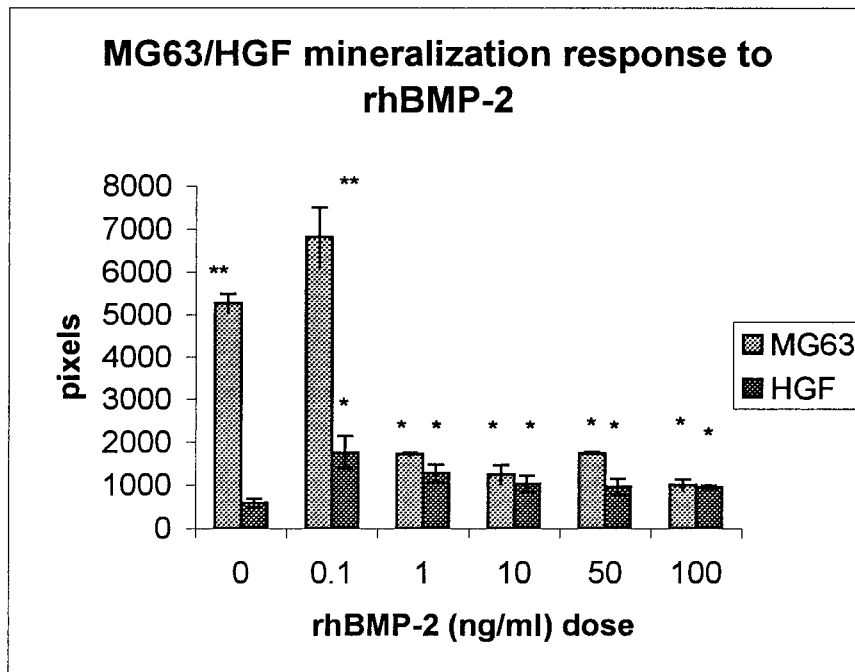


FIGURE 4. MG63/human gingival fibroblast (HGF) mineralization response to recombinant human bone morphogenetic protein-2 (rhBMP-2). Comparison of mineralization between MG63 cells and HGFs after 72 hours of exposure to various concentrations of rhBMP-2. Values are the means \pm SD of two separate experiments, each conducted in duplicate. * p < 0.05, treatment versus untreated control; ** p < 0.05, MG63 versus HGFs.

ly to find mature osteoblastic cells in the gingiva. The fact that these cells responded positively to rhBMP-2 stimulation with an increase in osteocalcin production adds credence to support the above conclusion. The temporal decline in osteocalcin production may be explained by the fact that over time, these mesenchymal cells are becoming more differentiated and are less capable of synthesizing osteocalcin or that rhBMP-2 downregulates osteocalcin production. Perhaps this question could be answered if osteocalcin levels were measured at later time intervals. Increasing concentrations of rhBMP-2 significantly inhibited osteocalcin levels. This might also be related to downregulation of osteocalcin production beyond a maximal dose that may be receptor related (*ie*, the receptors may be saturated at a certain concentration of rhBMP-2). One might argue that rhBMP-2, at a certain dose, is toxic to these cells; however, mineralization evaluation in these same cells did not bear out this notion.

Mineralization was evaluated indirectly by staining cell cultures with the Von Kossa method after 21 days in culture. The Von Kossa stain detects calcium deposits, which can then be semiquantitated by using densitometry. Hence, mineralization, *per se*, is not precisely measured but inferred from the amount of calcium deposition. Therefore, references to mineralization pertain to calcium deposition by the cells used in this investigation. Experimental cells were dosed for 72 hours with varying concentrations of rhBMP-2 to evaluate the effects rhBMP-2 had on mineralization. In this study, we found that rhBMP-2-exposed MG63 cells did not result in significantly greater amounts of mineralization when compared with controls. In fact, doses greater than 0.1 ng/mL appeared to inhibit mineral formation in culture when compared with the nonstimulated controls and low-dose rhBMP-2 cells. Interestingly, we found similar results when evaluating the effects of rhBMP-2 on osteocalcin production.

Within the limits of this study, it is hard to determine the exact relationship between mineralization and rhBMP-2 stimulation. It may well be that it is directly related to the suppression in osteocalcin production, but because of the timing of osteocalcin determination (days 10 through 12) and mineralization (day 21), this cannot be stated with certainty. It may be another mechanism entirely or some combination of mechanisms. It has been demonstrated that osteoblasts synthesize BMPs and that BMP-2 positive cells were located preferentially in mineralized nodules in a rat calvarial cell culture model.²⁹ It could be that the endogenous BMPs act in concert with exogenous rhBMP-2 to exert a feedback mechanism that results in a dedifferentiation of osteoblasts. This dedifferentiation could result in a decrease in the phenotypic expression of the more mature osteoblast (*ie*, a decrease in mineralization).

As with osteocalcin production, the HGF cells did exhibit a small amount of mineralization in the absence of rhBMP-2. RhBMP-2 stimulation significantly increased mineralization at all doses compared with the nonstimulated controls. Corresponding to the osteocalcin results, a decline in mineralization was noted over all doses of rhBMP-2 greater than 0.1 ng/mL, and it was dose dependent. The HGF and MG63 cells behaved similarly in their mineralization response to rhBMP-2. Therefore, it may be that the same mechanisms operating in the MG63 cells are at work in these gingival cells.

In summary, the results indicated that the pooled MG63 cells and the pooled HGF cells differed, at the lower doses of rhBMP-2, in their response to rhBMP-2 exposure. In MG63 cells, rhBMP-2 appeared to have an inhibitory effect at all doses and time points both on osteocalcin production and mineralization, as determined by Von Kossa staining, whereas osteocalcin production and mineralization appeared to be enhanced in the HGF cells. However, both cell types re-

sponded similarly to the highest doses of rhBMP-2 in that both mineralization and osteocalcin production were inhibited in both cell types.

These results suggest that the HGF cells are capable of expressing phenotypic markers of an osteoblastlike cell and hence may contribute to the mechanisms essential to periodontal regeneration. However, the results also suggest that exposure to rhBMP-2 may, in fact, be inhibitory to some of these same mechanisms.

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