Dexamethasone Enhances In Vitro Vascular Calcification by Promoting Osteoblastic Differentiation of Vascular Smooth Muscle Cells

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Abstract—Vascular calcification is often associated with atherosclerotic lesions. Moreover, the process of atherosclerotic calcification has several features similar to the mineralization of skeletal tissue. Therefore, we hypothesized that vascular smooth muscle cells might acquire osteoblastic characteristics during the development of atherosclerotic lesions. In the present study, we investigated the effect of dexamethasone (Dex), which is well known to be a potent stimulator of osteoblastic differentiation in vitro, on vascular calcification by using an in vitro calcification model. We demonstrated that Dex increased bovine vascular smooth muscle cell (BVSMC) calcification in a dose- and time-dependent manner. Dex also enhanced several phenotypic markers of osteoblasts, such as alkaline phosphatase activity, procollagen type I carboxy-terminal peptide production, and cAMP responses to parathyroid hormone in BVSMCs. We also examined the effects of Dex on human osteoblast-like (Saos-2) cells and compared its effects on BVSMCs and Saos-2 cells. The effects of Dex on alkaline phosphatase activity and the cAMP response to parathyroid hormone in BVSMCs were less prominent than those in Saos-2 cells. Interestingly, we detected that Osf2/Cbfa1, a key transcription factor in osteoblastic differentiation, was expressed in both BVSMCs and Saos-2 cells and that Dex increased the gene expression of both transcription factors. These findings suggest that Dex may enhance osteoblastic differentiation of BVSMCs in vitro. (Arterioscler Thromb Vasc Biol. 1999;19:2112-2118.)

Key Words: alkaline phosphatase | core-binding factor-α1 | atherosclerosis

Calcification is a common feature of advanced atherosclerotic lesions. Atherosclerotic plaque calcification, especially in the coronary arteries, is associated with clinical complications such as myocardial infarction, impaired vascular tone, dissection in angioplasty, poor surgical outcome, and coronary insufficiency due to loss of aortic recoil. Although the mechanism of vascular calcification remains to be established, recent evidence suggests that it has several features similar to mineralization in skeletal tissue, including the expression of bone morphogenetic protein-2 (BMP-2), osteocalcin, and osteopontin and the presence of the bone mineral hydroxyapatite and matrix vesicles.

To clarify the mechanism of vascular calcification, we developed an in vitro calcification system in which diffuse calcification can be induced by culturing bovine vascular smooth muscle cells (BVSMCs) in the presence of β-glycerophosphate (β-GP). In this model, alkaline phosphatase (ALP), which is 1 of the markers for osteoblastic differentiation, is critical for vascular calcification and the expression of osteopontin mRNA, which increases during the development of calcification. Furthermore, we identified a local calcium-regulating system in which parathyroid hormone (PTH)–related peptide plays an important role as an autocrine/paracrine regulator of vascular calcification. Through recent evidence demonstrated by us and other investigators, we hypothesized that VSMCs might acquire osteoblastic characteristics during the development of atherosclerotic lesions.

Osteoblastic differentiation is a multistep process, proceeding through defined stages of maturation from a committed progenitor cell of mesenchymal origin capable of proliferation to a postproliferative osteoblast expressing bone phenotypic markers. However, the molecular basis of osteoblast-specific gene expression and differentiation remains unclear. Recently, a key regulatory transcription factor in osteoblastic differentiation, osteoblast-specific transcription factor-2/core-binding factor-α subunit 1 (Osf2/Cbfa1), has been identified. The Osf2/Cbfa1 gene generates 2 types of transcripts, osteoblast-specific and T cell–specific isoforms. In the mouse, the osteoblast isoform is different from the T-cell isoform in that the former contains a unique 87–amino acid sequence at its amino-terminal end. However, the precise roles of the 2 transcripts in osteoblastic differentiation still remain unclear. The homozygous Osf2/Cbfa1 (−/−) mouse shows a total lack of bone and a retention of the partially calcified cartilaginous skeleton. In humans, mutations of this gene cause cleidocranial dysplasia, an autosomal dominant skeletal disorder. Moreover, overexpression of the...
osteoblast isoform in nonosteoblastic cells induces expression of the principal osteoblast-specific genes, such as α1(I) procollagen, osteopontin, bone sialoprotein, and osteocalcin. Therefore, Osf2/Cbfa1 (osteoblast isoform) is thought to be one of the “master genes” for osteoblastic differentiation.

Dexamethasone (Dex), a potent, synthetic glucocorticoid, is well known to promote differentiation of progenitor cells, such as bone marrow stromal cells, to the osteoblastic phenotype. Chronic treatment of osteoblast cultures with Dex increases the number of mineralized bone nodules in primary fetal rat calvarial osteoblast cultures. Glucocorticoid promotes phenotypic markers of osteoblast differentiation, such as ALP, cAMP responses to PTH, osteopontin, bone sialoprotein, and osteocalcin, while it depresses production of insulin-like growth factor-I and type I collagen by osteoblasts.

Although glucocorticoids have been shown to inhibit in vitro proliferation of VSMCs and prevent the development of atherosclerosis in experimental animals, retrospective studies including pathological findings obtained at autopsy have suggested that glucocorticoids adversely affect atherogenesis in humans. Moreover, the involvement of glucocorticoids in atherogenesis is supported by the strong correlation between an increased serum cortisol level in humans and the extent of coronary artery disease. Furthermore, long-term administration of glucocorticoids induces several metabolic and pathophysiological complications, such as insulin resistance, hypertension, and hyperlipidemia, which are thought to be coronary risk factors. However, the mechanism of glucocorticoid action in atherogenesis is poorly understood. Considering the fact that calcification is a common feature of advanced atherosclerotic lesions, it is important to evaluate the effect of glucocorticoids on vascular calcification.

In this study, we investigated the effect of Dex on vascular calcification by using an in vitro calcification model. We first demonstrated that Dex increased calcium deposition in a time- and dose-dependent manner. In this process, Dex increased ALP activity, its mRNA expression, and procollagen type I C-peptide (PICP) production and influenced cAMP responses to PTH. Finally, we demonstrated that Dex promoted expression of the Osf2/Cbfa1 gene. These results suggest that Dex may stimulate vascular calcification by promoting osteoblastic differentiation of VSMCs.

Methods

Reagents

Media, FCS, and sodium pyruvate were purchased from GIBCO. β-GP and Dex were obtained from Sigma Chemical Co. Unless otherwise mentioned, all other reagents were obtained from Wako Pure Chemical Industries, Ltd.

Cell Culture and In Vitro Calcification

BVSMCs were obtained from the media of aortas by an explant method as previously described. Cells that had migrated from the explants were collected and maintained in Dulbecco’s modified Eagle’s medium (DMEM; high glucose [4.5 g/L]) containing 15% FCS and 10 mmol/L sodium pyruvate supplemented with 100 U/mL penicillin and 100 μg/mL streptomycin (growing medium) at 37°C in a humidified atmosphere containing 5% CO2. The cells up to passage 8 were used for experiments. Human osteoblast-like cells (Saos-2) and Jurkat cells were obtained from the American Type Culture Collection (Manassas, Va) and maintained in DMEM supplemented with 15% FCS. Calcification of BVSMCs and Saos-2 cells was induced as previously described. After reaching confluence, the cells were incubated in DMEM containing 15% FCS in the presence of 10 mmol/L β-GP. The medium was replaced with fresh medium every 2 days. In the time-course experiments, the beginning day of culture in the calcification medium was defined as day 0.

Quantification of Calcium Deposition

The cells were decalcified with 0.6N HCl for 24 hours. The calcium content was determined with a bicinchoninic acid protein assay kit (Pierce). The calcium content of the cell layer was normalized to protein content.

ALP Assay

After the cells were washed twice with PBS, the cellular proteins were solubilized with 1% Triton X-100 in 0.9% NaCl and centrifuged, and the supernatants were assayed for ALP activity as described previously. One unit was defined as the activity producing 1 nmol of p-nitrophenol for 30 minutes. Protein concentrations were determined with a bicinchoninic acid protein assay kit (Pierce).

PICP Assay

The cells were plated into 24-well plates and grown to confluence. The medium was replaced with phenol red–free DMEM containing 0.2% FCS in the presence of Dex. After the indicated period of incubation, the supernatants were collected and stored at −20°C until assay. PICP secreted into the culture medium by BVSMCs and Saos-2 cells was assessed by measuring the PICP content of the culture supernatant with an enzyme immunosorbent assay kit (PIP EIA kit, Takara). The data were normalized to the protein content of the cell layer.

Measurement of cAMP

cAMP responses to PTH were assessed by measuring intracellular cAMP. The cells were plated into 24-well plates and grown to confluence before treatment with either vehicle or various concentrations of Dex (10−10 to 10−7 mol/L). After 4 days of treatment with Dex, the cells were washed twice with PBS and preincubated for 10 minutes with DMEM containing 0.1% BSA and 1.0 mmol/L 3-isobutyl-1-methylxanthine at 37°C. Human PTH(1–34) (10−7 mol/L) was then added to the medium, and the cells were incubated for an additional 15 minutes at 37°C. Thereafter the medium was removed, and the cell layer containing cAMP was extracted with 500 μL of 5% trichloroacetic acid. One-hundred-microliter aliquots of these samples were washed 3 times with 5 volumes of water-saturated ethyl ether and then dried. The extract was analyzed for cAMP by utilizing a cAMP radioimmunobassay kit (Yamasa Shoyu).

Preparation of cDNA Probes

The human ALP (liver/bone/kidney type) cDNA probe was obtained from the Japanese Cancer Research Resources Bank, Osaka, Japan. Human Osf2/Cbfa1 cDNA probes (585-bp fragments) were obtained by reverse transcription of an mRNA from Saos-2 cells, followed by polymerase chain reaction and subcloning into the TA cloning vector (Invitrogen). Sequences of the obtained cDNA were confirmed by the dideoxy sequencing method. The Osf2/Cbfa1 cDNA probe, which contains the carboxy terminus of the coding region (684 to 2113 bases), was used in the Northern blot analysis with polyA+ RNA of Saos-2 and of Jurkat (human T-cell line) cells, respectively.

RNA Isolation and Northern Blot Analysis

Total RNA was prepared using the acid-guanidinium-isoniodyocate-phenol-chloroform extraction method. PolyA+ RNA was obtained by use of an mRNA isolation kit (Microfast Track Kit, Invitrogen) by using oligo(dT) cellulose for absorption. Twenty micrograms of total RNA and 1 microgram of polyA+ RNA were denatured and
separated by electrophoresis on 1% agarose gels containing formaldehyde and transferred to a nylon filter (Hybond N, Amersham). Blots were prehybridized for 24 hours at 37°C in a buffer containing 50% formamide, 3× SSC (1× SSC is 0.15 mol/L NaCl and 15 mmol/L sodium citrate, pH 7.4), 50 mmol/L Tris-HCl (pH 7.5), 0.1% SDS, 20 μg/mL denatured salmon sperm DNA, and 1× Denhardt’s solution and then hybridized at 37°C for 48 hours with cDNA probes for human ALP and Osf2/Cbfa1, which had been labeled with [α-32P]dCTP (3000 Ci/mL, New England Nuclear) by use of a random priming method (Megaprime cDNA labeling system, Amersham). Blots were washed and autoradiographed with x-ray film at −70°C. The amounts of mRNA were quantified by densitometric scanning and normalized by comparison with glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

Measurements of DNA Synthesis
DNA synthesis of BVSMCs was evaluated by [3H]thymidine incorporation assays. The cells were grown in 24-well plates until confluent and then incubated in serum-free DMEM for 48 hours. After 48 hours, the medium was changed to DMEM containing 15% FCS or platelet-derived growth factor (PDGF)-BB (10 ng/mL) in the presence or absence of the indicated concentrations of Dex. The cells were subsequently incubated for 21 hours and then labeled with 1 μCi/mL [3H]thymidine (6.7 Ci/mmol, New England Nuclear) for an additional 3 hours. [3H]Thymidine incorporated into DNA was evaluated by trichloroacetic acid precipitation and counting in a scintillation counter (Beckman Instruments).

Statistics
In certain experiments, data were analyzed for statistical significance by ANOVA with post hoc analysis, unless otherwise stated. These analyses were performed with the assistance of a computer program (StatView version 4.11, Abacus Concepts).

Results
We first examined the effect of Dex on BVSMC calcification. As previously described, β-GP induced calcium deposition in a time-dependent manner. In the presence of β-GP, Dex (10−7 mol/L) significantly increased calcium deposition compared with calcified controls at each time point (Figure 1A). The calcium deposition in the Dex-treated group increased to 175% of the calcified control value on day 6. Likewise, Dex promoted calcium deposition in a dose-dependent manner on day 4, and the calcium deposition increased to 322% of the calcified control value at 10−7 mol/L (Figure 1B). These results suggest that Dex increases BVSMC calcification.

ALP is known to be 1 of the phenotypic markers of osteoblastic differentiation. Because we reported that ALP plays an important role in this calcification system, we next examined the effect of Dex on ALP activity in BVSMCs. As a positive control of Dex’s effect, we utilized human osteoblast-like (Saos-2) cells. In the absence of β-GP, Dex (10−7 mol/L) enhanced ALP activity in a time-dependent manner, and ALP activity had increased to 222% of controls on day 4 (Figure 2A). On day 4, Dex dose-dependently increased ALP activity in the absence of β-GP, and the maximal effect (436% of control) was observed at 10−7 mol/L (Figure 2B). In the presence of β-GP, Dex also increased ALP activity in BVSMCs, but the response to Dex was less prominent than that in its absence (Table 1). Furthermore, a greater response was observed in Saos-2 cells, both in the absence and presence of β-GP (Table 1). Next, we examined the effect of Dex on expression of the ALP gene in BVSMCs. Dex dose-dependently promoted the expression of ALP mRNA at 48 hours, and the maximal effect was observed at 10−7 mol/L (180% increase of control; Figure 2C). Together, these results suggest that Dex may accelerate BVSMC calcification partially through enhancing expression of the ALP gene and its activity.

Type I collagen is 1 of the early phenotypic markers for osteoblastic differentiation and may play an important role in this process. We utilized PICP as an index for type I collagen synthesis. We measured PICP contents of the cell layers were measured by the o-cresolphthalein complexone method, normalized to cellular protein content, and are presented as mean±SEM (n=3). Differences compared with calcified controls at each time point were statistically significant (P<0.05, Fisher’s protected least significant difference test [PLSD]). Uncalcified control group is indicated by open circles, calcified control group by open squares, and Dex-treated group by closed squares. β-GP (+) and (−) indicate the presence and absence of β-GP, respectively. B, Dose-dependent effect of Dex. Cells were cultured in calcification medium for 4 days in the presence of the indicated doses of Dex. Calcium contents of the cell layers were assessed as described above and are presented as mean±SEM (n=3). Differences compared with calcified controls with statistical significant (P<0.05, Fisher’s PLSD) β-GP (+) and (−) indicate the presence and absence of β-GP, respectively.
As another marker of osteoblastic differentiation, we investigated the effect of Dex on cAMP production in response to PTH stimulation. Dex stimulated cAMP responses to PTH in BVSMCs both in the presence and absence of β-GP (Table 2). The stimulatory effect of Dex in the absence of β-GP was greater than that in its presence. Moreover, Dex exerted greater responses in Saos-2 cells compared with BVSMCs (Table 2). These data suggest that Dex promotes cAMP responsiveness to PTH in BVSMCs as well as in Saos-2 cells.

We next assessed the effect of Dex on gene expression of Osf2/Cbfa1 in BVSMCs. The mRNA of Osf2/Cbfa1 expressed in BVSMCs was compared with that in Saos-2 and Jurkat cells (Figure 4A). The transcript in BVSMCs was larger than that in Saos-2 and Jurkat cells. The transcripts detected in Saos-2 and Jurkat cells are thought to be human osteoblast– and T cell–specific isoforms, respectively. Additionally, Dex (10⁻⁻ mol/L) increased Osf2/Cbfa1 mRNA expression 24 hours after treatment in Saos-2, but the presence of β-GP did not affect its expression (Figure 4B). In BVSMCs, Dex also enhanced mRNA expression in a time-dependent manner (Figure 4C). Furthermore, the presence of β-GP exerted no apparent effect on expression at 24 hours (data not shown).

Because it is likely that Dex may promote expression of osteoblastic markers in BVSMCs by inhibiting their proliferative capacity, we finally examined the effect of Dex on DNA synthesis in BVSMCs. The cells were incubated in DMEM containing 15% FCS or 10 ng/mL PDGF-BB in the presence or absence of the indicated concentrations of Dex for 24 hours. Both FCS and PDGF-BB stimulated DNA synthesis in BVSMCs, and Dex inhibited the stimulatory effect of PDGF-BB on DNA synthesis (Figure 5A). However, Dex did not affect DNA synthesis stimulated by FCS (Figure 5B), suggesting that Dex may not exert a direct effect on BVSMC proliferation in this calcification model.

### Discussion

Because glucocorticoids are well known to be potent stimulators of osteoblastic differentiation, we utilized Dex, a potent synthetic glucocorticoid, to induce BVSMCs to acquire osteoblastic characteristics. As shown in this study, Dex enhanced not only in vitro calcification but also several phenotypic markers for osteoblastic differentiation in BVSMCs, such as ALP expression, type I collagen production, and cAMP responsiveness to PTH (Figures 1, 2, and 3). Moreover, we confirmed the potency of Dex on osteoblastic differentiation by utilizing Saos-2 cells as a positive control. Regarding ALP activity and the cAMP response to PTH, the...
responsiveness of BVSMCs to Dex was less prominent than that of Saos-2 cells (Tables 1 and 2). The less potent effect of Dex on BVSMCs may be ascribed to a heterogeneous population of cells in the BVSMC culture. Interestingly, Dex exerted no effect on PICP production by Saos-2 cells, whereas Dex increased PICP secretion by BVSMCs in the absence of β-GP (Table 1). Early phenotypic markers for osteoblastic differentiation such as PICP may not be affected by Dex in well-differentiated osteoblastic cells. However, the precise mechanism by which such differences of responsiveness to Dex are induced remains to be clarified.

Several key factors in bone mineralization have been demonstrated in calcified lesions of arterial walls, such as matrix vesicles, BMP-2, osteopontin, matrix Gla protein, osteocalcin, and type I collagen.6–11,39 We previously dem-

responded by utilizing BVSMCs.12,13 Recently, a key regulatory factor was identified. BMP-7 induces expression of the osteoblastic isoform, Osf2/Cbfa1, has been identified. BMP-7 induces expression of the osteoblastic isoform, followed by its enhancement of the osteocalcin gene in nonosteoblastic cells.16 Therefore, the Osf2/Cbfa1 gene is thought to be one of the “master genes” of as well as a molecular marker for osteoblastic differentiation. In this study, we showed the presence of the Osf2/Cbfa1 gene in cultured BVSMCs as well as in Saos-2 cells (Figure 4A and 4B). Additionally, we cloned a 5’ partial sequence of the bovine osteoblast–specific Osf2/Cbfa1 transcript by reverse transcription–polymerase chain reaction by using total RNA from BVSMCs in preliminary experiments (K.M. et al,

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### TABLE 2. Effects of Dex on cAMP Production Stimulated by PTH in BVSMCs and Saos-2 Cells

<table>
<thead>
<tr>
<th></th>
<th>cAMP, pmol/mg Protein</th>
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<tr>
<td></td>
<td>β-GP−</td>
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<tr>
<td></td>
<td>Basal</td>
</tr>
<tr>
<td>BVSMCs</td>
<td></td>
</tr>
<tr>
<td>Ctl</td>
<td>2.5±0.1</td>
</tr>
<tr>
<td>Dex</td>
<td>3.4±0.3</td>
</tr>
<tr>
<td>Saos-2 cells</td>
<td></td>
</tr>
<tr>
<td>Ctl</td>
<td>6.7±0.5</td>
</tr>
<tr>
<td>Dex</td>
<td>7.2±0.4</td>
</tr>
</tbody>
</table>

Cells were treated with vehicle Ctl or Dex (10−7 mol/L) in the presence or absence of β-GP for 72 hours. cAMP contents in response to 10−7 mol/L PTH were determined as described in Methods, were normalized to cellular protein content and are presented as mean±SEM.

Differences compared with each control were statistically significant at *P<0.05 by Fisher’s protected least significant difference test.

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![Figure 3](image-url) Effects of Dex on PICP production by BVSMCs. A, Time-dependent effect of Dex. Cells were cultured for the indicated periods of time in the presence of 10−7 mol/L Dex as described in Methods. PICP contents of culture supernatants were measured by an enzyme immunosorbent assay and normalized to cellular protein contents. Data are presented as mean±SEM (n=3). Differences compared with control (CTL) at each time point were statistically significant (P<0.05, Fisher’s PLSD). Control group is indicated by open circles and Dex-treated group by closed squares. B, Dose-dependent effect of Dex. Cells were cultured for 4 days in the presence of the indicated doses of Dex as described. PICP contents of culture supernatants were measured by enzyme immunosorbent assay and normalized to cellular protein contents. Data are presented as mean±SEM (n=3). Differences compared with control were statistically significant (P<0.05, Fisher’s PLSD).

![Figure 4](image-url) Expression of Osf2/Cbfa1 mRNA. A, Northern blot analysis of Osf2/Cbfa1 in various cells. One microgram of polyA+ RNA from BVSMCs, Jurkat cells, and Saos-2 cells was electrophoresed, blotted, and probed with cDNAs of human Osf2/Cbfa1. B, Northern blot analysis of Osf2/Cbfa1 mRNA from BVSMCs and Saos-2 cells. C, Northern blot analysis of Osf2/Cbfa1 mRNA from BVSMCs and Saos-2 cells. Cells were cultured for the indicated periods of time in the presence of 10−7 mol/L PTH in BVSMCs as described. After treatment with Dex, cells were harvested for isolation of polyA+ RNA. One microgram of polyA+ RNA from BVSMCs was electrophoresed, blotted, and probed with cDNAs of human Osf2/Cbfa1. Top, Autoradiograph of Northern blot of Osf2/Cbfa1. Bottom, Densitometric analysis of autoradiograms was performed and results are presented as the ratio of Osf2/AML3/Cbfa1 to GAPDH (mean±SEM, n=3). Differences compared with control (CTL) at each time were statistically significant (P<0.05, Fisher’s PLSD).
drugs at high doses, glucocorticoids suppress the development of atherosclerosis in experimental animals, despite enhancement of hypertriglyceridemia and hypercholesterolemia. Glucocorticoids have also been shown to inhibit the proliferation of cultured VSMCs and the thrombin-induced expression of growth factors. On the other hand, glucocorticoids are capable of decreasing the expression of hepatic LDL receptors, stimulating the net synthesis of apoB-100 and apoB-48 and decreasing their intracellular degradation. These changes are potentially atherogenic, and the strong correlation between an increased serum cortisol level in humans and the extent of coronary artery disease has also been documented. In this study, we have shown that Dex increases in vitro calcification by promoting osteoblastic phenotypes in BVSMCs. Taking into consideration that chronic treatment with glucocorticoids induces osteoporosis and that vascular calcification is often associated with osteoporosis, it is suggested that Dex may develop and exacerbate vascular calcification. Further studies are necessary to clarify the long-term effect of glucocorticoid administration on the development of vascular calcification, especially calcified atherosclerotic plaque lesions.

Figure 5. Effects of Dex on PDGF-BB–stimulated (A) or 15% FCS-stimulated (B) DNA synthesis in BVSMCs. Cells were cultured in serum-deprived DMEM for 48 hours. Thereafter, the cells were incubated in DMEM containing PDGF-BB (10 ng/mL, A) or 15% FCS (B) for 21 hours in the presence or absence of the indicated concentrations of Dex followed by a pulse with 1 μCi/mL [3H]thymidine for an additional 3 hours. DNA synthesis was measured as trichloroacetic acid–insoluble radioactivity and data are presented as mean±SEM (n=4). Differences compared with PDGF-stimulated controls were statistically significant (*P<0.05, Fisher’s PLSD).

unpublished data, 1998). This evidence suggests that cultured VSMCs may be committed to differentiate into osteoblastic cells under certain conditions. However, whether the transcript detected in BVSMCs is the osteoblast-specific isoform remains to be confirmed. Furthermore, Dex enhanced the gene expression of Osf2/Cbfa1 in a time dependent manner in BVSMCs (Figure 4C). Therefore, it is likely that Dex may promote osteoblastic differentiation of VSMCs by increasing the expression of the Osf2/Cbfa1 gene.

Linkage of phenotypic gene induction to the downregulation of proliferation is the hallmark of differentiation in numerous cell types. Some agents inhibiting the proliferation of osteoblast-lineage cells, such as hydroxyurea, can induce osteoblastic differentiation. It is therefore possible that antiproliferative agents of VSMCs may induce osteoblastic differentiation under certain conditions. Moreover, 17β-estradiol has been reported to promote osteoblastic differentiation of bovine vascular cells and in vitro calcification without affecting cell growth. In this study, we examined the hypothesis that Dex may inhibit the proliferative capacity of BVSMCs, resulting in osteoblastic differentiation. Because Dex did not affect DNA synthesis in the presence of 15% FCS (Figure 5B), Dex may directly induce osteoblastic differentiation of BVSMCs without affecting their growth.

The mechanisms of glucocorticoid action on atherogenesis remain to be evaluated. When applied as anti-inflammatory drugs at high doses, glucocorticoids suppress the development of atherosclerosis in experimental animals, despite enhancement of hypertriglyceridemia and hypercholesterolemia. Glucocorticoids have also been shown to inhibit the proliferation of cultured VSMCs and the thrombin-induced expression of growth factors. On the other hand, glucocorticoids are capable of decreasing the expression of hepatic LDL receptors, stimulating the net synthesis of apoB-100 and apoB-48 and decreasing their intracellular degradation. These changes are potentially atherogenic, and the strong correlation between an increased serum cortisol level in humans and the extent of coronary artery disease has also been documented.

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References


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