Decreased Proliferation and Abnormal Differentiation of Human Mesenchymal Stromal Cells in Steroid-Induced Osteonecrosis of Femoral Head

Changdong Wang¹, Wei Huang², Xuan Gong³, Guoliang Ding⁴, Xi Liang² and Ning Hu^{2*}

¹Department of Biochemistry and Molecular Biology, Molecular Medicine and Cancer Research Center, Chongqing Medical University, Chongqing 400016, China

²Department of Orthopaedic Surgery, the First Affiliated Hospital of Chongqing Medical University, Chongqing 400016, China

³Department of outpatient, Chongqing Zhongshan Hospital, Chongqing, 400013, China

⁴Department of Orthopaedic Surgery, the Second Affiliated Hospital of Baotou Medical College of Inner Mongolia University of Science and Technology, Baotou, 014030, China

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***Corresponding author:** Ning Hu, Department of Orthopedic Surgery, Chongqing Medical University, Chongqing, China, Tel: 8618-6967-61022; Fax: 8623-8901-1212; E-mail: 1276321387@qq.com

Abstract

Objectives: To investigate if the pathogenesis of steroid-induced osteonecrosis of femoral head (ONFH) is associated with abnormal proliferation and differentiation of human mesenchymal stromal cells (MSCs) at the proximal femur.

Methods: Using isolated human MSCs and sections of whole femoral heads, we analyzed the proliferative capacity and osteogenic, angiogenic and adipogenic differentiation of human MSCs at the proximal femur.

Results: The proliferation of MSCs from patients with corticosteroid-induced ONFH is decreased. The down-regulated expression of BMP2, BMP7, BMP9 and Osteopontin provides supportive evidence corticosteroid-induced inhibition of osteogenesis. Down-regulation of HIF1 α , VEGF and VWF by glucocorticoids is directly responsible for decreased angiogenesis. Over-expression of PPAR γ 2 and (442)aP2 suggests that the corticosteroids may induce MSCs to differentiate into adipocytes.

Conclusions: Our findings suggest steroids may reduce the proliferative activity of MSCs, down-regulate the expression of osteoblast differentiation factors such as BMP2, BMP7, BMP9 and OPN, decrease angiogenesis by suppressing HIF1 α and VEGF, and up-regulate adipocyte transcription factor expression such as PPAR γ 2 and (442)aP2.

Keywords: Osteonecrosis; Mesenchymal Stromal Cells; Steroids; Bone Morphogenetic Proteins; Hypoxia-inducible factor1 α

Introduction

Osteonecrosis of femoral head (ONFH) is characterized by bone ischemia and microarchitectural deterioration, which lead a collapse of the femoral head during the late stage. ONFH represents a remarkable challenge to orthopedic surgeons and is a devastating disease for affected patients [1,2]. The use of corticosteroids such as dexamethasone and methylprednisolone for severe adult respiratory syndrome (SARS), rheumatoid diseases and organ transplantation has resulted in an increased

risk of ONFH [3]. Intraosseous hypertension, thrombotic intravascular occlusion and extravascular compression by progressive accumulation of marrow fat stores are commonly accepted theories [4-6]. Although it is recognized that steroids administration and the development of steroids-induced ONFH are related, the precise pathogenesis remains largely unknown [7]. The pathobiological mechanism underlying the induction of adipogenesis and suppression of osteogenesis and angiogenesis by steroids has not been elucidated.

Human mesenchymal stromal cells (MSCs) are multipotent progenitors which undergo self-renewal and differentiate into osteogenic, angiogenic and adipogenic lineages [8]. It is suggested that ONFH is a disease of MSCs, due to abnormal proliferation or differentiation of MSCs [9]. Osteogenic differentiation is a sequential cascade that recapitulates most of the molecular events occurring during bone development and remodeling. Bone morphogenetic proteins (BMPs) play an important role during development and have been shown to regulate stem cell proliferation and osteogenic differentiation [10]. BMP2, 7 and 9 are the most potent BMPs among the 14 types of BMPs in inducing osteogenic differentiation of MSCs [11]. Normal and pathological bone physiology is inexorably tied to angiogenesis. The vasculature plays an important role for the mechanism of coupling resorption by osteoclasts and bone formation by osteoblasts [11]. Angiogenesis, the development of a microvascular network for blood supply, is critical for the development, remodeling and healing of bone [11-13]. Hypoxia can induce both apoptosis as well as necrosis of cells and is associated with vascular disease [12]. Hypoxia-inducible factor1 α (HIF1 α) is a well established regulator of angiogenic cascade, which usually coordinates with skeletal development [11,14]. Vascular endothelial growth factor (VEGF), transcriptionally targeted gene of HIF1 α , is a key regulator of vasculogenesis in the embryo and angiogenesis in adult tissues [11,15,16]. Thus, it appeared promising to investigate the role of angiogenic factors

such as HIF1 α and VEGF. Small vessel occlusion by fatty emboli and the impedance of sinusoidal blood flow secondary to a rise in intraosseous pressure due to fatty infiltration following steroid therapy. In addition, steroid stimulates MSCs differentiation into adipocytes as well as the accumulation of fat in the marrow while suppressing cell differentiation into osteoblasts [17]. Peroxisome proliferator activated receptor γ 2 (PPAR γ 2) gene expression destines cells for adipocyte differentiation [18,19].

Here, we investigate if the pathogenesis of steroid-induced osteonecrosis is associated with decreased proliferative capacity and abnormal differentiation of human MSCs at the proximal femur. The proliferation of cultured human MSCs in patients with corticosteroid-induced ONFH is depressed. We report the identification of abnormal differentiation in isolated MSCs and sections of whole femoral heads obtained during total hip replacement for glucocorticoid-induced osteonecrosis. The decreased expression of BMP2, BMP7, BMP9 and Osteopontin provide supportive evidence corticosteroid-induced inhibition of osteogenesis. Down-regulation of HIF1 α , VEGF and VWF by glucocorticoids is directly responsible for disturbed angiogenesis. Over-expression of PPAR γ 2 and (442)aP2 suggests the corticosteroid-induced adipogenic differentiation. Taken together, our findings should not only expand our understanding of the molecular basis behind steroid-induced osteonecrosis, but also provide an opportunity to harness proliferative capacity and differentiation of MSCs in regenerative medicine.

Materials and Methods

Patients

The study was approved by the University Ethics Committee and informed consent was obtained from all patients. 12 ONFH patients (4 men and 8 women, aged from 38 to 65 years old) with history of corticosteroid usage for rheumatoid arthritis, systemic lupus erythematosus and uveitis were recruited. All Patients with stage III and stage IV ONFH underwent total hip replacement (THR). The exclusion criteria included metabolic bone diseases, such as Paget's disease, renal osteodystrophy, hyper- or hypoparathyroidism and malignant tumors. The necrotic bone in the center of split femoral head was harvested for study. Bone specimens from additional 12 patients who underwent THR for femoral neck fractures were harvested and enrolled as controls. No age-matched control was obtained because of the absence of surgical indication for THR in young patients with femoral neck fractures.

Harvest and identification of human mesenchymal stromal progenitor cells

Bone marrow was obtained at the femoral neck and adherent stromal cells were harvest and cultured in the conditions as described [20]. Cells were treated with 0.1% triton-X for 10 minutes and blocked with phosphate-buffered saline containing 1% bovine serum albumin and mouse serum for 30 minutes. Fluorescein isothiocyanate-labeled mouse anti-human CD29 antibody (1:100, Santa Cruz) and PE-labeled rat antihuman CD44 (1:100, Santa Cruz) were added simultaneously to 1 well. Mouse antihuman CD34, or CD14 antibodies (Santa Cruz)

were added; following incubation for 30 minutes, fluorescein isothiocyanate-labeled rabbit antimouse IgG antibody then was added. Identification of mesenchymal stem cells was performed as previously described [21].

Measurement of proliferation of human mesenchymal stromal cells using Flow Cytometry

When MSCs covered 90% of the flask, they were digested and resuspended at the density of 1×10^6 /mL. Cells were fixed in 80% pre-cooled alcohol at 4 overnight. After washing with PBS and centrifuged for 5 minutes, the supernatant was discarded. 10 μ L of RNase (100 μ g/mL), 10 mL of propidium iodide (100 μ g/mL) and 40 μ L of PBS were added, and the cells were kept in darkness for 30 minutes. The cell cycles were measured with a flow cytometry instrument. The proliferation index (PI) was used to assess the levels of proliferation.

RNA isolation and semi-quantitative RT-PCR

Total RNA was isolated from subconfluent MSCs using TRIZOL Reagents (Invitrogen) and used to generate cDNA templates by RT reaction with hexamer and M-MuLV Reverse Transcriptase (New England Biolabs, Ipswich, MA). The first strand cDNA products were further diluted five to ten folds and used as PCR templates. Semiquantitative RT-PCR (sqPCR) was carried out as described [11]. PCR primers (supplementary material Table) were designed by using the Primer3 program to amplify the genes of interest (150–180 bp). A touchdown cycling program was as follows: 94°C for 2 minutes for 1 cycle; 92°C for 20 seconds, 68°C for 30 seconds, and 72°C for 12 cycles decreasing 1°C per cycle; and then at 92°C for 20 seconds, 57°C for 30 seconds, and 72°C for 20 seconds for 20–25 cycles, depending on the abundance of a given gene. PCR products were resolved on 1.5% agarose gels. All samples were normalized by the expression level of GAPDH.

Western blotting analysis

Western blotting was carried out as previously described [11]. Briefly, tissues were collected in Lysis Buffer. Cleared total cell lysate was denatured by boiling and resolved by 10% SDS-PAGE. After electrophoretic separation, proteins were transferred to an Immobilon-P membrane. Membrane was blocked with SuperBlock Blocking Buffer, and probed with anti-BMP2 (Proteintech), BMP7 (Proteintech), BMP9 (Proteintech), Osteopontin (ab8448), HIF1 α (Proteintech), VEGF (Proteintech), PPAR γ 2 (Proteintech), vWF (Proteintech) and (442)aP2 (Proteintech) or anti- β -actin (Santa Cruz), followed by incubation with a secondary antibody conjugated with horseradish peroxidase. The proteins of interest were detected by using SuperSignal West Pico Chemiluminescent Substrate kit.

Immunohistochemical and Immunofluorescent staining

Immunohistochemical staining on paraffin-embedded tissues was carried out with an anti-BMP2, BMP7, BMP9, HIF1 α , VEGF, PPAR γ 2, vWF and (442)aP2 antibody. The presence of the expected protein was visualized by DAB staining and examined under a microscope as previously described [11]. The slides were then incubated with primary antibody diluted in PBS containing 1% BSA for 1h. The primary antibodies used were as follows:

anti-osteopontin, vWF and (442)aP2 antibody. After washing 3 times in PBS, AlexaFluor488, AlexaFluor555 (Invitrogen, Grand Island, NY) conjugated anti-rabbit or anti-mouse IgG was added in PBS with 1% BSA for 1h. After the final wash, 6-diamidino-2-phenylindole (DAPI) (Sigma) was added and used as a counterstain for nuclei. Fluorescence images were acquired using an Olympus microscope with DP manager software.

Test concentration of triglycerid reagent

The tissues of the ONFH and control groups were lysed with NP40 buffer (1% NP-40, 0.15 M NaCl, 50 mM Tris, pH 8.0) containing protease inhibitors (Sigma). Protein quantitation was performed with BCA protein assay reagent (Pierce, Rockford, IL, USA). Equal amounts of protein from the different groups were added into the reaction of triglyceride reagent KIT for 5min at 37. The extracted dye was transferred to 96-well plates, and the optical density was measured at 600nm.

Statistical analysis

All quantitative experiments were performed in triplicate and/or repeated three times. Data were expressed as mean $\pm$ S.D. Statistical significances between vehicle treatments versus drug-treatment were determined by one-way analysis of variance and the Student's t-test. A value of $P < 0.05$ was considered statistically significant.

Results

Clinical data of patients

Representative radiograph and MRI showed femoral head erosion and collapse in the ONFH group and femoral neck fracture in the control group (Figure 1A and 1B). Bone marrow edema and femoral head necrosis were visible in the ONFH group (Figure 1C). This revealed significant accumulation of marrow fat stores of ONFH relative to the control of normal femoral head.

Inhibited proliferation of MSCs in corticosteroid-induced osteonecrosis of femoral head

We first identified the expression of typical MSCs markers. Immunofluorescent staining showed the cultured cells expressed typical MSCs markers such as CD29 and CD44 (Figure 2A and 2B), but not typical hematopoietic cell markers including CD34 and CD14 (data not shown). To investigate the potential role of steroid in affecting proliferative capability of MSCs, we examined growth period of MSCs between the ONFH and control groups using flow cytometry. The percentage of cells in the $G_2/M+S$ stages is taken as the proliferation index (PI) to indicate the proliferation of cells. Compared with the control group, the percentage of cells in the G_0/G_1 stages in the ONFH group was increased significantly, whereas the percentage in $G_2/M+S$ stages (PI) was decreased significantly ($P < 0.01$) (Figure 2C and 2D).

Corticosteroid down-regulates the expression of BMP2, 7, 9 and late osteogenic marker in the ONFH and inhibits osteogenesis of MSCs in vivo

Bone morphogenetic proteins (BMPs) play an important role in regulating stem cell proliferation and osteogenic differentiation.

Figure 1: Clinical data of patients.

(A) MRI showed femoral head erosion and collapse in the ONFH group. (B) Representative radiograph showed femoral head necrosis in the ONFH group and femoral neck fracture in the control group. (C) The split femoral heads were harvested for study. In the ONFH group, Bone marrow edema and femoral head necrosis were visible. In the control group, bone specimens from the cases who underwent THR for femoral neck fractures were harvested.

BMP2, 7 and 9 are the most potent BMPs among the 14 types of BMPs in inducing osteogenic differentiation of MSCs. Compared with those in the control group, we found the transcriptions of BMP2, 7 and 9 were significantly reduced by 93.8%, 64.3% and 96.4% respectively in the ONFH group (Figure 3A). Similarly, the protein expression levels of BMP2, 7 and 9 were significantly reduced in the ONFH group (Figure 3B). Immunohistochemical staining also confirmed that corticosteroid inhibited expression

Figure 2: Decreased proliferation of MSCs in corticosteroid-induced ONFH.

The expression of the surface markers were determined by immunocytochemistry using the monoclonal antibodies CD29-FITC (A) and CD44-PE (B). Positive staining was observed in the cells ($\times 200$). Growth period of flow cytometry in the control group (C) and the ONFH group (D). The percentage of cells in $G_2/M+S$ stages was 59.54% and the percentage in the G_0/G_1 stages was 40.46% in the control group (C). The percentage in $G_2/M+S$ stages was 37.45% and the percentage in the G_0/G_1 stages was 62.55% in the ONFH group (D).

of BMP2, 7 and 9 at protein levels (Figure 3C). We also found that corticosteroid was able to suppress late osteogenic marker osteopontin (OPN) at mRNA (Figure 3A) and protein (Figure 3B) levels. Immunofluorescent staining results also confirmed that osteopontin protein was significantly decreased in ONFH group relative to the control (Figure 3D).

Corticosteroid down-regulates the expression of HIF1 α , VEGF and vWF in the ONFH and inhibits angiogenesis of MSCs in vivo

HIF1 α is a well established regulator of angiogenic cascade. VEGF, transcriptionally targeted gene of HIF1 α , is a key regulator of angiogenesis in adult tissues. And von Willebrand factor (vWF) is one of angiogenic markers. We found that mRNA of HIF1 α , VEGF and vWF were inhibited by 98.2%, 62.3% and 69.93% respectively in the ONFH group, compared with those in the control group (Figure 4A). Western blot detected that protein expression levels of HIF1 α , VEGF and vWF were inhibited significantly (Figure 4B). Immunohistochemical staining also confirmed that corticosteroid inhibited the expression of HIF1 α ,

VEGF and vWF at protein levels (Figure 4C). vWF protein expression level was also tested by Immunofluorescence and vWF was notably decreased in group of ONFH relative to the control (Figure 4D).

Corticosteroid upregulates the expression of PPAR γ 2, (442)aP2 and triglycerid in the ONFH and induced adipogenic differentiation of MSCs in vivo

The gene expression of PPAR γ 2 and (442)aP2 destine cells for adipocyte differentiation. We also found that mRNA level of PPAR γ 2 and (442)aP2 were augmented by 9.44 and 33.57 folds respectively in the ONFH group (Figure 5A). Protein expression of PPAR γ 2 and (442)aP2 were increased respectively (Figure 5B). Immunohistochemical staining also confirmed that corticosteroid augmented the expression of PPAR γ 2 and (442)aP2 at protein levels (Figure 5C). Immunofluorescent results displayed that (442)aP2 protein was significantly increased in ONFH group relative to the control (Figure 5D). In addition, triglycerid concentration was markedly increased in the ONFH (Figure 5E).

Figure 3: Corticosteroid down-regulates the expression of BMP2, 7, 9 and late osteogenic marker gene of osteopontin in the ONFH and inhibits osteogenesis of MSCs in vivo. (A) The transcription expression of BMP2, BMP7, BMP9 and OPN in MSCs of the ONFH group and the control group. Total RNA was isolated from subconfluent cells and subjected to semi-quantitative PCR (sqPCR) analysis using primers specific for human BMP2, BMP7, BMP9 and OPN. Expected PCR products were resolved on agarose gels (a). The signal intensities of the expected products were quantitatively analyzed using the NIH ImageJ software (b). Each PCR condition was done in triplicate. Representative results are shown. (B) The protein expression of BMP2, BMP7, BMP9 and OPN in MSCs of the ONFH group and the control group. Tissues were prepared in the same fashion as described in (a). Tissues were lysed and subjected to SDS-PAGE and Western blotting with anti-BMP2, anti-BMP7, anti-BMP9 and anti-OPN antibodies. Expression levels of β -actin was used to assess equal loading of total lysate. (C) Immunohistochemical staining of BMP2, BMP7 and BMP9 in the sections of the ONFH and control groups. Bone marrow was retrieved in a similar fashion as shown in Figure 1C. The retrieved tissues were subjected to BMP2, BMP7 or BMP9 antibody immunohistochemical staining. Isotype IgG was used as a negative control (not shown). Representative results are shown. Magnification, 40x. (D) Immunofluorescent staining results also confirmed that osteopontin protein was significantly decreased in ONFH group relative to the control. Each assay condition was done in triplicate. * $p < 0.05$; ** $p < 0.001$.

Table 1: PCR Oligonucleotides.

human BMP2 Fwd:	ACTCGAAATTCCTCGTGACC
human BMP2 Rev:	CCACTTCCACCACGAATCCA
human BMP7 Fwd:	GACTTCAGCCTGGACAACGA
human BMP7 Rev:	TGTAGGGGTAGGAGAAGCCC
human BMP9 Fwd:	CTGTGGAGAGCCACAGGAAG
human BMP9 Rev:	CTCCTTTTCCGCTGGCTAA
human OPN Fwd:	CATACAAGGCCATCCCCGTT
human OPN Rev:	TGGGTTTCAGCACTCTGGTC
human HIF1 α Fwd:	TGCATCTCCATCTCTACCC
human HIF1 α Rev:	CGTTAGGGCTTCTTGGATGA
human VEGF Fwd:	CCCACTGAGGAGTCCAACAT
human VEGF Rev:	TTTCTTGCGCTTTCGTTT
human vWF Fwd:	CCACTTGCCACAACAACATC
human vWF Rev:	TGGACTCACAGGAGCAAGTG
human PPAR γ 2 Fwd:	ATCTTTCAGGGCTGCCAGT

human PPAR γ 2 Rev:	GGAGGCCAGCATTGTGTAA
human (442)aP2 Fwd:	GGTGCAGAAGTGGGATGG
human (442)aP2 Rev:	TGGCTCATGCCCTTTCAT
human GAPDH Fwd:	GCATGGCCTTCCGTGTCCCC
human GAPDH Rev:	GAGGGCAATGCCAGCCCCAG

Discussion

Corticosteroid has been the focus of studies on the pathogenesis of ONFH. Patients receiving steroid therapy have an approximately 20-fold increase in their likelihood of developing ONFH [22]. Though the dose effect of corticosteroid therapy on osteonecrosis remains largely unknown, recent studies suggest that corticosteroid doses above 25-40mg/day are significant risk factors for nontraumatic ON in renal transplant and SLE patients [22]. The number of MSCs at the proximal femoral was significantly decreased in the corticosteroid-induced osteonecrosis compared to other kinds of osteonecrosis [23]. Corticosteroid can promote apoptosis of osteoblasts and

Figure 4: Corticosteroid down-regulates the expression of HIF1a, VEGF and vWF in the ONFH and inhibits angiogenesis of MSCs in vivo. (A) The transcription expression of HIF1a, VEGF and vWF in MSCs of the ONFH group and the control group. Total RNA was isolated from subconfluent cells and subjected to semi-quantitative PCR (sqPCR) analysis using primers specific for human HIF1a, VEGF and vWF. Expected PCR products were resolved on agarose gels (a). The signal intensities of the expected products were quantitatively analyzed using the NIH ImageJ software (b). Each PCR condition was done in triplicate. Representative results are shown. (B) The protein expression of HIF1a, VEGF and vWF in MSCs of the ONFH group and the control group. Tissues were prepared in the same fashion as described in (a). Tissues were lysed and subjected to SDS-PAGE and Western blotting with anti-HIF1a, anti-VEGF and anti-vWF antibodies. Expression levels of β-actin was used to assess equal loading of total cell lysate. (C) Immunohistochemical staining of HIF1a, VEGF and vWF in the sections of the ONFH and control groups. Bone marrow was retrieved in a similar fashion as shown in Figure 1. The retrieved tissues were subjected to HIF1a, VEGF or vWF antibody immunohistochemical staining. Isotype IgG was used as a negative control (not shown). Representative results are shown. Magnification, 40x. (D) vWF protein expression level was also tested by Immunofluorescence and vWF was notably decreased in group of ONFH relative to the control. Each assay condition was done in triplicate. * $p < 0.05$; ** $p < 0.001$.

osteocytes and suppress the proliferative capacity of MSCs, leading to a decrease in osteocytes [24-26]. Our results also confirmed that corticosteroid induces osteonecrosis through lowering the proliferative activity or differential capacity as well as altering the differentiation direction of MSCs.

Osteogenic differentiation is a sequential cascade that recapitulates most of the molecular events occurring during embryonic skeletal development [10]. BMPs belong to the transforming growth factor β (TGFβ) superfamily and consist of at least 14 members in humans [8]. Genetic disruptions of BMPs have resulted in various skeletal and extraskeletal abnormalities during development [11]. BMP2, 7 and 9 are the most potent BMPs in inducing osteogenic differentiation of MSCs by regulating several important downstream targets during BMPs-induced osteoblast differentiation [8,11]. Our results confirmed that corticosteroid down-regulates the expression of BMP2, 7 and 9, thus reduces late osteogenic markers and inhibits osteogenesis of MSCs.

The process of bone development and repair depends on adequate formation of new capillaries from existing blood vessel

[11,12]. It has been reported that angiogenesis and osteogenesis are well coordinated processes during bone development [11]. We have demonstrated that BMP9 directly upregulates HIF1α expression in MSCs, which in turn induces both osteogenic factors and angiogenic factor VEGF [11]. Thus, potent osteogenic factors, such as BMP9, may induce a tightly-regulated convergence of osteogenic and angiogenic signaling in MSCs, and subsequently lead to efficient bone formation. HIF1 regulates target genes such as VEGF and vWF that mediate adaptive responses, such as angiogenesis, to reduced oxygen availability [11]. HIF1α polymorphisms are associated with idiopathic ONFH in men; and variations in HIF1α play a role in the pathogenesis and risk factor for ONFH [27]. Osteoblasts derived from femoral heads have been found to exhibit downregulation of VEGF within 24 hours of incubation with corticosteroid [28]. Down-regulation of HIF1α, VEGF and vWF by corticosteroid is directly responsible for disturbed angiogenesis resulting in the defects in capillary architecture, which eventually lead to osteonecrosis.

Corticosteroid can induce differentiation of MSCs into adipose cells. Since adipocytes and osteoblasts share a common progenitor pool, when exogenous stimulators shift the differentiation of

Figure 5: Corticosteroid upregulates the expression of PPAR γ 2, (442)aP2 and triglyceride in the ONFH and induced adipogenic differentiation of MSCs in vivo.

(A) The transcription expression of PPAR γ 2 and (442)aP2 in MSCs of the ONFH group and the control group. Total RNA was isolated from subconfluent cells and subjected to semi-quantitative PCR (sqPCR) analysis using primers specific for human PPAR γ 2 and (442)aP2. Expected PCR products were resolved on agarose gels (a). The signal intensities of the expected products were quantitatively analyzed using the NIH ImageJ software (b). Each PCR condition was done in triplicate. Representative results are shown.

(B) The protein expression of PPAR γ 2 and (442)aP2 in MSCs of the ONFH group and the control group. Tissues were prepared in the same fashion as described in (a). Tissues were lysed and subjected to SDS-PAGE and Western blotting with anti-PPAR γ 2 and anti-(442)aP2 antibodies. Expression levels of β -actin was used to assess equal loading of total cell lysate.

(C) Immunohistochemical staining of PPAR γ 2 and (442)aP2 in the sections of the ONFH and control groups. Bone marrow was retrieved in a similar fashion as shown in Figure 1. The retrieved tissues were subjected to PPAR γ 2 or (442)aP2 antibody immunohistochemical staining. Isotype IgG was used as a negative control (not shown). Representative results are shown. Magnification, 40x.

(D) Immunofluorescent results displayed that (442)aP2 protein was significantly increased in ONFH group relative to the control.

(E) Detection of the concentration of triglyceride in bone tissues by TG reagent kit. The TG concentration of the control group was 0.87 ± 0.03 mmol/L and the concentration of the ONFH group was 9.07 ± 0.28 mmol/L. Each assay condition was done in triplicate. * $p < 0.05$; ** $p < 0.001$.

MSCs into the adipocyte lineage, the osteoprogenitor pool is not sufficient to provide enough osteoblasts in order to meet the need for bone remodeling and repair of ONFH [29]. Fat cell hypertrophy has been observed in histologic specimens of human femoral heads following treatment with dexamethasone for 5 days [30]. Dexamethasone affects osteoblasts by inhibit the expression of type-I collagen and osteocalcin, thereby inducing osteoblast and osteocyte apoptosis [31-33]. It also down-regulates the expression of Cbfa1/Runx2 and osteocalcin promoter activity while it increases the expression of PPAR γ 2 [29, 34].

Conclusion

There may be several mechanisms that are involved in the pathogenesis of osteonecrosis. Corticosteroid reduces the proliferative activity of MSCs. It down-regulates osteoblast transcription factor gene expression such as BMPs, decreases angiogenesis by suppressing HIF1 α and VEGF, and up-regulates adipocyte transcription factor expression. Consequently, the action impairs the differentiation of MSCs and decreases blood supply, leading to bone cell death.

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