Neuroendocrinology Letters Volume 42 No. 3 2021
ISSN: 0172-780X; ISSN-L: 0172-780X; Electronic/Online ISSN: 2354-4716
Web of Knowledge / Web of Science: Neuroendocrinol Lett
Pub Med / Medline: Neuro Endocrinol Lett

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ORIGINAL ARTICLE

Dexamethasone induce osteoblast apoptosis in a duration- and dose-dependent manner

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Submitted: 2021-05-09 Accepted: 2021-06-03 Published online: 2021-06-03

Key words: **Dexamethasone; apoptosis; duration-dependent; dose-dependent; osteoblast**

Neuroendocrinol Lett 2021; **42**(4):236–244 **PMID:** 34436844 NEL420421A03 © 2021 Neuroendocrinology Letters • **www.nel.edu**

Abstract

OBJECTIVES: Osteoblasts play an important role in the process of osteogenesis and prevention of osteonecrosis. Dexamethasone, a type of glucocorticoids (GCs), induce apoptosis of osteoblasts and lead to the occurrence of non-traumatic osteonecrosis. This study aimed to explore the effects of different doses and duration of Dexamethasone on osteoblast apoptosis of rats *in vitro*.

METHODS: Proliferation and apoptosis of osteoblasts after Dexamethasone treatment were detected using cell counting kit-8 (CCK-8) assay and FITC-Annexin V/PI staining. The expressions of caspase-3 and -9 in osteoblasts after Dexamethasone treatment were analyzed using western blotting and qRT-PCR. Dexamethasone remarkably inhibited proliferation and induced apoptosis of osteoblasts in a dose-and duration-dependent manner.

RESULTS: As the intervention time extended, the expression of caspase-3 mRNA and caspase-9 mRNA in different Dexamethasone groups gradually increased in a duration-dependent manner. With the same time of intervention (12h, 24h, 48h), the expression of caspase-3 and -9 mRNA gradually increased in a dose-dependent manner. After treated with $5 * 10^{-8}$M, $5 * 10^{-7}$M, $5 * 10^{-6}$M and $5 * 10^{-5}$M Dexamethasone for 24 hours, the expression of cleaved caspase-3 and -9 protein increased in a dose-dependent manner.

CONCLUSION: Dexamethasone can induce osteoblast apoptosis in a duration- and dose-dependent manner.

Abbreviations:

GIOP	- Glucocorticoid-induced osteoporosis
GC	- Glucocorticoid
Dex	- Dexamethasone
ALP	- Alkaline phosphatase
COL-1	- Collagen I
OB	- osteoblast
OC	- osteoclast
OCN	- osteocalcin

INTRODUCTION

Osteoporosis is a systemic metabolic disease characterized by low bone mass and destruction of bone microstructure. It can be divided into two major categories: primary and secondary osteoporosis. Glucocorticoid-induced osteoporosis (GIOP) is the most common form of secondary osteoporosis, and its incidence ranks the third among osteoporosis, second only to postmenopausal osteoporosis and senile osteoporosis (Soen

& Tanaka 2005; Kok & Sambrook 2009; Whittier & Saag 2016). Osteoporosis complicated with fracture seriously affects patient quality of life and longevity, and increases the burden on society and families.

Glucocorticoid (GC) is one of the most commonly used drugs in clinic. Because of its good anti-inflammatory, anti-rheumatic, anti-shock and immunosuppressive effects, it is widely used in the treatment of rheumatic diseases, respiratory diseases, skin diseases, kidney diseases and cancer, and also plays an important role in bone remodeling (Canalis *et al.* 2007; Buttgereit *et al.* 2008; Ferris & Kahn 2012; Bultink *et al.* 2013). However, long-term use of GC will lead to many complications, among which osteoporosis, especially osteoporotic fracture is the most serious complication. Bone mass loss began in a few weeks after GC treatment; in the first three months, the rate of bone mass loss was the fastest followed by continuous bone loss, then slowed down relatively one year later (Lo Cascio *et al.* 1984; LoCascio *et al.* 1990). GIOP is the most common cause of osteoporosis in people aged 20-45 years ([Alesci *et al.* 2005]). According to incomplete statistics, about 3% of the global population over 50 years old use GC, 80 to 5.2% (Kanis *et al.* 2004). In a large sample population survey abroad, it was found that an average of 0.75% of the people were treated with long-term oral GC, and more than 40% of the people who used GC had bone mass loss (den Uyl *et al.* 2011; Fardet *et al.* 2011). The occurrence of GIOP is closely related to the dose of hormone (Ruiz-Irastorza *et al.* 2012), and high-dose and long-term hormone therapy are more likely to develop osteoporosis and fracture (van Staa *et al.* 2002). With daily cortisone dose increased from 2.5mg to 7.5 mg, the corresponding risk of vertebral fracture increased from 1.55 times to 5.18 times (Dore 2013). However, even if a small dose of GC can also cause GIOP. Osteoporotic fractures of vertebrae and hip joints may also occur if taking 2.5mg prednisone daily (Van Staa *et al.* 2000; van Staa *et al.* 2005). At present, GIOP has become a serious public health problem all over the world with high lethality and disability rate.

Although the adverse effects of high-dose GC on bone have been confirmed for more than 60 years, the specific effect and mechanism of GC on osteoblasts and osteoclasts have not been fully clarified, so it is impossible to carry out effective prevention and treatment. It has been found that GC affects calcium and phosphorus metabolism in many ways. GC can reduce intestinal calcium absorption and renal calcium and phosphorus reabsorption, increase parathyroid hormone synthesis and sensitivity, and decrease calcitonin secretion and sex hormone levels. Bone mass is mainly controlled by the balance between bone formation by osteoblasts and bone resorption by osteoclasts, as well as any factor that leads to the decrease of bone formation or the increase of bone resorption will lead to osteoporosis. GC has a direct effect on bone, which can increase the activity of osteoclasts (osteoclasts, OCs), prolong the life span of osteoclasts, and enhance bone resorption, reduce the number and function of osteoblasts (osteoblasts, OBs), induce osteoblast apoptosis and inhibit bone formation at the same time (Pereira *et al.* 2002; Lane *et al.* 2006; Espina *et al.* 2008). Osteoblasts are the main targets of GC (Gu *et al.* 2005). Clinical studies found that the number of osteoblasts in bone tissue of GIOP patients decreased, and the apoptosis rate of osteoblasts increased compared with the control group (Weinstein *et al.* 1998). *In vivo* experiments also showed that the apoptosis rate of osteoblasts in CD-1 mice treated with 1 mg/kg/d of Dexamethasone (Dex) for 72 hours was 8 times higher than that of the control group (Gohel *et al.* 1999). *In vitro* experiments have confirmed that Dex can induce osteoblast apoptosis by the intervention of mouse calvaria osteoblast line MLO-Y426 (Plotkin *et al.* 2002). Another study reported that after the intervention of osteoblasts with Dex, the apoptosis of osteoblasts decreased (Davies *et al.* 2002). Therefore, the effect of GC on osteoblasts are yet to be fully elucidated. Most of the previous studies on osteoblasts by GC are aimed at a single hormone dose or a single action time, but it is not clear whether the apoptosis of osteoblasts will change under different hormone doses and different action time.

In order to clarify the specific effect of GC on osteoblasts, Dex was used to intervene with rat osteoblasts isolated and cultured *in vitro* with different doses and different action times. The changes of osteoblast function and the expression of genes and proteins related to osteoblast apoptosis were observed. Possible mechanism of osteoblast apoptosis in GIOP was discussed so as to provide a theoretical basis for seeking new methods of prevention and treatment of GIOP in clinic.

MATERIALS AND METHODS

Reagent

Dex (Sigma), BCIP-NBT staining kit, Annexin V-FITC/PI apoptosis detection kit of (Biyuntian Biotechnology) Co., Ltd. (Japanese colleague), skim milk powder (Inner Mongolia Yili Company), rabbit anti-rat caspase9 monoclonal antibody (abcam), rabbit anti-rat caspase3 monoclonal antibody (CST), rabbit anti-rat β-actin monoclonal antibody (CST) horseradish peroxidase labeled sheep anti-rabbit second anti-(CST), Trizol (Invitrogen company), reverse transcription kit (Fermentas), PCR primers of DreamTaq Green PCR Master Mix (TaKaRa Bao Biology Co., Ltd.: primers were designed according to the gene sequences of rat ALP, 0.1% collagenase (Sigma-Aldrich, MO, USA), caspase-3 and -9 in Gene Bank, which were designed and synthesized by Bao Bioengineering (Dalian) Co., Ltd. Dulbecco's modified Eagle's Medium/Nutrient Mixture F-12 (DMEM/F-12, Gibco, Life Technologies, CA, USA) fetal bovine serum (FBS, Hyclone, UT, USA), 100 U/ml penicillin100 μg/ml streptomycin solution (Hyclone, UT, USA) and 1 mM glutamine

(Sigma-Aldrich, MO, USA) cell counting kit-8 (Dojindo Molecular Technologies, Inc., Kumamoto, Japan)

Isolation and culture of osteoblasts

Primary osteoblasts were obtained from calvarial bones of newborn SD rat by tissue pieces-stick method. Briefly, the calvarial bones were dissected and chopped into pieces. The bone pieces were digested with 0.1% collagenase in a water bath for 2h at 37 °C. Cell suspension was harvested and washed with phosphate buffered saline (PBS) for three times. After that, the primary osteoblasts were cultured in Dulbecco's modified Eagle's Medium/Nutrient Mixture F-12 containing with 10% (v/v) fetal bovine serum, 100 U/ml penicillin, 100 μg/ml streptomycin solution and 1 mM glutamine at 37 °C with 5% CO_2 in a humidity incubator. After identified by Alizarin red staining and alkaline phosphatase staining, the third passage osteoblasts were used in this experiment. Osteoblasts were treated with 5 * 10^{-8}M, 5 * 10^{-7}M, 5 * 10^{-6}M and 5 * 10^{-5}M Dex in an environment of 37°C with 5% CO_2 for 12h, 24h and 48h, respectively. All procedures used in this study were approved by the Ethics Committee for Animal Experimentation at the Guangxi Medical University (SCXK Gui 2014-0002).

Cell proliferation assay

Cell proliferation was assessed using a cell counting kit-8. Briefly, osteoblasts ($2*10^4$) were seeded in 96-well plates, incubated overnight and treated with different concentrations of Dex for 12h, 24h, 48h. Ten microliters of cell counting kit-8 solution was added to the wells, and the cells were incubated for 1.5h at 37 °C in the incubator. The absorbance values at 450 nm were measured using a Multi-Volume Spectrophotometer System.

Apoptosis assay

Fluorescein isothiocyanate (FITC)-conjugated Annexin V and propidium iodide (PI) staining was used to determine the apoptosis of primary osteoblasts. Briefly, primary osteoblasts were seeded into 24-well plate with $1*10^6$ cells per well and treated by Dex for 24 h. After that, adherent and floating cells in each well were harvested and washed with PBS for three times. 100μL cells were incubated with Annex in V-FITC 5μL and PI 10μL (Beijing Bio sea Biotechnology, Beijing, China) for 10 min at room temperature in the dark. The rates of cell apoptosis were recorded using flow cytometer (Bender Metasystems, CA, USA).

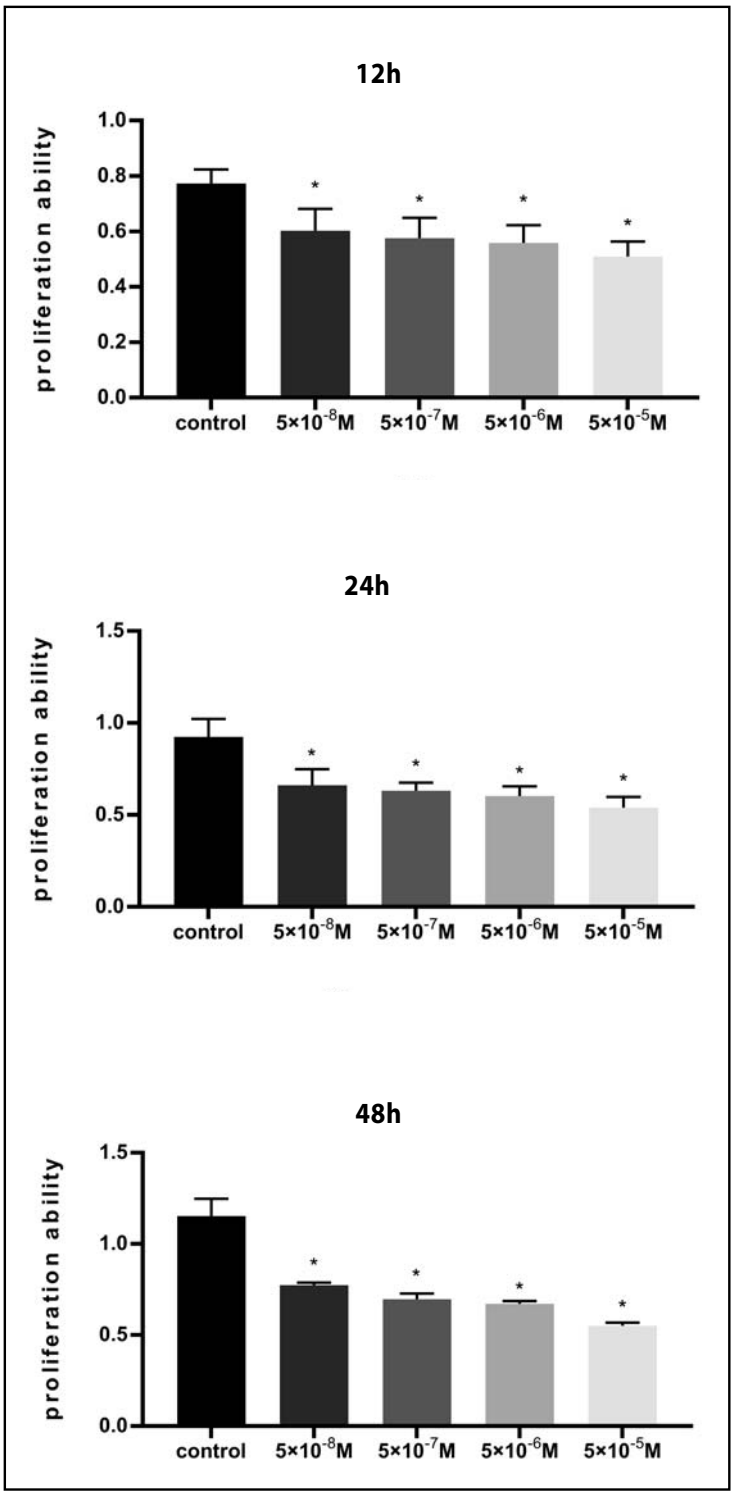

Fig. 1a. Dexamethasone inhibits proliferation of osteoblasts. CCK8 to study the changes of osteoblast proliferation of SD rats treated with different concentrations of 5 * 10^{-8}M, 5 * 10^{-7}M, 5 * 10^{-6}M and 5 * 10^{-5}M Dex for 12h, 24h and 48h. The proliferation of osteoblasts decreased gradually with the increase of intervention and concentration (b). *p<o.o5

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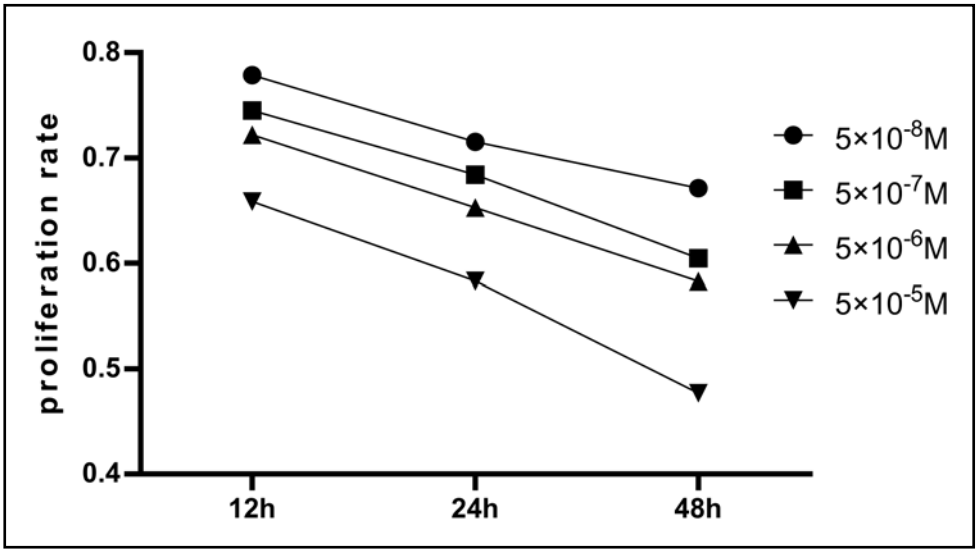

Fig. 1b.

Western blotting

Osteoblasts were treated with Dex (5 * 10^{-5}M) in an environment of 37°C with 5% CO_2 for 24h. the protein was extracted from osteoblasts using a mixture of radio immune precipitation assay (RIPA) buffer and phenyl methane sulfonyl fluoride (PMSF) (Beyotime, China). The BCA protein assay kit (Beyotime, China) was used to analyze the protein concentration. The SDS-PAGE loading buffer (4X) (Beyotime, China) was then mixed with the protein sample according to the instructions. 40ug of the total protein was separated into multiple target protein via 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS PAGE) and then transferred to a polyvinylidene difluoride membrane (Bio-Rad, USA). The 5% defatted milk was used to block membranes for 1h in room temperature, and then the membranes were immersed in the primary antibodies diluted solution overnight at 4 °C, including antibodies against β-actin (1:1000), Cleaved-caspase3 (1:1000), and Cleaved caspase9 (1:1000). Finally, the membranes were incubated with the respective secondary antibody (1:5000) at room temperature for 1 hours. We repeated the experiment three times.

Quantitative reverse transcription PCR (qRT-PCR)

Total RNAs in primary osteoblasts were isolated using Triazolo Reagent (TaKaRa) according to the manufacturer's instruction. Single stranded cDNA was subsequently synthesized using PrimeScript RT (TaKaRa). For detection the relative expression of caspase3 and caspase9, One-Step qRT-PCR kit (Invitrogen, CA, USA) was used following with the manufacturer's protocol. The expression of β-actin was acted as an internal control.

The primers for caspase3 were: forward primer 5′- CAGTCAGAGCGTAAGGAAAGGAG -'3, reverse primer 5′- GGACATCATCCACACAGACCA -'3. The primers for β-actin were: forward primer 5′- GGCACAGTCAAGGCTGAGAATG-'3, reverse primer 5′- ATGGTGGTGAAGACGCCAGTA. The primers for caspase9 were: forward primer5′-ACTGCTTCCCAGACCCACA-'3, reverse primer5′-CGAGACCTTGGAACACAGAGAA -'3.

Statistical analysis

All experiments in this paper were repeated at least three times. Data were presented as mean ± standard deviation (SD). SPSS 19.0 software (SPSS Inc, IL, USA) was used for statistical analysis. The significance between two groups was determined using Student t test. One-way analyses of variance were used for multiple comparisons.

RESULTS

Identification of osteoblasts

We used Alkaline phosphatase staining and calcium nodule staining of osteoblasts. Osteoblasts can synthesize alkaline phosphatase. BCIP/NBT (tetrazolitroblue) is the substrate of alkaline phosphatase. Under the catalysis of alkaline phosphatase, BCIP is hydrolyzed to produce a highly reactive product, which reacts with NBT to form insoluble dark blue or blue-black tetrazolium nitro-triphenylmethyl ester. Under the microscope, five evenly distributed visual fields were selected to count 400 cells, and it was found that the proportion of positive cells stained dark blue or blue-black accounted for 95%, as shown in figureS1A. Under the condition of continuous culture, osteoblasts will mineralize and form calcium nodules, which are orange-red by alizarin red staining, as shown in figureS1B.

Dex inhibited proliferation and induced apoptosis of osteoblasts

At first, we treated osteoblasts with different concentrations of Dex (5 * 10^{-8}M, 5 * 10^{-7}M, 5 * 10^{-6}M and

5 * 10^{-5}M) for 12h, 24h, 48h. The cell proliferation was examined. As shown in Fig1a, compared with the control group, osteoblasts proliferation was significantly reduced at each concentration with the same intervention time. We can see that Dex significantly decreased the number of surviving osteoblasts in a dose-dependent manner. At the same concentration of Dex, the proliferation rate of osteoblasts decreased gradually in a duration-dependent manner (Fig1b).

FITC-Annexin V/PI staining showed that the apoptosis rate of osteoblasts was remarkably enhanced after Dex treatment as evidenced by the rate of osteoblast apoptosis was increased (Fig2). Different concentrations of Dex (5 * 10^{-8}M, 5 * 10^{-7}M, 5 * 10^{-6}M and 5 * 10^{-5}M) were used to interfere with osteoblasts. The apoptosis rates of osteoblasts treated with 5 * 10^{-8} M Dex for 12 h, 24 h and 48 h were 7.8%, 11.3% and 26%, respectively; The apoptosis rates of osteoblasts treated with 5 * 10^{-7} M Dex for 12 h, 24 h and 48 h were 14%, 30% and 33.6%, respectively; 5 * 10^{-6} M Dex treated osteoblasts for 12 h, 24 h and 48 h, the apoptosis rates of osteoblasts were 21.6%, 45.7% and 42.4%, respectively; The apoptosis rates of osteoblasts treated with 5 * 10^{-5} M Dex for 12 h, 24 h and 48 h were 45.2%, 52% and 79%, respectively. The results showed that under the action of the same concentration of Dex, the apoptosis rate of osteoblasts increased gradually in a duration-dependent manner ($p< 0.01$). At the same time of intervention, the apoptosis rate of osteoblasts increased in a concentration-dependent manner ($p< 0.01$).

Dex induces caspase3 and caspase9 mRNA expression in osteoblasts in a duration-dependent and dose-dependent manner

Osteoblasts were treated with 5 * 10^{-5}M, 5 * 10^{-6}M, 5 * 10^{-7}M and 5 * 10^{-8}M Dex for 12h, 24h and 48h respectively. At the same time point, with the increase of Dex concentration, the expression of caspase3 mRNA increased gradually, and the expression of caspase3 mRNA in 5 * 10^{-5}m and 5 * 10^{-6}m Dex group was significantly higher than that in the control group. ($P<0.01$) Under the action of the same concentration of Dex, the expression of caspase3 mRNA increased gradually with the extension of intervention time, in a duration-dependent manner. ($p<0.05$) (Fig3a, 3b)

At the same time point, the expression of caspase9 mRNA increased gradually with the increase of Dex concentration. Under the intervention of the same concentration of Dex, the expression of caspase9 increased gradually with the extension of intervention time. (Fig. 3c, 3d)

Dex induces caspase3 and caspase9 expression in osteoblasts in a dose-dependent manner

Osteoblasts were treated with 5 * 10^{-8}M, 5 * 10^{-7}M, 5 * 10^{-6}M and 5 * 10^{-5}M Dex for 24 h. The expression of caspase-3 protein and caspase-9 protein increased in a concentration-dependent manner. (Fig 3e, 3f)

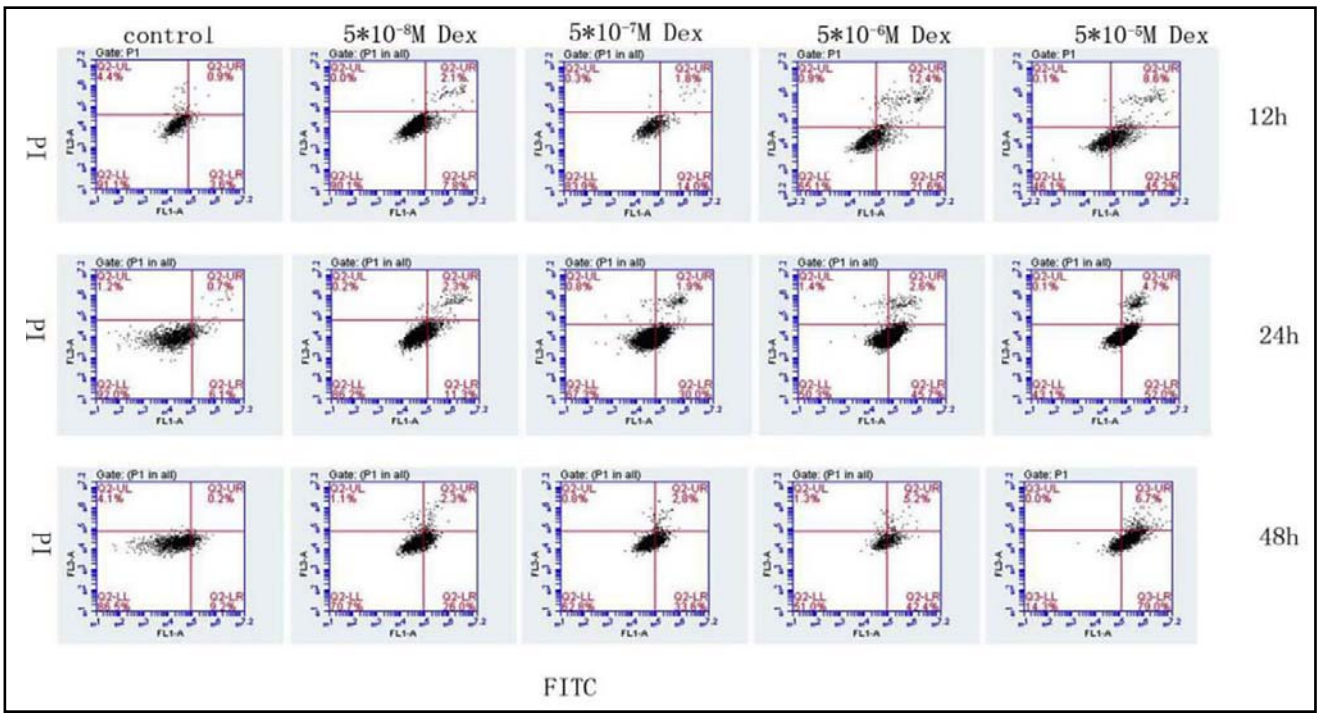

Fig. 2. Dexamethasone induces apoptosis of osteoblasts
FITC-Annexin V/PI staining showed that the apoptosis rate of osteoblasts was remarkably enhanced after Dex treatment. The apoptosis rate of osteoblasts increased with the increase of intervention and concentration. *p<o.o5

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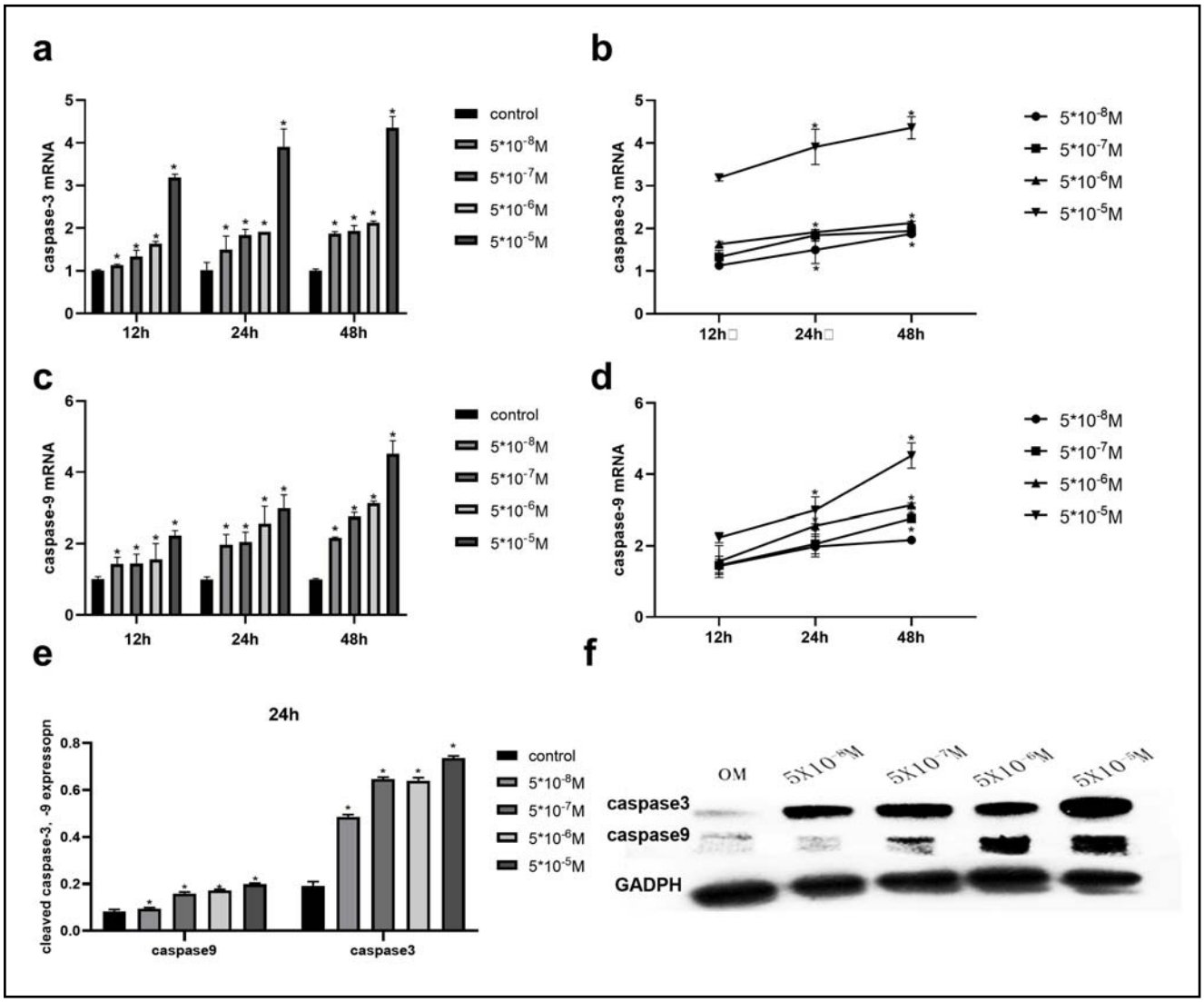

Fig. 3. Dexamethasone induces caspase3 and caspase9 expression in osteoblasts in a duration-dependent and dose-dependent manner

Osteoblasts were treated with 5 * 10^{-5}M, 5 * 10^{-6}M, 5 * 10^{-7}M and 5 * 10^{-8}M Dex for 12h, 24h and 48h respectively. the expression of caspase3 mRNA (a, b) and caspase9 mRNA (c, d) increased gradually with the extension of intervention time and concentration. The expression of caspase-3 protein and caspase-9 protein increased with the extension concentration after treated for 24h (e, f). *p<o.o5

DISCUSSION

With the advent of an aging society, osteoporosis and fractures caused by osteoporosis have become an important public health problem that needs to be solved urgently. Osteoporosis is defined as a systemic bone disease characterized by low bone mass, destruction of bone microstructure, increased bone brittleness and prone to fracture. GC can be secreted by the fascicular zone of the adrenal cortex and can also be synthesized by chemical methods (Kerr *et al.* 1972). In many medical fields, GC is a commonly used drug in clinical treatment of a variety of diseases. However, the use of GC in the treatment of diseases also leads to secondary osteoporosis, which is called glucocorticoid-induced osteoporosis (GIOP). GC is one of the main causes of drug-induced osteoporosis(Guanabens *et al.* 2014; Briot & Roux 2015). Osteoporotic fracture is the most serious complication caused by GIOP, and it is also the main cause of death in elderly women and men. (Johnell *et al.* 2005) Clinical observation showed that among patients with long-term exposure to high dose of GC, there are 30% developed osteoporosis and 20% developed osteoporotic fractures (den Uyl *et al.* 2011; Weinstein 2011).

A number of different approaches have been developed to study osteoblasts in vitro, including bone organ cultures, primary cell cultures, and immortalized osteoblast-like cell lines, and the isolation and culture of primary osteoblasts from calvaria of rats was the preferable way to study osteoblasts in vitro (Orriss *et al.* 2012). In this study, primary osteoblasts were extracted from the calvaria of SD rats by enzyme digestion. First of all, we used CCK8 to study the changes of osteoblast proliferation of SD rats treated with different concentrations

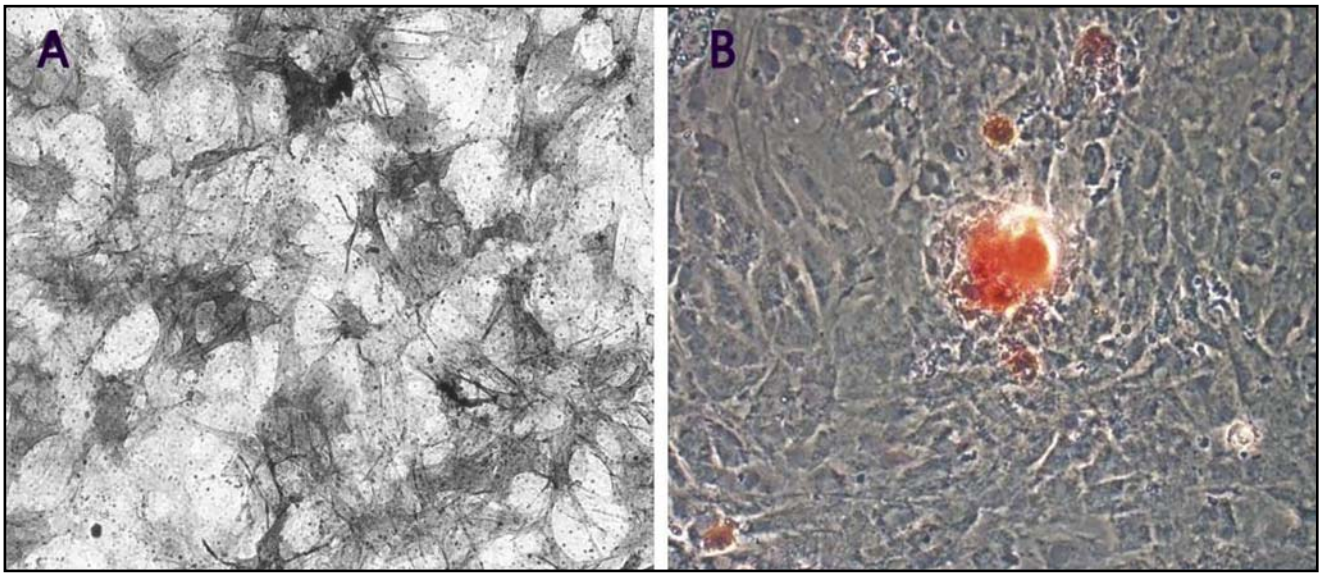

Fig. S1. A) Osteoblast alkaline phosphatase staining (inverted phase contrast microscope, (*100 times)
B) Osteoblast calcium nodules staining (inverted phase contrast microscope, (*200 times)

of 5 * 10^{-8}M, 5 * 10^{-7}M, 5 * 10^{-6}M and 5 * 10^{-5}M Dex for 12h, 24h and 48h. The results showed that Dex could inhibit the proliferation of osteoblasts in different degrees, and the proliferation of osteoblasts decreased gradually with the increase of intervention and concentration. Among them, 5 * 10^{-5}M Dex has the strongest inhibitory effect on the proliferation of osteoblasts. In previous studies, it has also been shown that the use of MTT the effects of 50uM, 125uM and 250uM DEX on MC3T3-E1 mouse osteoblast lines were observed. The results showed that the higher the concentration of Dex, the stronger the inhibitory effect on osteoblast proliferation (Kim *et al.* 2015). Above studies showed that Dex inhibited the proliferation of osteoblasts in a time-and duration-dependent manner.

Apoptosis, also known as type I programmed cell death, can be distinguished from other types of cell death by biochemical characteristics and cell morphology. These characteristics include cell contraction, chromatin condensation, nuclear fragmentation, membrane blistering and apoptotic body formation (Kerr *et al.* 1972). These apoptotic bodies are then swallowed by macrophages or adjacent cells. There is no cell leakage in the process of apoptosis and basically no inflammatory reaction, so apoptosis has little effect on nearby cells. Apoptosis plays an important role in maintaining the health and normal growth of tissue (Kale *et al.* 2012). Apoptosis also plays an important role in the occurrence and development of GIOP (Lin *et al.* 2014). It destroys the balance between cell death and cell proliferation, resulting in the decrease of proliferation and differentiation of normal osteoblasts, and the decrease of bone formation, which leads to the occurrence of osteoporosis. It has been found that long-term use or high dose of GC promoted osteoblast apoptosis (den Uyl *et al.* 2011)-(Weinstein 2011). In this study, Annexin V-FITC/PI double staining flow cytometry was used to detect the osteoblast apoptosis after treated with different concentrations of Dex for different time. The results showed that 5 * 10^{-8}M to 5 * 10^{-6}M Dex could induce osteoblast apoptosis after treated for 12h, 24h and 48h, and the apoptosis rate of osteoblasts increased gradually with the increase of Dex concentration and intervention time, indicating that glucocorticoid promoted osteoblast apoptosis in a time-and concentration-dependent manner. Our research results are consistent with the above research. However, some studies have pointed out that Dex had no effect on osteogenic apoptosis. 1 * 10^{-9}M to 1 * 10^{-6}M Dex was used to treat human osteosarcoma MG-63 cell line for 48 hours, and it was found that Dex failed to change spontaneous apoptosis of MG63 determined by 51Cr release assay (Nakashima *et al.* 1998). The results of Chae *et al.* also show that Dex can inhibit osteoblast apoptosis induced by TNF-α (Chae *et al.* 2000). Different studies have found that GC has different effects on osteoblast apoptosis, which may be related to the type, dose, action time, action environment of GC and methods of apoptosis detection.

Caspases is a group of aspartate proteolytic enzymes containing cysteine. Recent studies have found that the caspases family played a very important role in mediating apoptosis. The apoptosis of osteoblasts may be due to the activation of caspases. Caspase-3 exists in an inactive zymogen form and plays a key role in cell apoptosis after activation. It is a critical protease in apoptosis and is also the common downstream target of each apoptotic pathway (Guo *et al.* 2013). Caspase-9, first activated by cytochrome c in the cytoplasm, is the initiating caspase protein of the caspase cascade reaction, and the activated caspase-9 subsequently activates the executioner caspase-3. Some studies have shown

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that the activation of Caspase-3 is involved in the apoptosis of thymocytes induced by GC (Marchetti *et al.* 2003), suggesting that the activation of caspase-3 may also be involved in the apoptosis of osteoblasts induced by GC. We used real-time fluorescence quantitative PCR to detect the gene expression of apoptosis markers caspase-3 and -9. The results showed that the expression of caspase-3 and -9 mRNA increased gradually with the increase of concentration and time of Dex, indicating that Dex promoted osteoblast apoptosis in a concentration-and duration-dependent manner. Then Western blotting was used to detect the expression of cleaved caspase-3 and -9 in osteoblasts treated with different concentrations of Dex for 24 hours. The results showed that the expression of cleaved caspase-3 and -9 protein gradually increased in a concentration-dependent manner. Some studies have found that GC promotes the expression of cleaved Caspase-3 and -9, which leads to osteoblast apoptosis (Lin *et al.* 2014; Kim *et al.* 2015). Sun *et al.* showed that different concentrations of GC promoted the expression of cleaved caspase-3 and -9 in a concentration-dependent manner (Yun *et al.* 2009). Our results are consistent with those of Sun *et al.*

To sum up, GC may reduce the number of osteoblasts in a duration- and dose-dependent manner a by promoting osteoblast apoptosis, and lead to the occurrence of GIOP (Rat 200mg/ kg•d is equivalent to human 2000mg/ 70kg•d). At present, the commonly used drugs for the treatment of GIOP include bisphosphonates, teriprazeptide, denosumab and so on (Davies *et al.* 2002), but there are many side effects of bisphosphonates, while tripazeptide and denosumab are very expensive, so it is difficult to meet the treatment requirements of patients. It is necessary to develop drugs with low side effects and suitable price for the treatment of GIOP (Kim *et al.* 2014). In the future, it is expected to develop new drugs to inhibit osteoblast apoptosis so as to treat GIOP.

DISCLOSURE

None of the authors have any potential conflicts of interest associated with this research.

FUNDING

This study was supported by National Natural Science Foundation of China [Grant No. 81760165 and 81260142].

AUTHOR CONTRIBUTIONS

Xintong Guo:
Conceptualization, Methodology, Investigation, Formal analysis, Writing – Original Draft, Writing – Review and Editing.
Xiangying Ding:
Conceptualization, Methodology, Investigation, Formal analysis, Writing – Original Draft, Writing – Review and Editing.
Qinpei Ding:
Writing – Review and Editing.
Min Liang:
Writing – Review and Editing.

DATA AVAILABILITY STATEMENT

The datasets generated during and/or analyzed during the current study are available from the corresponding author on request.

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