SciMedCentral

JSM Clinical Pharmaceutics

Research Article

Reversing Effects of Bisphosphonates on Glucocorticoid-Induced Osteoporosis by Advancing Differentiation of Osteoblasts through Actions on PPAR γ and Hedgehog Pathways

Xiao Zhang[1]*, Ying Liu[2#], Ting Xu[1], Jian Chen[2], Yang Cui[1], Guang feng Zhang[1], Ling Li[1], and Liao Cui[2]

[1]Department of Rheumatology and Clinical Immunology, Guangdong General Hospital and Guangdong Academy of Medical Sciences, China

[2]Southern Medical University, Guang Zhou Avenue North, China

*Corresponding author

Xiao Zhang, Department of Rheumatology and Clinical Immunology, Guangdong General Hospital and Guangdong Academy of Medical Sciences, China, No. 106, Zhongshan, 2nd Road, Guangzhou, 510080, China, Email: 13922255387@163.com

Submitted: 17 July 2016

Accepted: 09 October 2016

Published: 18 October 2016

ISSN: 2379-9498

Keywords

- Glucocorticoid induced osteoporosis
- Bisphosphonates
- PPAR γ
- Hedgehog pathway

Abstract

Objective: To evaluate the effects of bisphosphonates on glucocorticoid-induced osteoporosis (GIOP) through PPAR γ and hedgehog pathways

Methods: Forty newly diagnosed systemic lupus erythematosus (SLE) patients were randomly divided into a control group (1mg/kg/d prednisolone) and an alendronate (ALN) group (1mg/kg/d prednisolone plus ALN 70mg/w). Expression of Gli1 and PPAR γ in bone marrow was detected before and after treatment. Human bone marrow stromal cells (hBMSC) were committed to either adipogenic or osteogenic differentiation, in the presence and absence of ALN, and supplemented with methylprednisolone (MP) (10^{-5} to 10^{-3}mmol/L). Untreated differentiating hBMSCs were used as the control. Expression of Gli 1/PPAR γ was measured by real-time quantitative RT-PCR and alizarin red/Oil red O staining was performed.

Results: Compared with the base line, the average integral optical density (IOD) of Gli 1 protein was significantly lower ($P<0.001$), while the average IOD of PPAR γ protein was significantly higher ($P<0.001$) after oral prednisolone therapy; compared with the control group, the average IOD of Gli 1 protein was significantly higher in the ALN group ($P=0.002$), while the average IOD of PPAR γ protein was significantly lower in the ALN group($P<0.01$). MP dose-dependently decreased Gli 1 expression ($P<0.05$) and mineralization, but the addition of ALN increased Gli 1 expression and mineralization. Dexamethasone stimulated PPAR γ expression and adipogenesis while ALN dose-dependently suppressed PPAR γ expression ($P<0.05$) and oil drop formation.

Conclusions: Glucocorticoids impaired osteoblasts differentiation through their suppression effects on the hedgehog pathway and stimulatory action on PPAR γ. Bisphosphonates reversed the osteogenesis impairment induced by glucocorticoids.

INTRODUCTION

Glucocorticoid-induced osteoporosis (GIOP) is one of the most prominent side effects of glucocorticoid (GC) therapy and is the main cause of secondary osteoporosis. Early epidemiological data revealed that 30% of patients on long-term GC therapy (>6 months) developed osteoporosis [1]. Although not all of the underlying mechanisms are precisely understood, it is generally accepted that reduced bone formation is the key process in GIOP. At the cellular level, GCs reduce bone formation by (1) inhibiting osteoblast differentiation, (2) inducing apoptosis of osteoblasts, and (3) stimulating bone marrow stromal cells (BMSC) to differentiate into adipocytes rather than osteoblasts.

Osteoblasts and adipocytes differentiate from a common pluripotent precursor, the mesenchymal stem cell (MSC). Studies have identified numerous transcription factors and multiple extracellular and intracellular signaling pathways that regulate the closely linked processes of adipogenesis and osteoblastogenesis, including the transcription factor peroxisome proliferator-activated receptor γ (PPAR γ), bone morphogenetic protein (BMP) pathway and the Wnt–β-catenin pathway [2,3]. However, few studies have investigated the effects of GCs on the hedgehog (Hh) pathway.

The Hh signaling pathway is indispensable for normal patterning of multicellular embryos and functions in

postembryonic development and in adult tissue homeostasis, including regulation of stem cell physiology [4,5]. It begins with the release of Hh protein that binds to the Patched (Ptch) receptor on Hh-responsive cells, relieving the suppression of Smoothened (Smo). Smo in turn activates an intracellular signaling cascade that results in activation of one or more Gli transcription factors, which in turn mediate the transcription of the Hh target genes, including Gli 1 and Ptch, the former one, transcription factor Gli 1 is the key regulator of the Hh signaling pathway. Although a direct promotive effect of Hh in rodent osteoblast differentiation has been documented [6-10], its effect on osteoblast differentiation in human cells is controversial. As far as we are aware, there are no published papers concerning the effect of Hh signaling on GIOP.

The American College of Rheumatology (ACR) has recommended bisphosphonates as first-line agents for the prevention and treatment of GIOP [11]. The benefits of bisphosphonates in GIOP have been attributed to their anti-resorptive effect, but there is little research focus on their effects in osteogenesis. A recent paper has reported that a reduction of bone formation plays an important role in GIOP and that bisphosphonates have an anabolic effect on osteoporosis [12, 13].Therefore, in the present study, the experiments were designed to elucidate the intervention effect of bisphosphonates on osteogenesis dysfunction GIOP, especially through PPAR γ and Hh pathways, to provide a further theoretical basis for their use in GIOP.

MATERIALS AND METHODS

Reagents and instruments

Rabbit anti-human Gli1 polyclonal antibody (Abcam Biotechnology UK ab92611); rabbit anti-human PPAR γ polyclonal antibody (Cell Signaling Technology); biotinylated sheep anti-rabbit IgG and diaminobenzidine (DAB)(Boster, Wuhan); indomethacin and dexamethasone (Sigma); fetal calf serum, insulin, 3-isobutyl-1-methylxanthine (IBMX)(Sigma Chemical); DAKO REAL En Vision kit (DAKO Corporation); normal goat serum (ZLI-9606 ZSGB-BIO, Beijing, China); antibody diluent (ZLI-9030 ZSGB-BIO, Beijing, China); hMSCs growth medium (OriCellTM, Cyagen); osteoinduction medium (Cyagen), sodium alizarin sulfonate (Cyagen); PrimeScript TM RT reagent Kit(Perfect Real Time)(TaKaRa Bio Inc); alendronate (ALN, Fosamax Merck); Leica fully automated microscope (model DMLA Leica Inc., Germany); Qwin software (ver. 2.3 Leica Inc., Germany); inverted microscope (IX70ISM, Olympus); CO_2 incubator, quantitative PCR (Thermal Cycler Dice™); Multi-Mode Micro plate Reader (SynergyTMH7 USA).

Patients and methods

After excluding the menopause, bone metabolic diseases, upper gastrointestinal disorders, diabetes and other ailments that can cause osteoporosis and severe disease activity (SLEDAI score ≥ 15), 40 patients admitted to the rheumatoid immunology department of Guangdong General Hospital from September 2010 to January 2012 with newly diagnosed systemic lupus erythematosus (SLE), with a base-line bone mineral density T score at both the lumbar spine and femoral neck >−2.0, that required long-term systemic GC therapy, were assigned to the trial. Diagnosis of SLE was made according to the 1982 criteria of the American College of Rheumatology (ACR). All patients provided written informed consent and the study protocol was approved by the research ethics committee of Guangdong General Hospital, Guangdong Academy of Medical Sciences (No. GDREC2010114H). After baseline bone specimens were collected from iliac crest, patients were randomly divided into two groups: the control group (n=20) received 1mg/kg/d prednisolone and the ALN group (n=20) received 1mg/kg/d prednisolone plus ALN (70mg/w) for 24 weeks. These drugs were administered at the same time that supplemental calcium and vitamin D therapy was initiated. After 24 weeks' therapy, a second biopsy was taken from the same region of each patient. The expression of PPAR γ/ Gli 1 was compared before and after GC therapy in the control group to investigate the effect of GC on osteogenesis. A similar comparison was also made between the control and ALN groups to evaluate the therapeutic effects of ALN on GIOP.

Bone tissue immunohistochemistry (IHC)

Bone specimens obtained from 2.2 were decalcified, embedded and 3 μm sections prepared. After incubation, dew axing, rehydration and antigen retrieval, the sections were rinsed in 3% hydrogen peroxide. Incubation was carried out in blocking serum and subsequently with the primary antibodies. The antibodies were diluted at 1:200 for Gli 1 and 1:100 for PPAR γ. Subsequently, slides were incubated with goat-ant rabbit IgG. The antigen-antibody binding was visualized with DAB acting as the chromogen. Sections were counterstained with hematoxylin. For image analysis, 4 sections were chosen from each patient and observed randomly at 400× magnification by 2 microscope operators. Absorbance (A) values of each section were detected by a LEICA Qwin microgramme analytical system (Leica Company, Shanghai, China), and the mean values were taken on behalf of the sample A value.

Differentiation of human bone marrow stromal cells (hBMSCs) and cell treatment

Human bone marrow was obtained from 5 consecutive traumatic patients (all were male, mean age 36 years, range 28-45) admitted to the orthopedics department of Guangdong General Hospital with approval and ethical clearance. Subjects did not have other bone disorders. Aspirated bone marrow was diluted with equivalent amounts of hBMSCs growth medium and incubated at 37°C with 5% humidified CO_2. To characterize the surface antigens of hBMSCs, immunostaining of CD29, CD34, CD44 and CD45 was performed on passage 2~4 cells. When cells reached 80-90% confluence and a purification rate of 99%, they were committed to osteogenicor adipogenic differentiation.

Identification of the effects of GCs and ALN on osteoblasts differentiation and activity: To investigate the effect of GCs on the Hh pathway, we used hBMSC special culture medium as the blank group, growth medium containing 0.1 μg/ml rh-SHHN cytokine as the control and rh-SHHN supplemented with increasing concentrations of methylprednisolone (MP) (10^{-5} to 10^{-3}mmol/L) as the treatment group.

Then we used osteogenesis induction media (OIM) instead of

rh-SHHN to study the intervention effect of ALN on GIOP. OIM supplemented with 10^{-3}mmol/L MP acted as the control group with the ALN treatment group exposed to10^{-9}mmol/L ALN.

Identification of the effects of GCs and ALN on adipocyte differentiation and activity: To study the effect of drugs on adipogenic differentiation, the culture medium was replaced with hBMSC growth media as the blank group, or using adipogenesis induction media (AIM, prepared with 1.0 μmol/L dexamethasone, 200 μmol/L indomethacin, 10 μg/ml insulin, 0.5 mmol/L 3-isobutyl-1-methylxanthine, 10% FCS) alone as the AIM group, or AIM with increasing concentrations of ALN. They were the low concentration (LC), 10^{-9}mol/L, moderate concentration (MC), 10^{-7}mol/L and high concentration (HC), 10^{-5}mol/L groups, respectively.

Quantitative real-time PCR

After osteogenic or adipogenic differentiation, total RNA was extracted with Trizol reagent according to the manufacturer's protocol. CDNA was synthesized using the Trizol protocol and quantitative real-time PCR was performed in triplicate by using SYBR Green I. The following primers were used: Gli 1 (5'-TGAACATTATGGACAAGTG-3', 5'-CCTGACTGGAGATATTGG-3') for all the groups in 2.4.1andPPARγ (5′-TGGAATTAGATGACAGCGACTT-3′, (5′-CTGGAGCAGCTTGGCAAACA-3′) for all the groups in 2.4.2

Alizarin red staining

Matrix mineralization of cell monolayers for all groups in 2.4.1 was detected by alizarin red staining. Twenty-one days after inducing osteogenic differentiation, hBMSCs were washed with PBS and fixed in 4% par formaldehyde in PBS for 15min. After washed with PBS, cells were stained with alizarin red dye 5 min and then copiously rinsed in PBS. The calcium phosphate precipitates were stained bright red.

Oil Red O Staining

After inducing adipogenic differentiation for 6 to 9 days, Oil red O staining to visualize adipocytes was performed for all the groups in 2.4.2. HBMSCs were washed with PBS and fixed in 10% formalin for 10min. After wash with PBS, cells were stained with Oil Red O for 15min and then washed with distilled water. Representative samples of treated cells were prepared, dipped in isopropyl alcohol and thoroughly rinsed. Absorbance (A) values were detected at a wave length of 515nm using a Multi-Mode Micro plate Reader.

Statistical analysis

SPSS ver. 17.0 software was used for statistical analysis. Kolmogorov-Smirnov (K-S) test was used for the normality test. Continuous and ordinal data are expressed as the mean ± standard error or the median and interquartile range. For *in vitro* studies, multi-group comparisons were made using analysis of variance (ANOVA) and two-group comparison of normally distributed data by a t-test. For *in vivo* study, a paired t-test was used to determine any significant differences of Gli 1 protein or PPAR γ protein between the baseline and the second biopsy readings within the control group; a 2-sample t test was performed on the mean a value of Gli 1 protein or PPAR γ protein between ALN group and control group. Values were considered to be significant when $P< 0.05$.

RESULTS

Baseline characteristics of patients

A total of 40 patients with recent-onset SLE were enrolled in the study. All patients were female, age range 16 to 51 years, with a SLEDAI score between 6 and 14. There were no significant difference in the age and SLEDAI scores between the control and ALN treatment groups (Table 1).

Expression of Gli 1 and PPAR γ in clinical samples

IHC showed that both the control and ALN groups had positive Gli 1 and PPAR γ protein expression in bone marrow. We first compared the protein expression before and after GCs therapy in the control group. The results showed that compared with base line, the average integral optical density (IOD) of Gli 1 protein was significantly lower (42.00 ± 5.83,n = 20, vs 60.80±12.08 n = 20, P<0.001), while the average IOD of PPAR γ protein was significantly higher (83.95±10.61, n = 20, vs 39.35 ± 11.50, n = 20,P<0.001) after oral prednisolone therapy. Next we compared the protein expression levels in the control and ALN treatment groups to investigate how ALN affected GIOP. As shown in Figure (1) and Figure (2), the bone tissue from the ALN treatment group had more Gli 1 protein particles but less PPAR γ protein particles than the control group. Compared with control group, the average IOD of Gli 1 protein was significantly higher in the ALN treatment group (51.48±5.61, n = 20, vs 42.00±5.83, n = 20, P=0.002), while the average IOD of PPAR γ protein was significantly lower in the ALN treatment group (73.62 ± 10.77, n = 20, vs 83.95±10.61, n = 20, P<0.01).

Effects of GCs and ALN on osteogenesis

We used rh-SHHN cytokine instead of OIM to investigated the

Table 1: Baseline characteristics of patients in the two groups.

Patient characteristics	Control	ALN	*P*
Female patients (*n*, %)	20(100)	20(100)	
Age(mean ± SD, years)	27.3±10.4	29.9±11.0	0.455
SLEDAI score	10.5±2.6	9.4±2.7	0.821

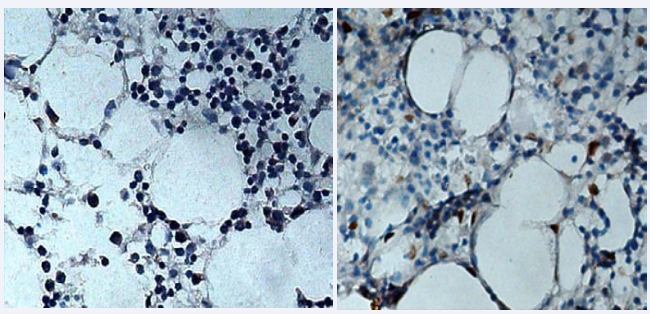

Figure 1 Immunohistochemistry results of Gli 1 protein in bone tissue. Gli 1 protein appears as the brown particles, mainly in the nucleus. A: bone tissue from the control group; B: bone tissue from the ALN treatment group (magnification, 400×).

potential effects of GCs on Hh signaling and mineralization. After 7 days treatment, compared with control group, the expression of Gli1 mRNA was significantly increased in SHHN group; adding MP into SHHN decreased in a concentration-dependent manner Gli 1 mRNA expression (P<0.05). This result correlated with alizarin red staining, which revealed much calcium nodus in the SHHN group, but no nodus in the control and MP groups (Figure 3).

Compared with the 10^{-3}mol/L MP group, the expression of Gli 1 mRNA significantly increased in the 10^{-3}mol/L MP+10^{-9}mol/L ALN group (Figure 4A) with alizarin red staining revealing that there was more calcium nodus in the ALN group (Figure 4B,C).

Effects of GCs and ALN on adipogenesis

GCs promoted the expression of PPAR γ and adipogenesis (Figure 5). Compared with the control group, the expression of PPAR γ mRNA was significantly increased in the AIM group (P<0.05, Figure (5A). Oil drops were found in the OIM group Figure (5B). Quantitative analysis of oil red O stain showed the mean A value in the AIM group was 0.46 ± 0.15. Also shown in Figure (5), compared to the AIM group, the expression of PPAR γ mRNA was significantly decreased in the high (10^{-5}mol/L) and moderate concentration (10^{-7}mol/L) ALN groups (P<0.05). Quantitative analysis of the oil red O stain showed that the mean a value of high, moderate and low concentrations in the ALN groups was 0.25±0.04, 0.37±0.14 and 0.40±0.14, respectively. Significant differences were found between the HC and AIM groups, and the MC and AIM groups (P<0.05).

DISCUSSION

Previous studies have reported that Hh promotes rodent osteoblast differentiation [6-10], but its effect on osteoblast differentiation in human cells remains controversial. It has been shown in humans that mutations in the Hh pathway, such as in brachydactyly type A1 and Gorlin syndrome, are associated with skeletal malformations [14]. Purmorphamine, an Hh agonist that directly targets smoothened transmembrane protein, increased ALP activity and bone-like formation in Osteoblasts differentiated from hBMSCs [15].However, a study conducted by Plaisant et al., found that osteoblast differentiation of hBMSCs was associated with a decrease in Gli 1 expression; the addition of purmorphamine to the osteogenic medium induced a 50% decrease in ALP activity and Gli 1 mRNA expression [16]. In a previous study, we investigated the effect of rh-SHHN on

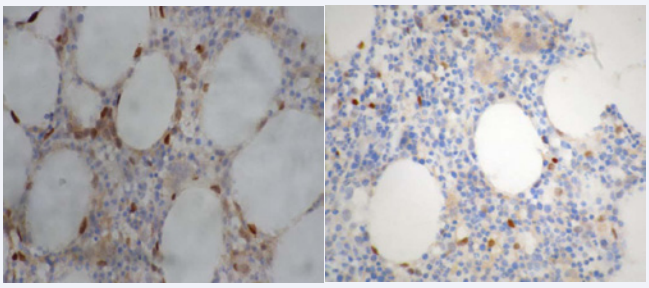

Figure 2 Immunohistochemistry results of PPAR γ protein in bone tissue. PPAR γ protein appears as the brown particles, seen mainly in the nucleus. A, bone tissue from the control group; B, bone tissue from the ALN treatment group (magnification 200×).

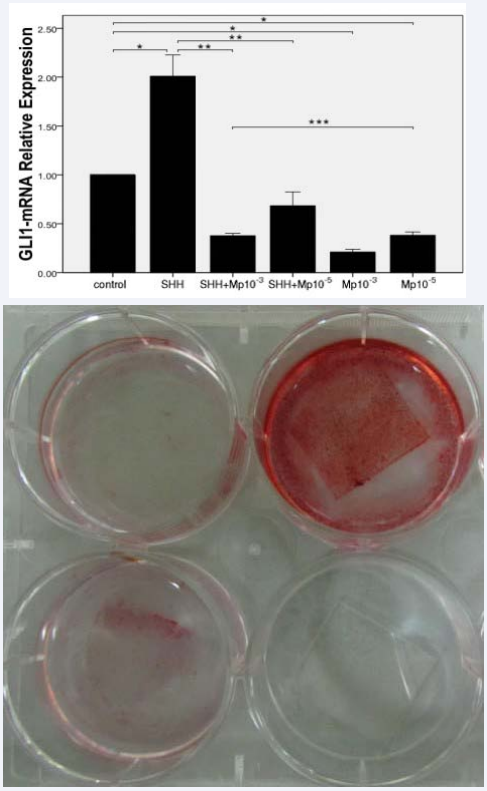

Figure 3 Effects of GCs on osteogenesis. A: Gli 1 gene expression analyzed by RT-PCR, data presented as the mean ± SEM (n=18; a: P<0.05 over control group; b: P<0.05 over rh-SHHN group; c: P<0.05 over 10^{-5}mol/L methylprednisolone (MP) group). B, C, D, E, mineralization was evaluated by alizarin red staining; B, control group; C, 10^{-3} mol/L MP group; D, rh-SHHN+10^{-3}mol/L MP group; E, rh-SHHN group.

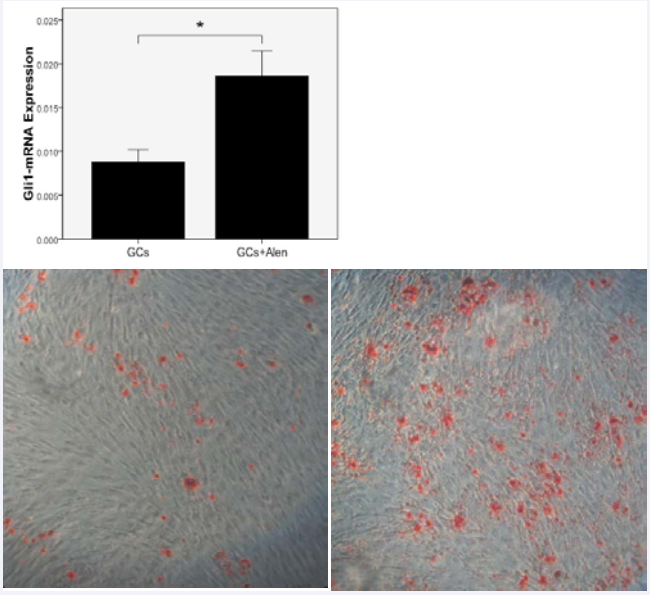

Figure 4 Effect of ALN on osteogenesis. A, expression of Gli1-mRNA by RT-PCR (* P<0.05). B and C, mineralization evaluated by alizarin red staining (magnification 100×); B, OIM+10^{-3}mol/L MP group; C, OIM+10^{-3}mol/L MP+10^{-9}mol/L ALN group.

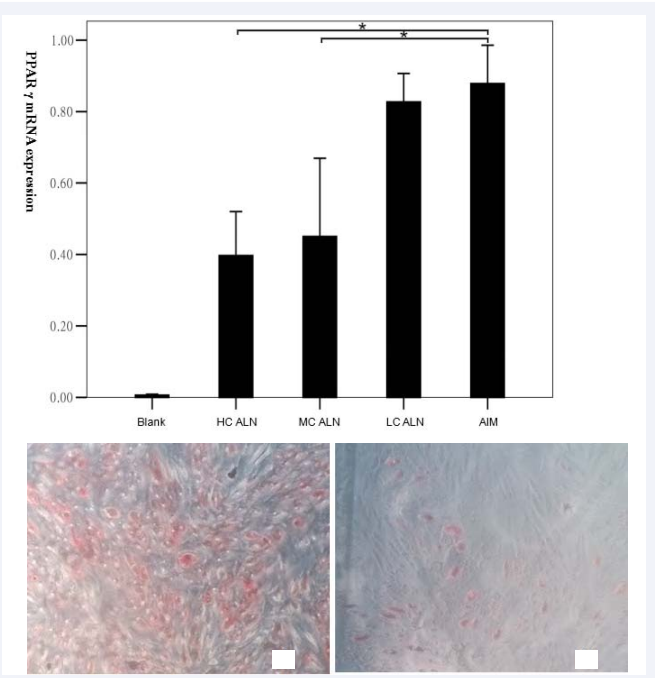

Figure 5 Effect of GCs and ALN on adipogenesis. A, PPAR γ gene expression analyzed by RT-PCR (n=18; $^{*}P$<0.05 compared to the adipogenesis induction media (AIM) group. HC, high concentration, 10^{-5}mol/L; MC, moderate concentration, 10^{-7}mol/L; LC, low concentration,10^{-9} mol/L. B and C, adipogenesis was evaluated by oil red O stain (magnification, 100×). B, AIM group; C, HC ALN group.

osteogenesis of hBMSCs. We found that as a key cytokine of Hh signaling, rh-SHHN alone significantly stimulated hBMSC to express the Gli 1 gene and form more calcium nodus. Since Gli 1 is the key regulator of Hh signaling pathway, this result suggested that the Hh pathway promoted the osteogenic differentiation of hBMSC [17]. In the present study, we compared Gli 1 gene expression in the control and MP group and found that GC significantly suppressed Gli 1 gene expression as well as calcium nodus formation. Subsequent *in vivo* experiments showed that GC therapy significantly decreased Gli 1 protein expression in the bone marrow of patients. These results provide both *in vitro* and *in vivo* evidence that GC reduced osteogenic differentiation of hBMSC by inhibiting the Hh pathway.

PPAR γ has been shown to be expressed early in the adipocyte differentiation program Increasing evidence indicate that it plays an important role in the regulation of bone metabolism [18-20]. In the present study, we found that adding AIM (containing dexamethasone) significantly increased PPAR γ gene expression and the number of oil drops, which was in line with a previous finding that GC induced PPAR γ expression and promoted adipogenesis [2]. Our subsequent *in vivo* study showed that GC therapy increased PPAR γ protein expression in the bone marrow of patients, confirming that GCs up-regulated PPAR γ expression.

Bisphosphonates are potent inhibitors of osteoclast activity and are widely used clinically to prevent bone loss associated with conditions such as osteoporosis, Paget's disease and metastatic bone disease [21]. Traditionally, the bone protective actions of bisphosphonates have been exclusively attributed to their interference with osteoblast [22]. The inhibitory effects of bisphosphonates on Osteoblasts, including suppressing osteoclastogenesis, osteoblast activity and shortening of the osteoblasts lifespan are well documented, both *in vivo* and *in vitro* [21]. However, their effects on osteoblast functions have been less well studied and are considered to be more complex. Nonetheless, in recent years, studies have provided evidence that bisphosphonates also have anabolic effects on osteoblasts and osteocytes that promote bone formation. Im et al., found that bisphosphonates could increase osteoblast and osteoblast progenitor numbers in primary human trabecular cultures [23] .A study by Follet et al., found that bisphosphonates acted directly on osteoblasts and osteocytes to prolong their life span [24]. A study by von Fu et al. found bisphosphonates facilitated the differentiation of stem cells into an osteoblast lineage [12].

In contrast, others studies have reported inhibitory effects of bisphosphonates on osteoblast function and the mineral apposition rate [25,26]. Short-term treatment with micro molar concentrations of zoledronate has been reported to decrease proliferation, mineralization, viability and alkaline phosphatase activity in MC3T3-E1 and MG-63 cells [27-29]. Long-term, intermittent exposure to nanomolar concentrations of pamidronate and alendronate has been shown to inhibit bone nodule formation by mouse calvarial osteoblasts *in vitro* [30].

The exact mechanism of action of bisphosphonates on osteogenesis has not been fully elucidated. Studies have found that bisphosphonates promote the gene expression of BMP-2, which is a key osteogenic transcription factors in the BMP pathway [23]. It has been reported that nanomolar concentrations of ALN promote the differentiation of human mesenchymal stem cells into an osteoblastic lineage [12]. In a previous study, we added rh-SHHN cytokine and increasing concentrations (from10^{-9} to 10^{-5}mol/L) of ALN to hBMSCs culture medium to test the effects of bisphosphonates on the Hh pathway. We found that a low concentration (10^{-9}mol/L) of ALN significantly increased the expression of Gli 1 and calcium deposition while a high concentration (10^{-5}mol/L) had the opposite effect [17].This finding is in line with previous studies that showed 10^{-8}mol/L ALN promoted osteogenic differentiation of hBMSCs while a high concentration of bisphosphonates (>10^{-5}mol/L) inhibited osteogenic differentiation of hBMSC and decreased mineral deposition [31]. Taken together, these results suggest that bisphosphonates have a biphasic effect on the Hh pathway, just like their effect on bone formation, i.e., stimulatory at low concentrations and suppressive at high concentrations. In present study, we assessed the intervention effect of different concentration of ALN on GIOP through PPAR γ expression and Hh pathway. Both *in vitro* and *in vivo* the data showed that adding ALN to GC promoted Gli 1 expression and mineralization but stimulated PPAR γ expression and adipocyte formation. These results suggest that bisphosphonates could reverse decreased osteogenesis and increased adipogenesis induced by GCs thus facilitating the differentiation of stem cells into an osteoblast lineage.

CONCLUSION

Taken together, the present study clearly demonstrates that GCs impaired the differentiation of osteoblasts through

their stimulatory effects on the expression of PPAR γ and suppression effect of the Hh pathway. We also established that bisphosphonates can fully reverse the impaired differentiation of osteoblasts induced by GCs through the PPAR γ and Hh pathways. To the best of our knowledge, this is the first demonstration of the stimulatory effect of bisphosphonates on bone formation in GIOP.

ACKNOWLEDGEMENTS

The study was supported by a grant from Guangdong science and technology plan projects (2010B080701023). We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work. There are no professional or other personal interests of any nature in any product, service and/or company that could be construed as influencing the position presented in, or the review of, the manuscript.

REFERENCES

1. Gudbjornsson U Juliusson B, Gudjonsson F. Prevalence of long term steroid treatment and the frequency of decision making to prevent steroid induced osteoporosis in daily clinical practice. Ann Rheum Dis. 2002; 61: 32-36.
2. Shi XM, Blair HC, Yang X, Mc Donald JM, Cao X. Tandem repeat of C/EBP binding sites mediates PPAR gamma2 gene transcription in glucocorticoid-induced adipocyte differentiation. J Cell Biochem. 2000; 76: 518-527.
3. Pereira RC, Delany AM, Canalis E. Effects of cortisol and bone morphogenetic protein-2 on stromal cell differentiation: correlation with CCAAT-enhancer binding protein expression. Bone. 2002; 30: 685-691.
4. Lai K, Kaspar BK, Gage FH, Schaffer DV. Sonic hedgehog regulates adult neural progenitor proliferation *in vitro* and *in vivo*. Nature Neuroscience. 2003; 6: 21-27.
5. Machold R, Hayashi S, Rutlin M, Muzumdar, Nery S, Corbin J, et al. Sonic hedgehog is required for progenitor cell maintenance in telencephalic stem cell niches. Neuron, 2003; 39: 937-950.
6. Nakamura Takashi, Aikawa Tomonao, Motomi Iwamoto-Enomoto, Masahiro Iwamoto, Yoshinobu Higuchi , Pacifici Maurizio, et al. Induction of osteogenic differentiation by hedgehog proteins. Biochemical & Biophysical Research Communications. 1997; 237: 465-469.
7. Kinto N, Iwamoto M, Enomoto-Iwamoto M, Noji S, Ohuchi H, Yoshioka H, et al. Fibroblasts expressing sonic hedgehog induce osteoblast differentiation and ectopic bone formation. FEBS Lett, 1997; 404: 319–323.
8. Spinel la-Jaegle S, Rawadi G, Kawai S, Gallea S, Faucheu C, Mollat P, et al. Sonic hedgehog increases the commitment of pluripotent mesenchymal cells into the osteoblastic lineage and abolishes adipocytic differentiation. J Cell Sci. 2001; 114: 2085-2094.
9. Vander Horst G, Farih-Sips G Löwik CWGM Karperien M. Hedgehog stimulates only osteoblastic differentiation of undifferentiated KS483 cells. Bone, 2003, 33: 899-910.
10. Shimoyama A, Wada M, Ikeda F, Hata K, Matsubara T, Nifuji A, et al. Ihh/Gli2 signaling promotes osteoblast differentiation by regulating Runx2 expression and function. Mol Biol Cell. 2007; 18: 2411-2418.
11. Grossman JM, Gordon R, Ranganath VK, Deal C, Caplan L, Chen W, et al. American College of Rheumatology 2010 recommendations for the prevention and treatment of glucocorticoid-induced osteoporosis. Arthritis Care Res. 2010; 62: 1515-1526.
12. Duque G, Rivas D. Alendronate has an anabolic effect on bone through the differentiation of mesenchymal stem cells. J Bone Miner Res. 2007; 22: 1603-1611.
13. Gao B, Guo J, She C, Shu A, Yang M, Tan Z, et al. Mutations in IHH, encoding Indian hedgehog, cause brachydactyly type A-1. Nat Genet. 2001; 28: 386-368.
14. Beloti MM, Bellesini LS, Rosa AL. Purmorphamine enhances osteogenic activity of human osteoblasts derived from bone marrow mesenchymal cells. Cell Biol Int. 2005; 29: 537-541.
15. Plaisant M, Fontaine C, Cousin W, Rochet N, Dani C, Peraldi P, et al. Activation of hedgehog signaling inhibits osteoblast differentiation of human mesenchymal stem cells. Stem Cells. 2009; 27: 703-713.
16. Liu Y, Xiao Zhang, Guang-feng Zhang, Yang CUI, Yun-xia LEI, Ling LI, et al. Effects of glucocorticoid and bisphosphonates on Hedgehog signaling pathway in human bone mesenchymal stem cells and bone tissues. Chinese Journal of Rheumatology. 2013; 17: 760-763.
17. Chawla A, Schwarz EJ, Dimaculangan DD, Lazar MA. Peroxisome proliferator-activated receptor (PPAR) γ adipose-predominant expression and induction early in adipocyte differentiation. Endocrinology. 1994; 135: 798-800.
18. Akune T, Ohba S, Kamekura S, Yamaguchi M, Chung UI, Kubota N, et al. PPAR γ insufficiency enhances osteogenesis through osteoblast formation from bone marrow progenitors. J Clin Invest. 2004; 113: 846-855.
19. Lecka-Czernik B, Gubrij I, Moerman EJ, Kajkenova O, Lipschitz David A, manolagas SC, et al. Inhibition of osf2/cbfa1 expression and terminal osteoblast differentiation by PPAR γ2. Journal of Cellular Biochemistry. 1999; 74: 357-371.
20. Russell RG. Bisphosphonates: From bench to bedside. Annals of the New York Academy of Sciences. 2006; 1068: 367–401.
21. Rogers MJ. From molds and macrophages to mevalonate: a decade of progress in understanding the molecular mode of action of bisphosphonates Calcif Tissue Int. 2004; 75: 451-461.
22. Im GI, Qureshi SA, Kenney J, Rubash HE, Shanbhag AS, et al. Osteoblast proliferation and maturation by bisphosphonates. Biomaterials. 2004; 25: 4105-4115.
23. Follet H, Li J, Phipps RJ, Hui S, Condon K, Burr DB, et al. Risedronate and alendronate suppress osteocyte apoptosis following cyclic fatigue loading. Bone. 2007; 40: 1172-1177.
24. Tobias JH1, Chow JW, Chambers TJ. 3-Amino-1-hydroxypropylidine-1-bisphosphonate (AHPrBP) suppresses not only the induction of new, but also the persistence of existing bone-forming surfaces in rat cancellous bone. Bone. 1993; 14: 619-623.
25. K Iwata, J Li, Follet H, Phipps RJ, Burr DB, et al. Bisphosphonates suppress periosteal osteoblast activity independently of resorption in rat femur and tibia. Bone. 2006; 39: 1053-1058.
26. A Schindeler, DG Little. Osteoclasts but not osteoblasts are affected by acalcified surface treated with zoledronic acid *in vitro*. Biochemical & Biophysical Research Communications. 2005; 338: 710-716.
27. Peter B, Zambelli PY, Guicheux J, Pioletti DP. The effect of bisphosphonates and titanium particles on osteoblasts: an *in vitro* study. J Bone Joint Surg Br. 1984; 87: 1157-1163.
28. Tenta R, Sourla A, Lembessis P, Koutsilieris M. Bone-related growth factors and zoledronic acid regulate the PTHrP/PTH.1 receptor bioregulation systems in MG-63 human osteosarcoma cells. Anticancer Research. 2006; 26: 283-291.

29. Idris AI, Rojas J, Greig IR, Van't Hof RJ, Ralston SH. Aminobisphosphonates cause osteoblast apoptosis and inhibit bone nodule formation *in vitro*. Calcif Tissue Int. 2008; 82: 191-201.

30. Ohe JY, Kwon YD, Lee HW. Bisphosphonates modulate the expression of OPG and M-CSF in hMSC-derived osteoblasts. Clin Oral Investig. 2011; 16: 1153-1159.

31. Adamson BB, Gallacher SJ, Byars J, Ralston SH, Boyle IT, Boyce BF, et al. Mineralisation defects with pamidronate therapy for Paget's disease. Lancet. 1993; 342: 1459-1460.

Cite this article

Zhang X, Liu Y, Xu T, Chen J, Cui Y, et al. (2016) Reversing Effects of Bisphosphonates on Glucocorticoid-Induced Osteoporosis by Advancing Differentiation of Osteoblasts through Actions on PPAR γ and Hedgehog Pathways. J Clin Pharm 2(1): 1009.