Int J Clin Exp Med 2020;13(5):3623-3629
www.ijcem.com /ISSN:1940-5901/IJCEM0107272

Original Article

GM6001 improves bone dysfunction and bone metabolism disorder in SINFH rats

Guancheng Li[1*], Xing Ma[2*], Ying Ye[1], Saifei He[1], Juhua Zhuang[2], Guoyu Wang[2], Jiening Wang[1], Wei Xia[2]

[1]Central Laboratory, [2]Department of Nuclear Medicine, Seventh People's Hospital of Shanghai University of Traditional Chinese Medicine, Shanghai, China. [*]Equal contributors and co-first authors.

Received November 18, 2019; Accepted January 13, 2020; Epub May 15, 2020; Published May 30, 2020

Abstract: Objective: To explore the effects of intravenously injected matrix metalloproteinase inhibitor GM6001 on serum calcium and phosphorus levels and bone metabolism markers in rats with steroid-induced necrosis of femoral heads (SINFH). Methods: Out of 90 2-week-old Sprague Dawley rats, 60 were randomly selected for SINFH modeling, and the other 30 were the control group. Of the 60 SINFH model rats, 30 were randomly selected as the GM6001 group. The other 30 were not treated, acting as a SINFH model group. Seven rats in each of the three groups were randomly selected at the second (T1), fourth (T2), sixth (T3), and eighth week (T4) after model establishment. Serum calcium and phosphorus levels were measured, and serum alkaline phosphatase (BALP) and osteocalcin (BGP) were detected by ELISA. Anti-tartaric acid phosphatase (StrACP) was detected by colorimetry. Bilateral femoral head tissues were obtained, and the femoral head density was measured using a dual-energy X-ray bone density detector. Osteoblasts and osteoclasts were microscopically examined and counted. Results: Serum calcium, phosphorus, and BGP levels, femoral head density, and osteoblast counts in the GM6011 group were higher than those in the model group ($P < 0.050$), which was lower than those in the control group ($P < 0.050$). Serum BALP and StrACP levels, and osteoclast counts at T2, T3, and T4 were lower than those in the model group ($P < 0.050$), which was higher than those in the control group ($P < 0.050$). Conclusions: GM6001 can effectively improve bone dysfunction and bone metabolism disorder in SINFH rats, which improves bone mechanical strength and promotes new bone formation. It is expected to be an effective drug for SINFH treatment.

Keywords: GM6001, SINFH, serum calcium phosphate, bone metabolism, animal

Introduction

Glucocorticoid is an adrenocortical hormone that plays an important role in a variety of autoimmune diseases, graft rejection, adrenal insufficiency, heart failure, cerebrovascular disease, nervous system inflammation, and chemotherapy for hematological malignancies [1, 2]. With the frequent use of glucocorticoids in the clinic, the incidence of side effects caused by glucocorticoids is also increasing [3]. Extensive use of glucocorticoids can lead to decreased anti-infective and anti-tumor immune functions, increased blood sugar, increased risk of hypertension and diabetes, osteoporosis, fetal malformations, and other hazards [3]. Among them, the incidence of glucocorticoid-induced femoral head necrosis has increased in recent years [4]. The number of patients with new steroid-induced necrosis of femoral heads (SINFH) exceeded two million in 2016 [5]. In areas with larger, older populations, such as China and India, the incidence of SINFH has exceeded 5% due to broader glucocorticoid application [6].

SINFH is not only very painful, but also affects normal patient functioning and can lead to more serious problems, such as permanent disability, collapse of the femoral head, deformation and joint inflammation [7]. The importance of glucocorticoids in clinical practice is undisputable. However, reducing the associated complications of glucocorticoid therapy is currently under active research.

Matrix metalloproteinases (MMPs) are members of the protease family involved in the degradation of extracellular matrices, such as bone [8]. They have been shown to be involved in tis-

sue development and repair, induction and development of inflammatory responses, and tumor development. MMPs may also play an important role in the development of SINFH. Li et al. showed that curcumin could improve bone microstructures in mice with secondary osteoporosis induced by glucocorticoid by regulating MMP-9 and activating micro365. Du et al. demonstrated that single nucleotide polymorphism in MMP-9 was associated with reduced SINFH risk [9-11] and it is speculated that MMP inhibitors may be therapeutic targets for the treatment and prevention of SINFH.

The use of MMP inhibitors for SINFH treatment has not been reported thus far. Therefore, in the current study we established a SINFH model in rats and treated them with an MMP inhibitor, GM6001, to examine the potential of MMP inhibitors in SINFH treatment.

Materials and methods

Animals

A total of 120 Sprague-Dawley rats (clean-grade, 2-week-old, weighing 180-250 g) were purchased from Shanghai Kaixue Science and Technology Co., Ltd. China. Rats were housed with free access to drinking water and food at 24 ± 2°C in 50 ± 5% humidity under natural light. This experiment was approved by the Seventh People's Hospital of Shanghai University of Traditional Chinese Medicine Animal Ethics Committee.

SINFH model

Sixty rats were randomly selected for SINFH modeling. Using methods based on Weinstein et al. [12], 20 mg/kg methylprednisolone (Shanghai Guangrui Biotechnology Co., Ltd., RB1336) was injected into the gluteal muscle. The left and right gluteus maximus were injected alternately once a day for a total of 6 weeks. One day after the last injection, two of the modeled rats were randomly selected for histopathological observation to confirm the successful induction of SINFH. Of the 60 SINFH rats, 30 were randomly selected as the GM6001 treatment group (SINFH + GM6001). These rats received a tail vein injection of 70 mg/kg GM6001 once a week for a total of eight injections. The 30 non-treated rats were used as a positive SINFH control group (SINFH). Another 30 rats treated with GM6001 only (WT + GM6001), and 30 untreated rats (WT) were used as negative control groups.

Detection methods

Seven rats in each of the four groups were randomly selected at the second (T1), fourth (T2), sixth (T3), and eighth (T4) week after model establishment (all rats were euthanized in week 8), and 6 mL of heart blood was drawn. After centrifugation for 10 min at 4000 rpm, the resulting serum was divided into two aliquots. One aliquot was used for automatic biochemical analysis to detect serum calcium and phosphorus levels using a CM-800 automatic biochemical analyzer (Jidan Biotechnology Co., Ltd.). The other serum aliquot was used for detection of alkaline phosphatase (BALP) and osteocalcin (BGP) by enzyme-linked immunosorbent assay (ELISA).

The expression of tartrate-resistant acid phosphatase (StrACP) was detected by colorimetry according to the manufacturer's instructions. Briefly, rat serum and R1, R2 reagents were mixed and incubated at 37°C in a water bath for 60 min. R3, R4 reagents were added and left to incubate at room temperature for 40 min, before centrifuging for 10 min at 3500-4000 rpm. A 0.5 ml aliquot of the resulting supernatant was mixed with the color developer and left to incubate for 10 min. Samples were read at 550 nm and 0.5 cm optical path.

The BALP ELISA kit was purchased from Shanghai Guduo Biotechnology Co., Ltd., GDBN6568. The BGP ELISA kit was purchased from Shanghai Crystal Anti-Bioengineering Co., Ltd., JK-(a)-5994. The StrACP kit was purchased from Shanghai Yaji Biotechnology Co., Ltd., A058.

After rats were sacrificed by cervical dislocation, blood was drawn by heart puncture and bilateral femoral head tissue was obtained. Femoral head density was measured using a dual energy X-ray bone density detector. After the density measurement, the femoral head was cut in the middle of the coronal plane, fixed with 4% formaldehyde, and allowed to stand for 24 h for decalcification treatment. After 24 h, the bone samples were dehydrated, embedded in paraffin, and stained with hematoxylin and eosin prior to light microscopy. Osteoblasts and

Table 1. Serum calcium levels (mmol/L)

	GM6001 group	Model group	Control group	Therapy group	F	P
T1	2.75 ± 0.20	2.74 ± 0.18	3.27 ± 0.25$^{\Delta,\triangledown}$	3.29 ± 0.24$^{\Delta,\triangledown}$	17.493	< 0.001
T2	2.83 ± 0.22	2.55 ± 0.20$^{\Delta}$	3.34 ± 0.31$^{\Delta,\triangledown}$	3.31 ± 0.29$^{\Delta,\triangledown}$	15.387	< 0.001
T3	2.94 ± 0.25	2.26 ± 0.17$^{*,\Delta}$	3.21 ± 0.29$^{\Delta,\triangledown}$	3.28 ± 0.26$^{\Delta,\triangledown}$	24.94	< 0.001
T4	3.08 ± 0.25*	2.17 ± 0.24$^{*,\#,\Delta}$	3.31 ± 0.24$^{\Delta,\triangledown}$	3.32 ± 0.22$^{\Delta,\triangledown}$	36.751	< 0.001

*Serum calcium expression level in the same group at T1, P < 0.050; $^{\#}$serum calcium expression level in the same group at T2, P < 0.050; $^{\Delta}$serum calcium expression level compared with the GM6001 group in the same period, P < 0.050; $^{\triangledown}$serum calcium expression level compared with the model group, P < 0.050.

osteoclasts were counted in five fields at 400-fold magnification using a microscope.

Outcome measures

Outcomes were based on: serum calcium and phosphorus levels in four groups at T1, T2, T3, T4; BALP, BGP, and StrACP activities; femoral head density and bone cell count.

Statistical analysis

The data were analyzed and processed using SPSS 24.0 statistical software (Shanghai Yuchuang Network Technology Co., Ltd.). All results were expressed as the mean ± standard deviation. A comparison between two groups was performed using independent t-test. For multiple-group-comparison, one-way ANOVA was used. Repeated measure ANOVA with post hoc Tukey test was performed for measurements with multiple time points. P < 0.050 was considered statistically significant.

Results

SINFH model

Observation of bone morphology was performed on each sacrificed rat to determine whether SINFH modeling was successful. The results showed that osteonecrosis occurred in 7 model rats sacrificed at T1, T2 and T3. Out of 7 model rats were sacrificed at T4, 6 models were successful. The overall modeling success rate was 98.33%.

Serum calcium and phosphorus expression levels

Serum calcium and phosphorus levels in the four rat groups at T1, T2, T3, and T4 were statistically significant (P < 0.001). There was no significant difference in serum calcium and phosphorus between the control group and the model group at any of the time points (P < 0.050). At T1, there was no significant difference in serum calcium and phosphorus expression between the GM6001 group and the SINFH model group (P > 0.050). The control and therapy groups showed higher expression of both serum phosphorous and calcium compared to the other two groups (P < 0.050). At T2, T3, and T4, the serum calcium and phosphorus levels were the lowest in the SINFH model group, followed by the GM6001 group. Levels in the control group as well as the therapy group were higher than those in the other two groups (P < 0.050). In the SINFH model group, serum calcium and phosphorus were highest at T1, and decreased at T2, T3, and T4 (P < 0.050). There was no significant difference in serum calcium and phosphorus expression levels between the control group and therapy group at each time point (P > 0.050) (**Tables 1** and **2**).

BALP, BGP, and StrACP expression levels

At T1, there was no significant difference in BALG, BGP or StrACP expression between the GM6001 group and the SINFH model group (P > 0.050) (**Tables 1**-**3**). At T2, T3, and T4, BGP levels in the control group were higher than those in the SINFH model group (P < 0.050), and lower than those in the control group and therapy group (P < 0.050) (**Table 2**). BALP and StrACP levels in the therapy group were lower than those in the SINFH model group (P < 0.050) and higher than those in the control group and therapy group (P < 0.050) (**Tables 1** and **3**). There was no significant difference in BALP, BGP and StrACP levels between the control group and therapy group at each time point (P > 0.050). At T2, T3, and T4, BALP, BGP and StrACP levels in the control group were not significantly different. However, BALP and StrACP levels were higher at T1 and BGP was lower at

Table 2. Serum phosphorus levels (mmol/L)

	GM6001 group	Model group	Control group	Therapy group	F	P
T1	1.12 ± 0.21	1.04 ± 0.16	1.68 ± 0.07$^{\Delta,\triangledown}$	1.71 ± 0.05$^{\Delta,\triangledown}$	34.234	< 0.001
T2	1.39 ± 0.14	0.87 ± 0.15$^{\Delta}$	1.62 ± 0.04$^{\Delta,\triangledown}$	1.73 ± 0.06$^{\Delta,\triangledown}$	70.950	< 0.001
T3	1.45 ± 0.21	0.70 ± 0.09$^{*,\Delta}$	1.69 ± 0.05$^{\Delta,\triangledown}$	1.71 ± 0.06$^{\Delta,\triangledown}$	102.413	< 0.001
T4	1.46 ± 0.12*	0.62 ± 0.05$^{*,\#,\Delta}$	1.66 ± 0.08$^{\Delta,\triangledown}$	1.74 ± 0.05$^{\Delta,\triangledown}$	274.520	< 0.001

*Serum calcium expression level in the same group at T1, P < 0.050; $^{\#}$serum calcium expression level in the same group at T2, P < 0.050; $^{\Delta}$serum calcium expression level compared with the GM6001 group in the same period, P < 0.050; $^{\triangledown}$serum calcium expression level compared with the model group, P < 0.050.

Table 3. Comparison of BALP expression levels

	Therapy group	GM6001 group	Model group	Control group	F	P
T1	55.13 ± 8.62	57.32 ± 10.24	47.16 ± 5.24$^{*,\#}$	48.08 ± 4.49$^{*,\#}$	3.164	0.043
T2	62.14 ± 6.79$^{\Delta}$	69.33 ± 10.68$^{*,\Delta}$	47.16 ± 4.96$^{*,\#}$	47.59 ± 4.71$^{*,\#}$	16.652	< 0.001
T3	64.13 ± 8.34$^{\Delta,\triangledown}$	76.14 ± 9.68$^{*,\Delta,\triangledown}$	47.23 ± 5.04$^{*,\#}$	48.37 ± 4.57$^{*,\#}$	25.463	< 0.001
T4	63.67 ± 5.69$^{\Delta,\triangledown}$	85.62 ± 8.68$^{*,\Delta,\triangledown,\square}$	47.14 ± 5.22$^{*,\#}$	48.62 ± 4.68$^{*,\#}$	57.020	< 0.001

Note: *represents a comparison with therapy group, P < 0.001; $^{\#}$represents a comparison with GM6001 group, P < 0.001; $^{\Delta}$represents comparison with T1 within the same group1, P < 0.001; $^{\triangledown}$represents a comparison with T2 within the same group, P < 0.001; $^{\square}$represents a comparison with T3 within the same group, P < 0.001.

Table 4. Comparison of BGP expression levels

	Therapy group	GM6001 group	Model group	Control group	F	P
T1	4.76 ± 0.55	4.98 ± 0.62	6.84 ± 0.41$^{*,\#}$	6.94 ± 0.35$^{*,\#}$	39.242	< 0.001
T2	4.51 ± 0.29$^{\Delta}$	3.72 ± 0.15$^{*,\Delta}$	6.77 ± 0.39$^{*,\#}$	6.89 ± 0.38$^{*,\#}$	178.124	< 0.001
T3	4.36 ± 0.31$^{\Delta,\triangledown}$	2.53 ± 0.20$^{*,\Delta,\triangledown}$	6.80 ± 0.35$^{*,\#}$	6.92 ± 0.30$^{*\#}$	357.329	< 0.001
T4	4.25 ± 0.34$^{\Delta,\triangledown}$	1.20 ± 0.14$^{*,\Delta,\triangledown,\square}$	6.82 ± 0.40$^{*,\#}$	6.87 ± 0.36$^{*,\#}$	475.207	< 0.001

Note: *represents a comparison with therapy group, P < 0.001; $^{\#}$represents a comparison with GM6001 group, P < 0.001; $^{\Delta}$represents comparison with T1 within the same group1, P < 0.001; $^{\triangledown}$represents a comparison with T2 within the same group, P < 0.001; $^{\square}$represents a comparison with T3 within the same group, P < 0.001.

Table 5. Comparison of StrACP expression levels

	Therapy group	GM6001 group	Model group	Control group	F	P
T1	72.37 ± 8.65	75.24 ± 10.33	32.76 ± 3.07$^{*,\#}$	32.15 ± 2.89$^{*,\#}$	80.273	< 0.001
T2	70.16 ± 10.24	89.57 ± 8.68$^{*,\Delta}$	33.42 ± 3.67$^{*,\#}$	32.37 ± 3.07$^{*,\#}$	110.325	< 0.001
T3	68.22 ± 7.67	104.04 ± 9.62$^{*,\Delta,\triangledown}$	34.16 ± 3.25$^{*,\#}$	33.08 ± 3.07$^{*,\#}$	185.214	< 0.001
T4	63.31 ± 6.12$^{\Delta}$	113.27 ± 12.04$^{*,\Delta,\triangledown,\square}$	33.04 ± 3.30$^{*,\#}$	32.52 ± 2.82$^{*,\#}$	200.842	< 0.001

Note: *represents a comparison with therapy group, P < 0.001; $^{\#}$represents a comparison with GM6001 group, P < 0.001; $^{\Delta}$represents comparison with T1 within the same group1, P < 0.001; $^{\triangledown}$represents a comparison with T2 within the same group, P < 0.001; $^{\square}$represents a comparison with T3 within the same group, P < 0.001

T1 compared to the levels at other time points (P < 0.05). BALP and StrACP levels increased and BGP levels decreased with time in the SINFH model group (P < 0.050) (**Tables 4** and **5**).

Femoral head density and bone cell count

At T1, there was no significant difference between the GM6001 group and the SINFH model group (P > 0.050). However, at T2, T3, and T4, femoral head density and osteoblast counts in the control group were higher than those in the SINFH model group (P < 0.050), and lower than those in the control and therapy groups (P < 0.050). Osteoclast counts in the GM6001 group were lower than those in the SINFH model group (P < 0.050) and higher than those in the control and therapy groups (P < 0.050). Comparison of femoral head density

Table 6. Comparison of femoral head density

	Therapy group	GM6001 group	Model group	Control group	F	P
T1	0.42 ± 0.08	0.42 ± 0.02	0.52 ± 0.02$^{*,\#}$	0.50 ± 0.02$^{*,\#}$	10.193	< 0.001
T2	0.45 ± 0.05	0.35 ± 0.07$^{*,\Delta}$	0.51 ± 0.03$^{*,\#}$	0.49 ± 0.01$^{*,\#}$	16.892	< 0.001
T3	0.45 ± 0.09	0.29 ± 0.05$^{*,\Delta,\triangledown}$	0.54 ± 0.02$^{*,\#}$	0.50 ± 0.01$^{*,\#}$	30.351	< 0.001
T4	0.47 ± 0.02	0.24 ± 0.08$^{*,\Delta,\triangledown,\square}$	0.52 ± 0.01$^{*,\#}$	0.48 ± 0.02$^{*,\#}$	61.720	< 0.001

Note: *represents a comparison with therapy group, P < 0.001; $^{\#}$represents a comparison with GM6001 group, P < 0.001; $^{\Delta}$represents comparison with T1 within the same group1, P < 0.001; $^{\triangledown}$represents a comparison with T2 within the same group, P < 0.001; $^{\square}$represents a comparison with T3 within the same group, P < 0.001.

Table 7. Comparison of osteoclast counts

	Therapy group	GM6001 group	Model group	Control group	F	P
T1	18.04 ± 3.51	18.23 ± 3.17	14.54 ± 2.16$^{*,\#}$	14.87 ± 2.25$^{*,\#}$	3.442	0.033
T2	20.62 ± 2.16	24.16 ± 3.55$^{*,\Delta}$	14.82 ± 2.05$^{*,\#}$	15.12 ± 2.07$^{*,\#}$	22.242	< 0.001
T3	21.17 ± 3.06	29.72 ± 4.21$^{*,\Delta,\triangledown}$	15.04 ± 1.76$^{*,\#}$	15.09 ± 1.89$^{*,\#}$	39.905	< 0.001
T4	23.00 ± 2.84$^{\Delta}$	36.95 ± 4.15$^{*,\Delta,\triangledown,\square}$	14.96 ± 2.00$^{*,\#}$	15.08 ± 1.92$^{*,\#}$	90.852	< 0.001

Note: *represents a comparison with therapy group, P < 0.001; $^{\#}$represents a comparison with GM6001 group, P < 0.001; $^{\Delta}$represents comparison with T1 within the same group1, P < 0.001; $^{\triangledown}$represents a comparison with T2 within the same group, P < 0.001; $^{\square}$represents a comparison with T3 within the same group, P < 0.001.

and bone cell counts between the control group and the SINFH model group at each time point showed no significant difference (P > 0.050). In the GM6001 group, femoral head density and osteoclast counts increased with time and osteoclast counts decreased with time (P < 0.050). In the SINFH model group, femoral head density and osteoclast counts decreased with time and osteoclast counts were increased with time (**Tables 6** and **7**).

Discussion

Serum calcium and phosphorus levels are important indicators of bone health, including femoral head health. Calcium and phosphorus levels determine the efficacy of bone resorption and remodeling and are affected by several factors, including BALP, BGP, and StrACP activity [13]. BALP is a marker of early bone cell maturation, which can provide inorganic phosphorus to the body, promote bone salt crystal formation, and is closely related to bone mineralization [14]. BGP is an osteoblast production factor, and its expression level directly reflects the rate of bone metabolism in the body [15]. StrACP is produced by osteoclasts, contrary to BGP, and is commonly used to monitor bone resorption [16]. GM6001 is a broad-spectrum matrix metalloproteinase inhibitor and currently the most potent synthetic MMP inhibitor known [17]. It is a dipeptide containing many carboxyl groups, which complex zinc ions required for protease activity upon binding to the MMP substrate recognition moiety, thereby inhibiting metalloproteinase activation [18]. Through the action of GM6001, MMP-induced collagen destruction can be reduced, and bone tissue repair can occur. Currently, the application of GM6001 in SINFH treatment has not been confirmed. In this study, we established a SINFH rat model and treated these rats with GM6001. Serum calcium and phosphorus levels and bone metabolism markers were measured to demonstrate the potential of GM6001 as a therapeutic option for SINFH.

Serum calcium and phosphorus levels, BGP levels, femoral head density, and osteoblast counts were all higher in the GM6001 group than in the SINFH model group. Conversely, BALP and StrACP levels and osteoclast counts were lower in the GM6001 group than the SINFH model group. This suggests that GM-6001 can effectively improve bone dysfunction and metabolism by promoting new bone formation and inhibiting osteoclast activation. These significant differences were not found in any of the indicators between the control group and the therapy group, which indicated that GM-6001 had no negative effect on the bone tissue of rats and could be used for the treatment of bone tissue diseases in humans.

Upon glucocorticoid treatment, a large amount of extracellular matrix accumulates. This accumulation causes a continual increase of the bone marrow fat system activity, destruction of intact and compact trabecular bone arrangement, and destabilization of local bone density, eventually leading to SINFH. After administering GM6001, fat differentiation was promoted, with an associated release of large amounts of many specific factors, such as BGP and osteoprotegerin. GM6001 promotes inorganic phosphorus release from hydrolyzed phospholipids, strengthens local phosphorus concentrations, and accelerates bone mineralization. GM6001 inhibits MMPs by binding to a polypeptide in the MMP, thereby inhibiting its activity [19]. Zinc ions chelated with GM6001 can induce calcium phosphate zinc formation in bone and increase BALP and BGP expression. By inhibiting MMPs, GM6001 treatment reduced the rate of apoptosis and bone cell degradation, effectively improving the bone tissue and restoring intact and compact trabecular bone arrangement. Martin-Martin *et al.* [20] produced a similar conclusion when studying GM6001 for treatment of joint inflammation, confirming the results of this study. However, further research on the mechanism of GM6001 on bone function is needed. Jasińska *et al.* [21] proposed that GM6001 can improve bone function and metabolism by regulating certain genes, such as PPAPγ and Runx2. In a study by Yao *et al.* [22] examining the effect of glucocorticoids on human bone function, glucocorticoids were shown to have no significant effect on serum calcium and phosphorus levels. The subjects included in the study by Yao *et al.* [22] were osteoporosis patients. Initial serum calcium and phosphorus levels of these patients were lower and no significant changes occurred upon glucocorticoid use. In this study, the significant difference in bone function between the SINFH model group and the control group demonstrated that the effects of glucocorticoids on bone tissue should not be underestimated.

However, the mechanism underlying the effect of GM6001 on bone metabolism needs further investigation. Moreover, there are always differences between animal models and human application. Human experiments will be pursued in future, and our experimental methods will be constantly improved to obtain optimal experimental results.

In summary, GM6001 treatment of SINFH model rats was shown to effectively improve bone dysfunction and disordered metabolism in rats. GM6001 treatment also improved bone mechanical strength and promoted bone formation in SINFH model rats. GM6001 thus has potential for use as an effective drug for the treatment of SINFH.

Acknowledgements

The study was supported by grants from Shanghai Municipal Commission of Health and Family Planning (No. 201740084), Key Specialty Construction Project of Pudong Health and Family Planning Commission of Shanghai (No. PWZxk2017-06), Science and Technology Development Fund of Shanghai Pudong New Area (No. PKJ2017-Y14) and Talents Training Program of Seventh People's Hospital of Shanghai University of TCM (XX2017-01).

Disclosure of conflict of interest

None.

Address correspondence to: Jiening Wang, Central Laboratory, Seventh People's Hospital of Shanghai University of Traditional Chinese Medicine, No. 358 Datong Road, Pudong, Shanghai 200137, China. Tel: +86-18930831118; E-mail: jnwangn@163.com; Wei Xia, Department of Nuclear Medicine, Seventh People's Hospital of Shanghai University of Traditional Chinese Medicine, No. 358 Datong Road, Pudong, Shanghai 200137, China. Tel: +86-1376-1295811; E-mail: awingxia@163.com

References

[1] Nair P, Wenzel S, Rabe KF, Bourdin A, Lugogo NL, Kuna P, Barker P, Sproule S, Ponnarambil S and Goldman M. Oral glucocorticoid-sparing effect of benralizumab in severe asthma. N Engl J Med 2017; 376: 2448-2458.

[2] Turecki G and Meaney MJ. Effects of the social environment and stress on glucocorticoid receptor gene methylation: a systematic review. Biol Psychiatry 2016; 79: 87-96.

[3] Caplan A, Fett N, Rosenbach M, Werth VP and Micheletti RG. Prevention and management of glucocorticoid-induced side effects: a comprehensive review: gastrointestinal and endocrinologic side effects. J Am Acad Dermatol 2017; 76: 11-16.

[4] Zhang M, Shi C, Zhou Z and Hou J. Bone characteristics, histopathology, and chondrocyte apoptosis in femoral head necrosis induced by

glucocorticoid in broilers. Poult Sci 2017; 96: 1609-1614.
[5] Wang X, Liu Y, Wang X, Liu R, Li J, Zhang G, Li Q, Wang L, Bai Z and Zhao J. The role of 99mTc-Annexin V apoptosis scintigraphy in visualizing early stage glucocorticoid-induced femoral head osteonecrosis in the rabbit. Biomed Res Int 2016; 2016: 7067259.
[6] Zhang Y, Yin J, Ding H, Zhang C and Gao YS. Vitamin K2 ameliorates damage of blood vessels by glucocorticoid: a potential mechanism for its protective effects in glucocorticoid-induced osteonecrosis of the femoral head in a rat model. Int J Biol Sci 2016; 12: 776.
[7] Fowler TW, Acevedo C, Mazur CM, Hall-Glenn F, Fields AJ, Bale HA, Ritchie RO, Lotz JC, Vail TP and Alliston T. Glucocorticoid suppression of osteocyte perilacunar remodeling is associated with subchondral bone degeneration in osteonecrosis. Sci Rep 2017; 7: 44618.
[8] Eckhard U, Huesgen PF, Schilling O, Bellac CL, Butler GS, Cox JH, Dufour A, Goebeler V, Kappelhoff R, Keller UAD, Klein T, Lange PF, Marino G, Morrison CJ, Prudova A, Rodriguez D, Starr AE, Wang Y and Overall CM. Active site specificity profiling of the matrix metalloproteinase family: proteomic identification of 4300 cleavage sites by nine MMPs explored with structural and synthetic peptide cleavage analyses. Matrix Biol 2016; 49: 37-60.
[9] Du J, Liu W, Jin T, Zhao Z, Bai R, Xue H, Chen J, Sun M, Zhang X, Wang G and Wang J. A single-nucleotide polymorphism in MMP9 is associated with decreased risk of steroid-induced osteonecrosis of the femoral head. Oncotarget 2016; 7: 68434-68441.
[10] Li G, Bu J, Zhu Y, Xiao X, Liang Z and Zhang R. Curcumin improves bone microarchitecture in glucocorticoid-induced secondary osteoporosis mice through the activation of microRNA-365 via regulating MMP-9. Int J Clin Exp Pathol 2015; 8: 15684-15695.
[11] Zheng L, Jiao W, Mei H, Song H, Li D, Xiang X, Chen Y, Yang F, Li H and Huang K. miRNA-337-3p inhibits gastric cancer progression through repressing myeloid zinc finger 1-facilitated expression of matrix metalloproteinase 14. Oncotarget 2016; 7: 40314.
[12] Weinstein RS, Hogan EA, Borrelli MJ, Liachenko S, O'brien CA and Manolagas SC. The pathophysiological sequence of glucocorticoid-induced osteonecrosis of the femoral head in male mice. Endocrinology 2017; 158: 3817-3831.
[13] Millán JL and Whyte MP. Alkaline phosphatase and hypophosphatasia. Calcif Tissue Int 2016; 98: 398-416.
[14] Li J, Zhang H, Yang C, Li Y and Dai Z. An overview of osteocalcin progress. J Bone Miner Metab 2016; 34: 367-379.
[15] Linder CH, Ek-Rylander B, Krumpel M, Norgård M, Narisawa S, Millán JL, Andersson G and Magnusson P. Bone alkaline phosphatase and tartrate-resistant acid phosphatase: potential co-regulators of bone mineralization. Calcif Tissue Int 2017; 101: 92-101.
[16] Serkies KB, Garcha R, Tam LE, De Souza GM and Finer Y. Matrix metalloproteinase inhibitor modulates esterase-catalyzed degradation of resin-dentin interfaces. Dent Mater 2016; 32: 1513-1523.
[17] Abdul-Muneer P, Pfister BJ, Haorah J and Chandra N. Role of matrix metalloproteinases in the pathogenesis of traumatic brain injury. Mol Neurobiol 2016; 53: 6106-6123.
[18] KingaVojnits CF, Hayes-Jordan A and Li Y. MMP inhibition induces mesenchymal-epithelial transition of human osteosarcoma cells. Cel Mol Biol 2016; 2.
[19] Ukaji T, Lin Y, Okada S and Umezawa K. Inhibition of MMP-2-mediated cellular invasion by NF-κB inhibitor DHMEQ in 3D culture of breast carcinoma MDA-MB-231 cells: a model for early phase of metastasis. Biochem Biophys Res Commun 2017; 485: 76-81.
[20] Martin-Martin B, Tovell V, Dahlmann-Noor AH, Khaw PT and Bailly M. The effect of MMP inhibitor GM6001 on early fibroblast-mediated collagen matrix contraction is correlated to a decrease in cell protrusive activity. Eur J Cell Biol 2011; 90: 26-36.
[21] Jasińska M, Miłek J, Cymerman IA, Łęski S, Kaczmarek L and Dziembowska M. miR-132 regulates dendritic spine structure by direct targeting of matrix metalloproteinase 9 mRNA. Mol Neurobiol 2016; 53: 4701-4712.
[22] Yao W, Dai W, Jiang L, Lay EA, Zhong Z, Ritchie R, Li X, Ke H and Lane N. Sclerostin-antibody treatment of glucocorticoid-induced osteoporosis maintained bone mass and strength. Osteoporos Int 2016; 27: 283-294.