Histol Histopathol (2016) 31: 647-656

http://www.hh.um.es

Histology and Histopathology

From Cell Biology to Tissue Engineering

Immunolocalization of MMP 2, 9 and 13 in prednisolone induced osteoporosis in mice

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Summary. Long-term use of glucocorticoids (GC) causes rapid bone loss and increases the risk of osteoporotic fractures. Matrix metalloproteinase (MMPs), the most prominent kind of proteases implicated in the proteolytic degradation of the extracellular matrix (ECM), have been reported to be involved in pathological process of GC induced osteoporosis. However, the underlining mechanisms are still unclear. The aim of this study was to investigate the spatial expression and the potential function of MMP 2, 9 and 13 in osteoporosis induced by prednisolone in the tibiae of mice. In this experiment, mice were given prednisolone (15 mg/kg body weight) in PBS intragastrically every other day, or only PBS as control. Two weeks later, mice were fixed with transcardial perfusion of 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4), and tibiae were extracted for histochemical analysis. Compared with control group, the number of TRAP-positive osteoclasts and the immunoreactivity of MMP 2, 9 and 13 were significantly increased in the trabecular bone of mice administered with prednisolone, leading to the decrease of trabecular bone volume. On the other hand, lighter eosin staining areas containing numerous empty lacunae of osteocytes and crevices were seen in the narrowing cortical bone. Furthermore, intense immunoreaction of MMP 2 and MMP 13 were found in the enlarged lacunae and the crevices, respectively. Taken together, we concluded that prednisolone administration induced the increase of MMP 2, 9 and 13 expressions, while MMP 2 and MMP 13 played essential roles in the osteocytic osteolysis and the early impaired areas in the cortical bone. Therefore, MMPs might be new potential therapeutic targets for prevention and treatment of glucocorticoid induced osteoporosis, especially osteocytic osteolysis.

Key words: Prednisolone, MMPs, Osteoclast, Osteocytic osteolysis, Osteoporosis

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DOI: 10.14670/HH-11-702

Introduction

Glucocorticoids (GCs) are widely used for the treatment of numerous immunologic, rheumatologic, neoplastic and allergic diseases. However, long term exposure to GCs is responsible for many adverse events such as osteoporosis, diabetes mellitus or infection (Fardet et al., 2011; Morin and Fardet, 2015). As the most common cause of secondary osteoporosis, glucocorticoid-induced osteoporosis (GIO) is characterized by the dynamic balance disturbances of bone remodeling leading to increased fracture risk (Carbonare et al., 2001; Roux et al., 2012). GCs increase bone resorption by stimulating osteoclastogenesis by increasing the expression of receptor activator of nuclear factor kB ligand (RANK-L) (Pichler et al., 2013; Shi et al., 2014) and decreasing the expression of its soluble decoy receptor osteoprotegerin (OPG) in stromal and osteoblastic cells (Sasaki et al., 2001, 2005). GCs also enhance the expression of macrophage colony-

stimulating factor (M-CSF), which in the presence of RANK-L induces osteoclastogenesis (Sivagurunathan et al., 2005). On the other hand, GCs also disturb biological functions of osteoblastic cells, thus facilitating GC-induced osteoporosis. GC directly inhibit cellular proliferation and differentiation of osteoblastic cells (Weinstein et al., 1998), reduce osteoblast maturation and activity, suppress the synthesis of insulin-like growth factor-1 (IGF-1) which is an agent that enhances bone formation (Pepene et al., 2010), and decrease the expression of Runx2 which is an osteoblast master regulator required for bone formation (Lin et al., 2010). Moreover, the suppression of osteoblast function by GCs is also reported to be associated with alteration of the Wnt signaling pathway (Ohnaka et al., 2005), a critical pathway for osteoblastogenesis (Krishnan et al., 2006; Yao et al., 2008)

In addition, it is worth noticing that GCs can induce osteocyte apoptosis or autophagy contributing to early fracture risk (O'Brien et al., 2004). Also, osteocytes are found to be able to induce the erosion and dissolution of osteocytic lacunae walls under the effects of GCs, which is called osteocytic osteolysis (Belanger, 1969; Tazawa et al., 2004). Enlarged osteocyte lacunae, reduced elastic modulus around the lacunae, and a "halo" of hypomineralized bone surrounding the lacunae was observed in the prednisolone-treated mice (Lane et al., 2006). Bone matrix harbors numerous osteocyte lacunae containing osteocytes. Each osteocyte extends numerous dendrites into canaliculi where dendrite tips make contact with dendrites of nearby neighboring osteocytes, osteoblasts, and osteoclasts, thereby establishing a communication network within the entire bone matrix (Matsuo, 2014). Several studies have suggested that osteocytes are central to bone homeostasis, regulating osteoblast and osteoclast activities through the bone canalicular network (Poole et al., 2005; Bonewald and Johnson, 2008). The estimated surface area of osteocyte lacunae and canaliculi is huge, and the lacuno-canalicular network is well connected to the blood circulation, suggesting that osteocytic osteolysis may function in pathogenesis and treatment of osteoporosis (Matsuo, 2014).

Extracellular matrix (ECM) production and degradation by bone cells are critical steps in bone metabolism and disturbed ECM turnover leading to bone diseases, such as dyschondroplasia (Shahzad et al., 2015), rheumatoid arthritis (Rong et al., 2014), chondrosarcoma (Stevens, 2013). Numerous proteases have been implicated in the proteolytic degradation of the ECM, most prominent among which are members of the matrix metalloproteinase (MMP) family, exerting their enzymatic activity at a neutral pH in a zinc ion-dependent manner (Inoue et al., 2006). Several MMPs are expressed in bone tissue. MMP 2 and MMP 13 are produced by osteoblasts and osteocytes, while MMP 9 is mainly expressed by osteoclasts (Vu et al., 1998; Nakamura et al., 2004). MMP 2 plays a crucial role in forming and maintaining the osteocytic canalicular network and MMP 9 participates in the degradation of organic components in bone matrix, including collagen and non-collagenous proteins (Reponen et al.; 1994, Engsig et al., 2001; Inoue et al., 2006). MMP 13 is mainly associated with mineralized bone matrix, and plays an important role in degradation of type I collagen in bone matrix in concert with cathepsin K and MMP 9 (Nakamura et al., 2004).

To date, there have been few studies focusing on the immunolocalization of MMPs in glucocorticoid-induced osteoporosis and osteocytic osteolysis. Thus, we set out to collect histochemical evidence of the expression of MMP 2, 9 and 13 using a prednisolone-induced osteoporosis mice model. Our data suggested that immunoreactivity of MMP 2, 9, 13 were obviously increased in prednisolone-induced osteoporosis, especially MMP 2 and MMP 13, which may play key roles in osteocytic osteolysis and lighter eosin staining impaired areas in the cortical bone.

Materials and methods

Animal and tissue preparation

All animal experiments in this study were conducted according to the Guidelines for Animal Experimentation of Shandong University. Twenty 7-week-old Kunming mice were obtained from Laboratory Animal Centre of Shandong University (Jinan, China) and kept in plastic cages (3-5 animals per cage) under standard laboratory conditions with a 12-h dark,12-h light cycle and a constant temperature of 20°C and humidity of 48%. All mice were fed a standard rodent diet *ad libitum*.

After 1 week acclimation, the mice were randomly divided into two groups (ten mice per group) and were given prednisolone (15 mg/kg body weight) in PBS intragastrically every other day, or only PBS as control. Two weeks later, the mice were anesthetized with an intraperitoneal injection of 10% chloral hydrate (400 mg/100 g body weight) and fixed with 4% para-formaldehyde in 0.1 M phosphate buffer (pH 7.4) by transcardial perfusion. After fixation, tibiae were removed and immersed in the same fixative for additional 24 h. Following that, samples were decalcified with 10% EDTA-2Na solution for 3 weeks at 4°C. Then the specimens were dehydrated through an ascending ethanol series and then embedded in paraffin using standard procedures. Serial longitudinal 5-μm-thick sections were prepared for following histological analysis using rotary microtome (LEICA SM 2010R, German).

Histological examination and image analysis

Hematoxylin and eosin (H&E) was performed to investigate the morphology of metaphysis in both groups. After being dewaxed and hydrated, the prepared sections were immersed in Ehrlich's haematoxylin for 15 minutes. Then the sections were washed with distilled

water and differentiated in 1% HCl in 70% alcohol for 1 minute and washed again for 2 minutes. After that, the sections were stained with 1% eosin for 10 minutes and washed with distilled water. Finally, all sections were dehydrated and mounted. The stained sections were observed and then digital images were taken with a light microscope (Olympus BX-53, Japan). With the aid of Image Pro Plus 6.2 software (Media Cybernetics, Silver Spring, MD), trabecular bone volume (BV/TV, trabecular bone volume/ tissue volume × 100%) was measured. Specifically, 10 slices of each sample were used for quantitative histomorphometric analysis to get the mean value.

Histochemistry (TRAP staining) for osteoclasts

To evaluate the osteoclast, tartrate-resistant acid phosphatase (TRAP) staining was performed as previously showed (Li et al., 2013). In brief, dewaxed paraffin sections were submerged in a mixture of 3.0 mg of naphthol AS-BI phosphate, 18 mg of red violet LB salt, and 100 mM L(+) tartaric acid (0.36 g) diluted in 30 ml of 0.1 M sodium acetate buffer (pH 5.0) for 15 min at 37°C. Counter staining was performed with methyl green.

Immunohistochemistry for MMP 2, 9 and 13

Prepared 5 μm thick paraffin sections were used for MMP 2, 9 and 13 immunolabeling. Briefly, after xylene treatment, dewaxed paraffin sections were pretreated with 0.3 % hydrogen peroxide for 30 minutes, and then with 1% bovine serum albumin (BSA; seologicals proteins Inc. Kankakee, IL, USA) in PBS (1% BSA-PBS) for 20 min to reduce non-specific staining. The treated sections were incubated with the primary

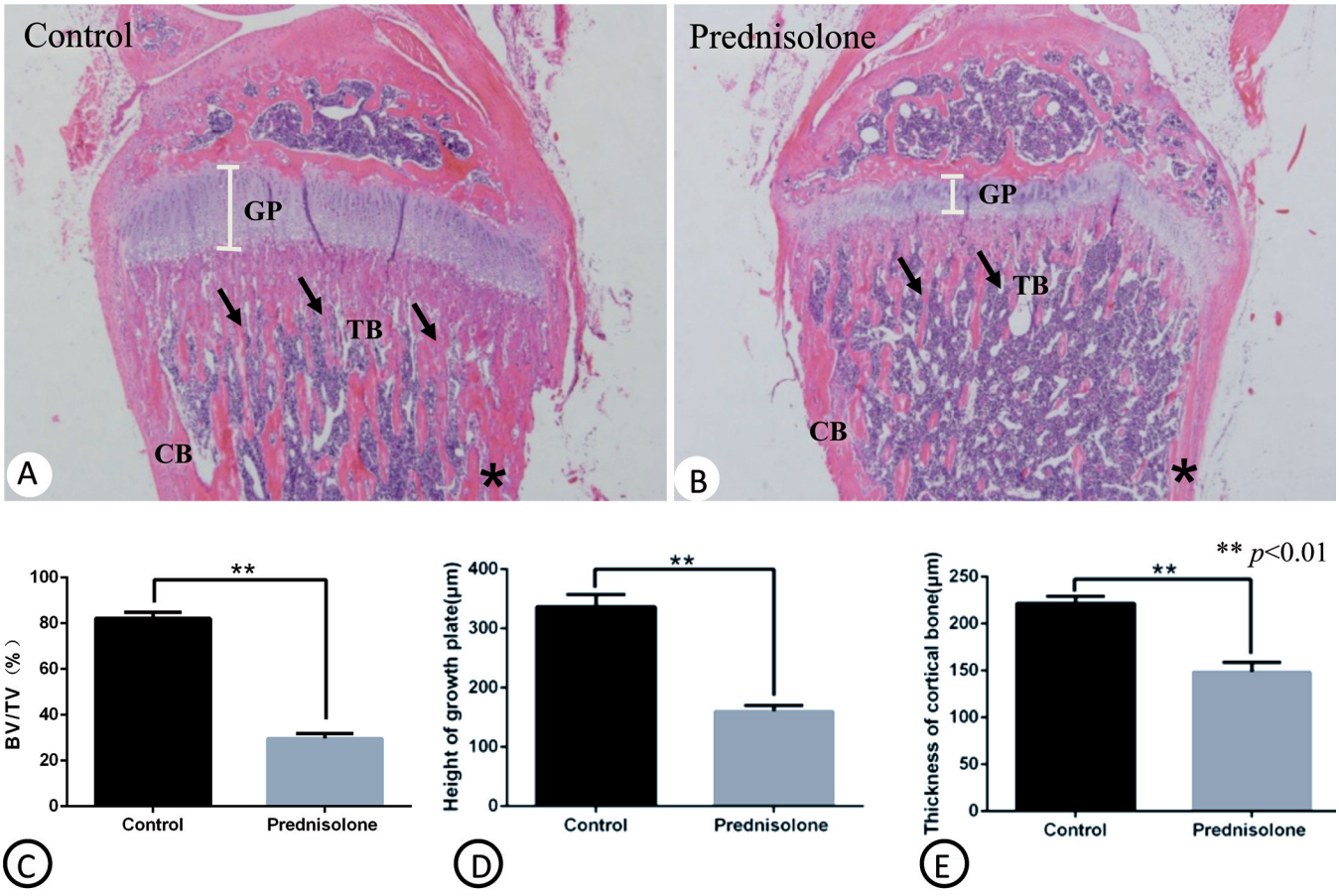

Fig. 1. Histological alterations and statistical analysis. Hematoxylin-eosin staining of tibiae reveals the differences between the two groups. **A, B.** Low magnification of tibiae of control group **(A)** and prednisolone- administered group **(B)**. **C-E.** The mean values of BV/TV **(C)**, thickness of growth plate **(D)** and width of cortical bone **(E)** were assessed for both groups by using statistics (n=10;** p<0.01). At 2 weeks after prednisolone administration, tibiae showed obvious histological manifestation of osteoporosis, including decreased trabecular bone volume, narrowed growth plate and cortical bone (asterisks and white lines). All corresponding numerical information is found in the results section. Error bars indicate ± SD. GP: growth plate; TB: trabecular bone; CB: cortical bone; BV/TV: trabecular bone volume/ tissue volume. A, B, x 40

antibodies of mouse antibody against MMP 2 (Millipore, USA) at a dilution of 1:50, goat antibody against MMP 9 (R & D Systems, Inc., Minneapolis, USA) at a dilution of 1:100 and goat antibody against MMP 13 (Millipore, USA) at a dilution of 1:50 with 1% BSA-PBS for 2 h at room temperature, respectively. After rinsing with PBS, they were immersed in horseradish peroxidase (HRP)-conjugated goat anti-mouse IgG for MMP 2, rabbit anti-goat IgG for MMP 9 and rabbit anti-goat IgG for MMP 13 (Jackson Immunoresearch laboratories, Inc., Baltimore, USA; Abcam, Hong Kong) respectively, at a dilution of 1:100 for 1 h at room temperature. The immunoreaction was visualized with diaminobenzidine (DAB) (Sigma-Aldrich, St. Louis, MO, USA). All sections were counterstained faintly with methyl green and observed under a light microscope (BX53, Olympus Corp., Japan). Specimens were scored according to the intensity of the dye color and the number of positive cells as previously described (Ma et al., 2010). The intensity of the dye color was graded as 0 (no color), 1 (light yellow), 2 (light brown), or 3 (brown), and the number of positive cells was graded as 0 (<5%), 1 (5-25%), 2 (25-50%), 3 (51-75%), or 4 (>75%). The two grades were added together and specimens were assigned to one of 4 levels: 0-1 score (Negative), 1-2 scores (Weak), 3-4 scores (Moderate), more than 5 scores (Intense). The positive expression rate was expressed as the percent of the addition of (Weak), (Moderate) and (Intense) to the total number.

Parameters measurement and statistical analysis

Image Pro Plus 6.2 (IPP 6.2) software (Media Cybernetics, Silver Spring, MD) was used for counting

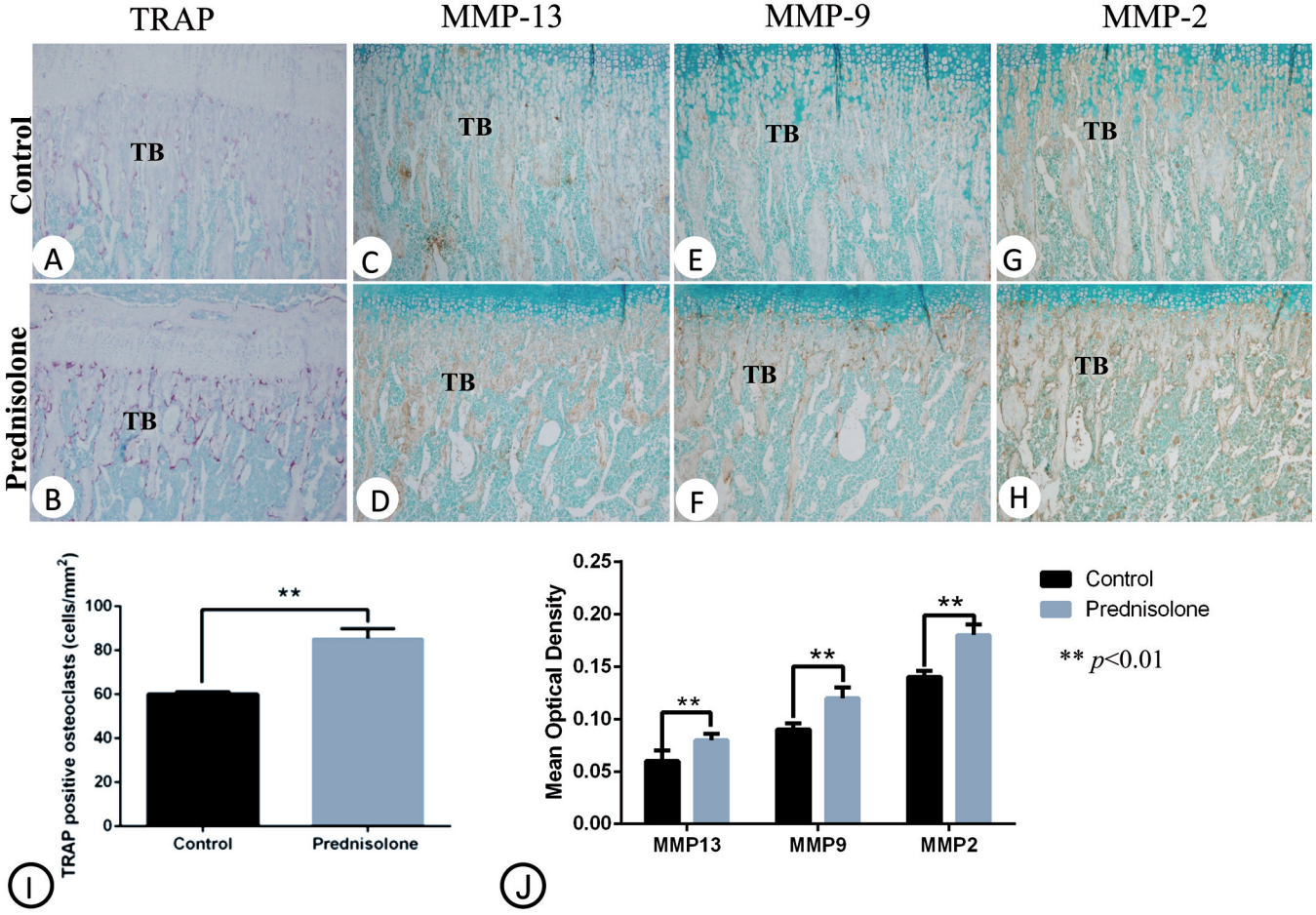

Fig. 2. TRAP staining for osteoclasts and immunolocalization for MMP 2, 9, 13 in the trabecular bone and statistical analysis. Upper line **(A, C, E, G)** control group; Lower line **(B, D, F, H)** prednisolone administration group. **A, B.** TRAP staining. **C, D.** Moderate expression of MMP 13. **E, F.** Intense expression of MMP 9. **G, H.** Moderate expression of MMP 2. More TRAP-positive osteoclasts (red) and intense expression of MMP 9 and moderate expression of MMP 2 and MMP 13(brown) were seen in the prednisolone administration group **(B, D, F, H)**. **I.** TRAP-positive osteoclasts were counted in the metaphysic of tibiae for both groups (n=10;** p<0.01). **J.** Mean optical density of MMP 2, 9, 13 immunoreactivity was assessed in the trabecular bone for both groups (n=10; p<0.01). All corresponding numerical information is found in the results section. Error bars indicate ± SD. SD: Standard deviation; TB: trabecular bone. A-H, x 100

the trabecular bone volume, thickness of growth plate, number of TRAP-positive osteoclasts in the trabecular bone and the cortical bone width, the number of vital osteocytes and empty lacunae in the cortical bone of the prednisolone-treated group and control. Because of the metabolic stability, diaphysis was chosen as the measurement area for the cortical bone width and the number of osteocytes. Immunostaining intensities (optical density, OD) also were analyzed using the same software. Positive reaction areas of MMP 2, 9 and 13 were manually selected in a color cube based manner. At least 10 sections from each sample were analyzed. All values are presented as mean ± standard deviation (SD). The differences between prednisolone administered group and control were assessed by student's t-test, and considered statistically significant at $p<0.05$ and $p<0.01$.

Results

Histological alterations and TRAP staining for osteoclasts

Two weeks after prednisolone administration, the tibiae of prednisolone administration group showed obvious histological manifestation of osteoporosis, including narrowed growth plate, decreased trabecular

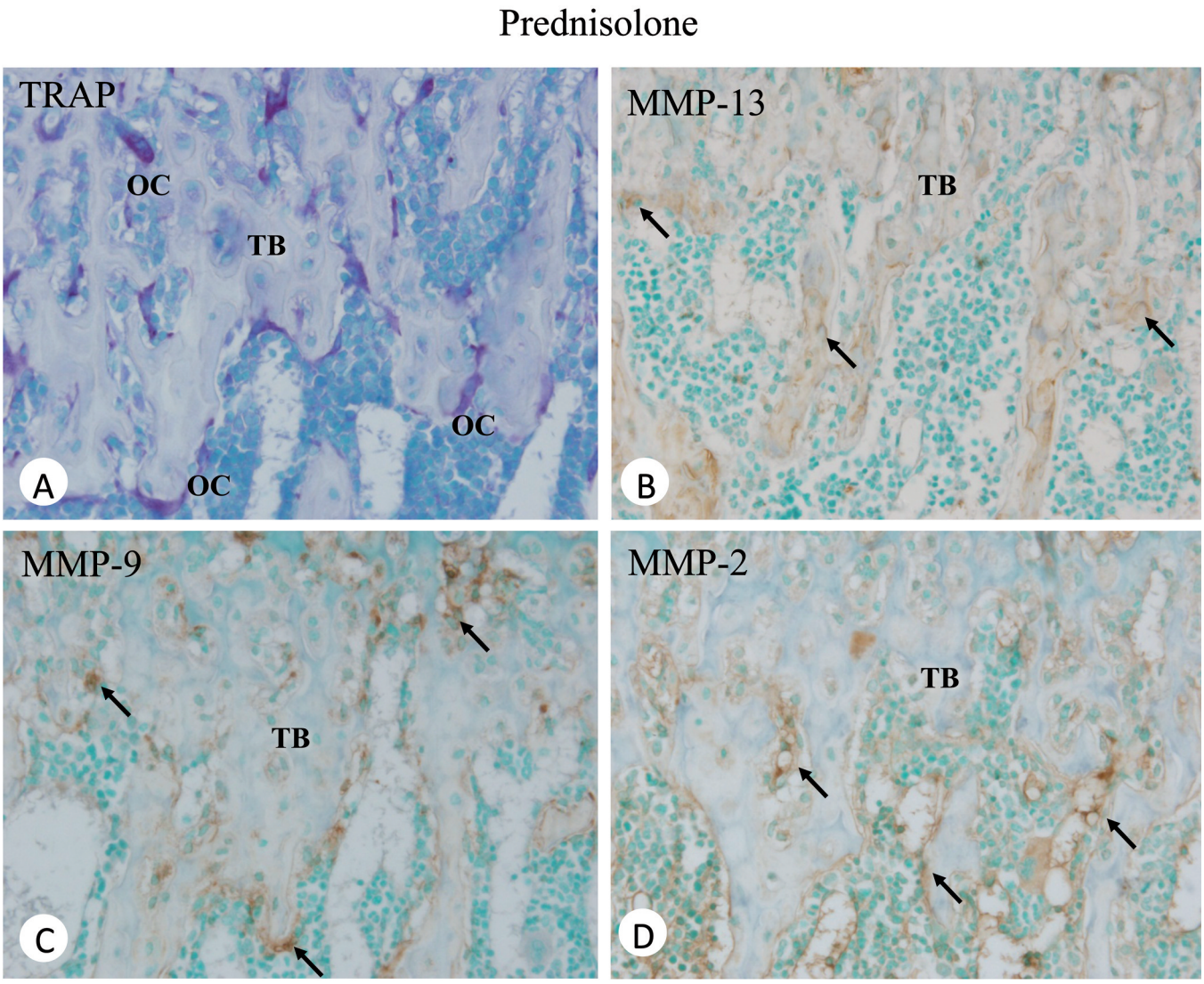

Fig. 3. TRAP staining for osteoclasts and immunolocalization for MMP 2, 9, 13 in the trabecular bone at higher magnification in prednisolone-administered group. **A.** A large number of TRAP-positive osteoclasts were seen on the surface of trabecular bone. **B.** Moderate expression of MMP 13 was found in the bone matrix indicated by black arrows. **C.** Intense expression of MMP 9 was seen on the surface of trabecular bone and adjacent to the growth plate indicated by black arrows. **D.** Moderate expression of MMP 2 was mainly seen on the surface of trabecular bone shown by black arrows. TB: trabecular bone; OC: osteoclast. x 400

bone volume and thinned cortical bone (HE staining, Fig. 1A,B). Statistical analysis revealed several significant differences between prednisolone-administered group and control with regard to BV/TV (29.710±1.240% in the prednisolone administration group vs 82.100±1.620% in control, p<0.01, Fig.1C), thickness of growth plate (160.000±5.770 μm in the experiment group vs 336.700±12.020 μm in control, p<0.01, Fig.1D) and width of cortical bone (148.700±5.930μm in the prednisolone-administered group vs 221.700±4.410 μm in control, p<0.01, Fig. 1E). In particular, lighter eosin staining areas can be distinguished easily in the cortical bone of prednisolone-administered group (Fig. 4B). More interestingly, a number of empty or enlarged lacunae were seen in the impaired areas induced by prednisolone. Statistical analysis revealed significant differences between prednisolone-administered group and control with regard to the number of osteocytes (396.700±12.020 cells/mm^2 in the prednisolone administration group vs 800.000±11.550 cells /mm^2 in control, p<0.01, Fig. 4C) and the number of empty lacunae (220.000±3.000 empty lacunae/mm^2 in the prednisolone administration group vs 80.000±2.500 empty lacunae/mm^2 in control, p<0.01, Fig. 4D).

Compared with control group, the number of TRAP-positive osteoclasts was significantly increased on the surface of trabecular bone in mice administered with prednisolone (Figs. 2A,B, 3A). Statistical analysis found a significant difference in osteoclastic numbers between

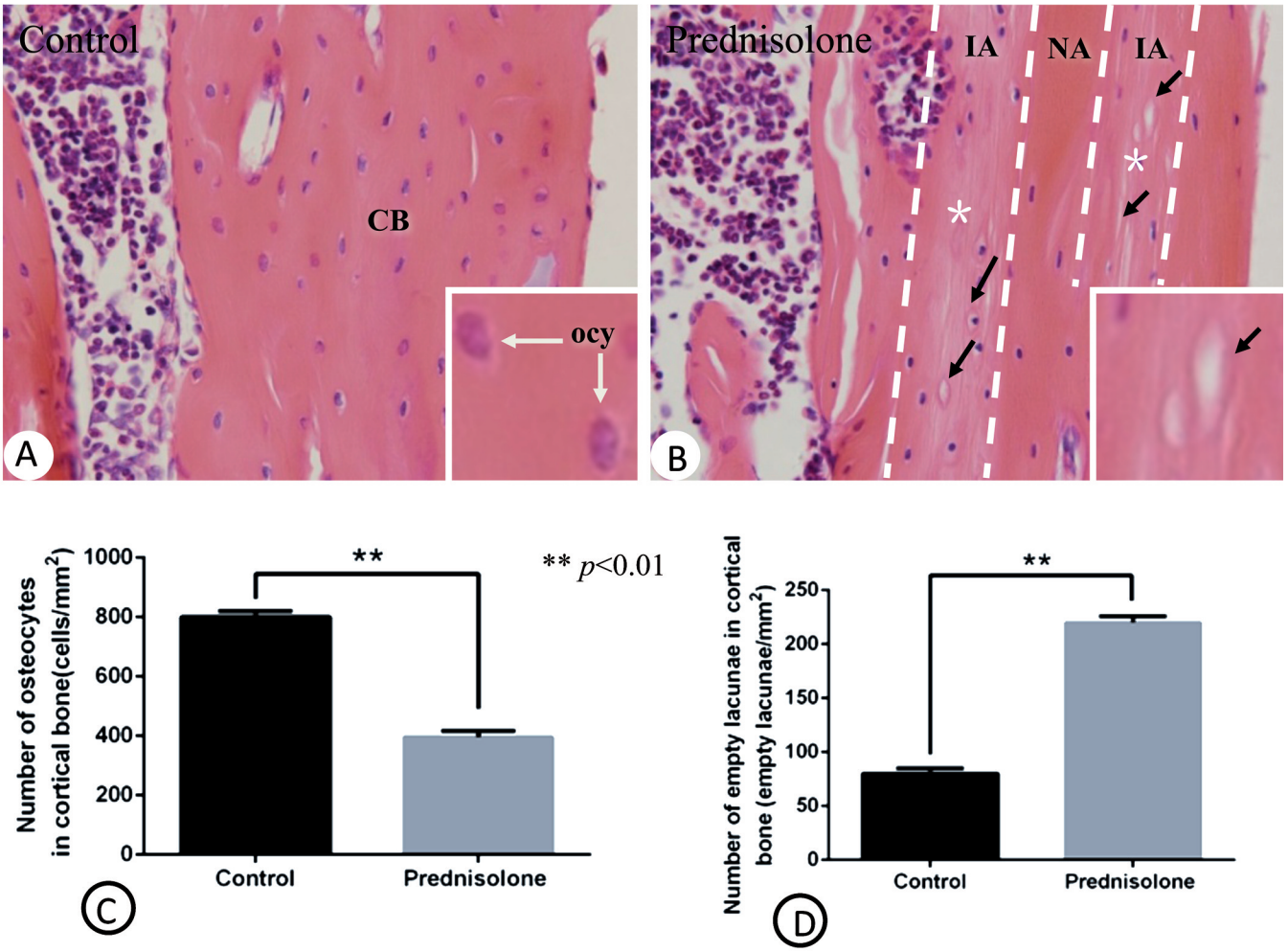

Fig. 4. Histological alterations in the cortical bone and statistical analysis. **A, B.** Histological imaging of cortical bone of control (HE staining) **(A)** and prednisolone treatment group **(B)**. **A.** Control group showed uniform eosin staining and osteocytes were seen within the lacunae indicated by white arrows in the bottom right panel. **B.** Lighter eosin staining areas could be found in the cortical bone of experimental group indicated by white asterisk. Furthermore, a lot of empty lacunae and atrophic osteocytes were seen in the impaired cortical bone areas indicated by black arrows. **C, D.** Number of osteocytes and empty lacunae were counted in the cortical bone for both groups (n=10;** p<0.01). All corresponding numerical information is found in the results section. Error bars indicate ± SD. SD: Standard deviation; CB: cortical bone; ocy: osteocyte; IA: impaired area; NA: normal area. A, B, x 100; inserts, x 400

prednisolone-administered group and control (85.400±2.540 cells/mm^2 in the prednisolone administration group vs 60.130±0.590 cells/mm^2 in control, p<0.01, Fig. 2I).

Immunolocalization of MMP 2, 9 and 13

In the prednisolone-administered group, trabecular bone of metaphysis showed the intense expression of MMP 9 and moderate expression of MMP 2 and MMP 13 (Figs. 2C-H, 3B-D). Statistical analysis revealed significant differences in MMP 2-, MMP 9-, and MMP 13-reactivity between prednisolone-administered group and control (MMP 2: 0.180±0.005 in the prednisolone administration group vs 0.140±0.002 in control, p<0.01; MMP 9: 0.130±0.004 in the prednisolone-treated group vs 0.080±0.002 in control, p<0.01; MMP 13: 0.080±0.003 in the prednisolone-administered group vs 0.050±0.002 in control, p<0.01, Fig. 2J). On the other hand, there is no significant difference between control and prednisolone administration group for MMP 9 expression (Fig. 5C,D,H), but intense expression of MMP 2 and moderate expression of MMP 13 can be seen in the cortical bone in prednisolone administration

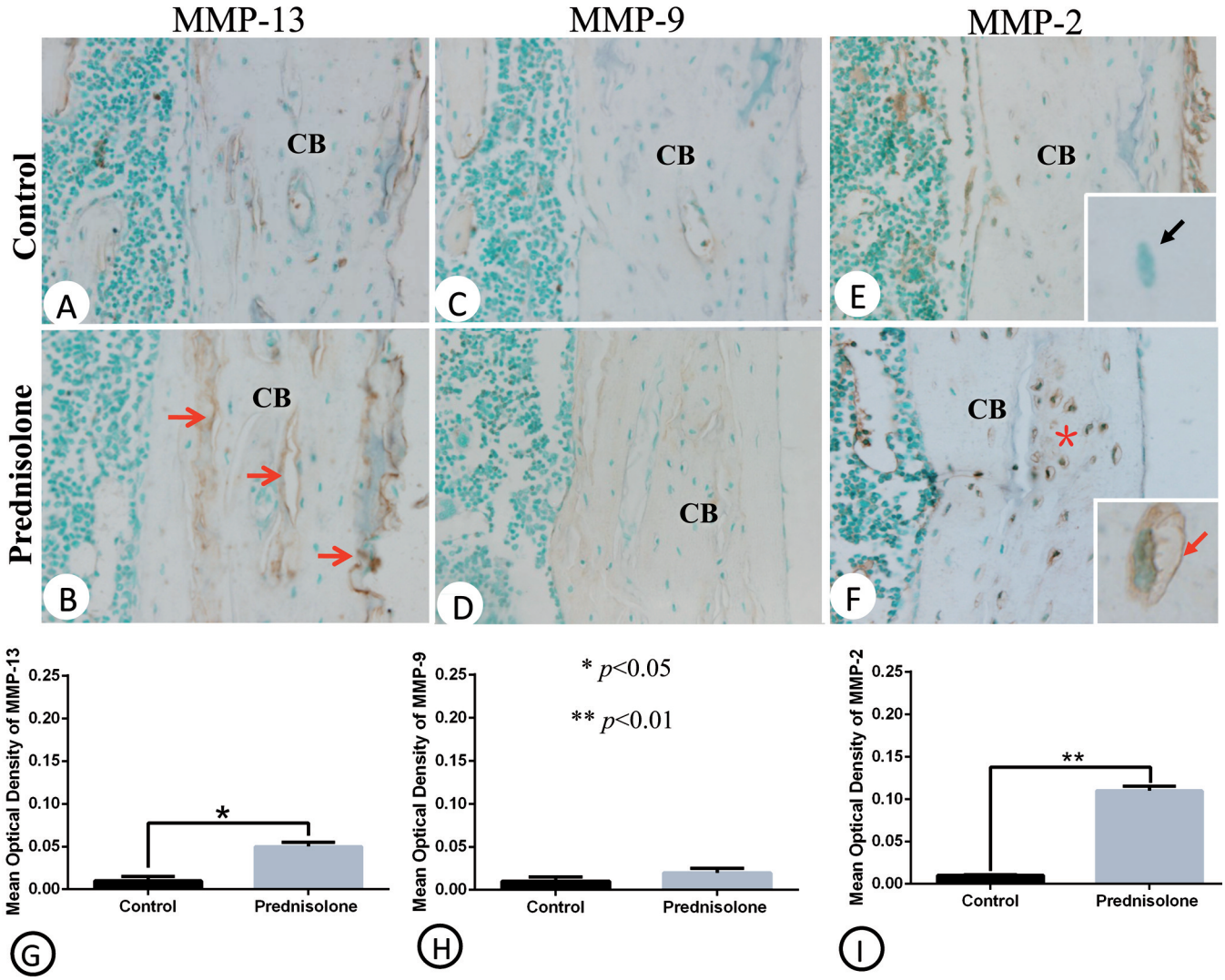

Fig. 5. Immunolocalization of MMP 2, 9, 13 in cortical bone and statistical analysis Upper line **(A, C, E)** control group; Lower line **(B, D, F)** prednisolone-administered group. **A, B.** Expression of MMP 13; **C, D.** Expression of MMP 9. **E, F.** Expression of MMP 2. There was no significant difference between control and prednisolone administration group for MMP 9 expression, but moderate expression of MMP 13 and intense expression of MMP 2 were seen in the crevices and around the lacunae indicated by red arrows and red asterisk in the prednisolone group. **G-I.** Mean optical density of MMP 2, 9, 13 expression was assessed for both groups (n=10;* p<0.05; ** p<0.01). All corresponding numerical information is found in the results section. Error bars indicate ± SD. SD: Standard deviation. CB: Cortical bone. A-F, x 100; inserts, x 400

group (Fig. 5A,B,G and Fig. E,F,I). Furthermore, MMP 2 expression mainly concentrated in the lacunae (Fig. 5F), while the expression of MMP 13 was found in the crevices and periosteum (Fig 5B). Statistical analysis revealed significant differences in MMP 13- and MMP 2-expression between prednisolone-administered group and control (MMP 13: 0.050±0.002 in the prednisolone administration group vs 0.010±0.002 in control, $p<0.05$, Fig. 5G; MMP 2: 0.110±0.002 in the prednisolone administration group vs 0.010±0.001 in control, $p<0.01$, Fig. 5I).

Discussion

In this study, we investigated the expression of MMP 2, 9 and 13 in prednisolone induced osteoporosis in mice. As shown in Fig.1, tibiae of prednisolone administered mice showed the obvious features of osteoporosis including decreased trabecular bone volume, narrowed growth plate and thinner cortical bone with empty lacunae and crevices. These findings are consistent with previous studies, which reported that GC administration reduced bone formation, increased bone resorption and increased apoptosis of osteocytes and osteoblasts both *in vivo* and *in vitro* (Lo Cascio et al., 1995; Weinstein et al., 1998; O'Brien et al., 2004). GCs affect bone cells by binding to glucocorticoid receptor (GR) to form ligand-receptor complex, regulating the expression of a wide array of target genes (Surjit et al., 2011; Karmakar et al., 2013). Our results showed that intense expression of MMP 2, 9 and 13 were seen in the trabecular bone of metaphysis. Furthermore, microscopic observation at higher magnification further verified the existing viewpoints in which MMP 2 and MMP 13 are produced by osteoblasts and osteocytes, while MMP 9 is mainly expressed by osteoclasts (Reponen et al., 1994; Vu et al., 1998; Nakamura et al., 2004; Inoue et al., 2006). Schema graph of prednisolone induces osteoporosis through affecting the expression of MMP 2, 9, 13 was shown in Fig. 6.

Recently, many researchers considered that the loss of trabecular mass, trabecular architecture, and deposition bone mass does not explain the increase in fracture risk from GCs, and the adverse effects of GCs on cortical bone quality may be an independent factor worth further investigation (Black et al., 2010; Jia et al., 2011). As a meaningful finding of this study, we observed two parallel belt-like lighter eosin staining areas containing abundant tiny strip-like crevices, increased number of empty or enlarged lacunae with nucleus condensed osteocytes in the cortical bone of prednisolone administered group. The finding may be new and valuable histological evidence for the adverse effects of GCs on cortical bone quality. In addition, previous studies demonstrated that MMP 13 was synthesized and secreted by osteoblastic cells and translocated to the resorption lacunae. Furthermore, MMP 13 was also shown to be expressed in cement lines (Dew et al., 2000; Stickens et al., 2004; Tang et al., 2012). We therefore also speculate that the increased expression of MMP 13 in the crevices of cortical bone may be related to the formation of the belt-like lighter eosin staining areas in the cortical bone in prednisolone-treated mice.

GC induced osteocytic osteolysis has been paid more and more attention because of its devastating side effects. Up to now, however, most research has focused on the description of this phenomenon; the exact mechanism has not been elucidated. MMP 2 is capable of cleaving native type I collagens and over expression of MMP 2 was reported to be related to osteolysis and arthritis (Aimes and Quigley, 1995; Martignetti et al., 2001). Furthermore, MMP $2^{-/-}$ mice exhibited opposing bone phenotypes featured by moderate disruption of the osteocytic networks and reduced bone density due to an impaired osteocytic canalicular network (Inoue et al., 2006). In the present study, intense immunoreactivity for MMP 2 was exhibited in the enlarged lacunae of

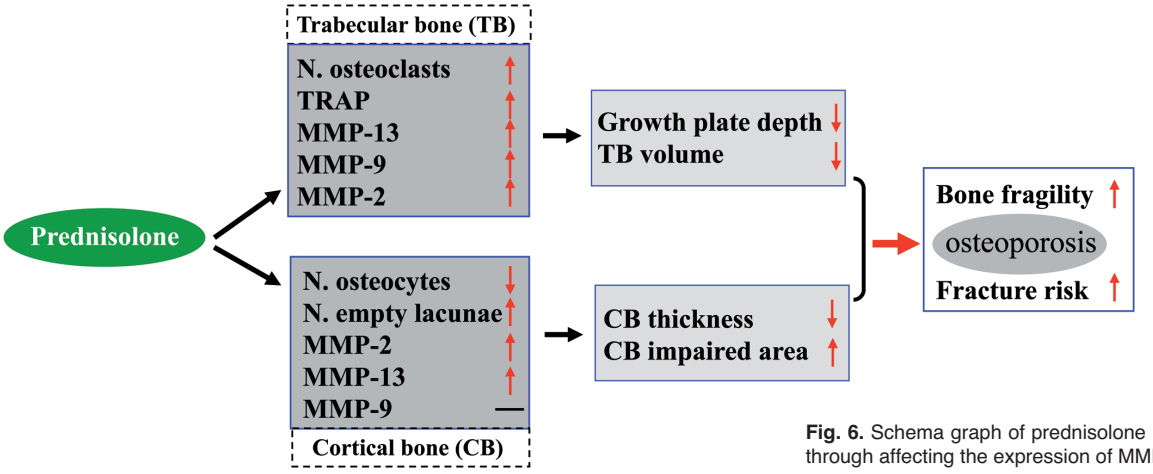

Fig. 6. Schema graph of prednisolone induces osteoporosis through affecting the expression of MMP 2, 9, 13.

osteocytes with condensed nucleus in cortical bone of diaphysis in prednisolone treated mice. The finding suggested that prednisolone administration induced high expression of MMP 2 in lacunae and demonstrated that MMP 2 may play a crucial role in osteocytic osteolysis. However, histological examination was not sufficient and further research might be necessary.

In conclusion, our data suggested that the expressions of MMP 2, 9, 13 were obviously increased in prednisolone induced osteoporosis, especially MMP 2 and MMP 13, which might play key roles in osteocytic osteolysis and the formation of lighter eosin staining impaired areas in the cortical bone (Figure 6). Concurrently, we considered that MMPs might be new potential therapeutic targets for glucocorticoid induced osteoporosis, especially osteocytic osteolysis.

Acknowledgements. This study was partially supported by the National Nature Science Foundation of China (No. 81271965; 81470719; 81311140173) and Specialized Research Fund for the Doctoral Program of Higher Education (No. 20120131110073) to Li M. The Shandong Province Science and Technique Foundation, China (No. 2014GSF118093) to Guo J.

References

Aimes R.T. and Quigley J.P. (1995). Matrix metalloproteinase-2 is an interstitial collagenase. Inhibitor-free enzyme catalyzes the cleavage of collagen fibrils and soluble native type I collagen generating the specific 3/4- and 1/4-length fragments. J. Biol. Chem. 270, 5872-5876.

Bélanger L.F. (1969). Osteocytic osteolysis. Calcif. Tissue Res. 4, 1-12.

Black D.M., Kelly M.P., Genant H.K., Palermo L., Eastell R., Bucci-Rechtweg C., Cauley J., Leung P.C., Boonen S., Santora A., de Papp A., Bauer D.C., Fracture Intervention Trial Steering C. and Committee H.P.F.T.S. (2010). Bisphosphonates and fractures of the subtrochanteric or diaphyseal femur. N. Engl. J. Med. 362, 1761-1771.

Bonewald L.F. and Johnson M.L. (2008). Osteocytes, mechanosensing and Wnt signaling. Bone 42, 606-615.

Carbonare L.D., Arlot M.E., Chavassieux P.M., Roux J.P., Portero N.R. and Meunier P.J. (2001). Comparison of trabecular bone microarchitecture and remodeling in glucocorticoid-induced and postmenopausal osteoporosis. J. Bone Miner. Res. 16, 97-103.

Dew G., Murphy G., Stanton H., Vallon R., Angel P., Reynolds J.J. and Hembry R.M. (2000). Localisation of matrix metalloproteinases and TIMP-2 in resorbing mouse bone. Cell Tissue Res. 299, 385-394.

Engsig M.T., Chen Q.J., Vu T.H., Pedersen A.C., Therkidsen B., Lund L.R., Henriksen K., Lenhard T., Foged N.T., Werb Z. and Delaisse J.M. (2001). Matrix metalloproteinase 9 and vascular endothelial growth factor are essential for osteoclast recruitment into developing long bones. J. Cell Biol. 152, 419-419.

Fardet L., Petersen I. and Nazareth I. (2011). Prevalence of long-term oral glucocorticoid prescriptions in the UK over the past 20 years. Rheumatology 50, 1982-1990.

Inoue K., Mikuni-Takagaki Y., Oikawa K., Itoh T., Inada M., Noguchi T., Park J.S., Onodera T., Krane S.M., Noda M. and Itohara S. (2006). A crucial role for matrix metalloproteinase 2 in osteocytic canalicular formation and bone metabolism. J. Biol. Chem. 281, 33814-33824.

Jia J.J., Yao W., Guan M., Dai W.W., Shahnazari M., Kar R., Bonewald L., Jiang J.X. and Lane N.E. (2011). Glucocorticoid dose determines osteocyte cell fate. FASEB J. 25, 3366-3376.

Karmakar S., Jin Y. and Nagaich A.K. (2013). Interaction of glucocorticoid receptor (GR) with estrogen receptor (ER) alpha and activator protein 1 (AP1) in dexamethasone-mediated interference of ERalpha activity. J. Biol. Chem. 288, 24020-24034.

Krishnan V., Bryant H.U. and Macdougald O.A. (2006). Regulation of bone mass by Wnt signaling. J. Clin. Invest. 116, 1202-1209.

Lane N.E., Yao W., Balooch M., Nalla R.K., Balooch G., Habelitz S., Kinney J.H. and Bonewald L.F. (2006). Glucocorticoid-treated mice have localized changes in trabecular bone material properties and osteocyte lacunar size that are not observed in placebo-treated or estrogen-deficient mice. J. Bone Miner. Res. 21, 466-476.

Li M.Q., Hasegawa T., Hogo H., Tatsumi S., Liu Z.S., Guo Y., Sasaki M., Tabata C., Yamamoto T., Ikeda K. and Amizuka N. (2013). Histological examination on osteoblastic activities in the alveolar bone of transgenic mice with induced ablation of osteocytes. Histol. Histopathol. 28, 327-335.

Lin L., Dai S.D. and Fan G.Y. (2010). Glucocorticoid-induced differentiation of primary cultured bone marrow mesenchymal cells into adipocytes is antagonized by exogenous runx2. APMIS 118, 595-605.

Lo Cascio V., Kanis J.A., Beneton M.N., Bertoldo F., Adami S., Poggi G. and Zanolin M.E. (1995). Acute effects of deflazacort and prednisone on rates of mineralization and bone formation. Calcif. Tissue Int. 56, 109-112.

Ma Y., Ma L., Guo Q.A. and Zhang S.L. (2010). Expression of bone morphogenetic protein-2 and its receptors in epithelial ovarian cancer and their influence on the prognosis of ovarian cancer patients. J. Exp. Clin. Cancer Res. 29-85.

Martignetti J.A., Aqeel A.A., Sewairi W.A., Boumah C.E., Kambouris M., Mayouf S.A., Sheth K.V., Eid W.A., Dowling O., Harris J., Glucksman M.J., Bahabri S., Meyer B.F. and Desnick R.J. (2001). Mutation of the matrix metalloproteinase 2 gene (MMP2) causes a multicentric osteolysis and arthritis syndrome. Nat. Genet. 28, 261-265.

Matsuo K. (2014). Glucocorticoid and bone. Osteocytic osteolysis: potential modulation by glucocorticoids. Clin. Calcium 24, 1337-1342.

Morin C. and Fardet L. (2015). Systemic glucocorticoid therapy: risk factors for reported adverse events and beliefs about the drug. A cross-sectional online survey of 820 patients. Clin. Rheumatol. 2119-2126.

Nakamura H., Sato G., Hirata A. and Yamamoto T. (2004). Immunolocalization of matrix metalloproteinase-13 on bone surface under osteoclasts in rat tibia. Bone 34, 48-56.

O'Brien C.A., Jia D., Plotkin L.I., Bellido T., Powers C.C., Stewart S.A., Manolagas S.C. and Weinstein R.S. (2004). Glucocorticoids act directly on osteoblasts and osteocytes to induce their apoptosis and reduce bone formation and strength. Endocrinology 145, 1835-1841.

Ohnaka K., Tanabe M., Kawate H., Nawata H. and Takayanagi R. (2005). Glucocorticoid suppresses the canonical Wnt signal in cultured human osteoblasts. Biochem. Biophys. Res. Commun. 329, 177-181.

Pepene C.E., Seck T., Diel I., Minne H.W., Ziegler R. and Pfeilschifter J. (2010). Effect of glucocorticoid-, parathyroid- and thyroid hormones excess on human iliac crest bone matrix insulin-like growth factor

(IGF)-I in patients with osteoporosis. Exp. Clin. Endocrinol. Diabetes. 118, 310-314.

Pichler K., Loreto C., Leonardi R., Reuber T., Weinberg A.M. and Musumeci G. (2013). RANKL is downregulated in bone cells by physical activity (treadmill and vibration stimulation training) in rat with glucocorticoid-induced osteoporosis. Histol. Histopathol. 28, 1185-1196.

Poole K.E.S., van Bezooijen R.L., Loveridge N., Hamersma H., Papapoulos S.E., Löwik C.W. and Reeve J. (2005). Sclerostin is a delayed secreted product of osteocytes that inhibits bone formation. FASEB J. 19, 1842-1844.

Reponen P., Sahlberg C., Munaut C., Thesleff I. and Tryggvason K. (1994). High expression of 92-kD type IV collagenase (gelatinase B) in the osteoclast lineage during mouse development. J. Cell Biol. 124, 1091-1102.

Rong X., Ni W., Liu Y., Wen J., Qian C., Sun L. and Wang J. (2014). Bufalin, a bioactive component of the Chinese medicine chansu, inhibits inflammation and invasion of human rheumatoid arthritis fibroblast-like synoviocytes. Inflammation 37, 1050-1058.

Roux C., Reid D.M., Devogelaer J.P., Saag K., Lau C.S., Reginster J.Y., Papanastasiou P., Bucci-Rechtweg C., Su G. and Sambrook P.N. (2012). Post hoc analysis of a single IV infusion of zoledronic acid versus daily oral risedronate on lumbar spine bone mineral density in different subgroups with glucocorticoid-induced osteoporosis. Osteoporos. Int. 23, 1083-1090.

Sasaki N., Kusano E., Ando Y., Yano K., Tsuda E. and Asano Y. (2001). Glucocorticoid decreases circulating osteoprotegerin (OPG): possible mechanism for glucocorticoid induced osteoporosis. Nephrol. Dial. Transplant. 16, 479-482.

Sasaki N., Kusano E., Takahashi H., Ando Y., Yano K., Tsuda E. and Asano Y. (2005). Vitamin K2 inhibits glucocorticoid-induced bone loss partly by preventing the reduction of osteoprotegerin (OPG). J. Bone Miner. Metab. 23, 41-47.

Shahzad M., Liu J., Gao J., Wang Z., Zhang D., Nabi F., Li K. and Li J. (2015). Differential expression of extracellular matrix metalloproteinase inducer (EMMPRIN/CD147) in avian tibial dyschondroplasia. Avian Pathol. 44, 13-18.

Shi C., Qi J., Huang P., Jiang M., Zhou Q., Zhou H., Kang H., Qian N., Yang Q., Guo L. and Deng L. (2014). MicroRNA-17/20a inhibits glucocorticoid-induced osteoclast differentiation and function through targeting RANKL expression in osteoblast cells. Bone 68, 67-75.

Sivagurunathan S., Muir M.M., Brennan T.C., Seale J.P. and Mason R.S. (2005). Influence of glucocorticoids on human osteoclast generation and activity. J. Bone. Miner. Res. 20, 390-398.

Stevens J.W. (2013). Swarm chondrosarcoma: a continued resource for chondroblastic-like extracellular matrix and chondrosarcoma biology research. Connect. Tissue Res. 54, 252-259.

Stickens D., Behonick D.J., Ortega N., Heyer B., Hartenstein B., Yu Y., Fosang A.J., Schorpp-Kistner M., Angel P. and Werb Z. (2004). Altered endochondral bone development in matrix metalloproteinase 13-deficient mice. Development 131, 5883-5895.

Surjit M., Ganti K.P., Mukherji A., Ye T., Hua G., Metzger D., Li M. and Chambon P. (2011). Widespread negative response elements mediate direct repression by agonist-liganded glucocorticoid receptor. Cell 145, 224-241.

Tang S.Y., Herber R.P., Ho S.P. and Alliston T. (2012). Matrix metalloproteinase-13 is required for osteocytic perilacunar remodeling and maintains bone fracture resistance. J. Bone. Miner. Res. 27, 1936-1950.

Tazawa K., Hoshi K., Kawamoto S., Tanaka M., Ejiri S. and Ozawa H. (2004). Osteocytic osteolysis observed in rats to which parathyroid hormone was continuously administered. J. Bone Miner. Metab. 22, 524-529.

Vu T.H., Shipley J.M., Bergers G., Berger J.E., Helms J.A., Hanahan D., Shapiro S.D., Senior R.M. and Werb Z. (1998). MMP-9/gelatinase B is a key regulator of growth plate angiogenesis and apoptosis of hypertrophic chondrocytes. Cell 93, 411-422.

Weinstein R.S., Jilka R.L., Parfitt A.M. and Manolagas S.C. (1998). Inhibition of osteoblastogenesis and promotion of apoptosis of osteoblasts and osteocytes by glucocorticoids. Potential mechanisms of their deleterious effects on bone. J. Clin. Invest. 102, 274-282.

Yao W., Cheng Z., Busse C., Pham A., Nakamura M.C. and Lane N.E. (2008). Glucocorticoid excess in mice results in early activation of osteoclastogenesis and adipogenesis and prolonged suppression of osteogenesis: a longitudinal study of gene expression in bone tissue from glucocorticoid-treated mice. Arthritis Rheum. 58, 1674-1686.

Accepted December 4, 2015