Contents lists available at ScienceDirect

Electronic Journal of Biotechnology

journal homepage:

Research Article

Protective effect of Sika Deer bone polypeptide extract on dexamethasone-induced osteoporosis in rats

Liping An [a], Liqiang Shi [a], Yujie Ye [a], Dankai Wu [b], Guangkai Ren [b], Xiao Han [a], Guangyu Xu [a], Guangxin Yuan [a,*], Peige Du [a,*]

[a] College of Pharmacy, Beihua University, Jilin, Jilin 132013, PR China
[b] Department of Orthopedics, Orthopedic Hospital, Jilin University Second Hospital, Changchun, Jilin 130041, PR China

ARTICLE INFO

ABSTRACT

Background: Osteoporosis attacks approximately 10% of the population worldwide. Sika Deer (*Cervus nippon*), one of China's precious traditional medicinal animals, has been widely recorded in ancient Chinese medical books and claimed for centuries to have numerous medical benefits including bone strengthening. This study aimed to find the use of Sika Deer bone in treating osteoporosis according to traditional records and to investigate the protective effect of Sika Deer bone polypeptide extract on glucocorticoid-induced osteoporosis (GIOP) in rats.
Results: Sika Deer bone polypeptide extract could increase serum Ca^{2+} and BGP, decrease serum P^{3+}, ALP, PTH, and CT, but had no effect on serum NO in rats with GIOP. The immunohistochemical iNOS results of the rats' distal femur were negative in each group. Besides the model group, the eNOS color reaction in osteoblasts was strongly positive in the other three groups.
Conclusions: Sika Deer bone polypeptide extract can improve pathological changes in the microstructure and stimulate the expression of eNOS in osteoblasts. The protective effect on bone might be mediated by eNOS-dependent NO generation.
How to cite: An L, Shi L, Ye Y, et al. Protective effect of Sika Deer bone polypeptide extract on dexamethasone-induced osteoporosis in rats. Electron J Biotechnol 2021;51. https://doi.org/10.1016/j.ejbt.2021.04.002

1. Introduction

Glucocorticoids are widely used for the treatment of various diseases such as chronic obstructive pulmonary disease, asthma, rheumatism, autoimmune diseases, inflammatory bowel diseases, and organ transplantation [1,2]. In addition to directly reducing the number and activity of osteoblasts, glucocorticoids can also reduce the absorption of calcium in the gastrointestinal tract, accelerate the excretion of calcium by the kidney, and inhibit insulin-like growth factor and prostaglandins by reducing the secretion of sex hormones; leading to osteopenia by affecting the parathyroid hormone [3,4]. With the wide application of glucocorticoids in clinics, a rise in the incidence of glucocorticoid-induced osteoporosis (GIOP) has been witnessed. The incidence ranks only second to that of postmenopausal and senile osteoporosis [5,6].

Owing to these reasons, effective prevention and treatment of osteoporosis induced by glucocorticoids have become an important issue to be resolved by clinicians and researchers. More attention needs to be paid to its pathogenesis.

Sika Deer bone, the skeleton of the Sika Deer (*Cervus nippon*), is one of the commonly used traditional Chinese medicines and can be used in tonifying bones. "*Dictionary of Traditional Chinese Medicine*" recorded that it has the pharmacological effect of invigorating asthenia, strengthening tendons and bones, and curing pain in limbs and arthritis and in tendons and bones caused by rheumatism. Several preclinical studies have demonstrated that deer bone-related products were effective in alleviating ovariectomy-induced osteoporosis in rats, and facilitating osteoblasts proliferation. Deer bone contains a large amount of protein, collagen, phospholipids, chondroitin, and phosphoprotein that are beneficial to bone formation, promote the synthesis of collagen and elastin, delay the bone resorption, and facilitate the process of endochondral ossification in the body [7,8]. In addition, deer bone also contains a lot of minerals such as calcium, magnesium, iron,

zinc, potassium, copper, phosphorus, and selenium, which help to maintain calcium, phosphorus, and magnesium ion concentrations and proportions in serum; providing a suitable microenvironment for bone regeneration [9].

Deer bone polypeptide, the main component in deer bone extracts and with features such as high water solubility and activities, has received much attention. It was used in the clinical treatment of rheumatism, rheumatoid arthritis, fracture, and trauma repair as injection and powder injection [10,11]. It has a significant effect, but its effect and mechanism on osteoporosis are seldom reported. In this study, a rat dexamethasone-induced osteoporosis model was established, and some related serum and morphological indexes were observed— to evaluate the therapeutic effect of bone polypeptides, such as delaying bone resorption, promoting bone formation, and improving osteoporosis—to explore the protective effect and mechanism of deer bone polypeptide on dexamethasone-induced osteoporosis, and to provide a theoretical basis for the development of deer bone polypeptide-related products.

2. Materials and methods

2.1. Materials

2.1.1. Animals

A total of 32 male Wistar rats aged 2.5 months were provided by Yisi Laboratory Animal Center of Jilin University (laboratory animal license number: SCXK-(Kat) 20140003).

2.1.2. Preparation of Sika Deer bone polypeptide extract

Sika Deer bone was purchased from Jilin City Longtan Mountain Deer Farm. Sika Deer bone polypeptide extract was prepared in the laboratory; after weighing, the fresh deer bone was cleaned, hair and fat were removed, and the bone was dried, put into water, and sterilized. Then, the bone grease was removed and the solution was filtered to collect the filtrate. The process was repeated one more time. The two filtrates were mixed, and the combined filtrate was concentrated. The concentrated filtrate was hydrolyzed with pepsin for 5 h; first under the enzymatic hydrolysis conditions at 1:50 ratio of pepsin and substrate (m/v), 37°C, and pH 2, and then with trypsin for 5 h again under the enzymatic hydrolysis conditions at 1:50 ratio of enzyme and substrate (m/v), 37°C, and pH 8; to obtain a Deer bone polypeptide extract. Furthermore, by using a HiTrap DEAE Sepharose Fast Flow column and a HiTrap Desalting Sephadex G-25 Superfine column to obtain a single-component Deer bone polypeptide extract by ÄKTA Pure protein purification instrument, the molecular weight was determined by Tricine-SDS PAGE. The chemical characterization of the deer bone polypeptide extract was further detected by UV spectrophotometry, Fourier transform infrared spectroscopy, and HPLC.

2.1.3. Main instruments

MicroCT scanners (Scanco microCT μ 100, Switzerland) and microplate reader (LabSystems Multiskan MS Company, Finland) were used in the study.

2.2. Methods

2.2.1. Animal grouping and administration

A total of 32 male Wistar rats were randomly divided into four groups: blank control group, model, Sika Deer bone polypeptide extract group, and positive control group (8 rats in each group). The rats in the blank control group were given an equal volume of saline solution intramuscularly, and those in the other three groups were given 2.5 mg/kg dexamethasone intramuscularly, twice a week, for establishing a rat GIOP model [12]. The rats in

the Sika Deer bone polypeptide extract group, positive control group, blank control, and model groups were intragastrically given 250 mg/kg of bone polypeptide, 540 mg/kg of Fufang Lurong Jiangu Jiaonang (Complex Antler Bone-invigorating Capsule, batch number: 14100231), and the same volume of saline, respectively, once daily for 75 d.

2.2.2. Detection of serum biochemical indexes

Blood samples were collected through the abdominal aorta of the anesthetized rats, left to stand for some time, and then centrifuged at 2000 rpm for 10 min to obtain the serum. The serum samples were stored at −20°C for use and kept at room temperature for thawing evenly. Ca^{2+}, calcitonin (CT), osteocalcin, bone-Gla-protein (BGP), parathyroid hormone (PTH), and nitric oxide (NO) levels in the serum samples were determined by the enzyme-linked immunosorbent assay (ELISA) method following the kit instructions. Phosphorus levels in the serum samples were detected with an automatic biochemical analyzer.

2.2.3. Detection by MicroCT

After the rats were sacrificed, their right tibias and soft tissues attached to the tibias were quickly removed, and then, the bone tissue samples were fixed in 4% formaldehyde solution. The bone samples placed and fixed well in sample cups were scanned (scanning voltage: 70 kVp, scanning current: 200 μA, layer spacing: 14.8 μm, dimensional resolution: 300 ms, and continuously scanning about 808 layers). After completing the scan, inflated and diaphyseal regions of the tibias were selected as the region of interest (ROI). The ROI of the specimens was reconstructed after three-dimensional reconstruction threshold values were set. The total volume (TV), bone volume (BV), bone volume/total volume (BV/TV), trabecula number (Tb.N), trabecula thickness (Tb.Th), trabecula separation (Tb.Sp), and bone surface/bone volume (BS/BV) were analyzed.

2.2.4. Hematoxylin and eosin staining

After the final administration, the rats were sacrificed and one-third of the distal parts of the femurs were taken. The surrounding tissues attached to them were cleanly removed. Then, the bone samples were fixed in 4% formaldehyde solution, embedded, sectioned, and stained for preparing undecalcified bone sections.

2.2.5. Immunohistochemical observation of femoral slices on inducible NO synthase and endothelia NO synthase

After placing in 10% formaldehyde solution for fixation, one-third of the distal parts of the femurs left were dehydrated step by step, immersed in butyl methacrylate and methyl methacrylate in the ratio of 3:7 for 24 h (vacuum negative pressure: 8.67 kPa), and embedded in semi polymerized methyl methacrylate. Each specimen was continuously cut with a YD-1508R paraffin slicing machine into several undecalcified 7-μm-thick sections. An immunohistochemical streptavidin–peroxidase method was used to detect the expression of inducible NO synthase (iNOS) and endothelial NO synthase (eNOS) according to the kit instructions.

2.2.6. Statistical analysis

SPSS statistics software V21.0 was used for data variance analysis and testing. The data were expressed as mean ± standard deviation ($\bar{X} \pm s$). A P value < 0.05 was considered to be statistically significant.

2.2.7. Ethical consideration

This study was approved by the Ethics Committee of the School of Basic Medical Sciences, Jilin University, and consent for each mouse was obtained in a written informed consent form.

3. Results

3.1. Preparation of Sika Deer bone polypeptide extract

The separation and purification results of deer bone polypeptide extract are shown in Fig. S1A: Sika Deer bone polypeptide extract was separated by DEAE Sepharose Fast Flow column and the elution peak was detected at 220 nm to obtain two components named: DBPE-A and DBPE-B. After freeze-drying, Sephadex G-25 superfine column was used to further separate and obtain the single component named DBPE-A-1; this single component is exhibited in Fig. S1B. Results of Tricine-SDS PAGE depicted that the molecular weight of DBPE-A-1 is greater than 3.3 KDa and less than 6.5 KDa (Fig. S2A). The UV full-wavelength scanning results exhibited that the maximum absorption wavelength of DBPE-A-1 was 208 nm, which was in the UV absorption range of polypeptide substance (Fig. S2B). The infrared spectrum scanning results demonstrated that DBPE-A-1 had the characteristic absorption peak of polypeptide and protein near 1,615 cm and 1,550 cm; and through the spectral database retrieval, five of the ten spectrums of substances with the highest hit rate were polypeptide (protein) spectrums (Fig. S2C). The optimized conditions of HPLC were as follows: chromatographic column: Alltima C18 (250 mm × 4.6 mm, 5 μm); mobile phase: 0.1% phosphate buffer-acetonitrile (80:20); flow rate: 0.500 ml/min; column temperature: 25°C; detection wavelength: 208 nm, 215 nm, and 220 nm; and injection volume: 10 μl. The results in 208 nm depicted that the retention time of DBPE-A-1-b was 6.581 s, the peak area was 24.8593%, and the separation degree was 4.562 > 1.5, Purity index of peak b was 99.99% (Fig. S2D). The results showed that DBPE-A-1 could be further separated.

3.2. Effects of Sika Deer bone polypeptide extract on the general state and body weight in rats with GIOP

During the experiment, the rats with GIOP were lazy and disliked eating; their fur and hair lacked luster. However, the rats in the blank control and Sika Deer bone polypeptide extract groups moved as usual, and their fur and hair showed a better glow. Table 1 illustrates that the body weights of the rats in the model group significantly decreased compared with those in the control group ($P < 0.05$); and the body weights of the rats in the Sika Deer bone polypeptide extract group were significantly higher compared with those in the model group ($P < 0.05$) (Table S1).

3.3. Effects of Sika Deer bone polypeptide extract on serum Ca^{2+} and P^{3+} levels and ALP activities in rats with GIOP

The results showed that serum Ca^{2+} levels in the rats in the model group decreased and serum ALP activities increased significantly compared with those in the blank control group ($P < 0.05$). Moreover, serum Ca^{2+} levels significantly increased ($P < 0.05$), and serum P^{3+} levels and ALP activities significantly decreased ($P < 0.05$) in the rats in the Deer bone polypeptide

extract group compared with those in the model group, as given in Table 1.

3.4. Effects of Sika Deer bone polypeptide extract on serum PTH, BGP, CT, and NO levels in rats with GIOP

As illustrated in Table 1, serum PTH levels decreased ($P < 0.05$) and serum BGP levels increased ($P < 0.05$) significantly in the rats in the Deer bone polypeptide extract group compared with those in the model group. Serum CT levels also presented a declining tendency, and serum NO levels slightly decreased, but without any statistically significant difference.

3.5. Effects of Sika Deer bone polypeptide extract on the bone microstructure in rats with GIOP

As shown in Table 2, and Fig. S1A–D, Tb.N and BV/TV in the proximal tibia of deer bone significantly increased ($P < 0.05$) and Tb.Sp and BS/BV significantly decreased ($P < 0.05$) in the rats in the Deer bone polypeptide extract group compared with those in the control group; however, Tb.Th showed no significant difference ($P > 0.05$). (Fig. 1)

3.6. Effects of Sika Deer bone polypeptide extract on the morphology of rats with dexamethasone-induced osteoporosis

The pathological examination of the slices of the rats' one-third of the distal parts of the femurs under a light microscope demonstrated that—the number of trabeculae decreased, they were sparse and ruptured, most of them could not connect with the mesh, the bone marrow cavity was enlarged, the trabecular structure appeared with larger blank regions, and a large number of fat cells could be found in the model group compared with the blank control group. Furthermore, the femoral trabeculae were wider and thicker with a significant increase in their number; the ruptured trabeculae and fat cells were rarely found, and the trabeculae were smooth in the Deer bone polypeptide extract group compared with the model group. All these findings were similar to those noted in the control group (Fig. 2A–D).

3.7. Immunohistochemical observation

The immunohistochemical observation revealed that the results of iNOS were negative, eNOS-immunoreactive substances were sepia precipitates, and eNOS was expressed in the trabeculae and bone marrow cavity; mainly distributed in osteoblasts and bone marrow stromal cells surrounding the trabeculae. The color reaction of osteoblasts in the model group was weakly positive, the cytoplasm was light gray, only a few regions demonstrated a brown precipitate, and the osteoblasts were dispersed with no dense particles in them. The color reaction of osteoblasts in the Deer bone polypeptide extract group was strongly positive, the cytoplasm was filled with brown precipitates, the osteoblasts were distributed uniformly, but the density was slightly lower than that in the control group. The color reaction of osteoblasts in the blank

Table 1
Effects of Deer bone polypeptide extract on serum ALP activities, Ca^{2+}, P^{3+}, PTH, BGP, CT, and NO levels in rats with GIOP ($\bar{X} \pm s$, $n = 8$).

Group	ALP (U/L)	Ca^{2+} (μmol/L)	P^{3+} (mmol/L)	PTH (pg/mL)	BGP (ng/mL)	CT (ng/L)	NO (ng/mL)
Blank control	16.29 ± 3.08	533.98 ± 106.72	1.58 ± 0.365	64.195 ± 13.77	4.67 ± 0.785	123.50 ± 16.76	10.59 ± 1.76
Model	23.7 ± 5.45[*]	292.95 ± 83.48[*]	2.22 ± 0.047[*]	100.797 ± 10.06[*]	2.85 ± 0.705[*]	122.79 ± 13.62	8.91 ± 1.70
Deer bone polypeptide extract	15.99 ± 2.53[#]	417.35 ± 88.74[#]	1.66 ± 0.293[#]	73.52 ± 11.35[#]	5.04 ± 0.257[#]	114.94 ± 13.40	10.88 ± 2.17
Positive control	21.75 ± 3.6	445.69 ± 84.51[#]	1.83 ± 0.37[#]	79.25 ± 17.77	3.97 ± 1.387	125.31 ± 9.98	11.01 ± 1.56

[*] $P < 0.05$ vs. the blank control group.
[#] $P < 0.05$ vs. the model group.

Table 2
Effects of Deer bone polypeptide extract on the bone microstructure in rats with GIOP ($\bar{X} \pm s$, n = 8).

Group	Tb.N	BV/TV	Tb.Sp	BS/BV	Tb.Th
Blank control	3.52 ± 0.45	0.33 ± 0.05	0.25 ± 0.03	21.27 ± 3.18	0.07 ± 0.01
Model	2.45 ± 0.02*	0.17 ± 0.04*	0.31 ± 0.02*	29.12 ± 4.69*	0.08 ± 0.02
Deer bone polypeptide extract	3.36 ± 0.38#	0.29 ± 0.06#	0.21 ± 0.04#	21.77 ± 2.4#	0.09 ± 0.01
Positive control	3.13 ± 0.29	0.23 ± 0.03	0.19 ± 0.03#	27.67 ± 1.3	0.1 ± 0.02

* $P < 0.05$ vs. the blank control group.
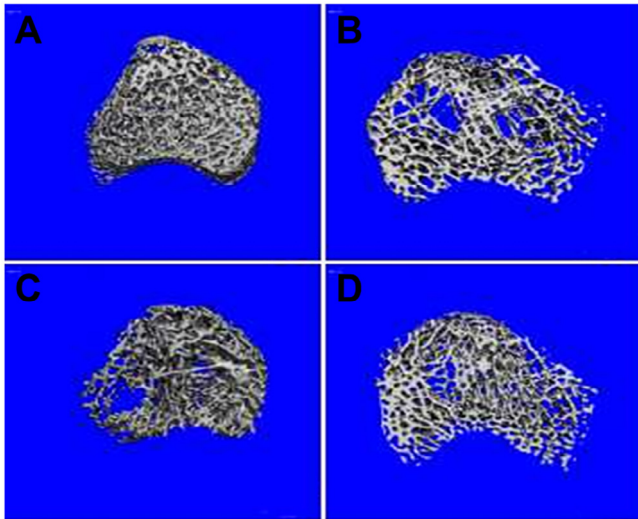# $P < 0.05$ vs. the model group.

Fig. 1. Effects of Deer bone polypeptide extract on the bone microstructure in rats with GIOP. (A) Blank control; (B) model; (C) Deer bone polypeptide extract; (D) positive control.

control group was strongly positive, and the whole cytoplasm was uniformly stained with a brown dense precipitate (Fig. 3A–D).

The statistical analysis demonstrated that the integral optical density values of femoral eNOS expression in the model group significantly decreased ($P < 0.01$) compared with those in the control group. After administering the Deer bone polypeptide extract, the optical density values of femoral eNOS expression in rats with GIOP were significantly higher than those in the model group ($P < 0.01$), as illustrated in Table 3.

4. Discussion

The application of long-term high-dose corticosteroids can not only—inhibit the proliferation of osteoblasts, promote their apoptosis, and reduce the components of active osteoblasts in the bone, resulting in decreased bone formation; but also allow the bone surface to be absorbed by osteoclasts, leading to bone loss and osteoporosis. Because the bone loss induced by glucocorticoids is primarily caused by the inhibition of bone formation, the key to prevent the bone loss induced by glucocorticoids is to stimulate bone formation and promote the increase in bone mass [13,14].

In this study, after injecting dexamethasone intramuscularly for 75 d, the serum BGP in rats in the model group was significantly lower, indicating that dexamethasone could promote the decomposition of minerals in the bone and increase the loss of calcium from the bone, leading to a bone metabolism disorder. The pathological results depicted that the trabeculae became sparse, thin, and ruptured; the bone marrow cavity expanded, the fat cells increased, and the bone density decreased, indicating that the rat GIOP model was successfully established. The serological results exhibited a significant decline in serum Ca^{2+} in the model group, which may be caused by the inhibition of calcium absorption induced by the application of a large dose of corticosteroids within a short time from different parts of the gastrointestinal tract—especially in the duodenum where glucocorticoids can inhibit the transport of calcium ions across cells, reduce the synthesis of
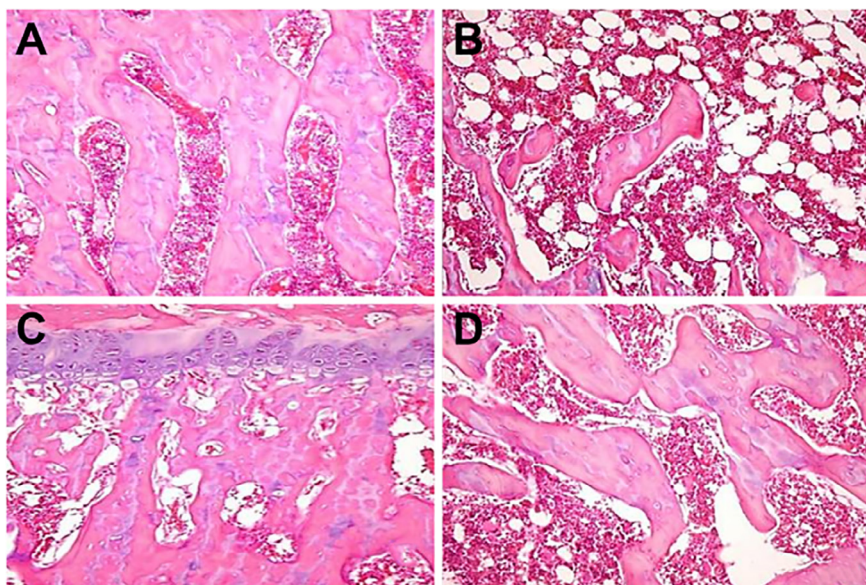

Fig. 2. HE staining of the rats' femurs (×100). (A) Blank control; (B) model; (C) Deer bone polypeptide extract; (D) positive control.
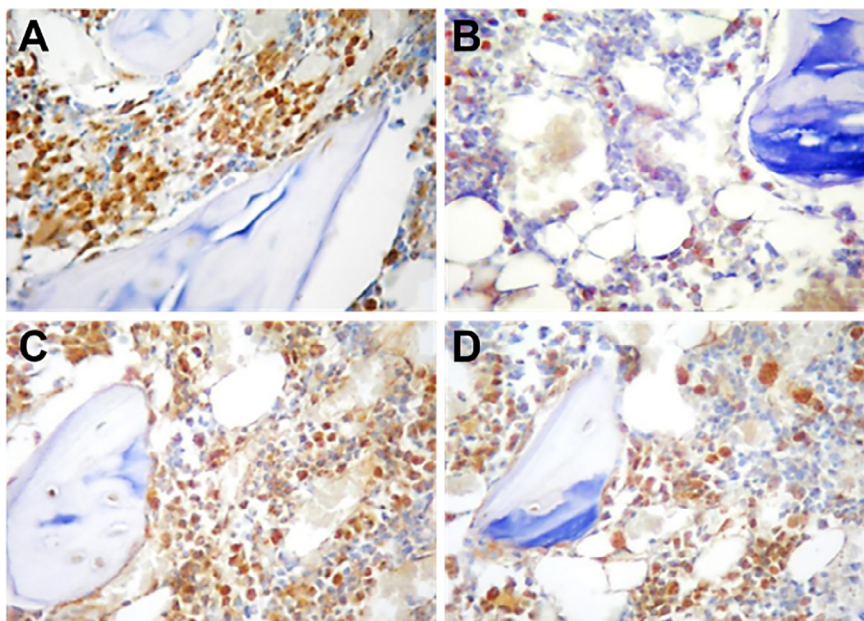
Fig. 3. Effects of Deer bone polypeptide extract on the eNOS expression in rats with GIOP ($\times$400). (A) Blank control; (B) model; (C) Deer bone polypeptide extract; (D) positive control.

Table 3
Effects of Deer bone polypeptide extract on the eNOS expression in rats with GIOP ($X \pm$ s, $n = 10$).

Group	Integral optical density values
Blank control	0.407 ± 0.038
Model	$0.198 \pm 0.059^{*}$
Deer bone polypeptide extract	$0.416 \pm 0.073^{\#}$
Positive control	$0.335 \pm 0.062^{\#}$

* $P < 0.05$ vs. the blank control group.
$^{\#}$ $P < 0.05$ vs. the model group.

calcium-binding proteins, and in turn reduce calcium absorption from intestines. Moreover, glucocorticoids can inhibit the reabsorption of calcium by the renal tubules to induce hypercalciuria, resulting in the increased excretion of calcium in the urine; this negative calcium balance can cause a compensatory increase in P^{3+} and PTH levels [15]. The serum biochemistry indexes, such as serum Ca^{2+}, P^{3+}, and PTH levels, significantly improved in rats with GIOP and treated with Deer bone polypeptide extract in this study, suggesting that Deer bone polypeptide extract can improve the dexamethasone-induced calcium and phosphorus metabolic imbalance.

ALP is a phosphomonoesterase primarily distributed in the bone, liver, kidney, small intestine, and lung tissues in the body; 50% of the ALP in serum is from the bone and another 50% of it is mainly from the liver. The ALP is secreted by osteoblasts in the bone, mainly concentrated in the ossification sites, that is, the epiphyseal line and subperiosteum [16]. BGP originates from bone tissues, synthesized and secreted by osteoblasts in the bone. It is the main component of noncollagenous proteins in the bone, accounting for about 3% of bone proteins, and its role is to regulate and maintain bone calcium. Two-third of osteocalcin is combined with light apatite crystals and deposited in the bone matrix, and one-third of osteocalcin enters the bloodstream so that serum osteocalcin concentrations can specifically reflect the activity of its "parent" osteoblasts [17,18,19]. The osteocalcin in the matrix can be released into the blood during bone resorption. Hence, osteocalcin is a specific indicator to evaluate bone formation and

turnover. Both ALP and BGP are biochemical indicators for bone formation, and the level of ALP and BGP may reflect the level of bone formation. CT is a hormone mainly synthesized and secreted by C cells of the thyroid gland, playing an important role in regulating bone metabolism and inhibiting the activity of osteoclasts through a receptor-mediated effect [20,21,22]. In this experiment, ALP, BGP, and CT contents in the serum of rats were detected by using the ELISA method. The results depicted that the serum ALP content decreased, the serum BGP content increased, and the serum CT content also exhibited a declining tendency in the Deer bone polypeptide extract group, suggesting that Deer bone polypeptide extract can inhibit bone resorption and reduce the rate of bone turnover to strengthen bone formation and alleviate the bone loss.

One of the features of osteoporosis is the degeneration of bone microstructure. Bone microstructure indexes such as the number, density, and spatial conformation of trabeculae can affect the biomechanical properties of the bone. Hence, changes in the bone microstructure can reflect the situation of osteoporosis directly. Pathological observation, the gold standard for evaluating the bone microstructure, is the most direct evidence to reflect changes in the bone microstructure [23,24,25]. MicroCT is an in vitro noninvasive measurement technology suitable for the three-dimensional analysis of trabecular structures following drug therapy. In this study, histopathological testing and MicroCT were simultaneously used for the analyses, and the results demonstrated that the trabeculae became thin and ruptured, their free ends increased, number decreased, space was widened, and the destruction of space structures increased. The intervention with Deer bone polypeptide extract could significantly improve the aforementioned bone microstructure indexes, suggesting that Deer bone polypeptide extract has a protective effect on the bone microstructure.

This study aimed to demonstrate that Deer bone polypeptide extract could inhibit bone resorption and promote bone formation, with a preventive and therapeutic effect on GIOP. The mechanism of GIOP was complicated. Glucocorticoid hormones mainly acted on the osteoblasts in the bone, and NO played a mediating role in a series of reactions induced by these hormones in osteoblasts [26,27,28]. NO carried biological information, serving as a second

messenger and neurotransmitter, and mediating and regulating a variety of pathological and physiological reactions. NOS was classified into three subtypes: neuronal NOS (nNOS), eNOS, and iNOS, with different genetic codes [29]. It was confirmed that eNOS and iNOS exist primarily in the bone tissue, and NO was secreted by osteoblasts and osteoclasts in autocrine and paracrine manners. It was involved in the functional regulation of osteoblasts and osteoclasts [30]. Under normal physiological conditions, eNOS generates a low concentration of NO (pmol) [31,32,33]. There were also some clinical studies on eNOS and osteoporosis proving that eNOS plays a key role in regulating osteoblast activity and bone formation [34,35,36]. In this study, the immunohistochemical results of eNOS in osteoblasts in the model group were weakly positive, whereas the reaction in the blank control group was strongly positive, indicating that the decreased expression of eNOS could play an important role in the pathogenesis of GIOP [37,38,39,40]. After administering the Deer bone polypeptide extract, the color reaction of eNOS in osteoblasts was strongly positive, indicating that Deer bone polypeptide extract could stimulate the osteoblasts to increase eNOS-dependent NO levels, and hence, promote bone formation [41,42,43].

5. Conclusions

Sika Deer bone polypeptide extract can inhibit the metabolism imbalances of calcium and phosphorus induced by glucocorticoids, reduce ALP, elevate BGP, and inhibit bone resorption and formation in rats with GIOP. In addition, they can improve pathological changes in the microstructure and stimulate the expression of eNOS in osteoblasts. Bone protection in GIOP rats might be mediated by eNOS-dependent NO.

Ethical approval and informed consent

All animal experiments comply with the ARRIVE guidelines and followed the U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines, EU Directive 2010/63/EU for animal experiments.

Conflict of interest

The author(s) declare no competing interests.

Financial support

Supplementary material

https://doi.org/10.1016/j.ejbt.2021.04.002.

References

[1] Buttgereit F, Burmester GR, Lipworth BJ. Optimised glucocorticoid therapy: the sharpening of an old spear. Lancet 2005;365:801–3. https://doi.org/10.1016/S0140-6736(05)17989-6.

[2] Den Uyl D, Bultink IE, Lems WF. Advances in glucocorticoid-induced osteoporosis. Curr Rheumatol Rep 2011;13:233–40. https://doi.org/10.1007/s11926-011-0173-y. PMid: 21365209.

[3] Hallberg I, Bachrach-Lindstrom M, Hammerby S, et al. Health-related quality of life after vertebral or hip fracture: a seven-year follow-up study. BMC Musculoskelet Disord 2009;10:135. https://doi.org/10.1186/1471-2474-10-135. PMid: 19886998.

[4] Kok C, Sambrook PN. Secondary osteoporosis in patients with an osteoporotic fracture. Best Pract Res Clin Rheumatol 2009;23:769–79. https://doi.org/10.1016/j.berh.2009.09.006. PMid: 19945688.

[5] Cauley JA, Thompson DE, Ensrud KC, et al. Risk of mortality following clinical fractures. Osteoporos Int 2000;11:556–61. https://doi.org/10.1007/s001980070075. PMid: 11069188.

[6] Briot K, Cortet B, Roux C, et al. 2014 update of recommendations on the prevention and treatment of glucocorticoid-induced osteoporosis. Joint Bone Spine 2014;81:493–501. https://doi.org/10.1016/j.jbspin.2014.10.001. PMid: 25455041.

[7] Zhang H, Zhao Y, Xu Y, et al. Study on effects of collagen of deer bone on osteoporosis in ovariectomized rats. Pharmacol Clin Chin Mater Med 2011;27:76–9.

[8] Wang MT, Huang Z, Yang R, et al. Disruption of the microfilament cytoskeleton induced by simulated microgravity affects NO/NOS system of osteoblasts. Nan Fang Yi Ke Da Xue Xue Bao 2010;30:1658–62. PMid: 20650794.

[9] Wang Q, Mao Y, Ma C. Treatment of osteoporosis by sika bone powder among rats after ovariectomy. Matern Child Health Care China 2015;17:2861–4.

[10] Sun SL, Xie YM, Li YY, et al. Clinical analysis of Cervus and Cucumis Polypeptide injection based on real world hospital information system. Zhongguo Zhong Yao Za Zhi 2016;41:4060–5. PMid: 28929696.

[11] Yang X, Niu K, Zhang X, et al. The effectiveness and safety of cervus and cucumis polypeptide injection in promoting fracture healing after bone fracture surgeries: Protocol for a systematic review and meta-analysis. Medicine (Baltimore) 2019;98(7):. https://doi.org/10.1097/MD.0000000000014571. PMid: 30762805e14571.

[12] An LP, Ren GK, Shi LQ, et al. Effect of deer bone polypeptides on bone microarchitecture of dexamethasone-induced osteoporosis rats. Chin Trad Herb Drugs 2016;47(22):4030–4.

[13] Chen Z, Xue J, Shen T, et al. Curcumin alleviates glucocorticoid-induced osteoporosis by protecting osteoblasts from apoptosis in vivo and in vitro. Clin Exp Pharmacol Physiol 2016;43:268–76. https://doi.org/10.1111/1440-1681.12513. PMid: 26515751.

[14] Chen Z, Xue J, Shen T, et al. Curcumin alleviates glucocorticoid-induced osteoporosis through the regulation of the Wnt signaling pathway. Int J Mol Med 2016;37(2):329–38. https://doi.org/10.3892/ijmm.2015.2432. PMid: 26677102.

[15] Whittier X, Saag KG. Glucocorticoid-induced osteoporosis. Rheum Dis Clin 2016;42:177–89. PMid: 26611558.

[16] Lindgren JU, Johnell O, DeLuca HF. Studies of bone tissue in rats treated by prednisolone and 1,25-(OH)$_2$D$_3$. Clin Orthop Relat Res 1983;181:264–8. https://doi.org/10.1097/00003086-198312000-00041.

[17] Canalis E, Mazziotti G, Giustina A, et al. Glucocorticoid-induced osteoporosis: pathophysiology and therapy. Osteoporos Int 2007;18:1319–28. https://doi.org/10.1007/s00198-007-0394-0. PMid: 17566815.

[18] Jeon J, Lee MS, Yang HS. Differentiated osteoblasts derived decellularized extracellular matrix to promote osteogenic differentiation. Biomater Res 2018;22(1):4. https://doi.org/10.1186/s40824-018-0115-0. PMid: 29484201.

[19] Furuya M, Kikuta J, Fujimori S, et al. Direct cell-cell contact between mature osteoblasts and osteoclasts dynamically controls their functions in vivo. Nat Commun 2018;9(1):300. https://doi.org/10.1038/s41467-017-02541-w. PMid: 29352112.

[20] Modi A, Siris ES, Tang J, et al. Gastrointestinal events and association with initiation of treatment for osteoporosis. J Clinicoecon Outcomes Res 2015;7:603–13. https://doi.org/10.2147/CEOR.S83227. PMid: 26648746.

[21] Chatmahamongkol C, Pravitharangul A, Suttapreyasri S, et al. The effect of compressive force combined with mechanical vibration on human alveolar bone osteoblasts. J Oral Biol Craniofac Res 2019;9(1):81–5. https://doi.org/10.1016/j.jobcr.2018.10.003. PMid: 30997326.

[22] Chen Z, Chen G, Zhou K, et al. Toxicity of food sweetener-sodium cyclamate on osteoblasts cells. Biochem Biophys Res Commun 2019;508(2):507–11. https://doi.org/10.1016/j.bbrc.2018.11.172. PMid: 30509495.

[23] Ritman EL. Micro-computed tomography-current status and developments. Annu Rev Biomed Eng 2004;6:185–208. https://doi.org/10.1146/annurev.bioeng.6.040803.140130. PMid: 15255767.

[24] Zhang J, Meng X, Ding C, et al. Effects of static magnetic fields on bone microstructure and mechanical properties in mice. Electromagn Biol Med 2018;37(2):76–83. https://doi.org/10.1080/15368378.2018.1458626. PMid: 29617158.

[25] Farias BDM, Schultz CL, Soares MB. Bone microstructure of the pareiasaur Provelosaurus americanus from the Middle Permian of southern Brazil. Hist Biol 2021;33(3):328–39. https://doi.org/10.1080/08912963.2019.1617288.

[26] Li Y, Li Y, Yang W. Preventive effects of nitroglycerine on glucocorticoid-induced osteoporosis in growing rats. J J Huazhong Univ Sci Technolog Med Sci 2007;27:528–31. https://doi.org/10.1007/s11596-007-0513-3. PMid: 18060627.

[27] Van't Hof RJ, Macphee J, Libouban H, et al. Regulation of bone mass and bone turnover by neuronal nitric oxide synthase. J Endocrinol 2004;145(11):5068–74. https://doi.org/10.1210/en.2004-0205. PMid: 15297441.

[28] Webster TJ, Ergun C, Doremus RH, et al. Enhanced functions of osteoblasts on nanophase ceramics. Biomaterials 2000;21(17):1803–10. https://doi.org/10.1016/S0142-9612(00)00075-2.

[29] Van't Hof RJ, Ralston SH. Nitric oxide and bone. Immunology 2001;103(3):255–61. https://doi.org/10.1046/j.1365-2567.2001.01261.x. PMid: 11454054.

[30] Rubin J, Murphy TC, Zhu L, et al. Mechanical strain differentially regulates endothelial nitric-oxide synthase and receptor activator of nuclear kappa B ligand expression via ERK1/2 MAPK. J Biol Chem 2003;278(36):34018–25. https://doi.org/10.1074/jbc.M302822200. PMid: 12824189.

[31] Ricciardolo FL, Nijkamp FP, Folkerts G. Nitric oxide synthase (NOS) as therapeutic target for asthma and chronic obstructive pulmonary disease. Curr Drug Targets 2006;7(6):721–35. https://doi.org/10.2174/138945006777435290. PMid: 16787174.

[32] Ding J, Tang Y, Tang Z, et al. Icariin improves the sexual function of male mice through the pi3k/akt/enos/no signalling pathway. Andrologia 2018;50(1):. https://doi.org/10.1111/and.12802. PMid: 28294371e12802.

[33] Cencioni C, Spallotta F, Savoia M, et al. Zeb1-hdac2-enos circuitry identifies early cardiovascular precursors in naive mouse embryonic stem cells. Nat Commun 2018;9(1):1281. https://doi.org/10.1038/s41467-018-03668-0. PMid: 29599503.

[34] Nursal AF, Yigit S, Tekcan A, et al. VNTR variant of eNOS gene and their relation with osteoporosis in postmenopausal Turkish women. Endocr Metab Immune Disord Drug Targets 2000;20:1. https://doi.org/10.2174/1871530320999200918120208. PMid: 32957896.

[35] Singh P, Singh M, Valecha S, et al. Investigation of eNOS gene polymorphism exposes a genetic association between endothelial dysfunction and osteoporosis in postmenopausal women. Menopause 2020;27(6):714–21. https://doi.org/10.1097/GME.0000000000001514. PMid: 32187129.

[36] Gu Z, Yu Z, Qiu G. Promoter polymorphism T-786C, 894G→T at exon 7 of endothelial nitric oxide synthase gene are associated with risk of osteoporosis in Sichuan region male residents. Int J Clin Exp Pathol 2015;8(11):15270. PMid: 26823879.

[37] Dinan TG. Glucocorticoids and the genesis of depressive illness a psychobiological model. Br J Psychiatry 2018;164(3):365–71. https://doi.org/10.1192/bjp.164.3.365. PMid: 7832833.

[38] Kamel M, Leue S. Formalization and validation of the general inter-ORB protocol (GIOP) using PROMELA and SPIN. Int J Softw Tools Technol Transfer 2000;2(4):394–409. https://doi.org/10.1007/s100090050045.

[39] Yin H, Shi ZG, Yu YS, et al. Protection against osteoporosis by statins is linked to a reduction of oxidative stress and restoration of nitric oxide formation in aged and ovariectomized rats. Eur J Pharmacol 2012;674(2–3):200–6. https://doi.org/10.1016/j.ejphar.2011.11.024. PMid: 22130356.

[40] Jintanaporn W, Cholathip T, Wipawee TM, et al. Morinda citrifolia L. leaf extract protects against cerebral ischemia and osteoporosis in an in vivo experimental model of menopause. Oxid Med Cell Longev 2018;2018:. https://doi.org/10.1155/2018/1039364. PMid: 297654881039364.

[41] Newman ED, Matzko CK, Olenginski TP, et al. Glucocorticoid-induced osteoporosis program (GIOP): a novel, comprehensive, and highly successful care program with improved outcomes at 1 year. Osteoporos Int 2006;17 (9):1428–34. https://doi.org/10.1007/s00198-006-0149-3. PMid: 16865312.

[42] Lee SJ, Namkoong S, Kim YM, et al. Fractalkine stimulates angiogenesis by activating the Raf-1/MEK/ERK- and PI3K/Akt/ENOS-dependent signal pathways. Am J Physiol Heart Circ Physiol 2006;291(6):H2836–46. https://doi.org/10.1152/ajpheart.00113.2006. PMid: 16877565.

[43] Wang Q, Wang G, Wang B, et al. Activation of TGR5 promotes osteoblastic cell differentiation and mineralization. Biomed Pharmacother 2018;108:1797–803. https://doi.org/10.1016/j.biopha.2018.08.093. PMid: 30372884.