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Variance of spinal osteoporosis induced by dexamethasone and methylprednisolone and its associated mechanism ☆

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ABSTRACT

Background: Glucocorticoid (GC) administration is the most common cause of secondary osteoporosis. Previous studies investigated GCs dose and frequency correlated positively with the side effects of glucocorticoid on bone health, however the impaired effect of various types of GCs on bone has not yet been reported.
Purpose: The aim is to compare the effect of long-acting (dexamethasone) and relatively short-acting glucocorticoid (methylprednisolone) on rat lumbar spine and try to explore the associated mechanism.
Method: Sprague Dawley rats ($N = 48$) were randomly divided into four groups: baseline group (BL), control group (CON), methylprednisolone group (MP) and dexamethasone group (DEXA). BL rats were euthanized to remain as baseline (M0) at the beginning of experiment. CON group were injected daily with vehicle, while the other groups were given a daily subcutaneous injection of 1 mg/kg methylprednisolone and were given a subcutaneous injection of 0.6 mg/kg dexamethasone per 3 days, respectively. CON, MP and DEXA groups were monitored at 4th week (M1), 8th week (M2) and 12th week (M3) after intervention. Dual-energy X-ray, micro-computed tomography, compressive test, enzyme-linked immunosorbent assay have been used for bone mineral density, microarchitecture, biomechanical property of vertebrae and levels of estrogen, PINP and β-CTX, respectively. mRNA expression analysis of Biglycan, Col1a1, MMP9, Cathepsin K, Runx2, OPG, LRP5, Sclerostin were performed.
Result: We found that the bone mineral density (BMD) was significantly lower in DEXA rats at M3 compared with MP rats. The relative surface and trabecular number were significantly lower in DEXA group than that in MP group at M2, while trabecular separation was significantly higher in DEXA group than that in MP group at the same point. The compressive strength was significantly lower in L4 of DEXA than that in MP rats at M2 and M3. The levels of both PINP and estradiol in DEXA group were lower than MP group at M3, even though without statistical significance. The expression of bone formation marker Runx2 was significantly down-regulated at M3 in DEXA group compared with MP, CON and BL groups, while the expression of Col1a1 was significantly up-regulated and biglycan, LRP-5, OPG were significantly down-regulated in GCs intervention groups compared with CON and BL groups. There were no statistical differences in MMP9, Cathepsin K, Sclerostin among CON, MP and DEXA groups.
Conclusion: These results indicate that dexamethasone, the long-acting glucocorticoid, generates more serious osteoporosis of rat lumbar spine than methylprednisolone, which is relatively short-acting glucocorticoid. The discrepancy between the two GCs inducing osteoporosis may be mainly caused by a decrease in bone formation. RUNX2 and Col1a1 may be the two of critical genes inducing the discrepant impairment.

1. Introduction

Epidemiologic studies showed that 1–3% of adults worldwide use glucocorticoids (GCs) at long time for inflammatory, immunologic, and allergic disorders such as renal, respiratory, and neural diseases [1], however, the long-term exposure to GCs

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simultaneously induce osteoporosis, which is characterized by rapid loss of bone mass, significant decrease of bone strength and increase of fracture risk at all ages. Since now, glucocorticoid-induced osteoporosis (GIOP) has been reported as the most frequent secondary osteoporosis and the third epidemic osteoporosis following postmenopausal and senile osteoporosis in rank [2]. Therefore, more attention should be paid for GIOP.

To explore the pathology and optimal treatment for GIOP, several studies have developed animal models by GCs administration to mimic GIOP in human being. However, findings are lack of consensus, in which bone loss and decreased bone strength occurred in GIOP could be replicated in animal models [3–5], while increased bone mineral density and bone volume haven't rarely been investigated [3,6–8], due to confounding factors existed in these researches, such as the age of the animals, the dose and type of GCs. The effect of different ages of rats and diverse doses of GCs on rat bone health have been analyzed in depth [3,6,9], while the distinction about impairment of various types of GCs on bone hasn't yet been referred. Therefore, it is critical to differentiate the effect of varied types of GCs on osteoporosis for development of standard GIOP animal models and guidance of GCs selection in clinical practice.

In order to compare the impairment of various GCs on bone quality and quantity, long-acting steroid, dexamethasone and relatively short-acting medicine, methylprednisolone could be representatives, which are the two common types of GCs used in clinical practice and animal studies, with half-life of 36–72 h and 12–36 h respectively [10,11]. Furthermore, it is reported in the previous pharmacological studies that dexamethasone is five times more potent than methylprednisolone, which could make adverse effect of these two GCs be compared under the prerequisite of equivalent pharmacological effect-dosage, and thus dexamethasone and methylprednisolone have been defined as interventions in this study.

Since the inhibition of bone formation and promotion of bone resorption induced by GCs mainly reduced trabecular bone mass [3,12,13], vertebral bodies, which are composed of 95% trabecular bone and 5% cortical bone [14–16], are more susceptible to GIOP than the long bones in the extremities [16]. To improve the sensitivity in this comparative study, we designated the bone quality and quantity of rat lumbar spine as the main study objects.

In the present study, to compare the effect of dexamethasone and methylprednisolone on rat lumbar spine, after subcutaneous injection of GCs [17] for 3 months, bone mineral density, microarchitectural, biomechanical characteristics of rat lumbar spine were evaluated. Moreover, this research might also provide new insight on the mechanism of the discrepancy existed between the osteoporosis induced by dexamethasone and methylprednisolone at the metabolic and molecular level through examination of the serum level of bone turnover marker and expression of genes related with bone formation, bone resorption and bone matrix.

2. Material and methods

2.1. Ethical approval

The approvals of the animals and all the experimental procedures were obtained from Ethics Committee of the First Affiliated Hospital of Guangzhou University of Chinese Medicine (License No: 20130425). Humane care was performed according to the Guide for the Care and Use of Laboratory Animals, published by the US National Institutes of Health.

2.2. Animals and study design

3-month-old female Sprague–Dawley rats ($n = 48$) were purchased from the animal experiment center of southern Medical University (Guangzhou, China), were housed in the First Affiliated Hospital of Guangzhou University of Chinese Medicine (SYXK[Yue]2013-0092). The average initial weight of rats at baseline was (212 ± 30)g. Following an acclimation period of a week, rats were randomly assigned to 4 groups as follow: (1) baseline group (BL, $n = 6$); (2) control group (CON, $n = 14$), (3) methylprednisolone group (MP, $n = 14$); (4) dexamethasone, group (DEXA, $n = 14$). All of rats received normal diet under standard laboratory condition in the whole experiment. Rats ($n = 6$) in BL group were euthanized to remain as baseline (M0) under general anesthesia by intraperitoneal injection using 10% chloral hydrate (0.3 ml/100 g body weight, Tianjin Guangfu Fine chemical research institute, China) at the start of this experiment. The CON group was subjected to inject subcutaneously with vehicle for eliminating system bias and interference factor of possible bone loss due to aging during the experiment. Group DEXA and MP received a subcutaneous injection of dexamethasone-21-isonicotinate (Guangzhou BaiyunshanTianxin Pharmaceutical CO. Ltd. Batch No: H44022091) at a dose of 0.6 mg/kg body weight, twice per week and methylprednisolone (Tianjin Pharmaceutical Jiaozuo Co., Ltd. Batch No: H20030727) at a dose of 1 mg/kg body weight, once a day, during 12 weeks, respectively.

Rats in the CON, DEXA and MP groups were euthanized for experimental analysis at the end of fourth week (M1, N = 4/group), eighth week (M2, N = 4/group) and twelfth week (M3, N = 6/group). En bloc lumbar vertebrates were isolated and devoid of soft tissues. Lumbar vertebrae 6 (L6) samples were preserved at −80 °C for mRNA expression analysis. Lumbar vertebrae 1–3 (L1–3) samples were stored at −20 °C for dual-energy X-ray absorptiometry. Lumbar vertebrae 4 (L4) samples were fixed in 4% phosphate-buffered paraformaldehyde for micro-CT and biomechanical analysis, and L5 samples were fixed in 4% phosphate-buffered paraformaldehyde for histomorphometry. Blood was collected from right heart and serum were store at −20°C prior to assessment of both level of estradiol and bone turnover markers including serum β-C-telopeptide of type I collagen (β-CTX) and serum amino-terminal propeptide of type I collagen (PINP). The adrenals and uterus were carefully excised, trimmed of fat, blotted dry, and weighed.

2.3. Measurement by dual-energy X-ray absorptiometry

The Bone mineral density (BMD, g/cm^2), bone mineral content (BMC, g) and bone area (BA, cm^2) of L1–3 separated from rats were analyzed using dual-energy X-ray absorptiometry with a small-animal high-resolution collimator (Discovery A/SL/W/C; Hologic, Bedford, MA, 01730 USA). After thaw, the samples were scanned successively under the model of the hand-regional high resolution. The regions of interest (ROI) were marked with respect to L1, L2, L3 and the whole L1–3 (Fig. 1). All samples were measured by the same technician at different time points. Analysis was performed using small animal mode of the software (Hologic, Bedford, USA, v.13.2:3) and was calibrated at each start of the experiment.

2.4. Micro-computed tomography examination

L4 of all group [$N = 6$/group (M0 and M3), $N = 4$/group (M2)] was measured by a cone-beam-type desktop micro-CT (μCT80, Scanco Medical, Brüttisellen, Zurich, Switzerland) and was evaluated by associated analysis software (μCT80 Evaluation Program v6.5-1, Scanco Medical, Switzerland). The X-ray system is based on a microfocus tube reaching a minimum spot size of 5 μm and generating X-rays in cone-beam geometry. The energy and intensity were equal to 55 kvp and 145 μA, respectively. The X-ray detector consists of 64-bit digital CCD high-resolution (512 × 512 to 4096 × 4096 pixel) array and the maximum spatial resolution