Differential Response of Risedronate on Tibial and Mandibular Bone Quality in Glucocorticoid-treated Growing Rats

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Abstract

Glucocorticoids induce bone loss and retard bone growth in children. In this study we investigated the effect of treatment with risedronate on glucocorticoid –prednisolone– induced decreases in bone density, quality, strength and growth of the tibia and mandible in growing rats. Trabecular and cortical bone structure was measured by peripheral quantitative computed tomography (pQCT) and three-dimensional (3D) micro-computed tomography (micro-CT). Indicators of bone strength were calculated from cortical bone density and the modulus of sections obtained from pQCT analysis. Tibial and mandibular bone sizes were also measured. Prednisolone decreased the bone growth of both tibia and mandible. It also caused deterioration of trabecular and cortical bone structure and strength in the mandible, and in cortical bone in the tibia, but had no effect on trabecular bone in the tibia. Risedronate inhibited the prednisolone-induced decreases in tibial width and mandibular length and height but did not improve the retardation of longitudinal bone growth. Risedronate prevented prednisolone-induced deterioration of trabecular and cortical bone architecture. In the mandible, this protective effect of risedronate was accompanied by an increase in cortical bone density and in bone strength. These findings show that risedronate inhibits prednisolone-induced loss of bone density, structure, decrease in bone strength, and retardation of bone growth in the mandible in young growing rats.

Key words: Glucocorticoid/Risedronate/Bone quality/Growing rats/3D micro-CT

Introduction

Bisphosphonates have been approved and are used for treatment of various metabolic bone disorders such as Paget's disease¹⁾, postmenopausal osteoporosis²⁾ and glucocorticoid-induced osteoporosis³⁾. In oral bone tissues, bisphosphonates prevented mandibular bone loss

in a rat model of experimental periodontitis⁴⁾ and had a positive effect on new bone formation in experimental mandibular bone distraction osteogenesis in rabbits⁵⁾. Risedronate is a recently developed bisphosphonate, which inhibits osteoclast-mediated bone resorption⁶⁾, and has been shown to promote osteoblastic bone formation *in vitro*⁷⁾. Risedronate has been reported to decrease the risk of vertebral fracture in the first year of treatment in postmenopausal women⁸⁾ and adult patients receiving glucocorticoid therapy⁹⁾. However, at present, no evidence has been presented that bisphosphonates are effective in glucocorticoid-induced bone loss in children and adolescents, although cyclic intravenous pamidronate is used in osteogenesis imperfecta¹⁰⁾.

Glucocorticoids are used to treat various diseases in children, such as asthma and juvenile rheumatoid arthritis^{11,12)}. During the past two decades, the prevalence of asthma and other allergic diseases among Japanese children has increased dramatically¹³⁾, and the use of glucocorticoids for asthma patients has also increased¹⁴⁾. However, long term administration of glucocorticoid is known to stunt bone growth, decrease bone mineral density, and increase risk of bone fracture^{11,12)}. Morris (1975)¹⁵⁾ demonstrated that growth retardation was found in 35 % of asthmatic children who had taken glucocorticoids daily for more than 2 years. Additionally, recent clinical data showed that although patients with glucocorticoid-induced osteoporosis were younger and had a higher bone mineral density, they had higher risk of fracture than those with other kind of osteoporosis¹⁶⁾.

In the National Institute of Health (NIH) consensus¹⁷⁾, bone strength is defined by not only bone mass but also bone qualities such as bone size and macro- and micro-architecture, suggesting that improvement in bone quality rather than bone mass might decrease the risk of bone fracture for young patients treated with glucocorticoids. However, there is little or no data on the effects of risedronate on bone quality and in glucocorticoid exposure during critical bone growth and building periods. In the present study, we evaluated effects of risedronate treatment on changes in tibial and mandibular bone density, strength and quality (in particular structural properties) in established glucocorticoid-induced osteoporosis in growing rats. For the analyses we used peripheral quantitative computed tomography (pQCT) and three-dimensional (3D) micro-computed tomography (micro-CT).

Materials and methods

I Materials

Experimental animals

48 male Wistar rats, 5 weeks of age with a body weight of approximately 135 g, were purchased from Charles River Japan (Kanagawa, Japan). The animals were housed individually under a 12-hour light-dark cycle, at a constant temperature of $22 \pm 1^\circ\text{C}$ and a humidity of $50 \pm 5\%$, and fed a standard pelleted chow containing 1.14 % calcium and 1.06 % phosphorus (CE-2; CLEA Japan, Tokyo, Japan). All animal procedures were approved by the Committee for Care and Use of Laboratory Animals of Kyushu Dental College.

II Methods

A Experimental design

Animals were randomly divided into 6 groups of 8 animals each as shown in Fig. 1. The six groups were designated as follows, (1) Control 6 wk; (2) Prednisolone (Pred) 6 wk; (3) Control 10 wk; (4) Pred + vehicle group; (5) Pred + Risedronate (Ris) 0.5 mg/kg; (6) Pred + Ris 1.0 mg/kg. In the Pred + vehicle and Pred + Ris groups, prednisolone was given for 6 weeks followed by vehicle or Ris for 4 weeks. Prednisolone sodium succinate (Prednine, Shionogi & Co., Ltd., Osaka, Japan) was administered orally, 30 mg/kg/day on alternate days. Risedronate sodium (Ajinomoto, Tokyo, Japan) was administered orally, 0.5 mg/kg/day or 1.0 mg/kg/day starting immediately after the 6 weeks of prednisolone administration. The doses of prednisolone and risedronate were determined based on the results of previous studies¹⁸⁻²⁰. Both compounds were dissolved in sterile saline, to deliver the required dose in a total volume of 10 ml/kg body weight. Prednisolone and prednisolone vehicle (saline) were administered in the morning. Risedronate and risedronate vehicle (saline) were administered in the afternoon: animals were fasted for 4 hours before dosing and 2 hours after dosing of risedronate. Water was available *ad libitum*¹⁹.

Body weights were measured weekly. At the end of the experimental period, all rats were deeply anesthetized with diethyl ether and given a lethal injection of thiamylal natrium (Isozole; Mitsubishi Pharma Co, Osaka, Japan) intraperitoneally at a dose of 90 mg/kg body weight. From each animal, the left tibia and mandible were removed for measurement of

Fig. 1 Experimental protocol.

Control 6 wk, treated with prednisolone vehicle (saline only) for 6 weeks; Pred 6 wk, treated with prednisolone 30 mg/kg/2days for 6 weeks; Control 10 wk, treated with prednisolone or risedronate vehicle (saline only) for 10 weeks; Pred + vehicle, treated with risedronate vehicle (saline only) for 4 weeks after 6 weeks prednisolone administration; Pred + Ris 0.5 mg/kg, treated with risedronate 0.5 mg/kg for 4 weeks after 6 weeks prednisolone administration; Pred + Ris 1.0 mg/kg, treated with risedronate 1.0 mg/kg for 4 weeks after 6 weeks prednisolone administration.

cephalometry, peripheral Quantitative Computed Tomography (pQCT), and three-dimensional (3D) micro-computed tomography (micro-CT).

B Tibial and mandibular bone size

The left tibia and left mandible were obtained from each rat and fixed in 70 % ethanol. After fixation, surrounding soft tissues around of them were carefully detached. The tibia and mandible were mounted to contact the dental occlusal film (FG; Fuji Film, Tokyo, Japan) surface, and radiographed using a SOFTEX ESM-2 (Softex Co., Tokyo, Japan) under both electrical conditions of 28 kv and 6 mA for 60 seconds. Developed radiographic images of both tibia and mandible were digitized using a GT-X900 scanner (Epson, Tokyo, Japan), connected to a VGN-S50B computer (Sony, Tokyo, Japan) and images were analyzed using Scion Image Beta 4.0.3 (Scion Co., MD, USA) according to the modified method of Morimoto *et al* (2000)²¹. The regions of measurement of the tibia

were total tibial length and tibial width in lateral planes at the midpoint (Fig. 2). For the mandible, the regions of the measurements were obtained as described by Eratalay *et al* (1981)²². Mandibular length was measured from the most anterior inferior bone point (Id) to the middle of the condyle (Cd). The base of the mandible was measured from the Id to the most posterior point of the mandible (Go), and the height of the mandible from the coronoid process (Cr) to the inferior border of the inferior ramus (Ag) was also measured (Fig. 3).

C Peripheral Quantitative Computed Tomography

After the cephalograms had been taken, all bones were prepared for pQCT (XCT Research SA + series; Stratec, Medizintechnik, Pforzheim, Germany). The tibial metaphysis was used to measure total bone density, total bone cross-sectional area, trabecular bone density, trabecular cross-sectional area, cortical bone density, cortical bone cross-sectional area, and

Fig. 2 Lateral radiograph of left tibia.

1, total length of tibia. 2, tibial width in lateral planes at the midpoint.

Fig. 3 Lateral radiograph of left mandible.

Ag, antegonion; Cd, the most superior posterior point of the condylar head; Cr, the most superior posterior point of the coronoid process; Go, gonion; Id, infradentale (labial side). Id-Cd, mandibular length; Id-Go, base; Cr-Ag, mandibular height

cortical thickness. The metaphysis tibial bone was scanned at a point 3 mm distal from the proximal growth plate, which consisted of a majority of the trabecular and adequate cortical components, with a voxel size of 0.12×0.46 mm (Fig. 4A). The trabecular region was defined by peel mode 2 at a threshold value of 395 mg/cm^3 . The cortical region was determined after setting a cortical mode 1 at a threshold value of 690 mg/cm^3 ²³. The mandibular bone was used to measure trabecular bone density, trabecular bone cross-sectional area, cortical bone density and cortical bone cross-sectional area. For those measurements, we scanned between the mesial and distal root of the mandibular first molar at 3 different positions with an interval of 0.1 mm, and measured the cortical component using a voxel size of 0.08×0.26 mm (Fig. 4B). The trabecular region was defined manually. The cortical region was defined by setting a threshold value of 690 mg/cm^3 ²⁴.

Fig. 4 Peripheral quantitative computed tomography scans of Wistar rat specimens. Left side image shows a tibial metaphysis specimen (a), and right side image shows a mandibular bone specimen (b).

D Bone strength (non-invasive assessment)

Bone strength of the tibial metaphysis and mandible was estimated from the strength strain index (SSI), which was determined using pQCT as a non-invasive assessment of mechanical properties at a threshold of 690 mg/cm^3 ^{23,24}. SSI was calculated using the equation $\text{SSI} = \text{CBD} \cdot \text{Z} / \text{NCBD}$, where CBD is cortical bone density (mg/cm^3), Z is the section modulus (mm^3), and NCBD is the normal physiological value of cortical bone density (1200 mg/cm^3).

CBD and Z were derived from the pQCT data.

E Three-dimensional (3D) micro-computed tomography

After pQCT measurements, trabecular microarchitecture of the tibial metaphysis and mandible was analyzed by 3D micro-CT (Scan Xmate-A090S; Comscantecno Co., Kanagawa, Japan). In the tibial metaphysis, volume data for 100 slices was obtained from each sample under the following exposure conditions: tube voltage, 31 kV; tube current, $100 \mu\text{A}$; and slice thickness $18.03 \mu\text{m}$. In the mandible, volume data for 20 slices was obtained from each sample under the following exposure conditions: tube voltage, 50 kV; tube current, $20 \mu\text{A}$; and slice thickness $22.68 \mu\text{m}$. 3D-BON software (Ratoc System Engineering, Tokyo, Japan) was used for measurement of the trabecular architecture, as described in Wakabayashi *et al* (2004)²⁵. The sites of the measurement by pQCT were selected for data analysis. The region of trabecular bone in the mandible is indicated in Fig. 5 (colored

white). As 3D architectural parameters, tissue volume (TV) and trabecular bone volume (BV) were measured directly, and trabecular bone volume fraction (BV/TV) was calculated. In addition, trabecular number (Tb.N), trabecular thickness (Tb.Th), and trabecular separation (Tb.Sp) were measured according to the parallel plate model²⁶⁾.

F Statistical analysis

Data are presented as the mean $\pm$ standard deviation, and statistical comparisons between the Control 6 wk and the Pred 6 wk groups were made using a two-sided *t*-test for unpaired samples. Multiple comparisons among the four 10 wk treatment groups were performed by a one-way analysis of variance (ANOVA) using Scheffe's method. The level of significance was set at 5 % on a two-tailed test.

Results

I Body weight and bone size

The initial body weight did not differ significantly among the groups (Table 1). Prednisolone: Pred for 6 wk significantly decreased body weight, tibial length and width, mandibular length (Id–Cd), base (Id–Go), and mandibular height (Cr–Ag) compared with the Control 6 wk group.

Risedronate completely reversed the prednisolone-induced decreases in tibial width, mandibular length and mandibular height.

Table 1 Body weight and bone growth

	Control 6 wk	Pred 6 wk	Control 10 wk	Pred + vehicle	Pred + Ris (0.5 mg/kg)	Pred + Ris (1.0 mg/kg)
Initial body weight (g)	132.00 $\pm$ 6.76	132.11 $\pm$ 3.40	136.11 $\pm$ 2.79	135.94 $\pm$ 4.82	137.30 $\pm$ 4.03	136.97 $\pm$ 4.32
Final body weight (g)	404.82 $\pm$ 22.29	348.93 $\pm$ 26.18 ^a	469.53 $\pm$ 19.95	432.84 $\pm$ 24.27 ^b	435.06 $\pm$ 22.08 ^b	433.52 $\pm$ 29.86 ^b
Tibial length (mm)	39.99 $\pm$ 1.03	38.87 $\pm$ 0.57 ^a	42.86 $\pm$ 1.10	41.13 $\pm$ 1.10 ^b	41.15 $\pm$ 0.62 ^b	41.16 $\pm$ 0.83 ^b
Tibial width (mm)	3.12 $\pm$ 0.19	2.77 $\pm$ 0.25 ^a	3.31 $\pm$ 0.21	2.90 $\pm$ 0.19 ^b	3.11 $\pm$ 0.13 ^{bc}	3.14 $\pm$ 0.17 ^c
Mandibular length (Id–Cd) (mm)	26.01 $\pm$ 0.37	25.08 $\pm$ 0.69 ^a	26.71 $\pm$ 0.75	25.74 $\pm$ 0.99 ^b	25.98 $\pm$ 0.57	26.05 $\pm$ 0.47
Base (Id–Go) (mm)	26.07 $\pm$ 0.65	24.63 $\pm$ 0.31 ^a	26.87 $\pm$ 0.82	26.12 $\pm$ 0.47	26.08 $\pm$ 1.00	26.51 $\pm$ 0.53
Mandibular height (Cr–Ag) (mm)	14.57 $\pm$ 0.30	14.04 $\pm$ 0.41 ^a	14.92 $\pm$ 0.29	14.45 $\pm$ 0.29 ^b	14.55 $\pm$ 0.25	14.72 $\pm$ 0.35

Data are expressed as means $\pm$ SD.

^a*P* < 0.05, vs Control 6 wk group (*t*-test).

^b*P* < 0.05, vs Control 10 wk group (ANOVA and Scheffe's test).

^c*P* < 0.05, vs Pred + vehicle group (ANOVA and Scheffe's test).

Pred, prednisolone; Ris, risedronate.

Fig. 5 The region of mandibular alveolar bone between the mesial and distal root of the first molar.

II peripheral Quantitative Computed Tomography (pQCT)

Tibia trabecular bone density (Table 2) was significantly higher in the Pred 6 wk group compared with the Control 6 wk group. Total bone and cortical bone cross-sectional areas in the Pred 6 wk group were significantly lower than those in the Control 6 wk group.

Table 2 Bone density, cross-sectional area, and Strength Strain Index (SSI) of tibial metaphysis as measured using pQCT

	Control 6 wk	Pred 6 wk	Control 10 wk	Pred + vehicle	Pred + Ris (0.5 mg/kg)	Pred + Ris (1.0 mg/kg)
Total bone density (mg/cm ³)	451.28±54.43	456.52±20.20	556.87±24.97	498.26±16.63 ^b	566.09±22.73 ^{bc}	608.03±29.06 ^{cd}
Total bone cross-sectional area (mm ²)	20.96±0.92	18.50±0.75 ^a	22.11±2.24	20.60±1.22	21.48±1.62	23.63±1.13
Trabecular bone density (mg/cm ³)	165.22±17.60	190.64±11.35 ^a	212.90±44.27	200.18±21.98	244.68±41.47	278.62±36.42 ^c
Trabecular bone cross-sectional area (mm ²)	9.85±0.84	10.40±1.16	11.51±1.83	10.07±0.57	9.21±1.86	7.97±0.33 ^b
Cortical bone density (mg/cm ³)	927.90±16.71	943.33±11.82	1045.68±27.51	1007.32±12.51	1004.58±39.10	1022.84±44.84
Cortical bone cross-sectional area (mm ²)	5.24±0.32	4.55±0.31 ^a	7.89±0.13	6.22±0.10 ^b	7.13±0.40 ^c	7.98±0.68 ^{cd}
Cortical thickness (mm)	0.35±0.03	0.32±0.03	0.54±0.01	0.42±0.01 ^b	0.47±0.03 ^{bc}	0.54±0.02 ^{cd}
xSSI	4.80±0.32	4.00±0.23 ^a	6.87±0.34	5.53±0.25 ^b	7.19±0.71 ^c	8.32±0.92 ^{bc}
ySSI	5.67±0.63	4.69±0.29 ^a	7.98±0.62	6.02±1.04 ^b	7.97±0.71 ^c	8.24±0.37 ^c
pSSI	9.66±0.87	8.19±0.47 ^a	13.94±0.77	10.43±0.78 ^b	12.35±1.28	15.15±0.51 ^{cd}

Data are expressed as means ± SD. Pred, prednisolone; Ris, risedronate.

^a*P*<0.05, vs Control 6 wk group (*t*-test).

^b*P*<0.05, vs Control 10 wk group (ANOVA and Scheffe's test).

^c*P*<0.05, vs Pred + vehicle group (ANOVA and Scheffe's test).

^d*P*<0.05, vs Pred + Ris 0.5 mg/kg group (ANOVA and Scheffe's test).

Table 3 Bone density, cross-sectional area and Strength Strain Index (SSI) of mandible as measured using pQCT

	Control 6 wk	Pred 6 wk	Control 10 wk	Pred + vehicle	Pred + Ris (0.5 mg/kg)	Pred + Ris (1.0 mg/kg)
Trabecular bone density (mg/cm ³)	732.65±41.65	765.40±39.94	827.50±34.92	743.50±22.97 ^b	781.17±23.02	799.27±28.87
Trabecular bone cross-sectional area (mm ²)	5.20±0.17	4.27±0.55 ^a	5.48±0.33	4.68±0.92 ^b	6.00±0.12 ^c	6.02±0.95 ^c
Cortical bone density (mg/cm ³)	1258.40±20.92	1255.66±18.69	1267.70±7.28	1273.38±2.85	1299.10±14.13 ^{bc}	1284.85±0.71
Cortical bone cross-sectional area (mm ²)	6.58±0.75	5.75±0.15 ^a	7.04±0.26	6.73±0.04 ^b	6.93±0.15	8.17±0.28 ^{bcd}
xSSI	3.96±0.36	3.23±0.12 ^a	4.58±0.33	4.28±0.25	4.20±0.25	4.60±0.15
ySSI	7.18±0.73	5.44±0.24 ^a	7.98±0.21	7.00±0.44 ^b	7.10±0.38	8.35±0.26 ^{bc}
pSSI	8.95±1.05	7.04±0.26 ^a	10.23±0.31	8.94±0.56 ^b	9.14±0.17 ^b	10.70±0.43 ^{cd}

Data are expressed as means ± SD. ^a*P*<0.05, vs Control 6 wk group (*t*-test).

^b*P*<0.05, vs Control 10 wk group (ANOVA and Scheffe's test).

^c*P*<0.05, vs Pred + vehicle group (ANOVA and Scheffe's test).

^d*P*<0.05, vs Pred + Ris 0.5 mg/kg group (ANOVA and Scheffe's test). Pred, prednisolone; Ris, risedronate.

Risedronate completely reversed the prednisolone-induced decreases in total bone density, cortical bone cross-sectional area and cortical thickness. Risedronate produced dose-related decreases in the trabecular bone cross-sectional area.

Mandible trabecular and cortical bone cross-sectional areas (Table 3) were significantly lower in the Pred 6 wk group compared with the Control 6 wk group.

Risedronate completely reversed the prednisolone-induced decreases in trabecular bone density and trabecular and cortical bone cross-sectional areas. Cortical bone density in the Pred + 0.5 mg/kg group and cortical bone cross-sectional area in the Pred + Ris 1.0 mg/kg group increased above the Control 10 wk level.

III Bone strength assessment (non-invasive)

SSI for the reference x (xSSI), y (ySSI) and polar (pSSI) axes in the tibia and mandible are summarized in Tables 2 and 3, respectively. For the tibia all strength values were significantly lower in the Pred 6 wk group than in the Control 6 wk group and this reduction was maintained with 4 wk vehicle treatment. Risedronate completely reversed the prednisolone-induced decreases in all SSI values. The high doses of risedronate significantly increased xSSI compared with the Control 10 wk group. For the mandible, all SSI values in the Pred 6 wk group were significantly lower in the Pred 6 wk group than those in the Control 6 wk group, and ySSI and pSSI continued to be reduced with 4 wk vehicle treatment. ySSI and pSSI in the risedronate treatment groups reached the Control 10 wk level. The high doses of risedronate significantly increased ySSI above the Control 10 wk level.

IV Three-dimensional (3D) micro-computed tomography (micro-CT)

The results for trabecular 3D micro-structural parameters of the tibia are summarized in Table 4. In the tibia, Tb.Th in the Pred 6 wk group was significantly higher than that in the Control 6 wk group. Risedronate induced a dose-related increase in BV/TV through an increase in Tb.N. Risedronate produced dose-related decrease in Tb.Sp. Figure 6 demonstrates representative features of the 3D images of the trabecular structure of the tibial metaphysis. The 3D images of the Pred + Ris 1.0 mg/kg group show significant formation of trabeculae when compared with the Control 10 wk group.

The results for trabecular 3D micro-structural parameters of the mandible are summarized in Table 5. Prednisolone induced a decrease in Tb.Th, and this was manifest as a decrease in BV/TV in the Pred + vehicle group. Risedronate completely reversed the prednisolone-induced decreases in BV/TV and Tb.Th, and showed an increase in Tb.Sp. Figure 7 demonstrates representative features of the 3D images of the trabecular structure of the mandible. The 3D images of the Pred + vehicle group show significant bone loss. The trabeculae in the Pred + Ris 0.5 mg/kg and 1.0 mg/kg groups were densely formed when compared with those of the Pred + vehicle group.

Table 4 Trabecular microstructural parameters of tibial metaphysis as measured using 3D micro-CT

	<i>Control 6 wk</i>	<i>Pred 6 wk</i>	<i>Control 10 wk</i>	<i>Pred + vehicle</i>	<i>Pred + Ris</i> (0.5 mg/kg)	<i>Pred + Ris</i> (1.0 mg/kg)
BV/TV (%)	9.41 ± 1.10	10.14 ± 1.45	12.02 ± 5.09	10.03 ± 0.64	18.94 ± 3.19 ^c	21.17 ± 1.80 ^{bc}
Tb.Th (μm)	42.74 ± 1.14	45.54 ± 0.90 ^a	53.88 ± 7.92	50.16 ± 1.49	51.17 ± 1.22	53.94 ± 3.19
Tb.N (1/mm)	2.20 ± 0.20	2.26 ± 0.26	2.42 ± 0.58	2.25 ± 0.22	3.40 ± 0.57	4.50 ± 0.59 ^{bc}
Tb.Sp (μm)	414.41 ± 63.99	400.79 ± 51.76	399.27 ± 69.68	412.05 ± 45.35	220.11 ± 38.43 ^{bc}	179.35 ± 31.25 ^{bc}

Data are expressed as means ± SD. Pred, prednisolone; Ris, risedronate. BV/TV, bone volume fraction (TV: total tissue volume); Tb.Th, trabecular thickness; Tb.N, trabecular number; Tb.Sp, trabecular separation.

^a $P < 0.05$, vs Control 6 wk group (t -test). ^b $P < 0.05$, vs Control 10 wk group (ANOVA and Scheffe's test).

^c $P < 0.05$, vs Pred + vehicle group (ANOVA and Scheffe's test).

Fig. 6 Three dimensional trabecular micro-CT images of the tibia. Representative images of Control 6 wk, Pred 6 wk, Control 10 wk, Pred + vehicle, Pred + Ris (0.5 mg/kg) and Pred + Ris (1.0 mg/kg) groups are shown.

Table 5 Trabecular microstructural parameters of mandible as measured using 3D micro-CT

	<i>Control 6 wk</i>	<i>Pred 6 wk</i>	<i>Control 10 wk</i>	<i>Pred + vehicle</i>	<i>Pred + Ris</i> (0.5 mg/kg)	<i>Pred + Ris</i> (1.0 mg/kg)
BV/TV (%)	29.03±2.33	31.56± 3.05	33.09± 1.52	27.48± 2.42 ^b	32.85±0.26 ^c	32.22±0.45 ^c
Tb.Th (μm)	57.08±1.74	50.62± 2.38 ^a	62.20± 2.11	54.80± 0.86 ^b	58.62±4.16	58.19±2.05
Tb.N (1/mm)	5.04±0.42	5.34± 0.57	5.75± 0.24	5.57± 0.18	5.72±0.39	5.71±0.26
Tb.Sp (μm)	136.49±9.17	126.68±21.49	115.46± 3.39	144.01±14.85 ^b	127.10±1.75	118.78±4.64 ^c

Data are expressed as means ± SD. Pred, prednisolone; Ris, risedronate. BV/TV, bone volume fraction (TV: total tissue volume); Tb.Th, trabecular thickness; Tb.N, trabecular number; Tb.Sp, trabecular separation.

^a $P < 0.05$, vs Control 6 wk group (t -test). ^b $P < 0.05$, vs Control 10 wk group (ANOVA and Scheffe's test).

^c $P < 0.05$, vs Pred + vehicle group (ANOVA and Scheffe's test).

Fig. 7 Three dimensional trabecular micro-CT images of the mandible. Representative images of Control 6 wk, Pred 6 wk, Control 10 wk, Pred + vehicle, Pred + Ris (0.5 mg/kg) and Pred + Ris (1.0 mg/kg) groups are shown.

Discussion

In the present study using growing rats, glucocorticoid (prednisolone) treatment had detrimental effects on bone structure and strength in the tibia and mandible, and these effects were inhibited by risedronate. The actions of glucocorticoids on bone are complex, and whether they result from changes in bone formation or resorption, or both, is not fully elucidated. In previous studies in mice, glucocorticoid treatment decreased bone formation by increasing osteoblast apoptosis, and increased bone resorption by decreasing osteoclast apoptosis^{27, 28)}. On the other hand, in another study using rats, glucocorticoid treatment inhibited bone resorption by increasing osteoclast apoptosis²⁹⁾.

In the present study, prednisolone treatment reduced growth in tibial width and mandibular length and height, and in tibial length. Our findings for growing rats were consistent with other studies that showed glucocorticoid treatment in growing animals reduced body weight and bone growth^{20, 30, 31)}. Therefore, we propose that prednisolone had a suppressive effect on bone growth in the both mandible and tibia of growing rats.

As determined using pQCT and 3D micro-CT, prednisolone caused deterioration of trabecular bone structure in the mandible but not the tibia. In the mandible, trabecular bone cross-sectional area and Tb.Th decreased, while bone density was unchanged. In the tibia, bone density and Tb.Th increased while bone cross-sectional area was unchanged. To explain these observations on trabecular bone, we propose that in addition to reducing bone formation as shown by reduced bone growth, prednisolone inhibited bone resorption. Thus, existing calcified cartilage matrix accumulated in primary spongiosa without taking the place of secondary spongiosa, resulting in an increased degree of mineralization and trabecular thickness in the tibial metaphysis. Our findings for the tibia are consistent with other studies that showed glucocorticoid treatment of growing animals reduced bone turnover and maintained or increased trabecular BV/TV^{30, 32)}. In some studies of humans¹¹⁾ and animals¹⁸⁾, glucocorticoid treatment reduced bone density. However, in the current study we used pQCT, which measures the degree of mineralization of bone³³⁾ rather than just bone mineral density. In contrast, prednisolone induced deterioration of cortical bone structure in both the tibia and mandible. This was seen as reduced bone cross sectional area, but with no change in bone density.

These prednisolone-induced changes in trabecular and cortical bone structure (mandible) or in cortical bone structure alone (tibia) resulted in decreased bone strength of both tibia and mandible as measured by SSI. SSI is determined by a non-invasive mechanical analysis of cortical bone density and modulus of sections. Ferretti *et al* (1996)³⁴⁾ and Jamsa *et al* (1998)³⁵⁾ demonstrated that SSI values and a three-point bending test had a high correlation for the long bones in animals, and SSI could be used as an indicator of bone strength.

Parfitt (1994)³⁶⁾ demonstrated that during adolescence (bone growth), increased cortical bone turnover increases cortical porosity, resulting in higher risk of fracture of the long bones. Therefore, in the long bones (tibial metaphysis) of growing animals, cortical bone

might be more responsive to prednisolone than is trabecular bone. Ikeda *et al* (2003)³⁰⁾ reported that in growing minipigs responsiveness of cortical bone to glucocorticoid was greater than that of trabecular bone, while Iwamoto *et al* (2006)³¹⁾ reported that glucocorticoid treatment deteriorated trabecular architecture in the tibia of adult female rats. Thus, in older animals with little or no longitudinal bone growth and with on-going remodeling³⁷⁾, as opposed to growth related modeling, glucocorticoid treatment induces loss of trabecular architecture. This is in contrast to younger animals with longitudinal bone growth, where suppression of the age-dependent augmentation of trabecular bone structure by glucocorticoid treatment might not lead to substantial deterioration of trabecular bone structure³⁰⁾. In summary, the reduction in bone strength by prednisolone was caused by a suppression of the bone growth and a deterioration of intra-bone structure without affecting bone mineralization of the tibia and mandible. In the mandible, there was deterioration of both cortical and trabecular bone, but in the tibia only cortical bone was affected.

The effects of risedronate have been studied in various animal models of bone loss. For examples, risedronate inhibited bone resorption leading to increases in bone mass and bone strength of long bones in ovariectomized rats³⁸⁾ and in a model of limb immobilization in rats¹⁹⁾. In addition, the combination therapy of risedronate and parathyroid hormone (PTH) promoted bone formation of the mandible in aged, ovariectomized rats³⁹⁾. However, there are very few studies examining the effects of risedronate on the mandible and long bones in a glucocorticoid-treated model of bone loss in growing rats. In the present study, both doses of risedronate reduced the prednisolone-induced decrease in the mandibular length and height, and the higher dose of risedronate (1.0 mg/kg) reduced the prednisolone-induced decrease in the tibial width but had no significant effect on the changes in body weight and tibial length.

As determined using pQCT and 3D micro-CT, risedronate inhibited the prednisolone-induced deterioration of trabecular and cortical bone structure both in the tibia and in the mandible, although there were differences in response to risedronate between the two sites.

In the trabecular bone, risedronate dose-dependently improved bone density and architecture in the tibia, while both dose levels inhibited the prednisolone-induced deterioration of density and architecture in the mandible. However, the higher dose increased BV/TV and Tb.N above untreated control levels in the tibia but not in the mandible, indicative of an effect on the growing skeleton. These findings were consistent with pQCT findings that showed risedronate dose-dependently increased the degree of bone mineralization, resulting in a decrease in cross-sectional area. The difference in response between the tibia and mandible may be due to the weaker effects of prednisolone on the trabecular bone in tibial metaphysis. Recent data has suggested that bisphosphonates inhibit glucocorticoid-induced osteoblast and osteocyte apoptosis *in vivo* in mice²⁸⁾, and this increased osteoblast survival may result in overall enhanced bone formation³⁸⁾. Our results for growing rats are consistent with risedronate reducing trabecular bone resorption/remodeling and lengthening the formation period, which allows an increased degree of bone

mineralization and improved bone architecture. In cortical bone, risedronate inhibited the prednisolone-induced decreases in the bone cross-sectional area and thickness but had no effect on density in the tibia. In contrast, the mandible was more responsive to risedronate. Both dose levels inhibited the prednisolone-induced deterioration of cortical bone structure. In addition, the low-dose risedronate increased bone density above untreated control levels, and the high-dose risedronate increased bone cross-sectional area above untreated control levels. An increase in cortical thickness and cortical bone density of the alveolar bone tissue depends on masticatory function and occlusal force in the young rats⁴⁰. Therefore, a high susceptibility of the mandible to risedronate might be related to the continuous mechanical stimulus. Furthermore, there is a higher percentage of cortical bone in the mandible than in the tibial metaphysis. Thus, the different responses of tibial and mandibular bone to risedronate in prednisolone-treated growing rats may be due to the different growth patterns, structure and functions.

These effects of risedronate on bone structure resulted in inhibition of the prednisolone-induced decreases in bone strength, as measured by SSI, and for some parameters risedronate increased levels above those in untreated controls. However, because of different effects of risedronate in the tibia and mandible, the improvements in SSI are likely related to different parameters at the two sites. In the tibia, the increase in SSI is likely related to geometric effects such as increased cortical bone cross-sectional area and thickness without any effect on bone density. In the mandible, the increase in SSI is probably related to improved trabecular and cortical architecture and to increased cortical bone density.

Conclusion

Risedronate treatment inhibited the reduction in bone density and deterioration of bone quality and strength, both of the tibia and mandible, which was induced by prednisolone administration in growing rats. Further, risedronate inhibited prednisolone-induced decrease in mandibular bone length and height and tibial width, although it did not improve the retardation of longitudinal bone growth. These findings suggest that systemic treatment with risedronate influences oral osseous tissues in glucocorticoid-induced osteoporosis, and indicate a its potential as a treatment for glucocorticoid-induced bone loss in young patients. Furthermore, we demonstrated that a combination of pQCT and 3D micro-CT is a useful method for non-invasive evaluation of the efficacy of drug therapy, as well as for diagnosis of osteoporosis and other oral diseases.

On the other hand, several studies reported cases of jaw osteonecrosis in patients receiving oral bisphosphonate therapy^{41, 42}. We need to carefully evaluate the effects of risedronate on mandibular bone metabolism and clarify the mechanisms of jaw osteonecrosis.

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グルココルチコイド投与成長期ラットの脛骨および 下顎骨の骨質にリセドロネート治療が及ぼす影響

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抄 録

小児期のグルココルチコイド投与が骨量の低下や成長遅延を引き起こすことはよく知られている。本研究では、グルココルチコイド（プレドニゾロン）投与によって骨密度、骨質、骨強度の低下、更に成長遅延が惹起された成長期ラットの脛骨、下顎骨に対してリセドロネート治療が及ぼすかについて比較、検討を行った。海綿骨および皮質骨の骨構造解析には、末梢骨骨塩定量専用 CT (pQCT) と 3D マイクロ CT を用いて行い、骨強度測定には、pQCT 解析によって得られた皮質骨密度と断面係数から計算された骨強度指数 (SSI) を用いた。また脛骨、下顎骨の骨サイズは、軟エックス線写真を用いて計測した。プレドニゾロン投与は脛骨、下顎骨の骨成長を抑制した。更に、下顎骨の海綿骨、皮質骨の構造を破綻させた結果、骨強度低下を引き起こした。一方脛骨に関しては、骨強度の低下は皮質骨の構造破綻のみによって惹起された。リセドロネート治療は、プレドニゾロン投与による脛骨幅と下顎骨長、下顎骨高の成長遅延を回復させたが、脛骨長の成長遅延を回復させることはできなかった。しかし、プレドニゾロン投与による骨構造破綻、骨強度低下を抑制した。特に下顎骨においては、これらの効果に加え、皮質骨密度の増加を伴っていた。以上の結果から、リセドロネート治療は、プレドニゾロン投与によって引き起こされた成長期ラット下顎骨の骨量、骨強度の低下、骨構造の劣化、成長遅延を回復させることが示唆された。

キーワード：グルココルチコイド/リセドロネート/骨質/成長期ラット/3DマイクロCT