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2）海外

口　頭　発　表	12件
原著論文による発表	20件
それ以外（レビュー等）の発表	件

そのうち主なもの

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G. 知的所有権の出願・取得状況

(予定を含む。)

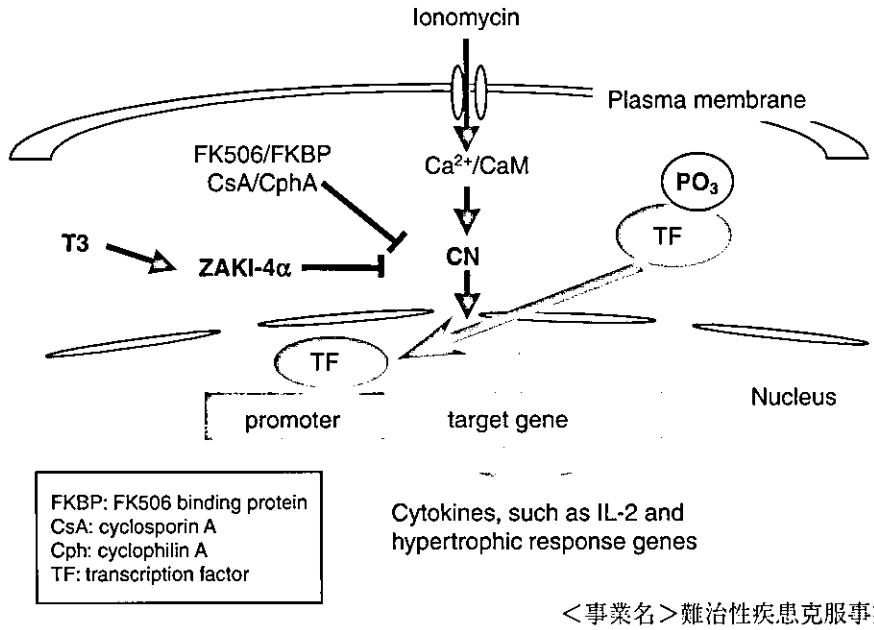
1) 特許取得

発明の名称：皮膚病関連遺伝子の用途
発　明　者：妹尾　久雄、村田　善晴
出　願　日：平成16年5月31日
出 願 番 号：特願2004-160953

2) 実用新案登録

3) その他

甲状腺ホルモン応答性遺伝子産物ZAKI-4αによるカルシニューリン活性の抑制：
カルシニューリン活性調節障害によるRTHの発症

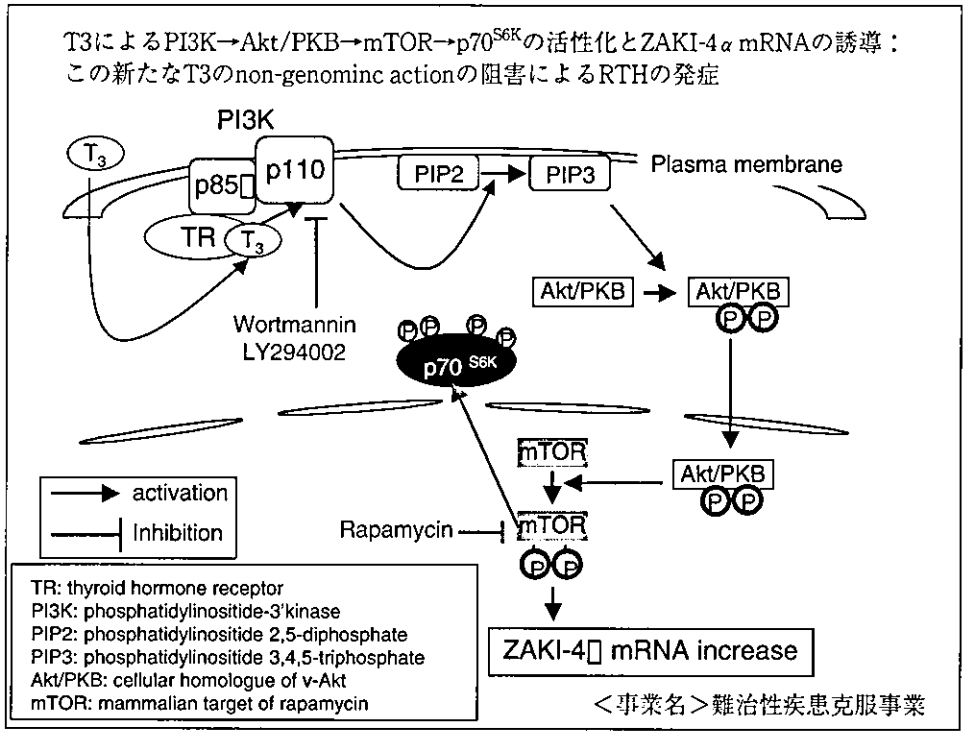


＜事業名＞難治性疾患克服事業

CNは、カルシウム-カルモジュリン(Ca2+/CaM)により活性化されるセリン・スレオニン脱燐酸化酵素である。免疫抑制剤であるFK506やcyclosporin A (CsA)やFK506がそれぞれの結合蛋白であるFKBP 、cyclophoilin と結合し、その活性を抑制することが発見され、その多彩な機能が明らかにされた。CNは脳に大量に発現されているが、他の組織にも広く分布し、 T細胞の活性化、神経の可塑性に関わる長期増強や長期抑制の制御や、神経細胞のアポトーシス、心筋や骨格筋の肥大などに関与している。こうした多彩な作用の一部は、種々のの転写因子(TF)の脱燐酸化を介した活性調節による。

厚生労働科学研究費補助金による成果として、我々がクローニングした甲状腺ホルモン応答性遺伝子ZKI-4の構造とその産物の機能を解明した。ZKI-4遺伝子は第6 染色体の短腕に位置し、２つのアイソフォームa、bをコードする。 a、bとも にカルシニューリン(CN)のcatalytic subunitと結合し、その活性を抑制する。一方、甲状腺ホルモンはaアイソフォームのみを増加し、CN活性を抑制する。この甲状腺ホルモンによるZKI-4 aの誘導とCN活性の抑制は、甲状腺ホルモン不応症患者からえられた皮膚繊維芽細胞には認められず、CNの多彩な機能を鑑みると、不応症患者の多彩な症状の発症機序の一因と考えられる。

甲状腺ホルモンによるCN抑制蛋白の誘導は、我々が世界に先駆けて発見した成果である。



甲状腺ホルモン(T3)によりCN活性を抑制するZKI-4aの発現調節機序を詳細に検討した。その結果、甲状腺ホルモン受容体は細胞膜に存在するPI3Kのcatalytic subunit、p85aと結合して存在し、TRにT3が結合するとPI3Kを活性化し、この活性化によりAkt/PKB→mTOR→p70S6Kが段階的に燐酸化を受け活性化され、ZAKI-4a mRNAが増加する。こうしたT3の作用はPI3Kの阻害剤であるwortmanninやLY294002やmTORの阻害剤であるrapamycinによって完全に阻害される。

T3によるPI3→ Akt/PKB→mTOR→p70S6Kシグナリングカスケードの活性化は、蛋白合成を必要とせず、5～10分と短時間に認められるため、転写を介さないT3のnon-genomic actionと考えられる。 T3とTRの結合を介したこのシグナリングカスケードの報告はこれまで認められない。

甲状腺不応症(RTH)患者では甲状腺ホルモン受容体(TR)のリガンド結合ドメインに変異があるため、TRとT3の結合が無く、患者から得られてた皮膚繊維芽細胞ではT3によるこのシグナリングカスケードの活性化が阻害されている。

PI3→ Akt/PKB→mTOR→p70S6Kシグナリングカスケードはインスリンやアミノ酸によっても活性化を受け、細胞の増殖、分化、機能維持に重要な役割をはたしている。特にAkt/PKB、mTORの活性化は蛋白合成に必要とされるリボゾーム蛋白の合成や種々の転写因子の燐酸化を介して多くの遺伝子発現を調節している。また、mTOR遺伝子の変異が終脳発育発育障害をもたらすことも知られている。従って、甲状腺ホルモンによるこのシグナリングカスケードの活性化はヒトの成長・発育に極めて重要な役割を果たしていると考えられ、RTH発症機序の解明に大きく貢献すると考えられる。

Ⅳ．研究成果の刊行に関する一覧表

刊行書籍又は雑誌名 (雑誌のときは雑誌名、巻頁数、論文名)	刊　行 年月日	刊 行 書 店 名	執 筆 者 名
Journal of Steroid Biochemistry & Molecular Biology 89-90:343-345. Direct action of 1,25-dihydroxyvitamin D on bone:VDRKO bone shows excessive bone formation in normal mineral condition.	2004	Elsevier Ltd.	Tanaka H, Seino Y.
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Journal of Steroid Biochemistry & Molecular Biology 89–90 (2004) 343–345
The Journal of Steroid Biochemistry & Molecular Biology
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Direct action of 1,25-dihydroxyvitamin D on bone: VDRKO bone shows excessive bone formation in normal mineral condition☆

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Abstract

In the present study, the direct role of Vitamin D in bone metabolism was investigated. Vitamin D has been suggested to be an important hormone for bone metabolism, but there has been little evidence that Vitamin D actively participates in this process. Here, we show the direct action of Vitamin D by transplanting the bone of the Vitamin D receptor null mutant mice (VDR−/−) to the wild-type mouse. This procedure allowed us to investigate the changes in the bone without VDR in the normal humoral environment. Unexpectedly, the volume and the density of the VDR−/− bone transplanted to the wild-type mouse were signi□cantly increased compared with the control (wild-type bone transplanted to the wild-type mouse). We show that Vitamin D has key roles in bone metabolism negatively.

Keywords: Vitamin D; Wild-type mouse; Vitamin D receptor null mutant mouse; Transplantation

1. Introduction

Vitamin D has been suggested to be an important hormone for calcium homeostasis and bone metabolism [1,2], but there has been little evidence that Vitamin D directly regulates bone formation. The direct role of Vitamin D in bone formation is still controversial. It was reported that Vitamin D increased bone remodeling via stimulating bone cells [3,4]. In contrast, it was also reported that Vitamin D did not need bone formation or calci□cation [5,6].

The Vitamin D receptor null mutant mouse (VDR−/−) has provided new insights into Vitamin D metabolism and its role in vivo [7]. Calcium-supplement experiments aimed at establishing physiological direct functions of VDR in many organs including bone have been inconclusive owing to the essential roles of calcium in biological function. Although calcium supplementation showed an apparent cure of rickets [8,9], we could not exclude a compensatory mechanism such as hyperparathyroidism in this process. To evaluate the direct action of Vitamin D on the bone without an in□uence of calcium homeostasis, it was necessary to investigate the bone of the VDR−/− under a normal environment. To this aid, we performed bone-transplantation of the VDR−/− to the wild-type mouse.

2. Materials and methods

2.1. Animals

VDR null mutant mice were generated by gene targeting as described previously [7]: the locus targeted for the disruption of the VDR gene included exon 2, and the mutant locus contained the neomycin resistant gene. Mice were weaned at 3 weeks of age, and were then fed distilled water and a chow diet ad libitum, (MF, Oriental Yeast, Tokyo, Japan; ingredients: 11.1 mg/g calcium, 8.3 mg/g phosphorous, 1.08 IU/g Vitamin D_3). The mice were maintained under speci□c pathogen-free conditions with a 12 h light, 12 h dark cycle. They were bred as heterozygotes. The VDR genotypes were determined by the previously reported methods [10]. The studies were reviewed and approved by the Institutional Committee of Animal Care and Use of Okayama University Graduate School of Medicine and Dentistry.

2.2. Bone transplantation

The femur and the calvaria prepared from the 2-week-old VDR−/− males were transplanted into the back muscle of the wild-type males and VDR−/− males. The femur and calvaria from the wild-type mice were also transplanted to the wild-type mouse and VDR−/− mice. VDR−/− bone was transplanted to one side of the back and wild-type bone was transplanted to the other side of the back in the same host at the same time. The parents were the same pair both

☆ Presented at the 12th Workshop on Vitamin D (Maastricht, The Netherlands, 6–10 July 2003).

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doi:10.1016/j.jsbmb.2004.03.021

in the donor and the host and the host mice were 6 weeks old. The host mice were maintained under the regular dietary conditions, and the transplanted bones were retrieved after 2, 3 or 4 weeks.

2.3. Analysis of skeletal morphology

Bone radiographs of the transplanted femur were taken with a micro-focus X-ray apparatus (25 kV, 80 μA, 5 s, μFX-1000 digital micro-radiography system; Fuji Photo Film Co., Ltd.). The projected bone area and the density of the femur were analyzed with an imaging analyzer (BAS-2000 II, Fuji Photo Film Co., Ltd.). For Villanueva–Goldner staining, transplanted femurs were excised, fixed with 70% ethanol, embedded in methyl methacrylate, and sectioned into 6 μm slices.

2.4. Serum chemistries

Calcium levels were measured using the *o*-cresol phthalein complexion method (Wako, Osaka, Japan). Phosphorous levels were measured using the *p*-methylaminophenol method (Wako).

2.5. Statistical analysis

Values are given as the means±S.E.M. Statistical analysis was performed using unpaired Student's *t*-test and ANOVA, followed by Fisher's protected least significant difference. $P < 0.05$ was considered significant.

3. Results

3.1. Serum calcium, phosphorous, and ALP activity in the wild-type host mouse

Serum calcium, phosphorous, and ALP activity in the wild-type host mouse were measured at pre-transplantation, and at 2, 3 and 4 weeks after transplantation. These parameter levels are shown in Table 1. There were no significant differences between the transplantation periods. The serum calcium and ALP levels were maintained constant during the transplantation period. The serum phosphorous level was slightly decreased as physiological natural course.

Table 1
Serum calcium, phosphorous, and ALP activity in the wild-type host mouse

Transplanted periods	Ca	P	ALP
Pre	8.31 ± 0.26	8.18 ± 0.68	9.34 ± 0.53
2 weeks	8.38 ± 0.18	7.72 ± 0.23	9.82 ± 0.39
3 weeks	8.17 ± 0.17	7.57 ± 0.38	9.63 ± 0.38
4 weeks	8.37 ± 0.27	6.93 ± 0.35	9.10 ± 0.45

Values are given as means ± S.E.M. (Ca, mg/dl; P, mg/dl; ALP, nmol *p*-nitrophenol/30 min). $n = 4$–5. Serum calcium, phosphorous, and ALP activity in the VDR−/− were 5.36 ± 0.25 mg/dl, 5.26 ± 0.37 mg/dl, and 19.05 ± 1.48 nmol *p*-nitrophenol/30 min, respectively. During the transplanted period, serum calcium, phosphorous, and ALP activity in the wild-type host mouse was not changed.

Table 2
The density and the projected area of the transplanted femur (transplanted period: 4 weeks)

KO-WT	WT-WT	KO-KO	WT-KO	
Density (%)	148 ± 29*	100 ± 5	58 ± 2*	35 ± 2*
Area (%)	104 ± 2	100 ± 2	82 ± 2	59 ± 2*

The images of the radiographs by micro-focus X-ray of the transplanted femur were analyzed using an imaging analyzer system. Values are given as the means ± S.E.M. (the mean values of the density and area in the WT-WT bone were expressed as 100% and those of KO-WT, KO-KO, and WT-KO bones were expressed as a relative value to the WT-WT bone). KO-WT, the bone of the VDR−/− transplanted to the VDR+/+ mouse; WT-WT, the bone of the VDR+/+ transplanted to the VDR+/+ mouse; KO-KO, the bone of the VDR−/− transplanted to the VDR−/− mouse; WT-KO, the bone of the VDR+/+ transplanted to the VDR−/− mouse.
* $P < 0.01$ vs. WT-WT; $n = 4$.

3.2. Analysis of the transplanted bone

Four weeks after the transplantation, bone radiographs of the transplanted femur were taken and the density of the femur was analyzed using a micro-focus X-ray apparatus, and the results obtained are shown in Table 2. The density of the bone of the VDR−/− transplanted to the wild-type mouse (KO-WT) was significantly higher than that of the wild-type bone transplanted to the wild-type mouse (WT-WT). The density of the VDR−/− bone transplanted to the VDR−/− (KO-KO) was low. The density of the wild-type bone transplanted to the VDR−/− (KO-WT) was markedly decreased compared with the WT-WT. The bone area showed a similar trend as the bone density.

The histology by Villanueva–Goldner staining showed that mineralized bone tissue was significantly increased in the KO-WT compared with the WT-WT. On the other hand, the amount of osteoid tissue was increased in the bone of the KO-KO. The WT-KO, mainly consisted of fibrous tissue, was fragile and did not show organized bone structure.

The calvaria at 2 weeks old of the VDR−/− and the wild-type mice were transplanted to the wild-type mouse for 2 weeks. The thickness of the calvaria of KO-WT was 180.5 ± 8.0 μm. This was 2.7-fold greater compared with the WT-WT.

4. Discussion

Bone transplantation caused radical changes in the original bones. Wild-type femur transplanted to the VDR−/− mice (WT-KO) did not show organized bone structure. The density was markedly decreased to 35% of that of the controls (wild-type bone transplanted to the wild-type

mouse; WT-WT). High levels of 1,25-dihydroxy Vitamin D_3[1,25$(OH)_2D_3$] and PTH in the humoral environment of the VDR−/− caused the decreased bone mineral density in the WT-KO. The histology revealed that bone resorption was increased and the demineralized bone area was replaced by fibrous tissue in the WT-KO. The increased bone resorption in the WT-KO suggested that the transplantation procedure did not destroy the cells related to bone resorption such as osteoclasts, short live cells and its precursor.

The density of the KO-WT was markedly increased (1.48-fold greater than the control). Mineralized bone tissue was also significantly increased in the bone compared with the controls. The histology showed that increased bone mass was normal bone tissue not abnormal pathological mineralization. The VDR−/− and wild-type bones were transplanted to the same wild-type mouse at the same time, therefore, both bones were under the same humoral condition. No abnormal changes were observed in the serum levels of the systemic humoral environment by the transplantation procedure. In the normal humoral environment, the VDR−/− bone increased in density, in mineralized bone area and in size. There was one study that reported demineralized rachitic bone implanted into normal host rats resulted in bone formation similar to that seen for normal bone implants [11]. The present results suggested that there was no difference between the bone matrix of the VDR−/− and the wild-type and the increasing of bone formation in the KO-WT was not caused by the original difference in the bone matrix.

The thickness of the calvaria of KO-WT was 2.7-fold greater compared with the WT-WT. This result suggested that the lack of Vitamin D function in the bone caused increased membranous ossification in the normal environment.

In this study, a simple and primitive procedure, transplantation of the VDR−/− bone to a wild-type mouse, allowed us to investigate the VDR-dependent action of Vitamin D to whole bone. In the present findings, the lack of VDR caused increased bone formation. These findings provide the first direct evidence that Vitamin D is essentially a negative regulating factor in bone formation.

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Calcif Tissue Int (2004) 75:338–343
DOI: 10.1007/s00223-004-0028-x

Calcified Tissue International

Osteoclastogenesis Inhibitory Factor/Osteoprotegerin Reduced Bone Loss Induced by Mechanical Unloading

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Received: 14 December 2003 / Accepted: 30 March 2004 / Online publication: 30 July 2004

Abstract. Skeletal unloading resulting from space flight and prolonged immobilization causes bone loss. Such bone loss ostensibly results from a rapid increase in bone resorption and subsequent sustained reduction in bone formation, but this mechanism remains unclear. Osteoclastogenesis inhibitory factor/osteoprotegerin (OCIF/OPG) is a recently identified potent inhibitor of osteoclast formation. We studied effects of OPG administration on tail-suspended growing rats to explore the therapeutic potential of OPG in the treatment and prevention of bone loss during mechanical unloading, such as that which occurs during space flight. Treatment with OPG in tail suspension increased the total bone mineral content (BMC g) of the tibia and femur and the total bone mineral density (BMD g/cm^2) of the tibia. Moreover, treatment with OPG prevented reduction not only of BMC and BMD, but also of bone strength occurring through femoral diaphysis. Treatment with OPG in tail-suspended rats improved BMC, BMD and bone strength to levels of normally loaded rats treated with vehicle. Treatment with OPG in normally loaded rats significantly decreased urinary excretion of deoxypyridinoline, but the effect of OPG in tail suspension was unclear. These results indicate that OPG may be useful in inhibiting bone loss-engendered mechanical unloading.

Key words: Osteoprotegerin (OPG) — Tail suspension — Rat — Bone mineral density — Bone strength

Skeletal unloading during space flight causes bone mass loss in humans and rats [1]. Calcaneus bone mineral density (BMD) changed in all nine astronauts of the 28-, 59-, and 84-day Skylab missions [2]. Among Skylab missions, bone density loss was greater during the 84-day mission than in shorter missions. Similar bone density changes were observed in Salyut-6 astronauts [2]. Studies using growing rats in space have revealed decreased trabecular bone volume in the tibial proximal metaphysis [3]. Such bone loss resulted mainly from bone formation inhibition [2, 3].

In terms of both size and scope, costs have limited the studies addressing bone metabolism in space. Therefore, ground-based models have been developed to study consequences of skeletal unloading similar to those that occur during space flight. The most popular model for human studies is bed rest with a head-down tilt. In animals, the tail suspension model is more useful and popular than models involving tenotomy, sciatic neurectomy [4], casts [5], and taping of the legs [6]. Tail suspension is not so stressful and it may simulate fluid shift conditions.

Various agents, including parathyroid hormone (PTH), growth hormone (GH), insulin-like growth factor-1 (IGF-1) and bisphosphonates have been used to prevent bone loss induced by skeletal unloading [7–10]. Intermittent administration of PTH did not significantly reduce the deficit in tibial fat-free weight induced by unloading, but increased periosteal bone formation at the tibiofibular junction and midshaft [7]. GH administration in young hypophysectomized rats increased bone mass, but responses in trabecular bone volume in the proximal tibia and bone formation at the tibiofibular junction to GH were reduced significantly by skeletal unloading [8]. Continuous infusion of IGF-I increased bone mineral density (BMD) at the metaphysical area in unloaded rats [9]. Treatment with a bisphosphonate, pamidronate, increased the total BMD in addition to that of the metaphysis of the femur, but it showed almost no effect on the BMD of the diaphysis in both control and tail-suspended rats [10].

Osteoclastogenesis inhibitory factor/osteoprotegerin (OCIF/OPG) is a recently identified potent inhibitor of osteoclast formation and activities. Recombinant human OPG specifically acts on bone tissues and increases BMD and bone volume associated with a decrease of active osteoclast numbers in normal rats. It also blocks bone loss in ovariectomized rats [11], indicating that OPG is a potent drug for bone loss prevention.

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This study was intended to explore the therapeutic potential of OPG in the treatment and prevention of bone loss during skeletal unloading, such as during space flight.

Materials and Methods

Animals

We examined 30 six-week-old male Wistar rats (Japan SLC Inc., Shizuoka, Japan). The animals were individually housed and fed standard laboratory rat chow (Oriental MF; Oriental Yeast Co. Ltd., Tokyo, Japan) containing 1.11% calcium, 0.83% phosphorus, and 108 IU/100 g of vitamin D_3. They were maintained on a 12 h light, 12 h dark cycle. After one week of acclimation, 24 animals were suspended by their tails and allocated randomly to the OPG-treated group (n = 12, the TS-OPG group) or vehicle-treated group (n = 12, TS-vehicle group). Six animals were normally loaded and randomly allocated to the OPG-treated group (n = 3, NL-OPG group) or vehicle-treated group (n = 3, NL-vehicle group). Data of the normally loaded groups were not diverse, therefore few animals were used in these groups.

A single strip of surgical tape was wrapped around the tail of tail-suspended rats. One end of a chain was fixed to the tape with a wire and the other end was fixed to a horizontal bar, the height of which was adjusted to maintain the rat in a position with about a 40° head-down tilt. Thereby, the hindlimbs were unable to contact any supportive surface [9, 12, 13].

The animals in the OPG-treated groups were injected intramuscularly with 500 μg/kg of body weight/day of recombinant human OPG (rhOCIF) (Snow Brand Milk Products, Co., Ltd., Tochigi, Japan). The animals in the other groups were injected with vehicle every day for 10 days after which they were sacrificed by exsanguination. Their hindlimb bones and humeri were excised and their length was measured using a vernier caliper. Immediately after excision of femora, tibiae and humeri, all bones were used for analysis of BMD. The femora were also used for bone strength analysis.

Plasma and Urine Biochemistry

Urine samples were obtained before tail suspension and at days 4 and 10. Blood samples were obtained from the inferior vena cava at exsanguinations. Serum calcium and phosphorus concentrations were determined by the orthocresolphthalein complexone (OCPC) method using the Wako kit 272-21801 (Wako Pure Chemical Industries, Ltd., Osaka, Japan) and the p-methylaminophenol reduction method using the Wako kit 270-49801.

Pyridinoline and deoxypyridinoline concentrations in urine were measured as reported previously at the Mitsubishi Laboratory [14]. The pyridinoline/creatinine and deoxypyridinoline/creatinine at pre-treatment were baseline, and the changes in bone marker values from the baseline at days 4 and 10 were expressed as a percentage of the baseline value.

Bone Analysis

Bone Mineral Density. The BMD (g/cm^2) and BMC (g) of the right femora, tibiae, and humeri were measured by dual-energy X-ray absorptiometry (DXA) using QDR-1000 (Hologic, Inc., Waltham, MA, USA) at high resolution mode. In addition, the BMD and BMC of the femora were determined in four divided regions with equal longitudinal length (region 1 representing the distal femur, regions 2 and 3 the midshaft femur, and region 4 the proximal femur). Regions 1 and 4 represented the proximal metaphysis and the distal metaphysis (containing a high percentage of trabecular structures). Regions 2 and 3 represented the diaphysis (mainly cortical structure [15]).

Table 1. Changes in weight of rats during experiment

Drug	Pre (g)	4 days (g)	10 days (g)
TS-vehicle	205.6 ± 18.4	218.9 ± 14.0	247.3 ± 16.6
TS-OPG	208.3 ± 19.1	219.7 ± 15.3	251.4 ± 14.6
NL-vehicle	195.3 ± 5.7	231.1 ± 5.6	274.3 ± 12.7[ab]
NL-OPG	196.5 ± 3.8	235.2 ± 5.8	280.0 ± 7.6[ac]

Values are mean ± SD
[a] $P < 0.01$, when compared with TS-vehicle (ANOVA Fisher's PLSD test)
[b] $P < 0.05$, when compared with TS-OPG (ANOVA Fisher's PLSD test)
[c] $P < 0.01$, when compared with TS-OPG (ANOVA Fisher's PLSD test)

Bone Strength. Mechanical strength of the right femur was measured by the three-point bending method using a Bone Strength Tester TK-252C (Muromachi Kikai Co. Ltd., Tokyo, Japan). Tests were conducted with the femur at the midlength supported on anterior sides. Distance between the bottom supports was 12 mm. Bending force was applied with a crosshead speed of 2.5 mm/min until fracture occurred. The ultimate force (N) was obtained from the load-deformation curve.

Statistical Analysis. Values are expressed as respective means ± standard deviation (SD). Data were analyzed by one-way analysis of variance followed by a *post hoc* test of Fisher's PLSD test to evaluate intergroup differences among all groups. $P < 0.05$ was considered to be statistically significant.

Results

Body Weight, Plasma and Urine Biochemistry

All rats remained healthy and their body weights increased gradually. There was no significant difference in the body weights of OPG-treated group rats and vehicle-treated group rats in the same condition, but the weight of normally loaded groups (the NL-vehicle group and the NL-OPG group) was increased significantly compared with tail-suspended groups (the TS-vehicle and the TS-OPG group) (Table 1). Serum calcium and phosphorus levels were not significantly different among the four groups (Table 2).

OPG is a bone resorption inhibitor [11]. The urinary excretion of pyridinoline and deoxypyridinoline, both markers of bone resorption, were determined for the purpose of evaluating total body bone resorption activities. Treatment with OPG significantly decreased urinary excretion of deoxypyridinoline in the normally loaded groups (NL-OPG vs NL-vehicle), but the effect of OPG in the tail-suspension groups (TS-OPG vs TS-vehicle) was unclear (Table 3).

Table 2. Effect of OPG on serum calcium and phosphorus

Group	Ca (mg/dl)	P (mg/dl)
TS-vehicle	9.81 ± 0.72	12.46 ± 2.39
TS-OPG	9.25 ± 0.69	11.83 ± 2.55
NL-vehicle	9.92 ± 0.76	12.40 ± 0.19
NL-OPG	9.47 ± 0.17	11.65 ± 0.67

Values are mean ± SD

Table 3. Percent change from pre-treatment value in pyridinoline/creatinine(pyr) and deoxypyridinoline/creatinine (dpyr) (%)

Drug	Marker	4 days	10 days
TS-vehicle	Pyr	17.90 ± 32.68	−11.23 ± 18.57[b]
	dpyr	−11.43 ± 11.02	−33.53 ± 12.12[ab]
TS-OPG	pyr	8.74 ± 24.87	−6.18 ± 16.30
	dpyr	−14.21 ± 13.44	−30.16 ± 9.12[ab]
NL-vehicle	pyr	−3.21 ± 11.65	1.62 ± 2.86
	dpyr	−6.91 ± 10.49	9.24 ± 4.41
NL-OPG	pyr	−15.20 ± 19.52	−8.79 ± 14.84
	dpyr	−27.65 ± 11.28[a]	−19.41 ± 10.77[a]

Values are mean ± SD
[a] $P < 0.05$, when compared with NL-vehicle
[b] $P < 0.05$, when compared with day 4

Bone Length and Bone Mineral Measurements. Tibia and femur length showed no significant differences among the four groups (Table 4). Humeri in the normally loaded groups (the NL-vehicle group and the NL-OPG group) were significantly longer than in the TS-vehicle group. Regarding the tibiae, the BMC and the BMD in the TS-OPG group were significantly higher than that in the TS-vehicle group; the BMD in the normally loaded groups (the NL-vehicle group and the NL-OPG group) was significantly higher than that in the TS-vehicle group. Femurs of the rats showed that BMD in the TS-OPG group did not increase significantly, but BMC in the TS-OPG group was significantly higher than in the TS-vehicle group. The BMDs in the normally loaded groups (the NL-vehicle group and the NL-OPG group) were significantly higher than those in the tail-suspended groups. For the humeri, BMC and BMD among the four groups were not significantly different. In the regional BMC and BMD of the femur, the BMC of regions 1–3 and the BMD of regions 1–2 in the TS-OPG group were significantly higher than in the TS-vehicle group. The BMDs of regions 1 and 4 in the normally loaded groups (the NL-vehicle group and the NL-OPG group) were significantly higher than in the tail-suspended groups (Table 5, Fig. 1). The BMDs of regions 1 and 4 in the TS-OPG group were significantly lower than in the NL-vehicle group. Notwithstanding, the BMDs of regions 2–3 in the TS-OPG group were nearly equal to those in the NL-vehicle group.

Bone Strength of Femoral Midshaft. Bone strengths of the femoral midshaft in the TS-OPG and the NL-vehicle group were significantly higher than in the TS-vehicle group. Bone strength in the NL-OPG group was significantly higher than in the other groups (Table 6).

Discussion

This study uses few animals in normally loaded groups (NL-OPG and NL-vehicle). Tables 1–5 show that data of normally loaded groups were not so diverse therefore few animals were used in these groups.

Bone formation is reportedly reduced in skeletal unloading models [16]. However, the role of bone resorption in bone loss induced by unloading appears inconsistent because of its transient nature. For example, most tail-suspension models [9, 17, 18] have failed to show increased osteoclast numbers and activity in cancellous tissue at day 7 [17] or day 14 [9, 18]. In contrast, urinary excretion of deoxypyridinoline in tail-suspended rats increased at day 1 and day 3 [19, 20].

OPG is a bone resorption inhibitor [11]. Bekker et al. [26] reported that OPG treatment in postmenopausal women inhibited bone resorption dose-dependently, as reflected by bone resorption marker profiles (urinary deoxypyridinoline and N-telopeptide). In the present study, urinary excretion of deoxypyridinoline in the NL-OPG group was significantly lower than in the NL-vehicle group at days 4 and 10, implying that OPG treatment reduced bone resorption in the normally loaded groups. Nevertheless, the effect of OPG was unclear in the tail-suspended groups. Deranged bone metabolism during tail-suspension, not only in the unloaded hindlimbs but also in the whole body (forelimbs were overloaded), may explain that insufficient clarity in results. Urinary excretions of pyridinoline and deoxypyridinoline in the TS-OPG group tended to be lower, but not significantly lower than those in the TS-vehicle group at day 4. Such was not the case at day 10. Therefore, we infer that the effect of OPG in the case of tail-suspension might be clearer in early phases.

Matsumoto et al. demonstrated that the decrease in BMD of the femoral diaphysis (rich in cortical bone) was smaller than that in BMD of the metaphysis (rich in trabecular bone) during 7-day tail-suspension. Although 14-day tail-suspension decreased the BMD of the diaphysis markedly, the decrease in the BMD at the metaphysis was not as advanced as with 7-day tail-suspension [21–23]. Bone strength of the femoral midshaft was not affected until 7 days, but was markedly reduced after 14 days [21]. Therefore, the trabecular bone was reduced during the early phase, but the decrease in the cortical bone was delayed. This delayed decrease in the BMD of the diaphysis in 14-day tail-suspension resulted mainly from impairment of cortical bone formation [21–