Effects of Oral Administration of Simvastatin on Bone Formation in Senile Osteoporosis Rat

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Abstract: The aim of this study was to examine the effects of orally administered simvastatin on the bone healing process in stroke-prone spontaneously hypertensive rat. Twenty-week-old female stroke-prone spontaneously hypertensive rats (SHRSP) and Wistar Kyoto rats (WKY) as a control were used. Bone defects were created in the femur of each rat. From the first day following surgery, half of each of the WKY and SHRSP rats were orally administered simvastatin (10 mg/kg/day). The WKY and SHRSP rats were each divided into experimental groups: 1) WKY simvastatin administration group (WKY-S), 2) WKY control group (WKY-C), 3) SHRSP simvastatin administration group (SHR-S), and 4) SHRSP control group (SHR-C). Experimental periods were set at 1, 2 and 4 weeks after surgery. After each experimental period, the animals were sacrificed. Radiographic analysis and histologic and histomorphometric examinations and immunohistochemical staining for BMP-2 were performed on the harvested samples. The data were statistically analyzed. The radiological analysis and histomorphometry parameters showed that the amount of newly formed bone in the trabecular bone area and cortical bone area increased significantly in the WKY-S group compared to that in the WKY-C group at 2 weeks. In addition, a positive immune reaction with BMP-2 was seen in osteoblasts located on the surface of newly formed bone and the outer periosteum around the marginal area of existing bone in WKY-S. In contrast, in both SHR-S and SHR-C 4 weeks after surgery, no differences in bone formation in the trabecular bone area and cortical bone area were recognized as being based on simvastatin administration. In addition, in the expression of BMP-2, there was no significant difference between the presence and absence of simvastatin administration. The oral administration of simvastatin under our experimental conditions had a facilitating effect on bone formation in WKY rats, whereas no effect was recognized in SHRSP rats.

Key words: Simvastatin, Senile osteoporosis, Oral administration, Bone formation, Micro-CT

Introduction

In recent years, implant treatment has been a well-evaluated prosthetic treatment providing high stability and maintenance. However, elderly implant patients with various systemic diseases are becoming a significant problem because they face some hazards from implant treatment including osteoporosis1,2. On the other hand, since the usefulness of implantation is widely known, there is great demand for implantation by patients with osteoporosis. Therefore, promoting bone healing in osteoporosis patients is considered an extremely important issue associated with implant treatment.

Osteoporosis is classified as postmenopausal osteoporosis: increasing bone resorption due to postmenopausal estrogen deficiency, and senile osteoporosis: reduced ossification associated with aging3. Postmenopausal osteoporosis is high-turnover osteoporosis resulting in trabecular bone reduction, while senile osteoporosis is low-turnover osteoporosis that affects both cortical and trabecular bone4.

Experiments on ovarioctomized (OVX) animals have been carried out as postmenopausal osteoporosis models. These model studies have reported high-turnover osteoporosis caused trabecular bone loss5, delayed healing of fractures6 bone defects7, and reduction of the contact ratio between the implant and bone8,9. In the rodent model for senile osteoporosis, senescence accelerated mouse prone 6 (SAMP6)10 have been used, and stroke-prone spontaneously hypertensive rats (SHRSP) showing a pathological condition similar to that of senile osteoporosis have also been used10,11. These low-turnover osteoporosis models have been reported to decrease both cortical bone and trabecular bone12 and peri-implant bone density13, but not enough examinations of the effects have been done on the healing process of defective bone.

Currently, therapeutic medicine for osteoporosis such as...
bisphosphonate, calcitonin, and selective estrogen receptor modulators are used. In addition, statins recognized as therapeutic drugs for hyperlipidemia have been reported to have a facilitating effect on bone formation and are gaining attention as a method to improve bone mass in osteoporosis.

Statins are known to inhibit HMG-CoA reductase resulting in lipid lowering, and also to improve vascular endothelial cell function and have a vascular regeneration effect\[14,15]\). In addition, statins are suggested as promoting bone formation by increasing the expression of bone morphogenetic protein 2 (BMP-2)\[16,17,18]\) and to suppress bone resorption by decreasing osteoclast activity\[19,20]\). In epidemiological studies, it was reported that the risk of hip and non-spine fractures were decreased in patients who used statins for the treatment of high cholesterol\[21]\).

Mundy G, et al.\[16]\) reported that simvastatin increased the expression of BMP-2 mRNA in murine (2T3) and human (MG-63) osteoblast cells in vitro, and promoted mouse calvarial bone formation in vivo. Nyan M, et al.\[22]\) suggested that simvastatin accelerated the healing process of calvarial bone defect in rats.

In implant research, it was reported that the bone contact ratio and bone mineral density surrounding the implant were increased by intraperitoneal administration of simvastatin in rats\[23]\).

In OVX animal studies, it was recognized that orally administrated statins increased the histomorphometrical and mechanical parameter of bone\[24]\) and the expression of osteogenic proteins\[25]\), and also accelerated bone regeneration\[26]\). However, there were no reports on the effects of statins on senile osteoporosis. It is important to establish a method to promote bone healing and improve bone quality in low-turnover osteoporosis because it occurs not only in female patients but also in elderly male patients.

This study aimed to elucidate the radiological and histological effects of orally administered simvastatin on the recovery process of defective bone in SHRSP.

Materials and Methods

Experimental animals

Twenty-week-old female stroke-prone spontaneously hypertensive Rats (SHRSP) (Sankyo Labo Service Co. Inc., Tokyo, Japan) and Wister Kyoto Rats (WKY) (Sankyo Labo Service Co. Inc., Tokyo, Japan) were used (each n=30). The rats were kept in metal cages under controlled conditions of 23 °C, were raised under a 12-hour light-dark cycle, given a standard diet, and had free access to water. The WKY and SHRSP rats were each divided into a simvastatin administration group and a non-simvastatin administration group as control. The four groups were 1) WKY simvastatin administration group (WKY-S), 2) WKY control group (WKY-C), 3) SHRSP administration group (SHR-S), and 4) SHRSP control group (SHR-C).

Surgical bone defects and oral administration of simvastatin

For surgery, the hind legs of the rats were shaved considerably and disinfected with ethanol while the animals were under general anesthesia with an intraperitoneal administration of pentobarbital sodium (Somnopentyl® 0.9µl/g, Kyoritsu Seiyaku Corporation, Tokyo, Japan). The outside skin of the distal femur was incised longitudinally, and the femur was exposed by stripping the periosteum. A bone defect was formed 3 mm mesially from the articular surface of the femoral knee using a round bar 1.6 mm in diameter (Fig. 1). During the bone defect formation, surgical treatment was carefully performed to avoid the feeding vessel and epiphysis damage; all experimental animals showed no abnormal bleeding. After the bone defect was formed, the periosteum was replaced and the surgical wound sutured.

In the administration groups (WKY-S and SHR-S), simvastatin (Wako Pure Chemical Industries, Osaka, Japan) diluted by physiological saline solution was administrated orally using a...
gastric tube. The dose of simvastatin was 10 mg/kg per day starting the first day after surgery. In the control groups (WKY-C and SHR-C), water was orally administered daily in the same way. The experimental protocol is shown in Fig. 2. The experimental periods were set at 1, 2, and 4 weeks after surgery, and the rats were humanely sacrificed with intraperitoneally administered overdoses of pentobarbital.

This study was conducted according to the Tokyo Dental College Regulations for Animal Experiments. (Approval number 243005)

**Radiological assessment and measurement of newly formed trabecular bone**

Radiological assessment was performed using an *in vivo* micro X-ray CT system R_mCT2 (Rigaku Corporation, Tokyo, Japan) and X-ray images were taken immediately before each group was sacrificed. The imaging conditions were: tube voltage, 90 kV; tube current, 160 µA; magnification, ×10; slice width, 20 µm; and scanning time, 2 minutes. After morphological analysis, micro CT X-ray image data was acquired. Subsequently, pseudo-color bone mineral density (BMD) images were made using 3D trabecular bone structure analysis software (TRI/3D-BON) (Ratoc System Engineering Corporation, Tokyo, Japan) and bone volume was calculated.

The region of interest for the radiomorphometry was designated as the center of the trabecular bone defect to avoid the effects of the existing bone. Namely, a φ1.0×1.0 mm cylindrical range 0.5 mm above the cortical bone at the base of the bone defect, and 0.3 mm inside of the periphery of the trabecular bone defect.

**Histomorphological assessment and measurement of newly formed cortical bone**

The removed femurs were immersed in 10% neutral buffered formalin for 7 days, and paraffin specimens were made after decalcification with 10% EDTA (pH 7.0-7.5) (Wako Pure Chemical Industries, Osaka, Japan) for 6 days at room temperature. The specimens were sliced into 3 µm-thick sections and stained with hematoxylin and eosin staining (H-E staining) according to standard protocols. These samples were morphologically observed using a Universal Photomicroscope (Axiophot 2, Carl Zeiss, Oberkochen, Germany).
The regions of interest for the histomorphometry were designated the rectangular area formed by both margins of the existing cortical bone, which was divided into three evenly, i.e., one central area and two marginal areas (Fig. 4). The ratio of newly formed bone in each area was calculated using Adobe Photoshop (Adobe Systems, San Jose, USA). The Mann-Whitney U-test was used for statistical analysis.

**Immunohistochemical staining**

For immunohistochemical staining, the paraffin sections were deparaffinized with xylene and rehydrated using an ethanol series. The sections were washed in 10 nmol/l pH 7.4 phosphate-buffered saline (PBS) and endogenous peroxidase activity was blocked by incubating sections with 0.3% H_2O_2 in methanol for 30 minutes. The sections were then reacted with the primary antibody, rabbit anti-BMP-2 (Bioss Inc, MA, USA), diluted 1:100 overnight below 4 °C. The sections were washed in PBS and then incubated with the secondary antibody, peroxidase-labeled anti-mouse IgG polyclonal antibody (Histofine Simple Stain Rat MAX-PO [MULTI]; Nichirei, Tokyo, Japan), for 30 minutes and washed with PBS. The sections were then stained with 3,3'-diaminobenzidine (DAB) (DAB Substrate Kit, Nichirei, Tokyo, Japan), washed in distilled water, and counterstained with hematoxylin. The sections were then dehydrated according to the established protocol, and enclosed sections were examined and photographed using a Universal Photomicroscope (Axiophot 2).

**Results**

**Radiological analysis of newly formed bone**

At 2 weeks after surgery, clear new trabecular bone structures in the trabecular bone defect area could be recognized in both the WKY and SHR groups (A-D). The cortical bone defect area in WKY-S was cross-linked with the newly formed bone and the recess of the bone defect area was barely visible (A). In contrast, a wide recess at the cortical bone defect area was still observed in WKY-C (B), SHR-S (C), and SHR-C (D). At 4 weeks after surgery, a reduction of the skeletal structure in the trabecular area and increasing impermeability of the newly formed bone in the cortical bone defect area compared to the micro CT X-ray images at 2 weeks after surgery was recognized in both WKY-S (E) and WKY-C (F). In particular, newly formed cortical bone in WKY-S had the same impermeability as the existing bone and the recess had disappeared (E). On the other hand, the defective cortical bone area was cross-linked with new, narrow bone that increased impermeability in both SHR groups, but a shallow recess remained at 4 weeks after surgery (G and H). No difference was seen in the amount of new bone formation in cortical and trabecular bone between SHR-S and SHR-C.

In the micro CT images at 1 week after surgery, no radiopacity was seen in the bone defects of the cortical and trabecular bones in both the WKY and SHR groups (data not shown). At 2 weeks after surgery, clear, new trabecular bone structures in the trabecular bone defect area could be recognized in both the WKY and SHR groups (Fig. 5A-D). The cortical bone defect area in WKY-S was cross-linked with the newly formed bone and the recess of the bone defect area was barely visible (Fig. 5A). In contrast, a wide recess at the cortical bone defect area was still observed in WKY-C (Fig. 5B), SHR-S (Fig. 5C), and SHR-C (Fig. 5D). At 4 weeks after surgery, reduction of the skeletal structure in the trabecular area and increasing impermeability of the newly formed bone in the cortical bone defect area compared to micro CT X-ray images at 2 weeks after surgery was recognized in both WKY-S (Fig. 5E) and WKY-C (Fig. 5F). In particular, newly formed cortical bone in WKY-S had the same impermeability as the existing bone, and the recess had disappeared (Fig. 5E). On the other hand, at 4 weeks after surgery, the defective cortical bone area was cross-linked with new, narrow bone that increased impermeability in both SHR groups, but a shallow recess remained (Fig. 5G, H). No difference was seen between SHR-S and SHR-C in the amount of new bone formation in cortical and trabecular bone.

**Analysis of newly formed bone in trabecular bone**

Fig. 6 shows the bone volume of newly formed bone in the bone defect area of trabecular bone. In the WKY group (Fig. 6A), the BV values at 1 week after surgery were 0.28×10^3 ± 0.55×10^-3 mm^3 for WKY-S and 0.11×10^2 ± 0.25×10^-3 mm^3 for WKY-C. At
2 weeks after surgery, the BV values had increased considerably to $0.14 \pm 0.08 \text{ mm}^3$ for WKY-S, and a significant increase in new bone was seen compared to the $0.08 \pm 0.07 \text{ mm}^3$ values for WKY-C ($p<0.05$). At 4 weeks after surgery, the values were $0.03 \pm 0.04 \text{ mm}^3$ for WKY-S and $0.03 \pm 0.03 \text{ mm}^3$ for WKY-C and there was no significant difference.

In the SHR group (Fig. 6B), the BV values at 1 week after surgery were $0.34 \times 10^{-3} \pm 0.83 \times 10^{-3} \text{ mm}^3$ for SHR-S and $0.10 \times 10^{-2} \pm 0.33 \times 10^{-2} \text{ mm}^3$ for SHR-C. At 2 weeks after surgery, the BV values were $0.11 \pm 0.12 \text{ mm}^3$ for SHR-S and $0.22 \pm 0.15 \text{ mm}^3$ for SHR-C, and it had increased in both groups from week 1, but no significant differences were seen between the groups. At 4 weeks after surgery, the values for SHR-S increased only slightly to $0.16 \pm 0.10 \text{ mm}^3$ compared to 2 weeks after surgery, and the values for SHR-C had decreased slightly to $0.20 \pm 0.13 \text{ mm}^3$, but no significant differences were seen between the groups.
Figure 8  Ratio of newly formed bone in bone defect area of cortical bone.
In the WKY group, WKY-S showed a significant increase in newly formed bone in the central area and marginal area compared to that in WKY-C at 2 weeks. In the SHR group, no significant difference was observed between SHR-S and SHR-C in both areas. (*p<0.05; Mann-Whitney U-test)

Figure 9  Immunohistochemistry of BMP-2 at 2 weeks.
A positive immune reaction with BMP-2 was seen in osteoblasts localized on the surface of newly formed bone and the outer periosteum around the marginal area of existing bone in WKY-S (arrowheads in A). In contrast, immunoreaction with BMP-2 was only recognized in newly formed bone in WKY-C and the expression was weaker than that in WKY-S (A and B). In the SHR group, no difference was seen in the BMP-2 expression trend in both SHR-S (C) and SHR-C (D). A positive reaction was observed along the newly formed bone trabeculae and on the recessed surface layer of the cortical bone, but the reaction was weaker than that in the WKY group.
EB = Existing bone; NB = New bone
Histomorphological analysis of newly formed cortical bone

At 1 week after surgery, a large amount of new, immature bone was seen in the trabecular bone defect area but was not observed in the cortical bone defect area in both the WKY and SHR groups (data not shown). At 2 weeks after surgery, new bone had formed up to the cortical bone defect area and the bone recess had become shallow in WKY-S (Fig. 7A). New bone had not formed in the cortical bone areas except along the margins of existing bone and a deep bone recess was visible in the WKY-C (Fig. 7B) and SHR groups (Fig. 7C, D). At 4 weeks after surgery, new bone in the cortical bone defect area was cross-linked with thick, mature bone, and the bone recess could barely be seen in WKY-S (Fig. 7E). In WKY-C (Fig. 7F), the new bone trabeculae of the cortical bone defect area increased in thickness and was cross-linked with the marginal area of existing bone, but the bone recess was still visible. In both SHR-S (Fig. 7G) and SHR-C (Fig. 7H), the thickness of the new bone trabeculae that had formed in the cortical bone defect area increased, but a deep recess could still be seen.

Analysis of newly formed bone in cortical bone

Fig. 8 shows the ratio of newly formed bone in the bone defect area of cortical bone. At 1 week after surgery, the amount of bone formation in the marginal area (WKY-S: 9.69 ±5.80%, WKY-C: 17.91 ± 5.94 %) and the central area (WKY-S: 7.94 ± 16.69 %, WKY-C:2.75 ± 4.57 %) was small in both WKY-S and WKY-C, and no significant differences were seen. At 2 weeks after surgery, WKY-S showed a higher amount of bone formation than did WKY-C in both areas, and significant differences were seen between the marginal area (WKY-S: 54.50 ± 6.87 %, WKY-C: 27.01 ± 9.63 %) and the central area (WKY-S: 41.80 ± 6.47 %, WKY-C: 7.64 ± 12.02 %) (p<0.05). At 4 weeks after surgery, the amount of bone formation had continued to increase in the marginal area (WKY-S: 68.40 ± 9.68 %, WKY-C: 72.98 ± 13.72 %) and in the central area (WKY-S: 50.34 ± 7.26 %, WKY-C: 53.83 ± 10.54 %), but no significant differences were seen.

In the SHR group, the amount of newly formed bone increased over time, but no significant difference was observed between SHR-C and SHR-S in any period.

Immunohistochemical staining for BMP-2

At 2 weeks after surgery, a positive immune reaction with BMP-2 was seen in osteoblasts located on the surface of newly formed bone and the outer periosteum around the marginal area of existing bone in WKY-S (arrowheads in Fig. 9A). In contrast, an immunoreaction with BMP-2 was only recognized in newly formed bone in WKY-C and the expression was weaker than that in WKY-S (Fig. 9A, B). In the SHR group, no difference was seen in the BMP-2 expression trend in both SHR-S (Fig. 9C) and SHR-C (Fig. 9D). A positive reaction was observed along the newly formed trabecular bone and on the recessed surface layer of the cortical bone, but the reaction was weaker than that in the WKY group. At 4 weeks after surgery, a weak positive reaction with BMP-2 was seen in part of the remaining trabecular bone structure and no positive reaction was recognized in the newly formed cortical bone in both WKY-S and WKY-C (data not shown). In both SHR-S and SHR-C, the positive reaction with BMP-2 around the newly formed bone had weakened, but the positive reaction on the recessed surface layer of the cortical bone was strong in both groups, in contrast to WKY-S and WKY-C (data not shown).

Discussion

In this study, we examined the effects of oral administrations of simvastatin on the healing process of bone defects radiologically and histomorphologically using SHRSP showing a pathological condition similar to that of senile osteoporosis.

In this experiment, the dose of simvastatin was set at 10 mg/kg with reference to the following reports. Oxlund H et al.27 described an increase in the amount of trabecular bone and bone strength with a 10 mg/kg oral administration of simvastatin in normal rats and Garrett IR et al.28 reported that an increase in trabecular bone was recognized with a 10 mg/kg oral administration of simvastatin in OVX rat. Additionally, Hata S et al.29 reported that a 10 mg/kg oral administration of simvastatin facilitated the healing of bone defects in OVX rat.

SHRSP were adopted as the senile osteoporosis model for experimental animals in this study. Osteoporosis in SHRSP was known to start from age 3 months, and pathological conditions similar to low turnover, decreasing bone mass and amount of mineral content with aging, and a decline in calcification and bone formation rate were seen. Therefore, 5-month-old age of the rats used in this study was considered highly valid.

The effect of simvastatin in normal rats

In this study, we considered WKY rat as normal rat. At 2 weeks after surgery, significant increased bone mass was seen in both cortical and trabecular bone in WKY-S compared to that in WKY-C. In contrast, there was no significant difference between the presence and absence of simvastatin administration at 1 week after surgery. In addition, in micro CT images and HE-stained sections at four weeks after surgery in WKY-C, the recess in the cortical bone area still remained. However, there were no significant differences in the amount of new bone formation regardless of the administration of simvastatin. These results show that statins facilitated the healing mechanism of bone defects, but that the final amount of bone formation was consistent. That is to say, in the region where bone formation actively occurs, statins facilitate bone formation, but the effect of statins is thought to decline in the defect area where bone formation is reduced towards the final
stage of bone healing.

These results were supported by previous reports. BMP-2 expression increases through the inhibition of HMG-CoA reductase and the differentiation of osteoblasts is facilitated in the bone-healing process. In addition, it was found that the increased periosteal bone formation in cortical bone with oral administration of simvastatin on OVX rats compared to that on the sham group. Hata S et al. reported that a 10 mg/kg oral administration of simvastatin using a gastric tube facilitated the healing of bone defects, together with a significant reduction in serum total cholesterol level. In this study, promotion of bone healing was seen in WKY-S, which experienced a similar condition, and it was thought that the oral administration of simvastatin in this experiment was expressed in systemic effects. Therefore, the results in the present study might indicate that the expression of BMP-2 was stimulated by the oral administration of simvastatin in the outer cortical bone periosteum in WKY-S, leading to the disappearance of the recess of cortical bone defect.

The effect of simvastatin in SHRSP

In this study, no differences in bone formation were recognized 4 weeks after surgery based on simvastatin administration in both SHR-S and SHR-C. In addition, a deep recess in the cortical bone defect area and higher expression of BMP-2 in newly formed bone and the recess surface layer was still recognized compared to that in the WKY groups at 4 weeks after surgery. These results might indicate that the bone healing process of the SHR group was delayed in comparison with that of the WKY group and bone healing between SHR-S and SHR-C was not affected by oral administration of simvastatin.

In the experimental postmenopausal osteoporosis model using OVX rat, Hata S et al. reported that oral administration of simvastatin (10 mg/kg/day) had a beneficial effect on the bone healing process. They reported that the number of positive cells for BMP-2 and osteocalcin was significantly higher in the simvastatin administration group than in the control group. The reason for our results, which showed there was no difference in the bone healing process between SHR-S and SHR-C, is the decreasing bone metabolism including bone formation and bone resorption in the low-turnover osteoporosis models in comparison to that in the high-turnover osteoporosis model of OVX rat. In addition, it has been reported that there is an impairment of vascular endothelial cells with age in SHRSP, peeling or an irregular sequence of vascular endothelial cells, and a decrease in the release of NO from the endothelium. In this study, a possible reason the bone healing process was delayed in the SHR rats but not in the WKY rats is that, in addition to a decrease in bone metabolism, there is dysfunction in the vascular endothelial cells.

Oral administration of simvastatin might affect not only the healing process of bone defects but also bone remodeling during aging in OVX rat. It was reported that oral administration of 10 mg/kg simvastatin, the same value as in our study, was effective in increasing the volume of cancellous bone compared to 12-week-old OVX rats, which were used as a control group. Oxlud H et al. also reported that simvastatin administration (20 mg/kg) in 16-week-old rats increased cortical bone formation and reduced the loss of cancellous bone induced by ovariectomy. They suggested that this effect was related to the reduced activity of osteoclast-derived TRAP-5b caused by simvastatin. In contrast, Yao W et al. reported that simvastatin did not prevent ovariectomy-induced bone loss. However, they used older OVX rats (about 32–weeks old) and lower simvastatin dosage (0.3–10 mg/kg) than in the two studies previously described. It was known that promotion of bone resorption and formation peaked at 2 to 3 weeks after ovariectomy, and declined with age on high-turnover osteoporosis. From this result, it was suggested that OVX rat with a long duration after surgical treatment showed little effect of simvastatin administration in changing from high-turnover bone metabolism to that of low-turnover metabolism caused by aging.

In clinical studies, it was reported that male patients with hypercholesterolemia who were administered atorvastatin showed a significant reduction of serum N-terminal telopeptide of type I collagen (NTX), known as a bone resorption marker, but no difference in the bone formation marker (bone-specific alkaline phosphatase; BAP). Furthermore, it was reported that atorvastatin and fluvastatin administration to both male and female patients reduced the urine bone resorption marker including NTX, but did not change the bone formation marker. These results indicate that the effect of statins appeared to be to inhibit bone resorption rather than promote bone formation in vivo. In addition, decreasing osteoblastogenesis, a decrease in the number of osteoclasts, and decreasing osteoclastogenesis were recognized in senile osteoporosis mouse models. Accordingly, we surmised that one reason for no difference in the bone healing process between SHR-S and SHR-C was due to not enough osteoclasts and/or osteoclastogenesis-related cells to appear the effect of simvastatin.

The reason for no effect in the SHR group also seems to be related to the oral administration dosage (10 mg/kg) of simvastatin in this study, suggesting that the dosage was insufficient to have an effect in low-turnover osteoporosis. Simvastatin does not have selective adsorption to bone matrix like bisphosphonates, and has a high affinity for the liver, with the result that the statin level of systemic circulation was reduced to less than 5% of the oral administration doses. Maritz FJ et al. reported that BMD in rat femur increased dose-dependently with an increase in simvastatin dosage (0–20 mg/kg). Therefore, the effective dosage of simvastatin for senile osteoporosis was considered to be higher than that in our experiment (10 mg/kg). Komatsu et al. reported that statins affect the differentiation of
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chondrocytes in femurs known as endochondral ossification. In addition, statin drugs stabilize mRNA of endothelium nitric oxide synthetase (eNOS) for parts where the bloodstream is weakened by ischemia. Furthermore, statins reinforce the activity of eNOS through protein kinase B (Akt), and it is reported that the nitric oxide produced promotes vasularization at the same time to increase blood flow by expanding the blood vessels\(^4\). The above reason stems from the result of promoting bone formation in WKY-S in this study. In addition, the arrival ratio to bone of oral statins is low\(^{40,41}\), and decreasing osteoblastogenesis,\(^{9,39}\) a decrease in the number of osteoclasts, and decreasing osteoclastogenesis were recognized in senile osteoporosis mouse models\(^9,39\). For this reason, in the pathogenesis such as SHRS, the effect of statin is not sufficiently exhibited, and this study found no difference in bone formation between SHR-C and SHR-S.

Excessive administration of simvastatin is known to carry a risk of myopathy and rhabdomyolysis\(^{42}\). Recently, local administration of simvastatin was employed in order to avoid these adverse effects. It was already revealed that a locally applied low dose of statin promoted bone healing compared to oral administration\(^{44,45}\). In addition, Wang JW et al.\(^{46}\) reported that local administration of simvastatin by subcutaneous injection also promoted the healing of bone fractures in OVX rat. Thus, it may be necessary to increase the dosage and/or apply local administration of simvastatin to promote bone healing in low-turnover osteoporosis.

In conclusion, under our experimental conditions, oral administration of simvastatin facilitated bone formation in WKY rats, whereas it had no effect in SHRS.

Acknowledgments

This research was supported by an Oral Health Science Center Grant hrc7 from Tokyo Dental College, a “High-Tech Research Center” Project for Private Universities: Matching Fund Subsidy from MEXT (Ministry of Education, Culture, Sports, Science and Technology) of Japan, 2006-2010, and a Grant-in-Aid for Scientific Research (Challenging Exploratory Research: 23659925) from the Japan Society for the Promotion of Science.

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