

Locally applied simvastatin promotes fracture healing in ovariectomized rat

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Abstract

Summary Simvastatin solution was injected subcutaneously to the site of fractured tibiae of ovariectomized rats. Afterwards healing quality was evaluated by morphologic, radiographic, biomechanical, histological and histomorphometric methods at 1, 2 and 4 weeks after fracture. Results showed that locally applied simvastatin improved fracture healing.

Introduction Many studies have documented an anabolic effect of hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, statins, on undisturbed bone. Reports of their effects, however, on fractured skeletal systems have been limited. A study was, therefore, conducted to check the effects of statins on fracture healing.

Methods Simvastatin (10 mg/kg/day) was injected subcutaneously to tissue overlying the site of fractured tibiae of ovariectomized rats for a treatment period of 5 days. Vehicle reagent was used as a control. Healing quality was evaluated at 1, 2 and 4 weeks after fracture.

Results Compared with that in the vehicle group, the callus cross-section area in simvastatin-treated rats was signifi-

cantly enlarged by 21.3% ($p < 0.05$) at 1 week and by 21.5% ($p < 0.05$) at 2 weeks; new woven bone was relatively substantive and arranged more tightly and regularly at 2 and 4 weeks; and maximal load was increased by 57.5% ($p < 0.05$) at 2 weeks and by 31.4% ($p < 0.05$) at 4 weeks. Histomorphometrically, simvastatin was associated with a significant ($p < 0.05$) increase of mineralization width (MLW), mineralization volume (MLV) and mineral apposition rate (MAR).

Conclusion The current study suggests that local application of simvastatin could promote fracture healing in ovariectomized rats.

Keywords Fracture healing · Hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase inhibitors · Osteoporosis · Rats · Simvastatin

Introduction

Current therapies for the treatment of osteoporosis, including estrogen and related compounds, bisphosphonates, calcitonin, vitamin D analogues and selective estrogen receptor modulators, are primarily based on blunting the resorption component of bone homeostasis – a tightly coupled process of bone formation and bone resorption. Drugs that could stimulate new bone formation remain desirable. A study conducted several years ago showed that statins, the widely used lipid-lowering hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, could dramatically stimulate bone formation in cultured osteoblasts, neonatal murine calvaria, and the cortical bone of mice [1]. Then, a number of experimental and clinical studies sustained that statins had positive effects on bone turnover parameters [2–4], bone mineral density (BMD)

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[5–8], and fracture risk [9–12]. These exciting findings strongly indicated that statins would be a potential approach for eliciting anabolic effects in the skeleton.

In subjects with osteoporosis, susceptibility of fracture increases and healing quality is considered to be impaired in osteoporotic fracture [13–15]. Although most anti-osteoporosis drugs have the ability to decrease the risk of osteoporotic fractures, fractures still occur in patients under medical intervention. For this reason, the safety and efficacy of most anti-osteoporosis drugs for fracture healing have been evaluated in order to decide whether they should be ceased or continued after fracture [16–20]. Moreover, it is well expected that the anti-osteoporosis drugs have the ability to promote fracture healing at the same time.

In terms of statins, although no consensus has been made for their clinical application of osteoporosis, they have been widely used in hypercholesterolemic patients – mostly in older populations who also have a high prevalence of osteoporosis and a high fracture rate. If statins can promote the healing quality of osteoporotic fractures, it must be favorable.

Till now, the extensive research on statins has, to a large extent, been carried out on undisturbed bone. Only a few studies have been carried out to investigate their effects on fracture healing. To the author's knowledge, statins in those existing studies were administered systemically in extremely high doses [21, 22]. It should be noted that most gastrointestinal absorbed statins will be stored in the liver, resulting in a much lower concentration at the fracture site [23], and that exceedingly high dose of systemic applied statins will raise the risk of liver failure, kidney disease, and rhabdomyolysis [24]. This prompted us to investigate whether statins could improve fracture healing when applied locally.

In the present study, an osteoporotic fracture model was established in ovariectomized (OVX) rats to imitate fracture in postmenopausal women. In order not to interfere with the healing process by reduplicative injection, which might result in atrophic pseudoarthrosis, the local application in the present study does not mean the direct injection of drugs into the fracture site, rather a subcutaneous injection to tissue in close proximity of the fracture. The aim of the present study was to test the hypothesis that locally applied simvastatin has the ability to improve fracture healing.

Materials and methods

Experimental subjects

The study was carried out on 2-month-old female Sprague-Dawley rats (purchased from the Experimental Animal Center of Zhejiang University, Hangzhou, China) with

initial body weight of 178–201 g. The rats were housed in conventionally controlled clean facilities with a temperature of 23°C and a 12-h light-dark cycle. The rats were fed on chow with 0.46% of calcium, 0.38% of phosphorus and tap water.

The study was approved by the local ethics committee for animal experiments in Zhejiang University, China and was conducted in compliance with national and international laws and guidelines for the Use of Animals in Biomedical Research [25].

Ovariectomy

Three days after arrival, all the rats were randomly subjected to either OVX (n=120) or sham surgery (n=10). For OVX, the animals were anesthetized with an intraperitoneal injection of 5% ketamine hydrochloride (2 ml/kg body weight). Bilateral dorsal incisions were made on the back; both of the ovaries were identified; the blood vessels were clamped and tied off; and the ovaries were removed. The muscle layer was tied and skin incision was closed with silk suture. Sham surgery was performed following the steps above with the visualization of the ovaries, but without clamping or removing of the tissue.

After the operation, the sham rats had free access to food throughout the experiment, while the food of OVX rats was restricted to that of sham group in order to minimize the increase of body weight associated with OVX.

Confirmation of osteopenia status

Twelve weeks after surgery, BMD of the right tibia was measured under anaesthetic condition (ketamine hydrochloride, same dosage as above) to document osteopenia status. Ten rats were sampled from OVX and sham group. Dual-energy X-ray absorptiometer (DEXA, LUNAR Radiation, USA) was employed using the small animal model (voltage:76 kv, current:150 μ A.). The accuracy of this equipment is 0.3%, with an inter-assay coefficient of variation (CV) of 1.3%.

Fracture

All the OVX rats that survived were further used to establish open fracture models and fixed with intermedullary nails. Under sterile conditions after anesthetization by ketamine hydrochloride (same dosage as above), a simple transverse open fracture model was made in the proximal one-third of the right tibia. To stabilize the fracture, a small incision was made on the medial aspect of the knee, and the patella was deflected laterally to expose the tibial nod where a small hole was drilled. Then a sterile stainless steel wire (\varnothing 0.5 mm, Shanghai Medical Needle Factory, China) was inserted through the hole to the distal end of the tibia. The

muscle layer was tied and the skin incision was closed with silk suture. Each animal received antibiotics (penicillium, 0.80 million units, im, BID) for three days postoperatively.

The OVX rats were then randomly, but equally allocated to OVX + vehicle and OVX + SVS groups.

Pharmaceutical intervention

Simvastatin or vehicle drugs were injected subcutaneously to the fracture site once on the day of fracture and twice in 5 days thereafter. A solution of 5% simvastatin (Hisun Pharmaceutical Co. Ltd. China) was prepared according to literature [1], i.e., simvastatin was diffused in PBS with 2% dimethylsulfoxide and 0.1% BSA. The vehicle with no simvastatin was also prepared. Each injection contained 5 mg/kg simvastatin of the SVS group and an equal volume (about 30 μ l–50 μ l) of vehicle solution to the vehicle groups. In this study, simvastatin was injected via the method described by Mundy. In order not to interfere the healing process by reduplicative injection, drugs were injected not directly into the fracture site, but to subcutaneous tissue overlying the fracture site. Solutions were delivered through a 0.4 mm gauge pinhead connected to a micro-syringe with maximal scale of 250 μ l and minimal scale of 10 μ l (Shanghai Medical Laser Instrument Co. Ltd). Care was taken not to disturb or damage the healing callus.

In order to achieve undecalcified histomorphometry parameters, ten rats in each group were double fluorochrome labeled by tetracycline (BioBasic Inc. Canada), which was injected subcutaneously 14, 13, 4, and 3 days before sacrifice in a dosage of 25 mg/kg.

Evaluation of fracture healing

Rats were killed in 1, 2 and 4 weeks after fracture, and healing quality was evaluated as follows.

Radiography

On the day before sacrifice, the rats were generally anaesthetized (ketamine hydrochloride, same dosage as above) to get posterior-anterior radiographs of the fractured tibiae. X-rays were taken by DMR⁺ Mo target mammography machine (22 KV, 250 mAS, GE, USA). Fracture callus was described by two independent observers blinded to the treatment groups using following parameters: (a) no bridging, (b) unilateral bridging, or (c) complete bridging. The mean of duplicate scores was used for statistical analysis.

Biomechanical analysis

Ten rats in each group were scheduled to be killed for biomechanical analysis. Bilateral tibiae of each rat were

harvested, wrapped in pair with saline-moistened gauze and reserved in -20°C circumstance.

The specimens were thawed at room temperature before the biomechanical test, and the intramedullary nails were removed. The tibia was then subjected to a three-point bending test on Zwick-Z010 testing systems (Zwick GmbH & Co. Ulm, Germany. CV: 0.5%), with a span of 30 mm, and at a displacement rate of 2 mm/min. The specimens were kept in a coincident position and were moist through examination. The load-displacement data and the failure load were recorded. The anteroposterior and transverse diameters of the callus zones were measured by a vernier caliper with a sensitivity of 0.02 mm.

Serum chemistry

Serum total cholesterol was measured to prove the lipid-lowering effect of locally delivered simvastatin, and serum alkaline phosphatase (ALP) was detected as a marker of bone formation. Blood samples were taken from the left ventricle after the rats were anaesthetised and stored at -80°C . Serum ALP and total cholesterol were analyzed by AU1000 automatic biochemical analyzer (Olympus, Japan) using the same kit (Wako Pure Chemical Industries, Ltd., Osaka, Japan). The intra-assay and inter-assay CVs were as follows: ALP, 1.8% and 2.2%; total cholesterol, 0.5% and 0.58%, respectively.

Histological and immunohistochemical analysis

Five rats from each group were killed for histological and immunohistochemical analysis. The right tibia was harvested without damaging the periosteum. After the steel wire had been drawn out, the middle part of the tibia with the callus in it was cut. The tissue blockers were then fixed in 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) for 24 hours at room temperature, and decalcified in ethylene diaminetetra-acetic acid (EDTA, 0.5 mol/L, PH 7.4) for 3–5 weeks. EDTA were refreshed every 5–7 days until the bone could be easily inserted with a fine needle. Decalcified specimens were then washed in a phosphate buffer, dehydrated in a graded series of ethanol and embedded in paraffin wax. Tissue was sliced (5 μ m) along the longitudinal plane, mounted on a 0.01% poly-L-lysine (Fuzhou Maixin Biotech Inc. China) coated glass slide and dried overnight at 60°C . Haematoxylin and eosin (HE) staining was performed. The slices were examined under a Olympus BH-2 light microscope.

Bone histomorphometry

Rats with tetracycline label were killed 4 weeks after fracture. The tibial mid-shaft (1 cm) was immersed in

100% acetone for 3 days and then embedded without decalcification in methylmethacrylate. A longitudinal section (5 μm) from the center of the callus block was cut with a Reichert-Jung microtome (Leica Instruments GmbH, Germany) and left unstained for dynamic measurement. An additional section was stained with toluidine blue in order to measure static parameters. The measurement was performed under light microscope and fluorescent microscope (BX51 systems, Olympus, Japan) with a CCD camera (DP11, Olympus, Japan) connected to an image analyzing system installed with Qwin and Qfish software (Leica Instruments GmbH, Germany).

For static measurement, mineralization width (MLW), mineralization volume (MLV), osteoid width (O.Wi), osteoid volume (OV), osteoid surface (OS) and active osteoblastic surface (AOS) were calculated in four fields of each section. For dynamic measurement, the mineral apposition rate (MAR) was calculated by dividing the inter-label distance by the time between the tetracycline labels.

Statistical analysis

All data are presented as mean and standard deviation. Evaluation was performed using the one-way ANOVA for paired and unpaired data. The radiological score was analyzed using the chi-square test. Statistical analysis was done using the SPSS 11.5 software program. $p < 0.05$ was considered to be statistically significant.

Results

During the experimental process after fracture, 4 animals died in the OVX + vehicle group and 3 in the OVX + SVS group. No infection occurred at the fracture site.

Tibial BMD turned out to be distinctly different between the two groups at 12 weeks after ovariectomy ($p < 0.01$), namely $0.241 \pm 0.010 \text{ g/cm}^2$ in the sham group and $0.219 \pm 0.005 \text{ g/cm}^2$ in the OVX group.

The subcutaneous injection was easily performed, with minimal leakage of the solution from the site of injection. No significant difference was found in body weight between the OVX + SVS and the OVX + vehicle rats.

Radiography

None of the fractures presented nonunion on X-ray radiograph. As expected, the amount of bridging bone formation increased with time in both groups. At 1 week, no difference could be noticed between the manifestations of the two groups. At 2 and 4 weeks, the OVX + SVS group presented enhanced consolidation of the fracture compared with the OVX + vehicle group (Fig. 1). This

qualitative impression was confirmed by radiological score analysis, although these differences were not statistically significant ($p = 0.077$ at 2 weeks; and $p = 0.062$ at 4 weeks. Chi-square test, data omitted).

Callus dimension

Substantial histological differences between the OVX + vehicle and OVX + SVS callus zones were visible at 1 and 2 weeks after treatment. The callus cross-section area in simvastatin-treated rats was significantly enlarged by 21.3% ($p < 0.05$) at 1 week ($20.22 \pm 3.42 \text{ mm}^2$ vs. $16.67 \pm 4.02 \text{ mm}^2$) and by 21.5% ($p < 0.05$) at 2 weeks ($23.63 \pm 3.25 \text{ mm}^2$ vs. $19.45 \pm 3.19 \text{ mm}^2$) than the vehicle group (Fig. 2a).

Biomechanical analysis

Values of biomechanical strength were presented as actual maximal load and relative maximal load (as percentage of nonfractured contralateral tibiae) (Fig. 2b). As expected, the mechanical strength increased with time during the evaluation period. Significantly higher mechanical strength was found in the OVX + SVS group compared with the OVX + vehicle group at 2 and 4 weeks. The tested tibiae of the OVX + SVS group demonstrated a mean actual maximum load of $24.87 \pm 6.67 \text{ N}$ (increased by 52.3% vs. $16.33 \pm 6.32 \text{ N}$, $p < 0.05$) and relative maximal load of $49.88 \pm 10.60\%$ (increased by 57.5% vs. $31.66 \pm 13.13\%$, $p < 0.05$) at 2 weeks. The mean actual maximum load at 4 weeks was $47.32 \pm 9.59 \text{ N}$ (increased by 24.4% vs. $38.05 \pm 8.51 \text{ N}$, $p < 0.05$), and relative maximal load was $87.25 \pm 19.93\%$ (increased by 31.4% vs. $66.41 \pm 13.25\%$, $p < 0.05$). No statistical difference was detected on mechanical strength at 1 week after fracture.

Serum chemistry

Changes of serum total cholesterol concentrations indicated that local application of simvastatin could also result in lowering of serum lipid. Simvastatin-treated rats presented a 23.0% ($p < 0.01$) reduction at 1 week ($118.0 \pm 11.58 \text{ mg/dL}$ vs. $90.6 \pm 11.57 \text{ mg/dL}$) and a 14.0% ($p < 0.05$) reduction at 2 weeks ($103.6 \pm 15.4 \text{ mg/dL}$ vs. $89.1 \pm 13.9 \text{ mg/dL}$) of total cholesterol over the OVX + vehicle animals (Fig. 3a).

Serum ALP in OVX + SVS rats showed a significant increase over that in OVX + vehicle animals. Namely, total ALP increased by 20.0% ($p < 0.01$) and 12.4% ($p < 0.05$) at 1 and 2 weeks, respectively. No difference presented at 4 weeks (Fig. 3b).

Callus histology

Light microscopy examination of H & E-stained histological sections showed callus formation at various stages of

Fig. 1 Radiographs of tibiae of Sprague-Dawley rats at 1 (**a, d**), 2 (**b, e**) and 4 (**c, f**) weeks after fracture. The progressed consolidation of the fracture gap in OVX + SVS (**e, f**) groups is clearly recognizable compared with the OVX + vehicle (**b, c**) group at 2 and 4 weeks



development, generally represented by a mixture of endochondral and intramembranous ossification. One week after fracture, cartilaginous callus formation was visible in the OVX + SVS group, whereas less cartilage formation

was visible in the OVX + vehicle group. Relatively more periosteal bone formation was presented in OVX + SVS sections than in OVX + vehicle sections. At 2 and 4 weeks, callus zones of both groups showed periosteal bone

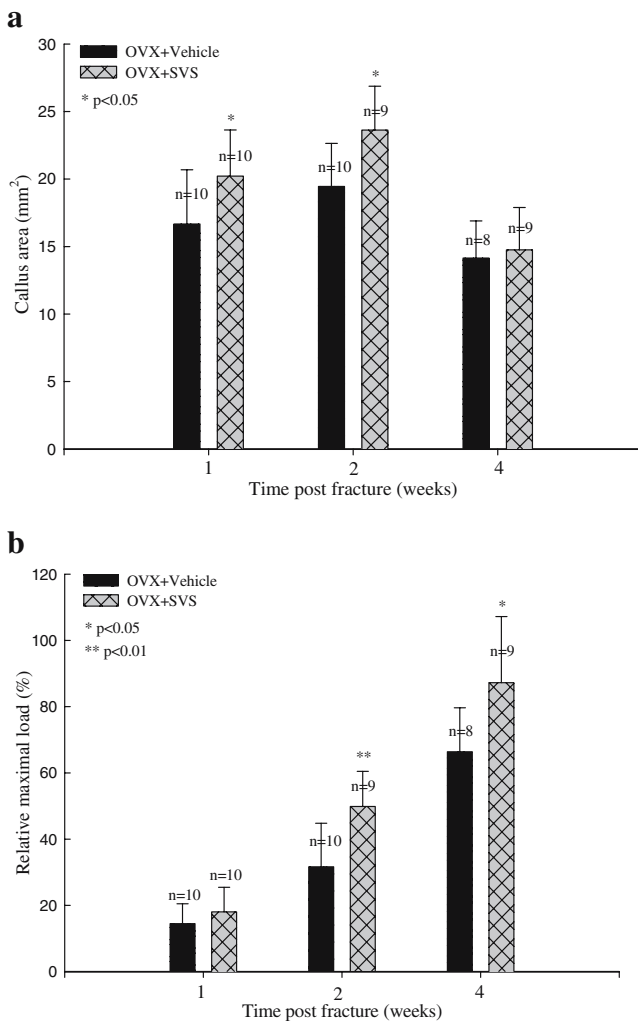


Fig. 2 Cross-sectional area (**a**) and biomechanical results (**b**) of callus at 1, 2, 4 weeks after fracture. (cross-sectional area = $\pi \times a/2 \times b/2$. **a**: Anteroposterior diameter; **b**: Transverse diameter)

formation at the edge and substantial cartilage formation in the central region. Compared with OVX + vehicle callus zones, new woven bone in OVX + SVS animals was relatively substantive and arranged more tightly and regularly (Fig. 4a–f).

There was no evidence of malignant transformation in any of the cell types in the calluses in simvastatin-treated groups over the study period.

Bone histomorphometry

Histomorphometric measurement was performed on undecalcified sections. In coincident with HE-stained sections, the toluidine blue-stained section also indicated a more impaired histological manifestation in OVX + vehicle sections than in OVX + SVS sections (Fig. 4g, j). An unstained undecalcified section of the fracture callus showed a mixture of single and double labels of tetracy-

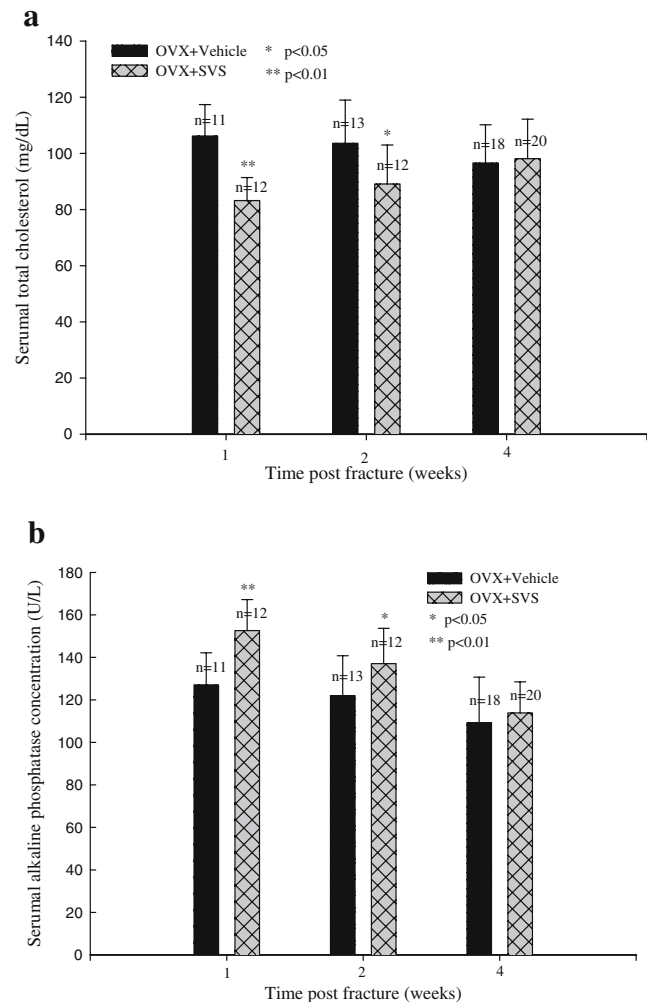


Fig. 3 Biochemical analysis of serum total cholesterol concentrations (**a**) and serum alkaline phosphatase concentrations (**b**) at 1, 2 and 4 weeks after treatment

cline. The single label line was mainly allocated in a newly formed woven region and double labels in relative mature trabecula. The intensity of tetracycline label indicated an increased osteogenic activity in simvastatin-treated animals (Fig. 4h, i, k, l).

Histomorphometric parameters revealed that simvastatin was associated with a statistically significant increase of MLW, MLV and MAR compared to results in the OVX + vehicle group. A larger increase in O.Wi, OV, OS and AOS was found in the OVX + SVS group compared to the OVX + vehicle group, although no statistical difference was detected (Table 1).

Discussion

The present study demonstrates the promotion of the healing process following subcutaneous injection of simvastatin to tissue in close proximity of the fracture. This is

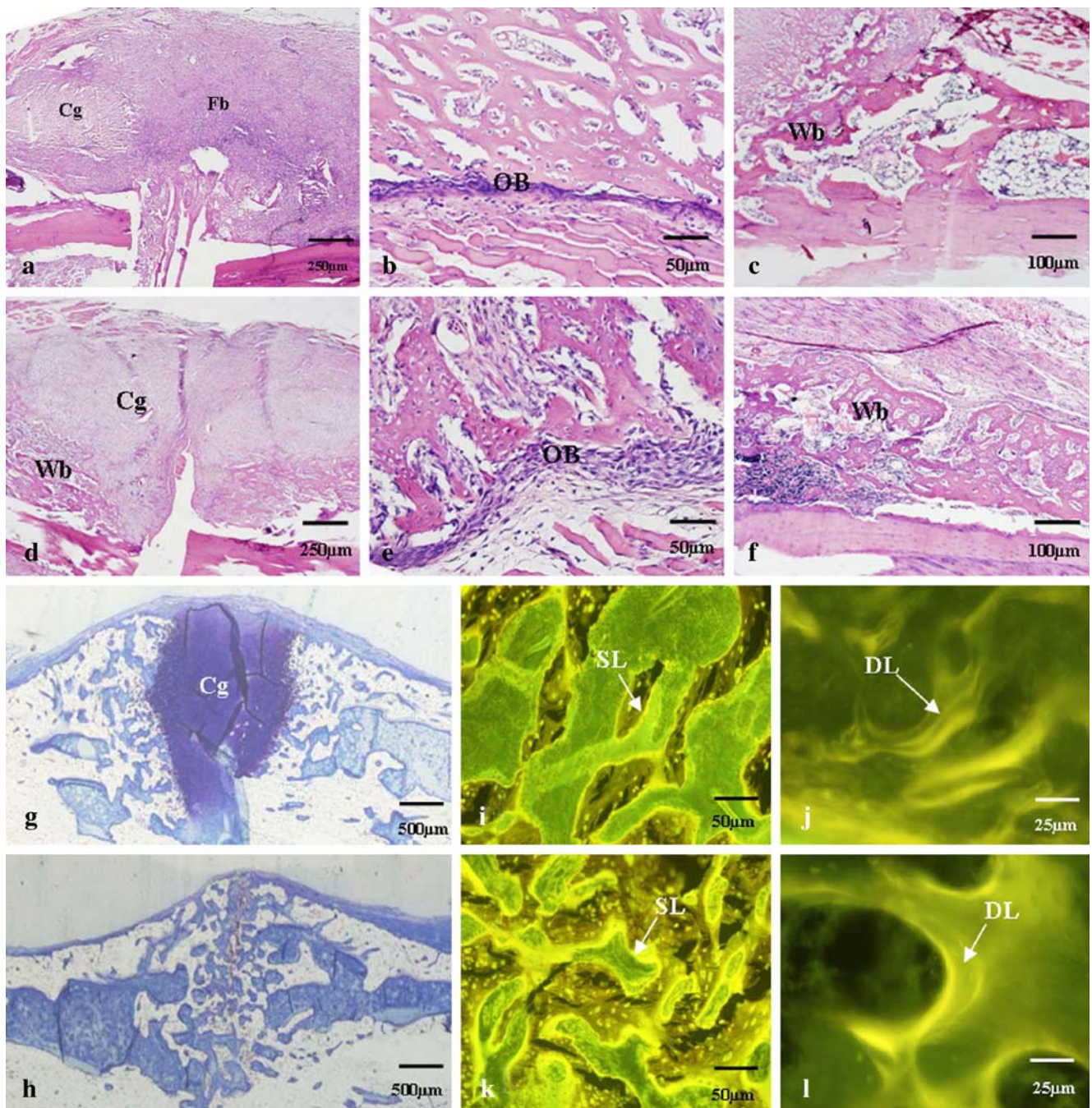


Fig. 4 Histological presentations of OVX + vehicle (a–c, g, i, j) and OVX + SVS (d–f, h, k, l) callus zones at 1 (a, d), 2 (b, e) and 4 (c, f, g–l) weeks after fracture. **a–f**: decalcified sections stained with haematoxylin and eosin demonstrates that callus of the OVX + SVS group presents more cartilage at 1 week; osteoblast cells in OVX + SVS sections are more distinct; and woven bones are relatively substantive and arranged more tightly and regularly in 4 weeks. **g, h**: toluidine-blue-

stained sections of undecalcified tibiae at 4 weeks after fracture. **i–l**: single (**i, k**) and double (**j, l**) fluorochrome labeled micrographs of undecalcified tibiae at 4 weeks after fracture. Original magnifications: **g, h**: $\times 20$; **a, d**: $\times 40$; **c, f, i, k**: $\times 100$; **b, e, j, l**: $\times 200$. Fb, fibrous tissue; Cg, cartilage callus; Wb, woven bone; OB, osteoblast; SL, single label; DL, double label

the first evidence indicating that locally applied simvastatin could promote the healing process in a fracture model.

After the discovery of Mundy et al., many studies have been conducted to evaluate the effects of statins on bone. Although in vitro and animal studies have shown positive

effects of statins on bone mineralization [5, 26], clinical data on surrogate markers and fracture rates are conflicting. Most observational studies showed positive results on bone mass [6–8] and fracture risk [9–12], while some controversial data existed [27–31]. Reasons leading to those differ-

Table 1 Effect of simvastatin on callus histomorphometric parameters

	OVX + vehicle	OVX + SVS
MLW (mineralization width, μm)	187.16 \pm 17.79	215.11 \pm 15.36**
MLV (mineralization volume, %)	31.22 \pm 3.19	37.02 \pm 5.06*
O.Wi (osteoid width, μm)	16.59 \pm 1.91	17.56 \pm 2.10
OV (osteoid volume, %)	4.44 \pm 0.45	4.81 \pm 0.68
OS (osteoid surface, %)	56.29 \pm 6.26	61.34 \pm 6.53
AOS (active osteoblastic surface, %)	51.60 \pm 4.87	53.21 \pm 5.39
MAR (mineralization rate, $\mu\text{m}/\text{d}$)	1.85 \pm 0.15	2.05 \pm 0.10**

* $p < 0.05$ vs. OVX + vehicle; ** $p < 0.01$ vs. OVX + vehicle

ences are complex. Residual confounding by matching variables, different definitions of the exposure time window, the lack of association in randomized trials, and the heterogeneity among observational studies may explain differences in those results. Besides, data suggested that statins affect bone, to a certain extent, in a dose-dependent manner. Animal testing by Frans et al. indicated that high-dose simvastatin (20 mg/kg/day) increases bone formation and resorption, while low-dose simvastatin (1 mg/kg/day) decreases bone formation and increases bone resorption [32]. For postmenopausal women, Tikiz et al. [30] found no change in BMD after a 1-year follow-up under 20 mg/day simvastatin medication, while Montagnani et al. [7] reported an increase of 2.8%, 1.0%, and 0.8% in lumbar, femoral neck and total femur BMD after one year under 40 mg/day simvastatin medication. And also under 40 mg/day simvastatin intervention, Rejnmark et al. found no effect on biochemical bone markers and BMD at the hip or spine [31]. The mixed results may be due to two factors. First, most of the orally administered simvastatin is stored in the liver, and only 5% of simvastatin will be distributed in the circulation [23]; even less will be allocated in bone. Such a low concentration is easily disturbed, resulting in a variance of clinical manifestation. Second, in normally remodeling skeleton, the skeletal effects of compounds probably would be confined to the relatively rare and small remodeling sites. While in healing bone, the entire region is involved in bone formation, so the presentation of skeletal response to treatments will be magnified. As a matter of fact, most previous studies were conducted on undisturbed bones and statins were orally administered; little literature was available regarding these two factors.

In this study, simvastatin was applied subcutaneously to tissue in close proximity of the fracture. This would yield high local concentration (much higher concentration than what could be obtained by oral administration) via simple

diffusion. The effect of locally applied drugs depends on an adequate delivery method. In this study, simvastatin was injected via a micro-injector. This technique requires reproducible placement of small volumes of factors or compounds adjacent to the bone. It is minimally invasive, and care should be taken not to disturb or damage the healing callus.

The history of statins as a therapeutic drug for hypercholesterolemia is long, and the toxicity of those drugs has been well examined. In this study, the dose of simvastatin was based on earlier safety and efficacy studies in rats [33] and with reference to previous studies conducted by Mundy in which simvastatin was also injected subcutaneously over the calvaria of mice [1]. Regarding pharmacokinetics, we considered a 10 mg/kg/day dose to rats about equivalent to 70 mg/day for humans, taking into account that metabolic processes in rodents are 10 times faster than in humans [34]. So, the current amount of simvastatin was higher than the routine dose in clinical applications (20–40 mg/day), while much lower than that administered orally in previous animal testing on fractures (120 mg/kg/day) [21]. In accordance with Mundy's study [1], no serious side effects were seen in the present study.

Fracture healing is a complex cascade of cellular events. The majority of fractures are healed by secondary fracture healing which involves a combination of osteoblast differentiation and ossification. In vitro studies have shown that simvastatin could promote osteoblast differentiation and mineralization in MC3T3-E1 cells and bone marrow stromal cells [35, 36]. Other studies have described a potential role of statins on chondrocytes and periosteum. For example, an in vitro study showed that mevastatin increased mRNA expression of BMP-2, aggrecan, type II collagen, and proteoglycan synthesized by cultured chondrocytes [37], and animal researches demonstrated a role of statins in cortical bone formation in ovariectomized rats [26, 38]. Those studies suggested that statins might play an important role in the variety mechanism of bone healing, including endochondral and intramembranous ossification. In current study, the increased area of calluses, together with the increased bone formation related histomorphometric parameters, proved that simvastatin had anabolic effect on bone formation. And the presentation of substantive new woven bone indicated an increased histological maturity of callus related to simvastatin treatment. Enhanced consolidation of the fracture healing was also found on X-ray radiography in this study.

In two previous experiments, the effects of statins on healing bone defects have been examined. The femur fracture model was made and stabilized with marrow-nailing in mature male BALB/c mice with normal bone quality, and simvastatin was administered systemically in an extremely high dose (120 mg/kg/day) [21]. After 14 days

of treatment, results showed that simvastatin was associated with a 53% increase of the callus area and a 63% increase of break force. Another histological study showed that orally administrated high-dose cerivastatin (1.0 mg/kg/day) induced the reconstruction of vascularized bone allograft in a rat tibia-fibula graft model [22]. In the present experiment, simvastatin was injected subcutaneously to the fracture site in a relatively lower dose, and increased callus area and enhanced fracture strength were found at 2 weeks post fracture. The value of fracture strength lies in the size and material properties of diaphysis. Interestingly, we also found increased fracture strength in the simvastatin-treated fractures at 4 weeks post fracture when the difference in callus size disappeared, thus pointing to an improved material property of fracture.

It is generally considered that the skeletal anabolic effect of statins is mainly to increase the gene expression of bone morphogenetic protein-2, which is an autocrine-paracrine factor for osteoblast differentiation. These agents stimulated BMP-2 transcription and also increased endogenous BMP-2 mRNA and protein expression in human osteoblastic cells [39, 40]. The exact mechanisms of statins on accelerating bone metabolism remain unclear. The reduction in mevalonate pathway intermediates with a subsequent inhibition of prenylation is responsible for a large proportion of the anabolic effects of these drugs [41]. Although these findings suggest direct effects on cellular function and acceleration of a normal physiological process, there was no evidence of malignant transformation in any of the calluses stimulated by simvastatin. The effect of statins on osteoclast is considered relatively unimportant compared to their effects on osteoblast. Their antiresorptive effect is thought to result from the enhanced production of OPG by osteoblast [42].

In conclusion, the present study clearly showed that simvastatin could promote fracture healing in OVX rats when injected subcutaneously into tissue in close proximity of the fracture. Limitations existed in our study. First, the doses used were higher than the normal dose in a clinical application. It is hard to predict the results in daily clinical practice, where much lower doses are used. Nevertheless, the current study showed an approach: it might be possible to use statins to improve healing of osteoporotic fractures. Second, simvastatin was delivered by local injection, an invasive approach which has the possibility of increasing the risk of infection. If it is administered via an adequate carrier (e.g., combined with fibrin glue, coated on an implant or mixed with poly D, L-lactide, etc.), we believe that local application of simvastatin could be a new option to improve the quality of fractures healing. Further studies need to be carried out to find an appropriate carrier and non-invasive delivery systems. Moreover, the potential side effects, i.e., carcinogenesis linked to rapid growth of bone cells, remain to be tested.

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