Histological study on bone structures and vessel arrangements of rats' patellae by tail suspension.

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Summary

In this study, we used patella of rats subjected to tail suspension, and observed proximal and distal of it as the effect of reduced traction and middle of it as the effect of decrease in compressive force.Moreover, we aimed to examine the structure change in relation to the arrangement of blood vessels.

Seven weeks old rats (wistar strain, male, n=56) were randomly assigned to the tail suspension group (TS) and the control group (CO). TS were subjected to a 14-day tail suspension. After the experiment period, the patellae were removed from each group. At the time, they were fixed in fixing solution and various specimens were made from them. And then, those were examined histologically.

In the cancellous bone at CO, trabecular bones were density, basically formed a network structure. Furthermore, there were thick trabeculae arranged in parallel to the long axis of the patella at the surface layer of it. At the deep layer of it, trabecular bones arranged radially from articular cartilage were observed. In TS, the trabecular bones of any part were thinner than CO and the bone mineral density was low. And, there were a lot of TRAP positive reaction at the region of embedded quadriceps tendon and patella ligament in TS. At the region where blood vessels and trabecular bone are close to each other, osteoclasts were observed between them.

In the patella, which is a non-weighted bone, bone mass were decreased due to weight reduction, suggesting that this bone structure change may be closely related to blood vessel orientation.

Keywords: patella, blood vessel, tail suspension

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1. Introduction

A patella is sesamoid, prevents an abrasion of a tendon, transmits suitably force of femoral quadriceps tendon to a tibial tuberosity, and permits smooth exercise of a knee joint.[1]

A bone density of a long bone that receives influences of weighting and mechanical load decreases by non-active life.[2] It has been shown that, in regards to the patella that is non-weighting bone, same changes are recognized. [3] Moreover, it has been reported that the blood vessels related closely to a bone formation and a process of healing bone after a bone fracture.[4] However, Effects of non-active life on a distribution and an orientation of the blood vessels haven't been also cleared.

Then, a purpose of this study was to investigate effects of a tail suspension on bone structures and on orientation of the blood vessels, using rats as materials.

2. Materials and methods

2.1 Materials

Thirty-six male rats (seven weeks old, wistar strain) were used as materials, and were divided into tail-suspended group(TS) and control(CO).

2.2 Methods

Tail suspension

Rats were anesthetized previously by pentobarbital sodium (40mg/kg, weight), so as not to give excessive sense of unease and pain. A tail of TS was suspended from a wire netting of a high-roof cage for fourteen days. Furthermore, a feed-box and a water bottle were laid on the floor to get foods and water easily.

Preparations of sample and fixation

After experimental period, rats of each group were euthanized by the absorption of CO2 to avoid excessive pain. Skin of the hindlimbs and soft tissues surrounding the patellas were removed and they were excised with tendons of femoral quadriceps muscle and patella ligaments. Those patellas were divided in the longitudinal direction of femur by a hand motor and were rapidly immersed in paraformaldehyde solution(4%, 4℃) for fixation.

Preparations of sample for macroscopic observation

Fixed samples were immersed in sodium hypochlorite solution to remove organic compornents.

Preparations of undecalcified resin-embedded ground sections

Undecalcified resin-embedded ground-sections were dehydrated by 70, 95 and 100% alcohol solutions (thirty minutes in each stage) and were cleared by aceton (thirty minutes, two times) after that. Then, those samples were immersed in mixtures (1:1, 1:3 and 1:7) of aceton and resin for 3, 5 and 12 hours, respectively. Furthermore, they were polymerized at 37, 45, 55 and 60℃ (one day in each stage) after immersing in pure fluid of the resin. Polymerized samples were trimmed by a band-saw, were put on the slide glasses and were ground carefully until thickness of 100-150 μm by stones and films for grounding. Surfaces of the ground sections were etched by 0.1N HCl and were stained by toluidineblue staining method.

Preparations of decalcified paraffin-wax embedded sections

Specimens fixed by 4% paraformaldehyde solution were immersed for decalcification in 8% EDTA solution (4℃), for six weeks. They were embedded in paraffin wax and were sectioned in 4 μm-thickness. And then, tartrate-resistant-acid-phosphatase were detected histochemically on those sections.

Bone morphometry

Patellas embedded in resin were cut in longitudinal direction, were stained by toluidine blue dye and were observed by light microscope. A bone density (BV/TV) and a thickness of bone trabecular (Tb. Th) of patella was measured, and difference of their averages were analyzed statistically by WinROOF V7.4. The reject rate was regarded as 5%.

3. Results

In CO, dense bone trabeculas existed in the cancellous bone of the patella and showed reticular structures, when observing that bone cut in longitudinal direction after treatment by hypochlorite solution. An aponeurosis of quadriceps muscle was observed at the surface side of patella and an articular cartilage was recognized at the femoral side of that bone. There were many thick bone trabeculas arranged in parallel direction of the long axis of patella at the surface side of the bone. On the other hand, there were bone trabeculas arranged radially from the articular cartilage.

Oppositely, in TS, the bone trabeculas decreased and those arranged in direction of surface-femoral sides of the bone showed remarkable decrease. (Fig.1)

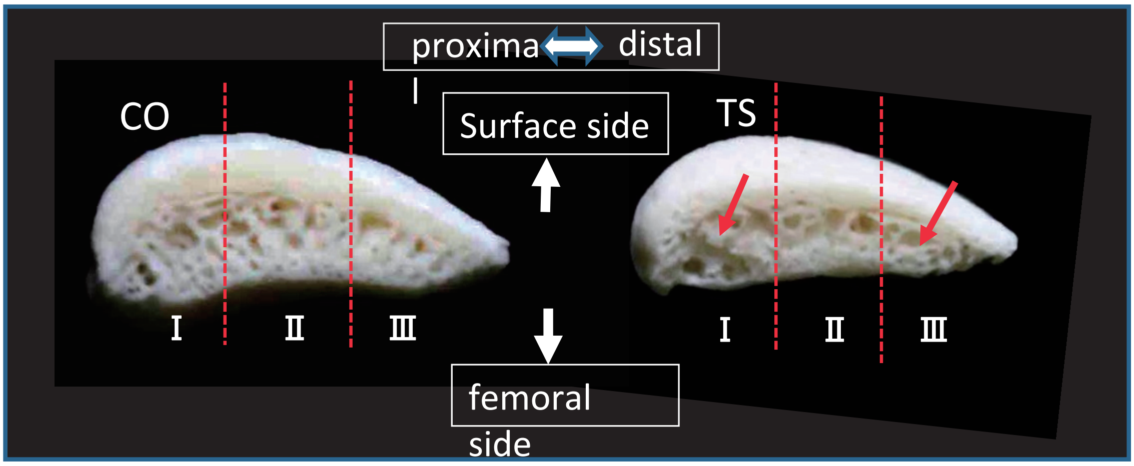

Fig1. Macro image of longitudinal section of patella
(Sodium-hypochlorite-treated specimens)
left: CO, right: TS, Ⅰ:proximal, Ⅱ:middle, Ⅲ:distal,
red arrows :bone trabeculas arranging in proximal and distal directions

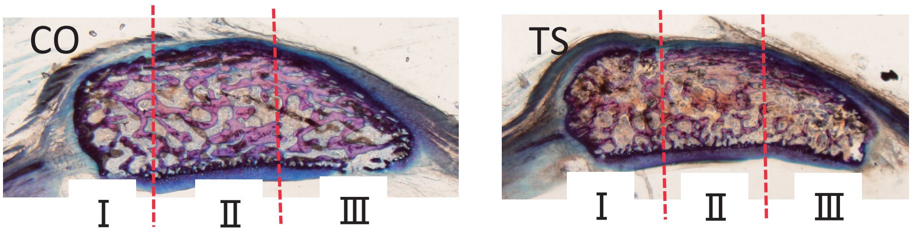

Fig2. Low magnified images of longitudinal sections of patellas in both groups
(Undecalcificated resin-embedded ground sections, toluidine blue stain)
Ⅰ:proximal, Ⅱ:middle, Ⅲ:distal

Fiber bundles of quadriceps tendon and patella ligament changed to a fibrocartilage at the both proximal and distal portions of patella and then, were embedded in the cortical bone. (Fig2) Significant ($p<0.05$) low values were found in both density and thickness of bone trabeculas of TS, compared to CO. (Fig3)

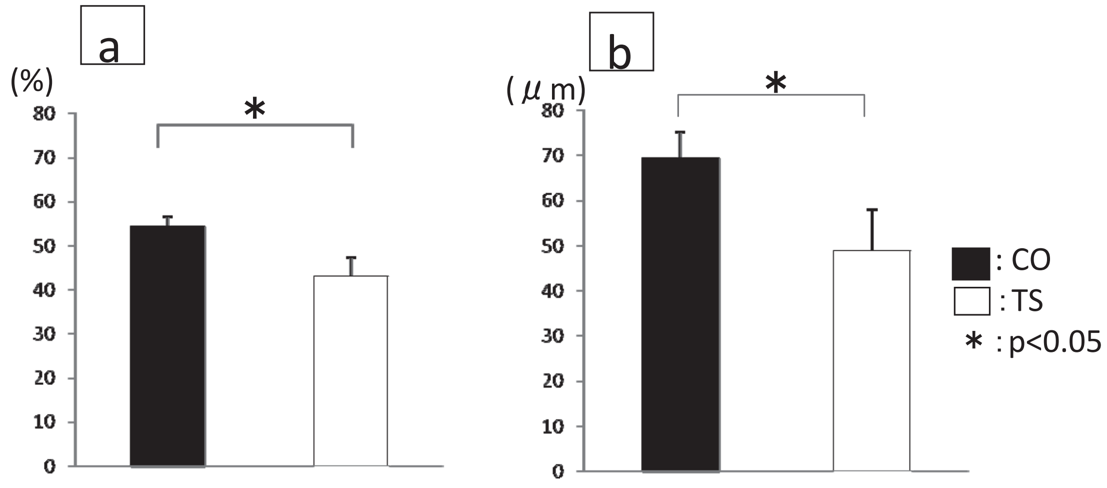

Fig3. Bone morphometry data in each group
a : bone volume(BV/TV) , b : thickness of bone trabecula (TB.TH.)

Osteoclasts were recognized at the surface of the bone trabeculas in whole of patella of CO, as observing decalcified-paraffin sections stained by TRAP staining method.

More osteoclasts appeared in TS, compared to CO, at the portion that the fiber bundles of the fibrocartilage were embedded in the bone, and at the middle portion of the articular cartilage particularly. (Fig.4)

Furthermore, vessels ran along the bone trabeculas, and at the portion that the vessels contacted to the surface of the bone particularly, the osteoclasts often appeared in the narrow space between the vessels and the bone trabeculas. (Fig5)

Discussion

In this study, an effect of mechanical loading on the structure of a patella that was non-weighting bone and the arrangements of vessels was investigated. Thick bone trabeculae were arranged in parallel to longitudinal axis of the patella in the superficial area of that bone. The bone trabeculae were arranged radially from an articular cartilage in the deep area of that bone. Those structural characteristics were observed in human patella, too[5].

It was showed that an arranging direction of the bone trabeculae was agreed with the direction of a line of strength given to a bone[6]. Then, it was thought that results of this study indicated that the bone trabeculae of the superficial area showed high resistance to tractive forces from both a femoral quadriceps muscle and a patellar ligament and those of the deep area showed the resistance to pressure from the femur.

In this study, it was recognized that a fibrous cartilage existed at the portions that a

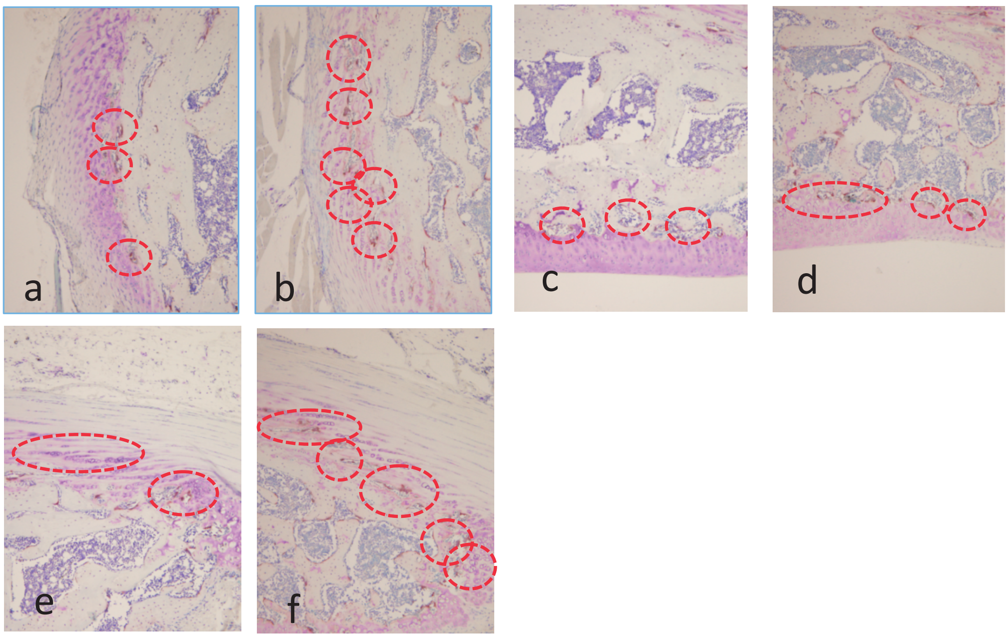

Fig4. Localizations of osteoclasts on each part of patella
（decalcified paraffin sections stained by TRAP）
a,b: proximal, c,d: middle, e,f: distal, a,e,d : CO, b,d,f : TS
red circles: osteoclats

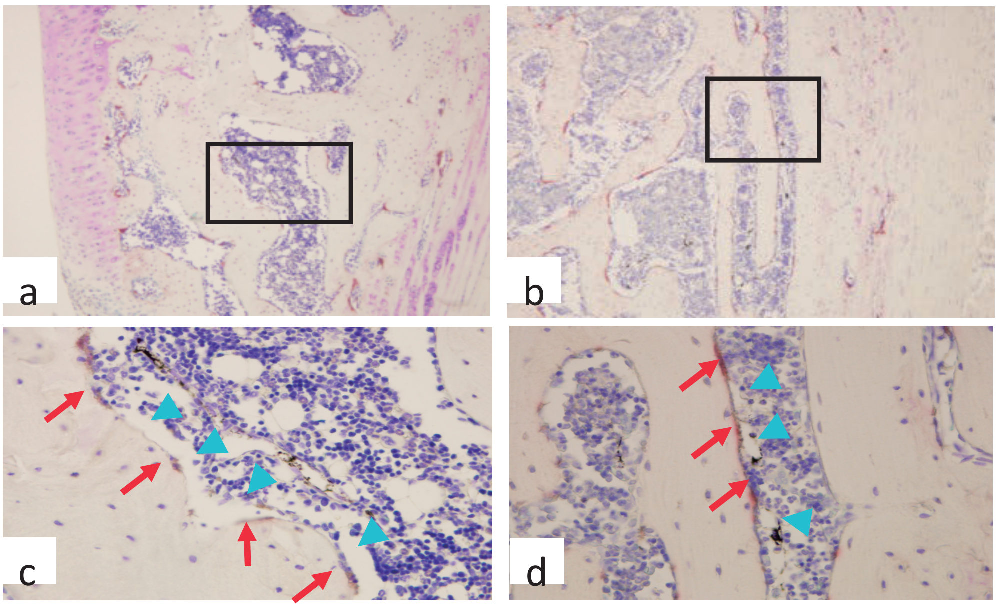

Fig5. Relationships of localization between in osteoclasts and blood vessels in bone marrow.
a,b: low-magnified images of middle portions in both groups
c,d: magnified images of squares of Fig. a and b, a,c : CO, b,d : TS,
red arrows : osteoclasts, blue arrowheads : blood vessels

tendon and the ligament were embedded in the patella. This result was agreed with report of Wanqwinyuvirat et al.[7] that, using human patella, observed the portions that a tendon of the femoral quadriceps muscle and the patellar ligament were embedded. The portion that the tendon and the ligament attached was divided into a non-calcified tendon, a fibrous cartilage, a calcified fibrous cartilage and a bone[8].

Benjyaminet et al.[9] investigated about humerus that the tendon of a supraspinous muscle attached. As a results, it was reported that a contraction at the interface between the tendon and the bone was possible by a existence of the non-calcified fibrous cartilage. It was supposed that the patella was pulled by both the tendon of the femoral quadriceps muscle and the patella ligament and the fibrous cartilage that

the tendon and the ligament attached played a role of resisting to the pulling force by absorbing the force.

The bone structures are changed by the force added to the bone according to the law of wolff[6]. It was reported, from previous studies using tail suspended models[10, 11] that assumed microgravity environment of the space and immobilized models[12, 13] that assumed a rest state in a certain period, that a bone density (BV/TV) and a thickness of the trabecular bone (Tb.TH) decreased in the case of the long bone like the tibia and the femur. Schwarth et al.[14] reported that the collagen fibers of a tendon of supraspinatus muscle arranged irregularly by decrease in activity with injection of a botulin toxin A. In this study, the bone trabeculae of TS were thinner and decreased at not only the superficial area but also the deep area of the patella, compared to those of CO. It was thought, as described above, that the bone trabeculae of the superficial area were arranged in direction pulled by the tendon of femoral quadriceps and the patellar ligament and those of deep area were arranged in direction for resistance to pressure from femur.

It was speculated that the decrease in the bone density and in thickness of the bone trabeculae at the superficial area derived from a decline in the pulling force of both the femoral quadriceps and the patellar ligament by the tail suspension. On the other hand, it was thought, as for the bone trabeculae at the deep area, that the decrease in pressure to femur caused decline in the bone density and in the thickness of the bone trabeculae.

A bone modeling always performed for maintaining a bone structures and functions. Moreover, a portion that the ligament attached, that is enthesis, was always remodeled, too [15]. It had been already reported that many osteoclasts appeared at the cancellous bone of the tibia in the inactivity condition by the tail suspension[16].

In this study, the osteoclasts were recognized on the surface of the bone trabeculae in everywhere of proximal, medial and distal portions, in CO. To the contrary, in TS, more osteoclasts appeared than CO everywhere. Moreover, those cells were observed remarkably at proximal and distal portions that the fibrous cartilage was embedded in the patella and at medial portion just under the articular cartilage, in TS.

A previous study[17] had been showing that a growing of the tendon was investigated using immobilized neonatal mouse, as a results, many osteoclasts appeared at tendon-attaching portion and a bone density decreased in experimental group. Nomura et al.[18] reported that the osteoclasts increased at the calcified layer and the junction between the calcified layer and subchondral bone of the tibia and the femur in rats that their hindlims were immobilized. Same results were also obtained in this study. The osteoclasts appeared at the portion except for the tendon-attaching portion in CO, and more those cells were observed in TS. It was thought that the increase in those cells resulted from the decline in the pulling force of the femoral quadriceps and the patellar ligament and from the decrease in pressure to the femur. Moreover, it seemed that the facts that many osteoclasts existed especially at the tendon-attaching portion of the patella and the middle portion of the articular cartilage meant that large pulling force and pressure ordinarily brought to those portions. It was thought that the structures at every portions were maintained by those mechanical forces but changed by loss of balance between a bone formation and resorption.

It was shown that the bone formation was related closely to orientations of blood vessels in the endochondral ossification[19,20]. Furthermore, it was also reported that the blood vessels ran along the bone surface of the cancellous bone that was remodeling and the osteoclasts existed at the portion between the blood vessels and the bone[21].

A vascular endothelium growth factor(VEGF) was secreted from both osetoblasts and osteoclasts, promoted angiogenesis[22], and enhanced a bone resorption ability of the osteoclasts[23]. In this study, it was observed that the blood vessels ran along the surface of the bone trabecula at the middle portion of the patella. Especially, the osteoclasts existed in the narrow space between the blood vessels and the bone in the case that they approached considerably. It was supposed that VEGF was secreted by the osteoblasts and the osteoclasts at the portion remodeled and that the blood vessel was renewed. From the report[21] that the angiogenesis was performed at the active or resorbing phase of remodeling period, it could be speculated that the osteoclasts increased at those phases.

Conclusion

It was suggested that the patella was non-weighting bone but their bone structures became fragile by decrease in mechanical loading and those changes related closely to orientation of the blood vessels.

Committee of Animal Experiment and Ethics

This study was approved by committee of Animal Experiment and Ethics for the research, Graduate School of Welfare Society design, TOYO University.

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尾部懸垂に伴うラット膝蓋骨の構造と血管配向の変化に関する組織学的研究

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要　旨

これまでに非活動的な生活を想定し、ラットに尾部懸垂を施すことによって、膝蓋骨の骨量減少を認めている。しかしながら、尾部懸垂による膝蓋骨の血管分布おび配向性に及ぼす影響については報告がない。本研究はラット膝蓋骨を用いて、尾部懸垂による骨梁構造や、血管の分布および配向性にいかなる影響をもたらすかについて検討することを目的とした。

材料として7週齢のウィスター系雄性ラット56匹を用い、2群(尾部懸垂群(TS)および対照群(CO)）に分類した。TSは14日間尾部懸垂を行い、実験期間終了後に両群の膝蓋骨を摘出し、すみやかに浸漬固定を行った。種々の標本を作製し、光学および電子顕微鏡による組織学的観察および骨形態計測を行った。

COでは、膝蓋骨内の海綿骨の中でも表層部に位置する骨梁は太く、膝蓋骨の長軸に対してほぼ平行に配列した。深部のものは関節軟骨に向かうにしたがって徐々に細くなった。TSの膝蓋骨の骨梁はCOより菲薄化し、密度も低下した。両群の血管分布には差異を認めなかったが、血管配向性の面では、TSはCOに比べて蛇行するものが多かった。

非加重骨である膝蓋骨は、加重低減によって骨構造が脆弱化し、その変化には血管の配向性が密接に関連していることが示唆された。

キーワード:膝蓋骨、血管、尾部懸垂