Comparison of effects of electrical stimulations in various conditions on femoral bone structures in rats.

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Abstract

A purpose of this study was to compare and investigate effects of electrical and no-electrical acupuncture simulations and transdermal electrical stimulation on bone loss using hindlimb-immobilized rats. Forty-five male rats (wistar strain, seven-week-old) were used as materials and were divided into five groups randomly: immobilized group (IM), immobilized and acupunctured group (IMA), immobilized and transdermal electrical-stimulated group (IMTE), immobilized and electrical acupuncture stimulated group (IMEA) and control group (CO). Hindlimbs of rats were immobilized for two weeks in group except for CO. Stainless needles were inserted until femoral periosteum in IMA and IMEA simultaneously with immobilization, and electrodes were pasted to the skin surface in IMTE. Conditions of those electrical stimulations were the continuous alternating current (250 μ sec, 50Hz, 0.02mArms), 10min/day, every day for two weeks. Femurs were excised from each group after an experimental period and these specimens were analyzed histologically. Many bone resorption images and large osteocytic lacunae were observed at the periosteum side of a cortical bone, in IM. Those images were hardly recognized in IMA, IMEA and IMTE. It was understood that the acupuncture stimulation to the periosteum and the transdermal electrical stimulation had inhibiting effects of decrease in the bone density, caused by immobilization.

Keywords: bone structure, hindlimb immobilization, electrical acupuncture stimulation

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1. Introduction

A bone strength declines accompanied with an aging or a long period bedrest, and the bone fractures easily by low-intense external force[1]. This is caused by decrease in a bone formation and increase in a bone resorption. To the contrary, it was shown that a recovery of a bone intensity is promoted by an electrical acupuncture stimulation[2].Author et al.[3] already recognized, differed from the report of Lam&Qin[4], that electrical acupuncture stimulation during hindlimb immobilization inhibited the bone resorption of a femur. Effect of electrical stimulation using surface electrode on the bone structure wasn't cleared. As focusing the difference on the methodology of an electrical stimulation between the acupuncture or the transdermal, this study aimed to compare and investigate effects of those electrical stimulations on the cortical bone.Furthermore, effect of inserting acupuncture was also investigated as non-electrical stimulation.

2. Materials and methods

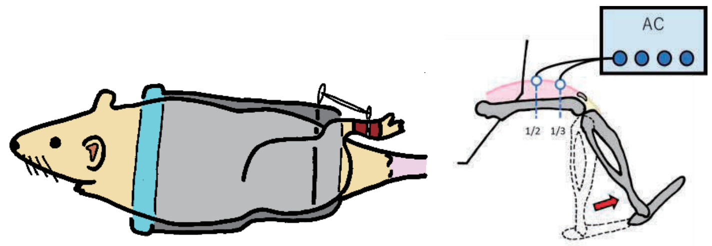
2.1 Materials

Forty-five male rats (wistar strain, seven-week-old) were used as material and were divided into five groups randomly: immobilized group (IM), immobilized and acupunctured group (IMA), immobilized and transdermal electrical-stimulated group (IMTE), immobilized and electrical acupuncture stimulated group (IMEA) and control group (CO). Hindlimbs of rats were immobilized in IM and stainless needles were inserted in their thigh during the immobilization in IMA. Rats of IMTE were electrical stimulated trans-dermally and those of IMEA were acupunctured electrically, during the hindlimbs immobilizations. Rats of CO were fed normally in cages.

2.2 Condition of experiment

Hip and knee joints were immobilized at extended position by a jacket-type immobilization equipment to restrict abduction and adduction of the hip joint (Fig.1-A).

An acupunctured part was middle and distal areas of the anterior surface of femur (Fig.1-B). The needle was inserted to a periosteum by ordinary method after shaving and disinfecting that part. An electrode was attached to surface of skin at the same part in IMTE. Acupunctured or trans-dermal electricity was performed 10min/day, in everyday, by an alternating current at the condition of 250 μ sec, 50Hz, 0.24mA. Femurs were excised from each group at the end of the experimental period. A middle potion of the femur of a left leg was observed and measured histologically and morphometrically and bone strength was measured by three-point-bending test.



(A) **(B)**

Fig.1 Condition of experiment

(A) Jacket-type immobilization equipment, **(B)** Point of intervention

Femurs were excised after confirming rat's death by suctioning of CO_2gas. Right legs were rapidly ruptured after a sampling and were used for bone-strength-test. Left legs were used for histological observation and bone morphometrical measurement after the sampling and a fixation. A middle point between great trochanter and lateral condyle was defined as a middle potion of the femur and the point was analyzed.

2.3 Histological preparation and observation of specimen

Femurs excised were immersed in Karnovsky fixation solution buffered by 0.1M sodium cacodirate (pH7.4, 4℃, overnight) and were rinsed after that. They were dehydrated by alcohol without decalcification and cleared by acetone.Rigolac resin was infiltrated to those specimens and they were polymerized at the temperature of 37, 45, 55 and 60℃ for one day, respectively. Those blocks were trimmed by a band-saw and were ground using whetstone until thickness of 150 μm. Ground sections were etched by HCl solution and were stained by toluidinblue solution(70℃).They were observed histologically by a light-microscope and each bone morphometrical data were measured.

2.4 Bone morphometric

Ground sections embedded in rigolac resin were mounted by water. They were photographed by a light-microscope and were measured by image analyzing application[5] (WinROOF v.7.4). Averaged and maximal areas of twenty osteocytic lacunae cut in longitudinal direction were measured at the front surface of the middle potion of the femur.

2.5 Condition of three-point-bending test

The femur was set on the supporting stand of three-point-bending test apparatus and a crosshead was hit at the front surface of the middle potion of the femur. And then, a bone strength in each group was measured according to an ordinal fracture condition[3,6] (Fig.2).

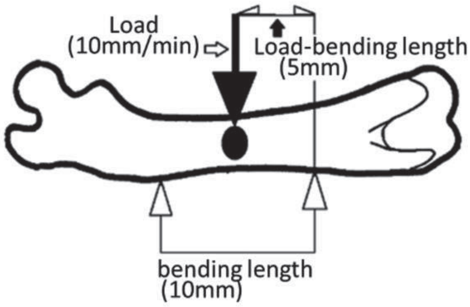


Fig.2 Condition of strength test

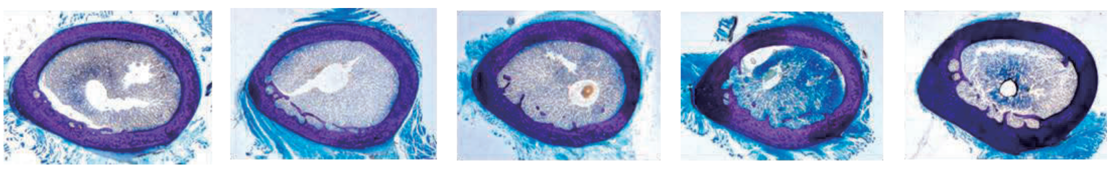
2.6 Statistics

Averages and standard deviations of bone morphometrical data were acquired and were analyzed by SPSS application. Those parameters were analyzed by One-way analysis of variance. Multiple comparisons between each group were performed by Turkey-HSD in the case that significance was found.

3. Observation

3.1 Microscopic observations

When observing cross-sectioned femur of CO, many small blood-vessel-cavities were recognized on the endosteum face of posterior-lateral side at the middle potion of a diaphysis but the cortical bone was compact on the whole. On the other hand, in the groups immobilized, large bone marrow cavities were found on the endosteum face in spite of electrical or non-electrical stimulation and the cortical bone of IM was thinnest. (Fig.3)



(A) (B) (C) (D) (E)

Fig3. Morphology of femur at the middle portions in each group

(A)CO, (B)IM, (C)IMA, (D)IMTE, (E)IMEA

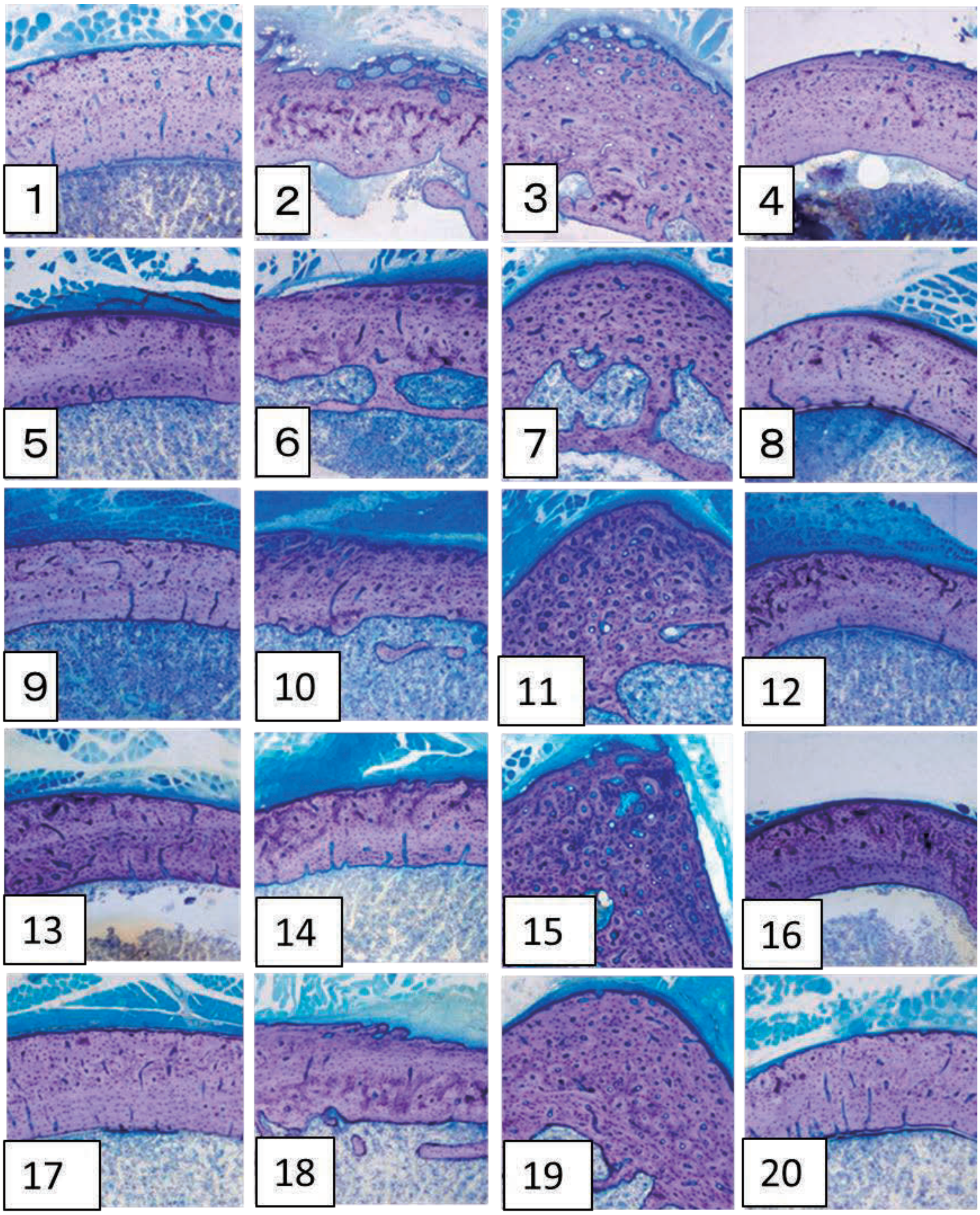


Fig.4 Comparison of structures of the cortical bone magnified in each group

1-4 : CO　5-8 : IM　9-12 : IMA　13-16 : IMTE　17-20 : IMEA

1,5,9,13,17 : Ant. 2,6,10,14,18 : Post. 3,7,11,15,19 : Lat. 4,8,12,16,20 : Med.

Moreover, a calcified cartilage matrix and an immature bone that showed high calcifying degree occupied 2/3 deep area of the cortical bone. They made core of the cortical bone, and there were circumferential lamellar bones on the periosteum and periosteum sides of them. The circumferential lamellar bone of the periosteum side was thin in CO and was same as IMEA but wasn't recognized in IM, IMA and IMTE. The bones making core of the cortical bone bared from the surface of the cortical bone, and many bone resorbing grooves were found at their surface in IM especially. Furthermore, many large osteocytic lacunae and canaliculi were observed on the anterior surface at the middle portion of diaphysis in IM and IMA, compared to CO (Fig.4).

3.2 Bone morphometry

Anterior-posterior and medial-lateral diameters showed significant low values when comparing outer diameters in each group, but differencs between each group weren't significant. Anterior-posterior and medial-lateral diameters of the cortical bone and cross-sectioned areas in IM were lower significantly than CO. The groups treated by the acupancture or transdermal stimulations indicated higher values than IM, but no significant differences were found between groups (Fig.5). As comparing a ratio that the lamellal bones occupied in the cortical bone between groups, raito of CO was close tothat of IMEA but their values was lower than other groups (Fig.6).

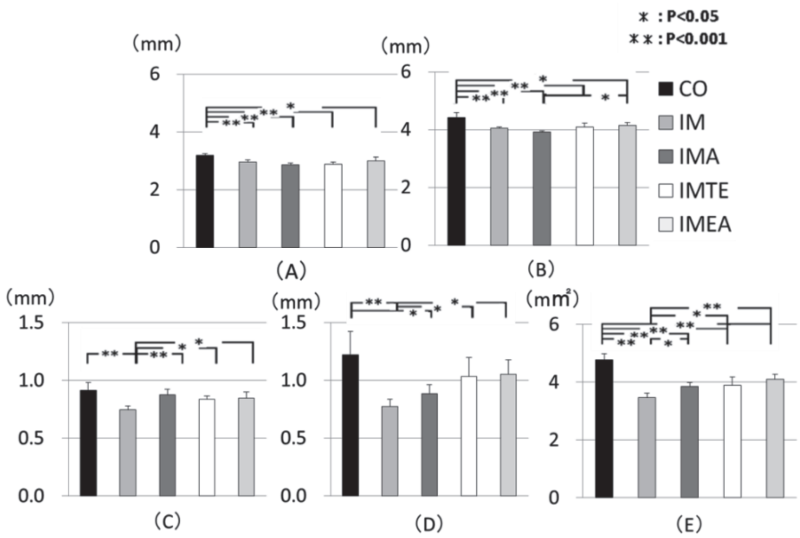


Fig.5 Bone morphometric data in each group

(A) Ant.-Post. diamater, (B) Mid.-Lat. diamater,

(C) Thicness (Ant.+Post.), (D) Thicness (Mid.+Lat.), (E) Area.

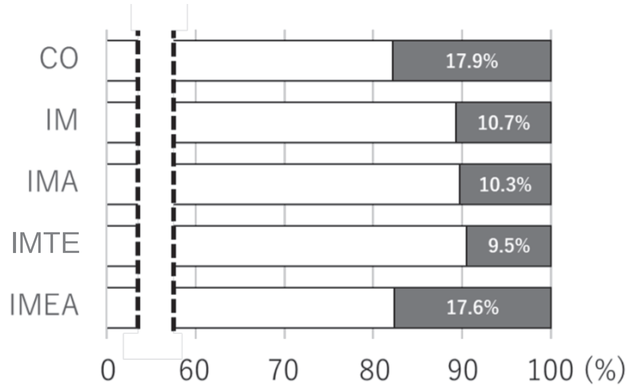


Fig6. Ratio of Layer structure in the cortical bone in each group

The areas of osteocytic lacunae of IM, IMA and IMTE were large significantly (P<0.001), but no differences were recognized between IMEA and CO (Fig.7).

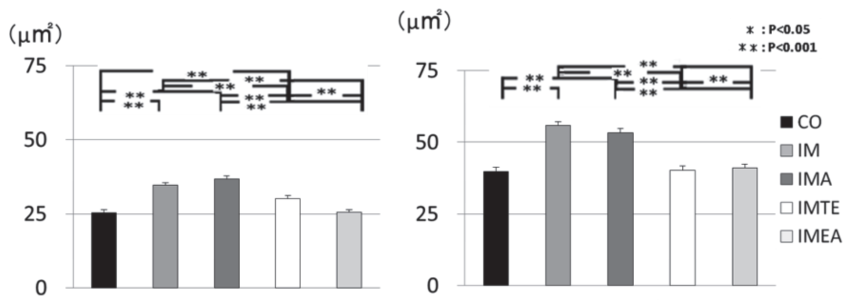


Fig.7 Comparison of osteocytic lacunas in each group

3.3 Bone strength test

Most groups, except for IMTE, showed significant high Stiffness compared to CO, and a difference among IMTE and CO wasn't significant. Significant low Deformations were found in IM and IMTE, but the differences between IMA, IMEA and CO weren't significant. Strength was lower in all groups immobilized than CO. A difference in Strength between IM and IMTE was no significant and Strength in IM was lower significantly than other groups.

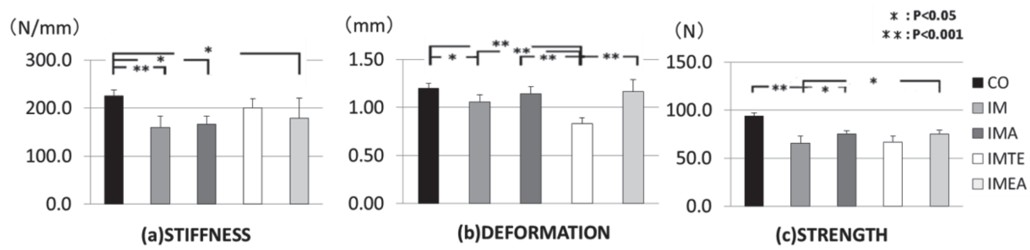


Fig8. Comparison of data of bone strength test in each group

4. Discussion

It was shown, from the study using rats, that a decline of a mechanical load by caused a decrease in the thickness of the cortical bone[8]. It was also shown that a longer period was needed for recovery from that condition[8].In this study, hindlimbs of rats were immobilized for two weeks and an acupuncture inserting and electrical stimulation were performed. A bone was resorbed remarkably, by immobilization, on the posterior-lateral face of a femur, compared to anterior-medial face of that, and bone marrow cavities were formed there[3]. Because a grate trochanter protuberated and the cortical bone grew toward posterior-lateral direction in CO, a bone formation was active at the periosteum side and a bone resorption was promoted at the endosteum side. To the contrary, from the facts that there were large bone marrow cavities at the endosteum side and the cortical bone was thinnest in IM, it was speculated that the bone formation at the periosteum side was inhibited, and at the same time, the bone resorption at the endosteum side was conspicuously restricted. The large bone marrow cavities were formed at the endosteum in the acupuncture inserting and electrical stimulating groups, too. Therefore, it was thought that the bone resorption from the endosteum side by the immobilization wasn't inhibited. The cortical bone in every treated groups were thick, compared to IM. That of IMA was thinnest and that of IMEA was thickest.Like above, it was understood that the inhibition of the bone formation at the periosteum side by the immobilization was found in IMEA obviously and its effect was hardly recognized in IMA.

Author et al.[3] had already studied about structural changes in the bone structure at the middle and distal portions of rat's femur. It was recognized, from this study, that the lamellar bone was formed at the anterior-medial face of middle portion of diaphysis but non-lamellar thick bone was formed at the posterior-lateral face of that. It was described that a deferential degree of the typical lamellar bone was high and a long time was spent on forming that bone, according to the previous study[9] that investigated about bone formation rate and degree of histological differentiation from a density and arrangement of the matrix fibers. When thinking of the result of this study, adapting it to this report, the former bone was high differentiated bone that was formed slowly and the later bone was comparatively formed in short time. A ratio that the lamellar bone occupied in the cortical bone in CO was same as IMEA. It was thought, from those results, that CO and IMEA were same as for the thickness of the cortical bone and the degree of maturation. The cell body and processes of the osteocyte were going to be embedded in osteocytic lacunae and canaliculi, respectively, when the osteoid calcified and the cell body and the processes were surrounded by the bone matrix. It was described that a cell size and an amount of organelles of the osteocyte decreased after the osteoblasts were embedded in the bone matrix, and function of the bone formation also declined[10]. Little differences in the thickness of the cortical bone was found between the experimental groups and CO. The osteocytic lacunae were larger in the experimental groups, compared to CO. No significant differences, as for the sizes of those lacunae, was recognized among IMEA and CO. Same tendency were also recognized as for the osteocytic canaliculi. It was known, in the case of an osteoporosis, that the size of the osteocytic lacunae become large by dissolving of inorganic materials from and it was called osteocytic osteolysis[11,12].Those changes were also observed at the condition of the osteocytic lacunae and canaliculi in this study. It was thought that the osteocytic osteolysis progressed by the immobilization but inhibited in IMTE and IMEA. On the other hand, Strength in IMEA showed middle value between CO and IM. It was thought, from these results, that Stiffness was related to the ratio that the lamellar bone occupied in the cortical bone, and the sizes of the ostecytic lacunae.

5. Conclusion

It was understood that the effect of the immobilization on the structure and the bone strength was inhibited by the electrical acupuncture.

6. 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.

7. Acknowledgements

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ラット大腿骨の骨構造に種々の電気刺激が及ぼす影響の比較

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要　旨

本研究は、後肢不動化ラットを用いて、骨量減少抑制に及ぼす通電および非通電鍼刺激ならびに経皮的な電気刺激の影響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45匹を用い、それらを不動群（IM)、不動・置鍼群（IMA)、不動・鍼通電刺激群（IMEA)、不動・経皮電気刺激群（IMTE）および対照群（CO）に分類した。CO以外の群には後肢膝関節に不動装置を2週間装着した。IMAおよびIMEAには、不動処置と同時に大腿骨の骨膜まで刺入し、IMTEは不動処置と同時に皮膚表面に電極を貼った。いずれも遠位部を陰極、中央部を陽極とした連続的交流鍼通電刺激(刺激条件:幅250μsec、50Hz、0.02mArms)を10分／日、不動期間中毎日行った。いずれの群も実験期間終了後に大腿骨を摘出し組織学的に観察した。IMでは、皮質骨の骨膜側に吸収像が多く認められ、皮質骨内の骨小腔の増大化がみられた。IMA、IMEAおよびIMTEではそのような骨構造の変化が抑制された。骨膜への鍼刺激および経皮的な電気刺激は、不動化によって引き起こされる骨量減少を抑制する効果があることが理解された。

キーワード：骨構造、後肢不動化、鍼電気刺激