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Effect of Estrogen on Decalcified Bone Matrix-induced Endochondral Bone Formation in Rats

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脱灰骨基質誘導性軟骨内化骨に及ぼすエストロジェンの影響

松　井　　徹

Summary

The influence of deficiency and administration of estrogen on the discrete phases of decalcified bone matrix induced endochondral bone formation was investigated. In ovariectomized (Ovx), sham operated (Sham) and sham operated and estrogen administrated (Sham+E) rats, chondrogenesis, cartilage degeneration and bone formation, and bone remodeling were occurred 7, 14 and 21 days after implantation of bone matrix, respectively. Calcium contents and alkaline phosphatase activity were less in Ovx than in the other groups during the experiment. Alkaline phosphatase activity was not different between Sham and Sham+E rats but calcium content was more on day 14 and less on day 21 in Sham+E than in Sham group. Calcium dependent ATPase activity was increased in Sham on day 21 but the activity was decreased in Sham+E animals. It is suggested that cartilage and bone differentiation were occurred notwithstanding estrogen deficiency and administration. Though estrogen deficiency suppresses calcification of bone and cartilage, exogenous estrogen induces rapid calcification of cartilage and suppresses calcium deposition of bone which may be related to calcium dependent ATPase activity.

Introduction

The exogenous hormonally active agents, which mimic estrogen, are common practice to improve the productive efficiencies of beef cattle because estrogen and it's analogue increase feed efficiency and rate of gain.[1-3] On the other hand, it was reported that estrogen analogue hasted skeletal maturity and reduced the length of the femur in bull.[4]

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Histological study shows that long bone growth depends on chondrogenesis and endochondral bone formation in the epiphyseal growth plate and cessation of long bone growth is due to epiphyseal fusion, namely, calcification of cartilage in the epiphyseal growth plate.[5] However there is few chemical research about the epiphyseal growth plate because discrete stages of cartilage and bone in the epiphyseal growth plate are difficult to evaluate. On the other hand, the usage of decalcified bone matrix induced bone formation overcomes these difficulties since chondrogenesis occurs 7 days after the matrix implantation which is followed by bone formation on day 14 and bone remodeling on day 21.[6]

The objective of this study is to determine the changes of decalcified bone matrix induced bone formation by estrogen administration and deficiency in rats.

Materials and Methods

Twenty four Wister rats, weighing about 250g, were used. All rats were fed on a commercial diet (MF, Oriental Yeast, Tokyo) *ad libitum*. Eight rats were ovariectomized (Ovx) and the other rats were sham operated (Sham). Eight of Sham rats were intramuscularly injected with 5 μg estradiol (Sigma Chemical Co., St Louis, Mo. USA.) (Sham+E). Decalcified bone matrix was prepared according to the method of Reddi.[6] All animals were implanted 15 mg of decalcified bone matrix into 6 places of abdominal muscle one week after the operations. Two plaques were harvested from each animal 7, 14 and 21 days after implantation between 9 : 30 and 11 : 30.

Portions of each plaque from every animal at each stage were fixed with buffered formaldehyde and embedded in paraffin. Sections were stained by hematoxylin and eosin or stained calcium by the method of Kossa.[7] The other portions were homogenized in saline by Polytron (PT 30/35) on ice. After centrifugation, protein concentration in supernatant was measured by the method of Lowery et al.[8] and alkaline phosphatase (AP) activity was measured by the method of Bessey et al.[9] AP activity was expressed as μ mol p-nitrophenol production per mg protein for 30 min. For determining calcium dependent ATPase activity, supernatant was incubated with 5 mM ATP (disodium salt), 5 mM $CaCl_2$ and 5 mM levamisole in 75 mM Tris-HCl buffer (pH 8.5) for 30 min. The hydrolysis was stopped by ice cold 10% trichloroacetate. ATPase activity was measured by the release of Pi and expressed as μg Pi release per mg protein for 30 min. Released Pi was analyzed by Takahashi's method.[10] The other plaques were ashed at 600°C and calcium contents were measured by atomic absorption spectrophotometry. Statistical differences were analyzed by Student's t test.

Results

Fig. 1 shows changes of calcium content in plaques. Calcium content was gradually increased in all groups. On day 14, calcium content tended to be greater in Sham+E than in Sham rats. But the accumulation of calcium in Sham animals exceeded that in Sham+E on day 21. On the other hand, calcium content was less in Ovx than in the other groups during the experiment.

AP activity increased by day 14 and then reduced in all groups (Fig. 2). There was no difference of AP activity between Sham and Sham+E groups during the experimental periods. The activity was less in Ovx group compared with the other groups during the experiment.

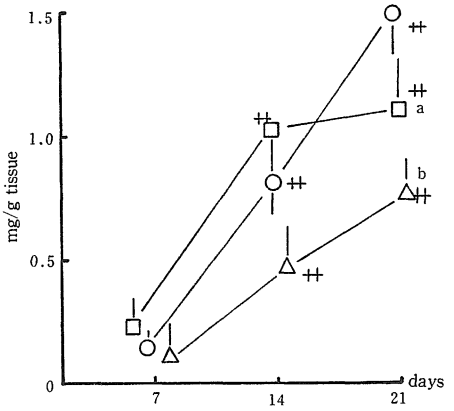


Fig. 1. Changes in calcium content during decalcified bone matrix induced endochondral bone differentiation in sham operated (○), sham operated and estrogen administrated (□) and ovariectomized (△) rats.
The bars represent the S. D. of 8 observation.
a ; $P<0.05$, b ; $P<0.01$, Significantly different from the value of sham operated rats.
++ ; $P<0.01$, Significantly different from the value on day 7.

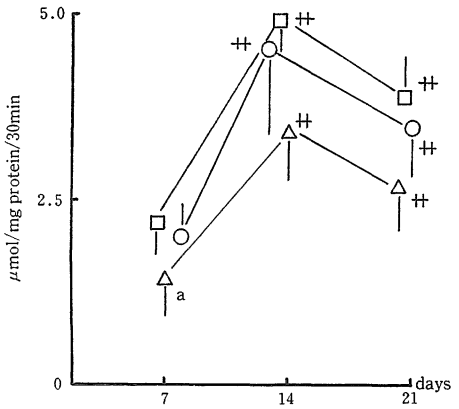


Fig. 2. Changes in alkaline phosphatase activity during decalcified bone matrix induced endochondral bone differentiation in sham operated (○), sham operated and estrogen administrated (□) and ovariectomized (△) rats.
The bars represent the S. D. of 8 observation.
The activities are exressed as μmol p-nitrophenol production per mg protein for 30 min.
a ; $P<0.05$, Significantly different from the value of sham operated rats.
++ ; $P<0.01$, Significantly different from the value on day 7.

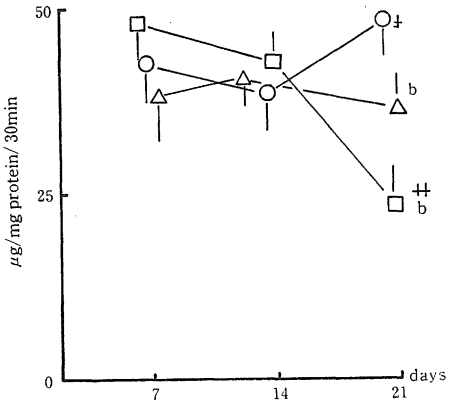
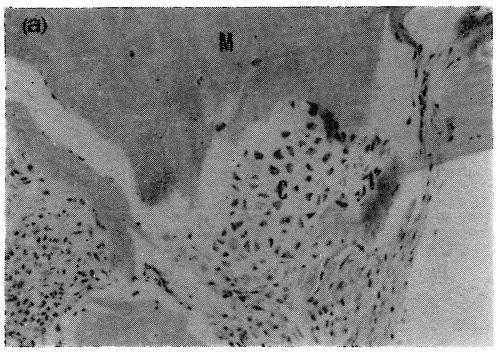
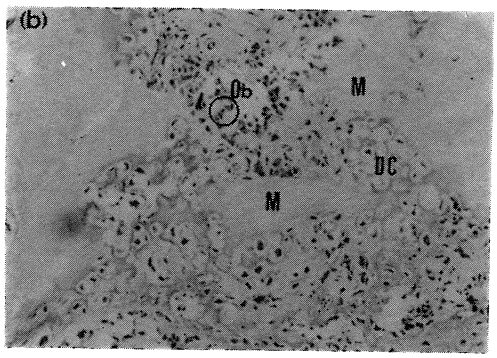


Fig. 3. Changes in calcium dependent ATPase activity during decalcified bone matrix induced endochondral bone differentiation in sham operated (○), sham operated and estrogen administrated (□) and ovariectomized (△) rats.
The bars represent the S. D. of 8 observation.
The activities are exressed as μg Pi release per mg protein for 30 min.
b ; $P<0.01$, Significantly different from the value of sham operated rats.
+ ; $P<0.05$, ++ ; $P<0.01$, Significantly different from the value on day 7.





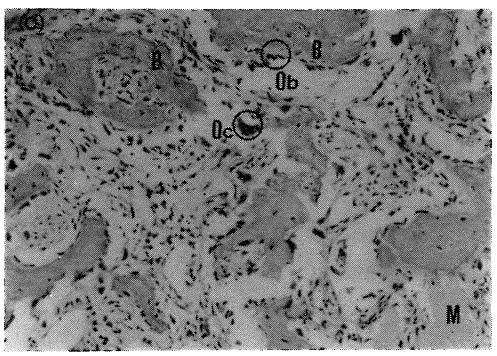
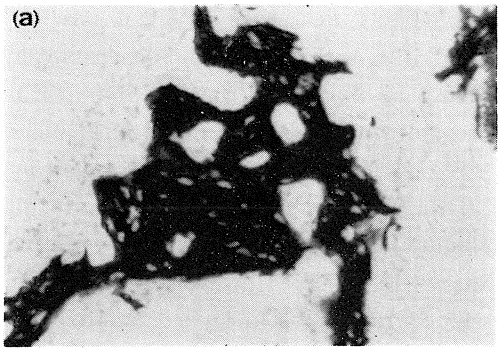
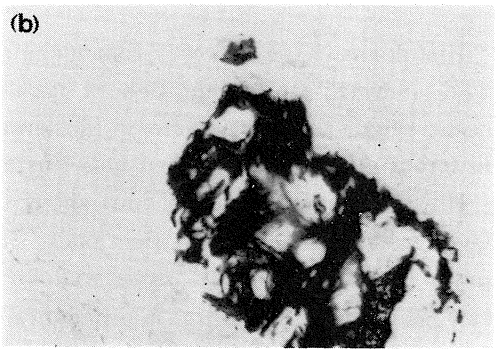


Fig. 4 Histological changes of plaques induced by implantation of decalcified bone matrix in sham operated rats. (X 100)
(a) On day 7, plaque contains cartilage (C).
(b) On day 14, plaque contains degenerated cartilage (DC) and osteoblasts (Ob).
(c) On day 21, plaque contains newly formed bone (B), osteoblasts (Ob) and osteoclasts (Oc).
M indicates implanted matrix.
Sections were stained by hematoxline and eosin.





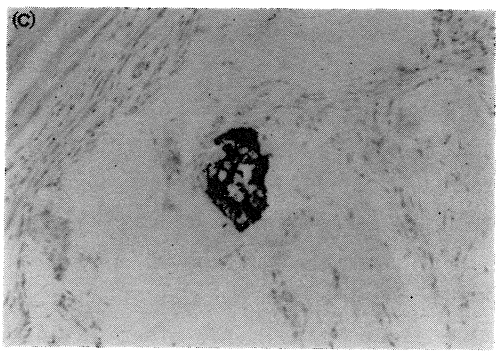


Fig. 5. Plaques induced by implantation of decalcified bone matrix on day 21 in sham operated (a), sham operated and estrogen administrated (b) and ovariectomized (c) rats. (X 100)
Sections were stained calcium.
Calcium deposition is less in ovariectomized than in the other rats.

Fig. 3 shows changes of calcium dependent ATPase activity. The activity was remarkably decreased between day 14 and 21 in Sham+E animals. On the other hand, the activity was increased on day 21 in Sham animals and the activity became higher in Sham rats than in Sham+E ones on day 21. In Ovx group, ATPase activity was not changed during the experiment.

Fig. 4 shows histological changes in the plaques in sham group. Chondrogenesis, cartilage degeneration and bone formation, and bone remodeling were occurred in plaques on day 7, 14 and 21, respectively. And same results were found in Ovx and Sham+E group. However, sections stained calcium showed that calcium deposition seemed to be less in Ovx compared with the other 2 groups on day 21 (Fig. 5).

Discussion

Histological study showed that chondrogenesis, bone formation and bone remodeling were induced by implantation of decalcified bone matrix. And these changes were occurred at the same time in all groups, which suggested that cartilage and bone differentiation were occurred notwithstanding estrogen deficiency and administration.

However, staining of calcium showed that calcium deposition seemed to be less in Ovx groups than in the other ones. Furthermore calcium content was less in Ovx group on day 14 and 21. It is well known that ovariectomy induces osteoporosis and estrogen is necessary for maintenance of bone mass.[11] These results suggested that estrogen deficiency suppressed not only calcification of bone but also that of cartilage. On the other hand, estrogen may secondary affect calcification of cartilage because estrogen receptor has not been found in the tissue.[12]

Skeletal AP mainly exists in osteoblast and the AP activity is increased when bone calcification is stimulated.[5] And the inorganic pyrophosphatase activity is an important part of AP because AP can catalyze hydrolysis of inorganic pyrophosphate.[13] Furthermore pyrophosphate is known as a inhibitor of crystallization of hydroxy apatite and it is suggested that bone calcification is regulated by the metabolism of this compound.[14] In this experiment, AP activity was less in Ovx than in the other groups. These results were consistent with results of the calcium content which is less in Ovx group than in the others. On the other hand, AP activity was not different between Sham+E and Sham animals during the experiment though calcium content was more on day 14 and less on day 21 in Sham+E than in Sham group. It is possible that factors other than pyrophosphate regulate calcification of cartilage and bone during estrogen administration.

Caicium dependent ATPase is suggested to be important in calcium transport in many tissues.[15] Moreover active transport of calcium is necessary for calcification in cartilage and bone.[13] Seven days after the implantation, calcium dependent ATPase activity tended tobe higher in Sham+E than in Sham animals which may reflect that the rapid transport of calcium was occurred during estrogen administration in cartilage. The higher ATPase activity may induce the more calcium content of Sham

+E rats on day 14. On the other hand, the ATPase activity was strikingly decreased in Sham+E rats on Day 21. It is possible that the suppression of calcium content in bone by exogenous estrogen is related to the reduction of calcium dependent ATPase activity in bone.

From these results, it is shown that estrogen stimulates calcification of cartilage and bone. Furthermore exogenous estrogen induces rapid calcification of cartilage but suppresses the increment in calcium deposition of bone which may be related to the activity of calcium dependent ATPase.

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摘　　要

エストロジェン欠乏とエストロジェン投与が脱灰骨基質誘導性軟骨内化骨に及ぼす影響を検討した．8匹のラットに卵巣摘出手術を，16匹に疑似手術を行った．また疑似手術を行ったラット8匹にはエストラジオールを1日当り 5μg 筋注投与した．いずれの区においても骨基質移植後7日目に軟骨形成，14日目に軟骨吸収と骨形成，21日目には骨リモデリングが認められた．試験期間を通じカルシウム含量とアルカリ性フォスファターゼ活性は他の区と比べ卵巣摘出区で低かった．アルカリ性フォスファターゼ活性は疑似手術区とエストロジェン投与区で差は認められなかったが，カルシウム含量はエストロジェン投与区のほうが疑似手術区と比較し14日目では高く，21日目では低かった．カルシウム依存性 ATP アーゼ活性は21日目に疑似手術区で上昇し，逆にエストロジェン投与区では低下した．以上の結果より，エストロジェン投与，エストロジェン欠乏にかかわらず，骨基質移植による軟骨と骨の誘導は生じることが示された．一方エストロジェン欠乏は軟骨と骨の石灰化を抑制した．またエストロジェン投与は軟骨における急速な石灰化を生じさせ，骨の石灰化を抑制した．このエストロジェン投与による変化にはカルシウム依存性 ATP アーゼが関与していると考えられた．