Microanalytic and Densitometric Study of Effect of Calcium Supplementation on Rat Models of Bone Loss: On Mandibular Alveolus

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Key words: Microanalysis/Microdensitometry/Calcium supplementation/Alveolus/Bone loss

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Introduction

The importance of puberty has been emphasized on the development of peak bone mass. Glastre et al. reported, in their study of 135 healthy caucasian children aged 1–15 years, the increase of bone mineral density measured by dual energy X-ray absorptiometry was steeper at the time of puberty, reaching values above 0.80 g/cm² after puberty was achieved. Peak bone mass reached at skeletal maturity and bone loss in later life are believed to be two major determinants of bone mass measured in the elderly. The prevention of future osteoporosis has been suggested to start in children during their maximum growth, in order to maximize the peak bone mass in adolescence. There are little experimental data on the role of calcium supplementation in bone status during this crucial period. Here we present the results of densitometric and microanalytic study of mandibular alveolar bones after 2-week administration of UNICAL calcium supplement in rat model of bone loss of 5 weeks of age, which corresponds to early childhood in human beings.

Materials and methods

Ten male Wistar rats of 5 weeks old, weighing about 40 g, were randomly subdivided into control and experiment groups as follows.

Control group was fed with standard feed (Oriental Yeast Co., LTD. Japan) and freely supplied with tap water.

Low Calcium–UNICAL group was fed with low calcium diet (Oriental Yeast Co., LTD. Japan) for the first three weeks and supplemented with UNICAL calcium supplement for another two weeks.

Low Calcium–standard diet group was fed with low calcium diet for the first three weeks
and supplemented with standard diet for another two weeks.

Calcium deficiency-UNICAL group was fed with calcium deficiency diet (Oriental Yeast Co., LTD. Japan) for the first three weeks and supplemented with UNICAL calcium supplement for another two weeks.

Calcium deficiency-standard diet group was fed with calcium deficiency diet for the first three weeks and supplemented with standard diet for another two weeks.

The mineral content of the feed was shown in Table 1.

The rats were sacrificed under anesthesia with intraperitoneal injection of pentobarbital sodium (Abott Laboratories, U.S.A.). The heads were removed and sagittally separated and fixed in 10 % neutral buffered formalin solution. The mandibular alveoli were extracted and debrided of soft tissue. The animals were handled and the experiment was carried out with adherence to the Guidelines on Animal Experiments outlined by Kyushu Dental College.

The samples were imaged with Softex CSM at 35 kVp, 5 mA, 60 s and focus–film distance of 70 cm with Fuji sofex film FG and aluminum reference wedge attached. Densitometric measurement was performed with microphotometer (Densitometer PDS–15, Konica, Japan). Scanning was carried out with a slit of 10×500 micron at a speed of 0.1 mm/s. The results were expressed as mean of measurement at three differing sites.

60–80 micron undecalified sections, sagittally cut, were examined by a scanning electron microscope equipped with an energy–dispersive X-ray microanalyzer (SED 800, SEIKO EG&G, Japan) was used to determine the calcium and phosphorus content of the alveolar bones.

Statistical analysis for differences between the groups was done by F test.

Results

1. Densitometric findings

The results of densitometric measurement in control and experimental groups, expressed as mm aluminum equivalence, are presented in Table 2. UNICAL supplement groups showed significantly higher density compared with standard diet groups in both calcium deficiency
Table 2  Densitometric measurement results

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Low ∙ UNICAL</th>
<th>Low ∙ Standard</th>
<th>Deficiency ∙ UNICAL</th>
<th>Deficiency ∙ Standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration</td>
<td>1.89±0.09</td>
<td>1.72±0.06</td>
<td>1.65±0.06*</td>
<td>1.51±0.04</td>
<td>1.41±0.07**</td>
</tr>
<tr>
<td>* :</td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>** :</td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

Mean±S. D.

Table 3  X-ray microanalytic results

<table>
<thead>
<tr>
<th></th>
<th>Concentration (Mean±S. D.)</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ca</td>
<td>P</td>
</tr>
<tr>
<td>Control group</td>
<td>66.63±0.57</td>
<td>34.37±0.57</td>
</tr>
<tr>
<td>Low calcium ∙ UNICAL</td>
<td>66.28±1.11</td>
<td>33.72±3.56</td>
</tr>
<tr>
<td>Low calcium ∙ Standard diet</td>
<td>65.65±3.56*</td>
<td>34.35±1.11*</td>
</tr>
<tr>
<td>Calcium deficiency ∙ UNICAL</td>
<td>66.88±0.25</td>
<td>33.82±0.25</td>
</tr>
<tr>
<td>Calcium deficiency ∙ standard diet</td>
<td>65.13±0.80*</td>
<td>34.87±0.80*</td>
</tr>
</tbody>
</table>

*: Not significantly different from the result of low calcium ∙ UNICAL group (p>0.05)
*: Significantly different from the result of calcium deficiency ∙ UNICAL group (p<0.05)

(p<0.01) and low calcium feeding groups (p<0.05).

2. X-ray microanalysis findings

The results of the energy-dispersive X-ray microanalysis of control and experimental groups are presented in Table 3. Of calcium deficiency groups, UNICAL supplement group showed significantly higher calcium and phosphorus concentration compared with standard diet group (p<0.05). No significant differences were detected between UNICAL supplement and standard diet groups in low calcium feeding groups.

Discussion

Multiple factors have been reported to be related to bone health, including genetic differences, physical activity, postmenopausal estrogen loss, and dietary factors. Among the dietary factors thus far investigated, calcium has long been considered to be the most important. It is an essential nutrient for growth during infancy and childhood. The concept of reduced calcium intake from either dietary deficiency or intestinal malabsorption causing osteoporosis was proposed many years ago. Since then considerable evidence for a strong positive association between dietary calcium intake and bone density has been provided by various cross-sectional studies, longitudinal studies, and calcium-supplementation studies. Inadequate calcium intake also has been linked in case-control studies with increased risk of osteoporosis in osteoporotic subjects when compared with their normal counterparts. The ultimate test of the hypothesis on the positive association between calcium intake and bone health would...
be the prevention of bone loss by high dietary intake of calcium, as some studies have suggested\textsuperscript{[10,11]}. Calcium therapy would be convenient, because it is easy and safe when taken in recommended doses. In contrast, other investigators have reported no protective effects of high calcium intakes\textsuperscript{[12,13]}. Riis \textit{et al.} (1987)\textsuperscript{[13]} pointed out that dietary calcium supplementation in the dosage they used, oral calcium of 2000 mg daily, can not be regarded as an effective alternative to estrogen or progestogen hormone replacement in the prevention of early postmenopausal bone loss. These conflicting data have led to the importance of calcium intake being questioned. The results of the experiment reported here should help to clarify the effects of calcium supplementation on recovery of bone loss in early childhood by comparing bone density and calcium and phosphorus content in mandibular alveolus of standard diet group with that of UNICAL calcium supplements group.

1. Densitometric measurement

A series of physical methods have been applied to the quantitative study of bone mineral. The amount of mineral in bone, its topographical distribution and fine structure, are important parameters which are of interest for many reasons, and may have a significant place in the study of bone physiology and pathophysiology. Microphotometric measurement was used here in the present study to investigate the density changes of alveolar bone after calcium supplementation. Chan (1991)\textsuperscript{[10]} reported a study of 164 healthy, white children aged 2 to 16 years, and found children ingesting more than 1000 mg of calcium daily had higher bone mineral content, determined by the method of single photon absorptiometry, than those ingesting less. Supplementary dietary calcium has also been shown to prevent bone loss during lactation in adolescents\textsuperscript{[11]} and there is preliminary evidence to suggest that sufficient intake of calcium and phosphate increases the mineral content in children's bones and might decrease the risk of accidental fractures\textsuperscript{[12]}. Our study showed that UNICAL calcium supplementation help with the recovery of bone density of alveolar bone from bone loss resulting from calcium insufficiency. Heaney \textit{et al.} (1997)\textsuperscript{[13]}, (1998)\textsuperscript{[14]} found a positive correlation between calcium intake and calcium balance in both premenopausal and postmenopausal women, and claimed that daily calcium intakes of 1000 and 1500 mg respectively would achieve calcium balance and that larger intakes would result in positive calcium balance, which must lead to increased bone calcium. Some other studies also indicated that a sufficiently high calcium intake can reverse a negative calcium balance and thereby suppress bone loss\textsuperscript{[13,14]}.

2. Microanalytical investigation

Densitometric measurement only indicates the level of bone mineralization, and gives no information about chemical composition. Since bone is a composite tissue, it is important to know the chemical composition at the level of microscopic structures. Such an investigation has been made possible by the use of the electron probe X-ray microanalyzer. With this instrument, a precise non-destructive elemental analysis can be performed on localized regions with diameters as small as 1 micron and the detectable concentration of elements is very low, less than 0.01%. The results concerning the quantitative analysis of calcium and
phosphorus, the principal components of bone mineral, have confirmed that high calcium intake resulted in higher calcium and phosphorus ratio in condylar bone than standard diet[^10]. Our study showed that UNICAL calcium supplementation resulted in a higher concentration of calcium in mandibular alveolus, and the difference between the UNICAL supplement group and standard diet group was proved statistically significant in calcium deficient groups, but not in low calcium feeding groups.

**Summary**

Results of densitometric and microanalytic study of mandibular alveolar bones were reported after 2-week administration of UNICAL calcium supplement in 5-week rat model of bone loss resulting from low calcium and calcium deficient feeding, which corresponds to early childhood in human beings.

1. Of calcium deficiency groups, UNICAL supplement group showed significantly higher calcium concentration in X-ray microanalysis compared with standard diet group (p<0.05). No significant differences were detected between UNICAL supplement and standard diet groups in low calcium feeding groups.

2. UNICAL supplement groups showed significantly higher density in densitometric measurement compared with standard diet groups in both calcium deficiency (p<0.01) and low calcium feeding groups (p<0.05).

The foregoing results implied that calcium is the agent of choice for treatment of bone loss. A long term preventive study with calcium in human beings, however, calls for a valid estimate of long term calcium balance.

**References**


幼児期ラットの食飼療法に関する Ca, P の定量分析および骨塩量—虚弱下顎歯槽骨—

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春岡 誠・矢原 健考・龔 瑞泰
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平成 10 年 6 月 25 日受理

生後 5 週齢の Wistar 系雄ラットに低カルシウム食とカルシウム欠乏食を与え、骨の過剰状態を惹起後、カルシウム含有量の高い UNICAL 混合食と標準食を与え、成長期下顎歯槽骨に及ぼす影響について検索し、次のような結果を得た。

1. 骨密度所見についてみると、低カルシウム・UNICAL 混合食群、低カルシウム・標準食群、カルシウム欠乏・UNICAL 混合食群、カルシウム欠乏・標準食群の順に骨構築状態が優れていた。

2. X 線マイクロアナライザーについてみると、相対 Ca 量比、相対 P 量比ともカルシウム欠乏・UNICAL 混合食群とカルシウム欠乏・標準食群との間有意差が認められ、低カルシウム・UNICAL 混合食群と低カルシウム・標準食群との間有意差が認められなかった。

以上のことから、低カルシウムとカルシウム欠乏により骨虚弱状態に陥った成長期下顎歯槽骨に UNICAL 投与は骨構築促進効果を認められるが、成長期における虚弱骨の治療ではカルシウム過含有量が重要であると示唆された。