Ultrastructural Study of the Effects of Ipriflavone and 1α-OH-D₃ on Debilitated Mandibular Condyle in Growing Rats

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Abstract

We studied three-week-old male Wistar rats, corresponding to the age of newly weaned human children, to understand the effect of a dietary therapy of ipriflavone combined with 1α-OH-D₃ on debilitated mandibular condyles resulting from insufficient calcium intake by observing ultrastructural alterations.

Light microscopy findings demonstrated calcification and a reduction of the formation of secondary spongy bone in the low calcium diet group, increases in the formation of secondary spongy bone in the low calcium diet and standard diet group, and calcification of the chondral matrix and active ossification along with normal growth of the mandibular condyle in the low calcium diet and standard diet with supplementary ipriflavone and 1α-OH-D₃ group.

In the low calcium diet group, the chondral lacunae were shaped irregularly, with sparse calcospherites and irregular collagen fibrils noted among the calcospherites. There were abundant vacuoles and lysosomes in the cytoplasm of osteoclasts, which were often active. In the low calcium diet and standard diet group, calcospherite agglutination was incomplete and some of the chondral lacunae borders were distinct. The number of osteoblasts was increased and evidence of their active transition to osteocytes was observed. In the low calcium diet and standard diet with supplementary ipriflavone and 1α-OH-D₃ group, territorial matrixes between chondral lacunae were often seen, and the chondral lacunae borders were distinct. Characteristically, most of the osteoclasts in this group were inactive and had parted from the bone matrix, while active osteoblasts lined the surface of new bone formations that were separated by a thick osteoid layer.

Our results showed that insufficient calcium raised the level of debilitated bone, while supplemental ipriflavone and 1α-OH-D₃ promoted its recovery.

Key words: Ipriflavone/1α-OH-D₃/Mandibular condyle/Growth rats
The Effects of Ipriflavone and 1α-OH-D₃ on Debilitated Mandibular Condyle (Kakiuchi et al.) — 53 —

Introduction

Vitamin D is known to be an antirachitic factor, that is necessary for bone formation and metabolism⁵ or, in humans, vitamin D₃ can be synthesized from a provitamin in skin by sunlight and can also be taken through diet. For vitamin D₃ to play a role in bone metabolism, it must first be activated into 1α-OH-D₃, the active type. In the present study, vitamin D₃ was metabolized to 25-hydroxy-vitamin D₃ in the liver then to 1α, 25-dihydroxy-vitamin D₃ in the kidney to form active vitamin D₃. When 1α, 25-dihydroxy-vitamin D₃ combines with the receptor in the nucleus of a cell, a specific gene transcription is activated. The effect of 1α-25(OH)₂D₃ is to promote calcium and phosphorus absorption in the intestine, so it is thought that 1α-25(OH)₂D₃ has an indirect relationship to bone formation. Recently, the receptor in the nucleus of osteoblasts was found, opening the possibility to directly study the relation of 1α-OH-D₃ with bone formation⁶.

The effect on bone of ipriflavone (IF), which comes from beans and alfalfa and acts as an inducer of isoflavone, has also been the focus of recent bone metabolism research. IF has been shown to be an inhibitor of bone resorption⁷, as well as a promoter of calcitonin secretion,⁸ which has an inhibiting effect on bone resorption and bone mass decrease. To our knowledge, these are the first experimental study results on the effect of 1α-OH-D₃ and IF on debilitated mandibular condyles in growing rats.

Materials and methods

Animals and treatment

Twenty 3-week-old male Wistar rats, corresponding in age to newly weaned human children, were randomly divided into 4 groups of 5. They were housed in small cages individually under similar conditions of 22±2°C and a humidity of 50±5%, with a 12 hour/12 hour light/dark cycle.

Control group rats were fed a standard diet and given tap water freely for 6 weeks. In the low calcium diet group, rats were fed a low calcium diet (30% calcium of the standard diet) and distilled water freely for 6 weeks. Rats in the low calcium diet and standard diet group were fed the low calcium diet and given distilled water freely for 3 weeks, followed by the standard diet and tap water for next 3 weeks. Further, olive oil at 2 ml/kg of body weight was given to all rats in these 3 groups, 3 times each week. In the low calcium diet and 1α–OH–D₃ with IF supplemented standard diet group (1α–OH–D₃ and IF group), rats were fed the low calcium diet and given distilled water freely for 3 weeks, followed by an IF supplemented standard diet with tap water for the next 3 weeks. They were also given an oral administration of a 1α–OH–D₃ solution at 2 ml/kg of body weight 3 times each week for the final 3 weeks. To prepare the 1α–OH–D₃ solution, we dissolved 5μg of 1α–OH–D₃ (Chugai Pharmaceutical Co., Ltd., Tokyo, Japan) in 100 ml of olive oil. All diets were prepared and provided by Oriental Yeast, Tokyo, Japan, and the components of each are presented in Tables 1, 2, and 3.
Sample preparation

Following the 6 week feeding protocol, all rats were killed under deep anesthesia (pentobarbital sodium, 250 mg/kg, Nippon Pharmacy) and the mandibular condyles were removed. Samples for light microscopy were fixed in 10% neutral buffered formalin, decalcified in 5% nitric acid, dehydrated through a graded ethanol series and embedded in paraffin. Successive sections 7 μm in width were made, and stained with hematoxylin and eosin.

Samples for scanning electron microscope (SEM) observation were immersed in 2.5% glutaraldehyde for 12 hours at 4°C, then cleaned with 10% sodium chlorite solution and super sonic waves to dispose of the adhesive residue. They were then rinsed with buffered phosphate acid (pH 7.2) before post-fixation, which was performed in 1% osmic acid buffer solution (pH 7.2) at 4°C for 2 hours. After fixation, the samples were dehydrated through a graded ethanol series, then treated with t-butanol and dried by freeze-drying method. The cracked surfaces were coated with a layer of aurum, before being observed under an SEM (S-3300N, Hitachi, Ltd., Japan).

Transmission electron microscope (TEM) samples were immersed in a fixative mixture of 1% paraformaldehyde and 3% glutaraldehyde in 0.05 M sodium cacodylate buffer (pH 7.2) at 4°C for 1 week, then rinsed with a 0.05 M sodium cacodylate buffer solution (pH 7.2, 3 × 1 hour) before decalcification, which was performed in a 5% EDTA–2Na solution for 2 weeks. The samples were then rinsed with 0.05 M sodium cacodylate buffer solution (pH 7.2) again and dissected into prismatic blocks of tissue.

Postfixation was performed with a 2% osmic acid buffer solution (pH 7.2) for 2 hours, followed by dehydration through a graded ethanol series and diaphanization in propylene oxide, before being embedding in epoxy resin. Semithin sections were cut at a thickness of

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**Table 1** Composition of experimental diets (%)

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Standard diet</th>
<th>Low Calcium diet (Cal44 mg/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-corn starch</td>
<td>38.00</td>
<td>37.64</td>
</tr>
<tr>
<td>Vitamin-free casein</td>
<td>25.00</td>
<td>25.00</td>
</tr>
<tr>
<td>α-potato starch</td>
<td>10.00</td>
<td>10.00</td>
</tr>
<tr>
<td>Cellulose powder</td>
<td>8.00</td>
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</tr>
<tr>
<td>Soy bean oil</td>
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<td>6.00</td>
</tr>
<tr>
<td>Mineral mixture</td>
<td>6.00</td>
<td>6.00</td>
</tr>
<tr>
<td>Granulated sugar</td>
<td>5.00</td>
<td>5.00</td>
</tr>
<tr>
<td>Vitamin mixture</td>
<td>2.00</td>
<td>2.00</td>
</tr>
<tr>
<td>CaCO₃</td>
<td>0.36</td>
<td></td>
</tr>
</tbody>
</table>

**Table 2** The origin of element content from the mineral mixture of the diet (mg/100 mg)

<table>
<thead>
<tr>
<th></th>
<th>Standard diet</th>
<th>Low calcium diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca</td>
<td>480</td>
<td>144</td>
</tr>
<tr>
<td>P</td>
<td>650</td>
<td>612</td>
</tr>
<tr>
<td>Mg</td>
<td>87</td>
<td>87</td>
</tr>
<tr>
<td>Na</td>
<td>220</td>
<td>293</td>
</tr>
<tr>
<td>K</td>
<td>440</td>
<td>746</td>
</tr>
<tr>
<td>Fe</td>
<td>32</td>
<td>32</td>
</tr>
<tr>
<td>Cu</td>
<td>0.46</td>
<td>0.5</td>
</tr>
<tr>
<td>Zn</td>
<td>3.4</td>
<td>3</td>
</tr>
<tr>
<td>Mn</td>
<td>1.6</td>
<td>2.6</td>
</tr>
<tr>
<td>I</td>
<td>0.46</td>
<td>0.3</td>
</tr>
<tr>
<td>Cl</td>
<td>170</td>
<td>174</td>
</tr>
</tbody>
</table>

**Table 3** Composition of experimental diets (%)

<table>
<thead>
<tr>
<th>Standard diet with supplementary IF</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard diet</td>
<td>91.5</td>
</tr>
<tr>
<td>Ipriflavone</td>
<td>8.5</td>
</tr>
</tbody>
</table>
0.35 µm and stained with toluidine blue, prior to examination with a light microscope to choose target areas for TEM observation. Ultrathin serial sections were then cut at a thickness of 70–90 nm using a diamond knife on an ultramicrotome, after trimming the blocks. The ultrathin sections were placed on Cu/Rh grids, stained with uranyl acetate and lead citrate, as described by Watson and Reynolds, and later observed using a TEM (JEM-1200EX, Japanese Electric Co., Ltd.).

The study was approved by the Committee for the Use of Laboratory Animals of Kyushu Dental College, Japan.

Results

1. Light microscopy

We observed the mandibular condyles with a light microscope. In the control group, undifferentiated chondrocytes in the stationary zone had a high nucleus–to–cytoplasm ratio, and were ovoid in shaped, with the long axis parallel with the articular surface. Adjacent to this zone was the proliferative zone, in which the chondrocytes were undergoing division and organizing into distinct columns. Next to that was the hypertrophic zone, in which the chondrocytes were greatly enlarged and the matrix had become mineralized. In the cytoplasm of the chondrocytes, there were abundant matrix vacuoles. Beyond the chondrocytes was the erodent zone, in which chondroclasts were often seen, newly formed capillaries were penetrating the bone matrix, and osteoblasts were found. The process of endochondral ossification could be seen here and trabeculae were formed (Fig. 1a, b).

Characteristic features of the low calcium diet group were an increase in the thickness of the hypertrophic zone, along with decreases in calcification of chondral matrix as well as the amount of primary spongy bone (Fig. 2a, b). In the low calcium diet and standard diet group, osteoblasts were often noted in the erodent zone, and bone formation was active, as

Fig. 1 Mandibular condyle of the control group. a. H&E stain (×20)  
CSZ: Chondrocytes in stationary zone  
CPZ: Chondrocytes in proliferative zone  
CHZ: Chondrocytes in hypertrophic zone  
Tr: Trabecula
b. H&E stain (×100)  
CEZ: Chondrocytes in erodent zone  
MC: Multinuclear cell  
Ob: Osteoblast  
CM: Chondral matrix
Fig. 3 Mandibular condyle of IF and 1α-OH-D₃ group. 
H·E stain (×200) 
CHZ: Chondrocytes in hypertrophic zone 
CEZ: Chondrocytes in erodent zone 
MC: Multinuclear cell 
Ob: Osteoblast 
CM: Chondral matrix

compared with the low calcium diet group. 
In the IF and 1α-OH-D₃ group, calcification of the chondral matrix and the number of chondroclasts were similar to those seen in the control group, while endochondral ossification had recovered from the debilitated state and growth of the mandibular condyle was also seen (Fig. 3).

2. SEM findings 
Using the SEM, we mainly observed the ultrastructure of hypertrophic chondrocytes in the mandibular condyle.

In the control group, the chondral lacunae, approximately 20–25μm inside the hypertrophic zone, showed distinct borders, and multiple distinct calcospherites that were about 1–2 microns in size. Collagen fibers in the chondrocytic lacunae walls were regular and dense with clear territorial matrixes, and calcospherites were dense in most of these. In the bone matrix, the osteocyte lacunae diameters were approximately 10–15μm, and most were distinct with abundant bone canaliculi open within their mouths, which were rounded by regular collagen fibrils, in the walls (Fig. 4a, b).

Compared with the control group, chondral lacunae in the low calcium diet group were irregularly shaped, and calcospherites were sparse. In some areas, collagen fibers connecting with calcospherites were found, whereas only calcospherites were noted in other areas. Bone resorption was more often seen than bone formation, which was a characteristic feature of this group. Most of the resorption bay borders were indistinct, though osteocyte lacunae
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were occasionally seen under the resorption bays. Further, canaliculi mouths and collagen fibers were seen on the osteocyte lacunae walls (Fig. 5a, b).

In the low calcium diet and standard diet group, the number of calciospherites and collagen fibers in the chondral lacunae were increased, as compared with the control group, however, calciospherites were unequal in size, their agglutination was incomplete, and the osteocyte borders were indistinct. Most of the territorial matrixes of the chondral lacunae were fragmented. The area of bone formation area had increased greatly as compared to that of the low calcium diet group. The characteristic feature of this group was that areas of bone formation were found more often than those of bone resorption (Fig. 6a, b).

In the IF and 1α-OH-D₃ group, calciospherites were greater in number and their agglutination was more obvious than in the low calcium diet and standard diet group. Further, the territorial matrix and distinct chondral lacunae were easily found. Although some fragmentation of some of the chondral lacunae was observed, the growth of the
Fig. 6 SEM images of low calcium diet and standard diet group (a.: ×3,000, b.: ×750)

a. Cal: Calcospherite
   CF: Collagen fibrils
b. La: Lacuna
   BM: Bone matrix

mandibular condyle was similar to that in the control group. In the bone matrix, areas of bone formation were often seen as was recovery of bone formation, whereas bone resorption was seldom found. In addition, some of the collagen fibers were running irregularly and some of the osteocyte lacunae borders were indistinct (Fig. 7a, b).

3. TEM findings

In the control group, osteoblasts were recognized by their mononuclear cells and cuboidal or polygonal shape, and osteoprogenitor cells were often seen adjacent to the osteoblasts. The osteoblasts were secreting both collagen and a ground substance that constituted the initial non-mineralized bone and osteoid layer. Most osteoblasts in this group had well-developed mitochondria (Mt) (Fig. 8a). Active osteoblasts were often seen, with abundant Golgi apparatus (Go) and Mt in their cytoplasm, with desmosomes between the osteoblasts as well. Osteoclasts were seldom seen, though in their cytoplasm were abundant

Fig. 7 SEM images of IF and 1α-OH-D<sub>3</sub> group (a.: ×2,000, b.: ×2,000)

a. Cal: Calcospherite
   TM: Territorial matrix
   CL: Chondral lacuna
b. La: Lacuna
   BM: Bone matrix
   BC: Bone canaliculi
The Effects of Ipriflavone and 1α-OH-D₃ on Debilitated Mandibular Condyle (Kakiuchi et al.)

Fig. 8 TEM images of control group (a.: ×3,000, b.: ×2,500)

a. Ob: Osteoblast
   Ost: Osteoid
   POb: Pre-osteoblast

b. Oc: Osteocyte
   Rb: Ruffled border
   BM: Bone matrix
   V: Vacuole
   Mt: Mitochondria
   N: Nucleus
   Ocl: Osteoclast
Mt and vacuoles. Ruffled borders (Rb) were adjacent to absorbed bone, with many vacuoles and lysosomes in the cytoplasm nearby. The osteocytes were small and had a high nucleus-to-cytoplasm ratio, with poorly developed organelles and gap junctions between the cytoplasmic processes (Fig. 8b). Young osteocytes, which were as small as the osteoblasts, had a well-developed rough endoplasmic reticulum (rER) and Go. Osteoprogenitor cells, which showed a spindle shape and had aggregated into a single layer, were also noted.

In the low calcium diet group, the proportion of cuboidal or polygonal osteoblasts was reduced and of most of their organelles were poorly developed. In some places, osteoclasts intruding into the line of osteoblasts were found, with abundant Go and well-developed Mt, along with abundant chromatin in their nuclei. The Rb had well-developed microvilli and the clear zone (Cz) in this area, which more or less demarcated the limits of the bone area being absorbed, contained abundant microfilaments, though it was essentially lacking in other organelles. There were many vacuoles in the cytoplasm, with collagen fibrils fragments found in some (Fig. 9). In the cytoplasm, there were abundant free lysosomes

Fig. 9 TEM image of low calcium diet group. (×4,000)
Ocl: Osteoclast
Rb: Ruffled border
Cz: Clear zone
BM: Bone matrix
V: Vacuole
Mt: Mitochondria
N: Nucleus
Go: Golgi apparatus
Ly: Lysosome

Fig. 10 TEM images of low calcium and standard diet group (a.: ×2,000, b.: ×20,000)
a. Ob: Osteoblast
Ost: Osteoid
N: Nucleus
Go: Golgi apparatus
b. Oc: Osteocyte
Ob: Osteoblast
BM: Bone matrix
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Fig. 11 TEM image of IF and 1α-OH-D₃ group (×2,000)
Ob: Osteoblast
Ost: Osteoid
N: Nucleus
BM: Bone matrix
Oc: Osteocyte

and ribosomes.

In the low calcium diet and standard diet group, active osteoblasts were often seen and desmosome junctions were noted between them. Most of the osteoblasts had well-developed free ribosomes, Go, and Mt, while abundant chromatin was apparent in their nuclei. The characteristic feature of this group was that the number of osteoblasts had significantly increased (Fig. 10a). As compared with the low calcium diet group, the number of osteoclasts were reduced, as was the proportion of active osteoclasts. However, in this area Rb, vacuoles, lysosomes and collagen fibril fragments were found. Osteocytes in the bone matrix were seen connected to each other with gap junction, and some had a few rER and Mt (Fig. 10b).

In the IF and 1α-OH-D₃ group, most of the osteoblasts and osteoclasts were active. The characteristic feature of this group was that the osteoclasts were different from those in the low calcium diet and standard diet group, as they were located away from the bone surface and closed to the osteoblasts or osteoprogenitor. The Rb were poorly developed, and there were few Mt and rER, however, while there were many lysosomes and vacuoles in the cytoplasm, with some material located in the vacuoles. The osteoblasts were cubical, spherical or conical in shape and had aggregated into a single layer. The number of Mt in
the osteoblasts was increased, with active calcification and a thick osteoid layer. Chromatins in the nucleus was abundant, with many collagen fibers around them. The young osteocytes, were similar to those in the osteoblasts, however, they were smaller and located in the bone matrix, with well-developed rER and Mt in their cytoplasm. In the area of ossification, osteocytes in the newly formed bone matrix showed poorly developed organelles, which was similar to those seen in the control group (Fig. 11).

Discussion

Recently, estrogen, vitamin D₃, vitamin K and calcitonin have been utilized as drugs for osteoporosis, as each is able to inhibit bone resorption or activate bone formation. Estrogen in particular has a remarkable effect on osteoporosis, however, because of its high risk of carcinogenicity it is not routinely given and other drugs with less serious side effects are used. Vitamin D promotes calcium absorption, and normalizes the metabolism of bone. However, these benefits are only seen in the form of 1α₂5(OH)₂D₃, which affects calcium absorption in the intestine, activation of calcification and remodeling of osteoblasts, inhibition of the production and secretion of parathyroid hormone (PTH), and proliferation of parathyroid cells. Moreover, a deficiency of 1α-OH-D₃ will result in bone calcification disorders and insufficient calcium absorption in the intestine. It has, been reported in a clinical trial that calcitriol, the active metabolite of vitamin D₃, may directly suppress osteoblastic activity in patients with secondary hyperparathyroidism when given in large doses to those undergoing peritoneal dialysis.

For many years alfalfa has been added to the feed of domestic animals to make them strong. Recently, research regarding IF, one of the flavonoids, has been conducted, as it has a close relation to bone metabolism. It is also known that beans also contain IF, whose molecular structure is similar to that of estrogen. However, since IF does not show carcinogenicity, it is often used as a drug for osteoporosis, and there are many reports of its effectiveness when used in menopausal osteoporosis therapy. Single therapy of 1α-OH-D₃ or IF in our previous serial observations indicated that 1α-OH-D₃ accelerates the growth of mandibular condyle cartilage, metaphysis tibia bone, and IF in mixture with standard calcium diet or with high-calcium diet promotes the bone formation of metaphysis tibia. In contrast, there is no known study regarding the effect of IF combined with 1α-OH-D₃ on debilitated mandibular condyles, thus, we observed the effect of that combination by histopathological and ultrastructural methods.

1. Light microscopy findings

It has been reported that calcification of the rat mandibular condyle is similar to that in other long bones until 5-days-old, and that cartilage at that time is retained on the surface of the condyle. It was also shown that enchondral ossification occurs in the condyle. In our series of studies, we have found that in the mandibular condyle of rats fed with a low calcium diet the hypertrophic zone is thickened, and the numbers of trabeculae and chondroclasts are reduced, which are a result of calcification that has become inactive. In
the present study, inactive calcification and a reduction of trabeculae were noted in the low calcium diet group. In the low calcium diet and standard diet group, recovery was seen, which showed that a debilitated mandibular condyle caused by insufficient calcium intake can recover with adequate calcium supplementation. We also found that a combination of 1α-OH-D₃ and IF promoted calcification of the cartilaginous matrix, and recovery of enchondral ossification, which led to near normal growth of the mandibular condyle and suggested that this combination could have a significant effect on the recovery of debilitated bone in subjects.

2. SEM findings

A few results have been reported regarding calcification of the cartilaginous matrix in the mandibular condyle(29-31), which was found to be nearly the same in the present control group. The debilitated state of the low calcium diet group was clear, especially our findings at the sites of bone matrix formation, which suggested that the calcium had been taken from bone in order to maintain the stable levels in rats that suffered from insufficient calcium intake. In mammals, a stable level of calcium in serum is very important, and, since bone plays a role in its storage, when serum levels decline, calcium will be taken from bone. In the present study, the low calcium diet and standard diet group showed good bone formation, and calcospherites, which have a close relation with primary calcification(32), were dense. However, compared with the control group, the condyle was still in a debilitated state. In the 1α-OH-D₃ and IF group, a territorial matrix was noted and the chondral lacunae borders were distinct, which showed the recovery of bone, though to our knowledge this result has not been reported in other studies. We considered that the IF had a great effect on the recovery of debilitated bone, while 1α-OH-D₃ also played a role with bone modeling and remodeling, so the multiplier effect was evident and the results satisfactory.

3. TEM findings

In our observations of the low calcium diet group with the TEM, the number of osteoblasts were decreased, whereas osteoclasts were increased and most were active were, which suggested that the calcium was taken from storage in bone. In the low calcium diet and standard diet group, the number of osteoblasts was increased and most were active, which suggested recovery from a low calcium state. In the 1α-OH-D₃ and IF group, in contrast to the low calcium and standard diet group, osteoclast activities were inhibited, and osteoblast differentiation and activites were activated by IF, thus the bone mass equilibrium was changed into a positive state. Takehu et al(33) found that IF combined with PTH or calcitonin, whose mechanism is well known, had a direct inhibiting effect on bone resorption. Further, a cell culture study, reported that IF directly stimulates markers of the osteoblast phenotype at a certain stage in bone formation(34). In the present study, we found that the effect of IF was to activate osteoblast differentiation and inhibit osteoclast activity, so that the debilitated mandibular condyles were able to recover.
Conclusion

Insufficient calcium intake during the developmental period can result in the mandibular condyle being debilitated. However, dietary supplementation with ipriflavone and 1α-OH-D3 showed no negative results and had a positive effect on promoting bone recovery.

References

16) Sato, Y., Kuno, H., Kaji, M., Saruwatari, N. and Oizumi, K.: Effect of ipriflavone on bone in elderly
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ラット成長期の虚弱下顎頭軟骨に対するイブリフラボン・1α-OH-D₃による食餌療法に関する研究
—超微形態ならびに微細構造—

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西岡孝浩・日高彰子・木村光孝

九州歯科大学小児歯科学講座（主任：木村光孝教授）

ヒトの離乳期に相当する生後3週齢のWistar雄系ラットを用いて、低カリウム食により虚弱骨を惹起させ、直ちにイブリフラボン（IF）と1α-OH-D₃を併用した食餌療法により軟骨内骨化による骨形成について超微形態ならびに微細構造的に検索し、次の結果を得た。

光顕レベルでは低カリウム食群では軟骨基質の石灰化の減少に伴って二次海綿骨の減少がみられた。

低カリウム食・標準食群では二次海綿骨は増加していた。

低カリウム食・IF・1α-OH-D₃群は軟骨基質の石灰化の促進がみられ、軟骨内骨化機構は回復し、下顎頭軟骨の成長がみられた。

超微形態的では低カリウム食群では軟骨小腔の形成は不規則で、石灰化小球が散在し、コラーゲン原線維で連絡している部分もみられた。

低カリウム食・標準食群では軟骨小腔の部では石灰化小球は癒合不完全な所もみられ、その区画も不明瞭な部分もみられた。

低カリウム食・IF・1α-OH-D₃群では軟骨小腔には縦走基質がみられるようになり、軟骨小腔の区画が明瞭になっていった。

著者らは低カリウム食・IF・1α-OH-D₃群では主として吸収窩を形成する破骨細胞が減少し、その部には骨芽細胞がみられず微細構造的特徴的所見であった。

以上のことを考えると、低カリウム食では虚弱骨を惹起する。しかし、直ちにIF・1α-OH-D₃に切り換ええた食餌療法により軟骨内骨化機構を阻害することなく、下顎頭の成長を促進することが明らかとなった。