Effects of Dietary Gizzerosine on Contents of Ash and Calcium in the Femur of Young and Ovariectomized Mice

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Abstract Gizzerosine (2-amino-9-(4-imidazolyl)-7-azanonanoic acid) is a causative substance for the gizzard erosion in chicks. Small amount of dietary gizzerosine causes significant increase in the contents of ash and calcium (Ca) in the femur of chicks. If gizzerosine enhances the bone calcification in mammals as in chicks, it may be effective for prevention of the fragility in pigs and osteoporosis in postmenopausal women. In this study, in order to clarify the effect of dietary gizzerosine on the calcification of bone in growing and ovariectomized mice, as model animals of osteoporosis, two experiments were conducted. In experiment 1, the young mice were fed the diets containing gizzerosine at 0, 2 and 10 ppm *ad libitum*. In the mice fed 10 ppm gizzerosine diet, though body weight and femur weight were not affected, the contents of ash and Ca in the femur increased significantly. In experiment 2, ovariectomized mice were fed diets containing gizzerosine at 0, 10, 30 and 100 ppm *ad libitum* and non-ovariectomized mice were fed a diet without gizzerosine. Though the body weights of ovariectomized mice fed the diet without gizzerosine were significantly heavier, the contents of ash and Ca in the femur of ovariectomized were lower than those of non-ovariectomized mice. The body weights and contents of ash and Ca in the femur of ovariectomized mice increased with increasing dietary gizzerosine levels, reached to maximum and then decreased slightly. Dietary gizzerosine levels did not affect concentrations of plasma Ca and pH of the digesta in the duodenum of ovariectomized mice. These results suggested that gizzerosine was effective for acceleration of bone calcification in ovariectomized mice as in chicks. In the case of chicks, gizzerosine decreased pH of digesta in the gizzard, the low pH increased the solubility and absorption of Ca and resulted in enhancement of bone calcification. However, pH of the digesta in the duodenum of mice was not affected by gizzerosine. This showed that the enhancement of bone calcification in mice was affected by different mechanism in chicks.

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Key words : gizzerosine, calcium content, femur, ovariectomized mice

It has been reported that a small amount of dietary gizzerosine increases contents of ash and Ca in the femur of chicks²⁾. Since these effects of gizzerosine are diminished by adding

cimetidine, which is a H₂-receptor antagonist of histamine and gizzerosine⁵⁾, it is suggested that gizzerosine acts to the stimulation of gastric acid secretion. Gastric acid secretion

increases acidity and causes increase of solubility of Ca in the digestive tract. As observed in chicks, if gizzerosine enhances the bone calcification in mammals, it may be effective for prevention of the bone fragility in pigs and osteoporosis in postmenopausal women. Because, recently, osteoporosis is an increasingly important public health problem in Japan. In the postmenopausal women, bone mass is lost in the same way as patients after ovariectomy elapsed after the loss of estrogen⁸⁾. Therefore, in order to certify the effect of gizzerosine on the calcification of bone in mammals, two experiments were conducted.

In experiment 1, the effect of gizzerosine on the calcification in growing mice was studied and in experiment 2, the effect of gizzerosine on the calcification in ovariectomized mice as an osteoporosis model animal was studied.

Materials and Methods

Experiment 1: Effects of gizzerosine on contents of ash and Ca in the femur of growing mice:

Directly after weaning on 21st day after birth, female ICR mice were weighed and allotted to 3 groups of 4 mice with 2 replication each. They were housed in a thermo-controlled room ($22.5 \pm 3^\circ\text{C}$) and fed diets *ad libitum*. The basal diet was obtained from Oriental Yeast Co., Ltd. It contained 24.6% crude protein, 1.2% Ca, 0.9% P and 800 IU/kg VD_3 . The basal diet was crashed with an ultra centrifugal mill (Mitamura Riken Kogyo) and was screened through the sieve of 1 mm mesh. After then 2 or 10 ppm of gizzerosine (Ajinomoto Co., INC.) were added to the diet. On the 20th day, mice were weighed individually and sacrificed by chloroform anesthesia. The right femur was taken and weighed after removing the meat. The content of ash in the femur was measured by heating at 500°C for 2 hrs in an electric furnace. The ash was added 10 ml of 6N HCl and diluted to the adequate concentration for analysis of Ca with an atomic absorption

spectrophotometer (Hitachi 170-50 A).

Experiment 2: Effects of gizzerosine on contents of ash and Ca in the femur of ovariectomized mice:

Female 50-day-old ICR mice were weighed, allotted to 5 groups of 4 mice with 2 replication each. The mice of 4 groups were ovariectomized and another one group was not operated. The ovariectomized mice of 4 groups were fed *ad libitum* the same mash diet as in experiment 1, to which none, 10, 30 or 100 ppm gizzerosine were added. Non-operated group was fed *ad libitum* a diet without gizzerosine. For ovariectomizing operation, the mice were anesthetized with nembutal. Nembutal (50 mg/ml) was diluted to 10 folds by 0.9% NaCl solution and was injected intraperitoneally (0.08 ml/g body weight). After being anesthetized, the waist position of mice were opened by surgical scissors and ovaries with a part of uteri were excised. The diets were supplied after 3 hrs of ovariectomizing operation. On the 21st day, mice were weighed individually and sacrificed. The right femur was taken and weighed after removing the meat. The contents of ash and Ca of the femur were determined in the same way as in experiment 1. Digesta in the duodenum of 4 mice were collected as one sample and were washed out by deionized water and made up to 100 ml. The pH of the solution was measured with a glass electrode pH-meter (Horiba, F-16). Concentrations of plasma Ca of 8 mice in each group were measured with a clinical chemistry analyzer (Shimazu CL-7000).

Statistical analysis: Each value was subjected to one-way analysis of variance⁶⁾. DUNCAN's multiple range test⁷⁾ was used to the test for the significant differences among treatment means.

Results

The results in experiment 1 are shown in Table 1. Supplementation of 2 ppm gizzerosine did not affect significantly the body weight and

Table 1. Effect of gizzerosine (GZ) on ash and calcium contents in femur of mice in experiment 1

Group	GZ (ppm)	Body weight(g)	Femur		
			Weight(g)	Ash(%)	Calcium(%)
1	0	22.8	0.064	32.7 ^a	11.15 ^a
2	2	22.0	0.061	32.6 ^a	11.04 ^a
3	10	23.7	0.065	34.7 ^b	11.84 ^b

Means for 8 mice each.

Values in the same column with different superscript letters differ significantly at 1% level.

Table 2. Effect of gizzerosine (GZ) on ash and calcium contents in femur of ovariectomy mice in experiment 2

Group	Ovary	GZ (ppm)	Body weight(g)	Weight(g)	Femur Ash(%)	Calcium(%)
1	—	0	28.3 ^b	0.0721	34.07 ^a	12.54 ^a
2	—	10	29.6 ^{bc}	0.0736	34.41 ^a	13.07 ^a
3	—	30	30.5 ^c	0.0684	33.86 ^b	14.27 ^{bc}
4	—	100	29.2 ^{bc}	0.0654	36.97 ^b	13.54 ^{ab}
5	+	0	26.1 ^a	0.0676	33.46 ^b	14.71 ^c

Means for 8 mice each.

Values in the same column with different superscript letters differ significantly at 5% level.

Table 3. Effect of gizzerosine (GZ) on plasma calcium concentrations and pH of digesta in duodenum of ovariectomy mice in experiment 2

Group	Ovary	GZ (ppm)	Plasma Ca(mg/dl) ¹⁾	Duodenal pH ²⁾
1	—	0	8.63	6.45
2	—	10	8.78	6.34
3	—	30	8.82	6.27
4	—	100	8.70	6.37
5	+	0	9.25	6.37

¹⁾ Means for 8 mice each.

²⁾ Means for 2 sample each. One sample was pooled digesta in the duodenum of 4 mice.

femur weight, respectively. However, supplementation of 10 ppm of gizzerosine to the diet caused significant increase in contents of ash and Ca in the femur of young mice.

The results of experiment 2 are summarized in Tables 2 and 3. The heavier body weights were observed in ovariectomized mice than in non-ovariectomized mice. The body weights of ovariectomized mice increased furthermore by dietary supplementation of gizzerosine.

They increased until 30 ppm level and then decreased slightly until 100 ppm level. In contrast to the body weights, the contents of ash and calcium in the femur of ovariectomized mice were lower than in the non-ovariectomized mice. The decrease of contents of ash and Ca in the ovariectomized mice was recovered by supplementation of gizzerosine. Both contents of ash and Ca in the ovariectomized mice increased until 30 ppm level and then decreased

slightly until 100 ppm level in the same pattern as observed in the body weights. As shown in Table 3, the concentrations of plasma Ca of non-ovariectomized mice were higher numerically, but not significantly than in the ovariectomized mice. Supplementation of gizzerosine had no effect on the concentration of plasma Ca of ovariectomized mice. There was also no difference in pH of digesta in the duodenum among the dietary groups.

Discussion

Gizzerosine induces the gizzard erosion by accelerating gastric acid secretion in broiler chicks⁵). But the sensitivity of rats for gizzerosine was lower in rats than in chicks. The heated whole fish meal, which contained high levels of gizzerosine and caused gizzard erosion in chicks, had little influence on rat⁴). The supplementation of 2 ppm of dietary gizzerosine caused significant increase in contents of ash and Ca in the femur of chicks²). Though no significant effect was found in the body weights and contents of ash and Ca of a young mice fed the diet supplemented with the same amount gizzerosine, the contents of ash and Ca in the femur increased significantly by 10 ppm of dietary gizzerosine. This result showed clearly that supplementation of dietary gizzerosine caused the acceleration of bone calcification even in growing mice at a higher level of dietary gizzerosine compared to in chicks.

The body weight of ovariectomized mice fed a diet without gizzerosine was significantly higher. Adversely, contents of ash and Ca in the femur of ovariectomized mice were significantly lower than those of the non-ovariectomized mice. However, the supplementation of dietary gizzerosine caused significant increase in ash and Ca in the femur of ovariectomized mice.

Since the acceleration of bone calcification by gizzerosine are diminished by adding cimetidine, it is suggested that the effect of gizzerosine acts to the stimulation of gastric acid secretion

in chicks. However, there is no report compared the activity of gastric acid secretion of the poultry with that of the mammals. In this report, the acidity of the digesta in the duodenum and concentration of plasma Ca were not affected by dietary gizzerosine levels. This result suggested that the acceleration of bone calcification by gizzerosine in the ovariectomized mice was controlled by the different mechanism found in chicks. Therefore, it is necessary to clarify this mechanism in mice, and also to study the effect of cimetidine on the bone calcification in mice.

It has been reported that gizzerosine raises the intracellular cyclic adenosine-3'-5'-monophosphate (c-AMP) level in the isolated chicken proventriculus³) and osteoclastic bone resorption activity is inhibited by c-AMP¹). Therefore, it is necessary to study the interrelationship of the bone cell c-AMP level and bone calcification enhanced by gizzerosine in mammals and chicks. Gizzerosine might be directly influenced on the osteoblast or the osteoclast activity. And it is also necessary to further investigate that gizzerosine is effective for improvement of the bone fragility in pigs and osteoporosis in postmenopausal women.

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成長中ならびに卵巣摘出したマウスの大腿骨の灰分および カルシウム量に及ぼす食餌ジゼロシンの影響

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ジゼロシン (2-アミノ-9-(4-イミダゾリル)-7-アザノナ酸) は雛の筋胃潰瘍原因物質で、ヒスタミンの H_2 -受容体に作用し、胃酸分泌を刺激する。このジゼロシンを少量給与すると雛の大腿骨中の灰分とカルシウム量が有意に増加することが明らかにされている。この効果が哺乳動物においても認められれば豚の骨弱や閉経後の女性の骨粗しょう症に対しても有効であることが考えられる。その可能性を確かめるため、成長期および卵巣摘出マウスを用いて2つの試験を実施した。試験1では成長中のマウスにジゼロシンを0, 2 および 10 ppm 添加した飼料を給与した。10 ppm 飼料では体重、大腿骨重に差はみられなかったが、大腿骨中の灰分量およびカルシウムが有意に増加した。試験2では骨粗しょう症のモデル動物としての卵巣摘出したマウスにジゼロシンを0, 10, 30, 100 ppm 添加した飼料を給与した。対照として卵巣を摘出しなかったマウスにジゼロシン無添加の飼料を給与した。ジゼロシン無添加飼料を給与した卵巣摘出マウスでは未摘出区に比べ体重は有意に重かったが、大腿骨中カルシウムおよび灰分量は有意に低かった。卵巣摘出区のマウスではジゼロシンの含量の増加に伴って、体重、大腿骨灰分およびカルシウム量は増加し、30 ppm を越すとやや減少する傾向がみられた。しかし、ジゼロシンを給与しても十二指腸の内容物中の pH に有意差は認められなかった。これらの結果から、卵巣摘出マウスでも雛同様ジゼロシンによる骨石灰化促進効果が認められた。しかし、雛ではジゼロシンにより、胃内容物中の pH が下がり、そのため、Ca の可溶化と吸収を促進し、その結果、骨石灰化が促進されると解釈されるのに対し、マウスでは十二指腸内容物の pH は変わらないことから、マウスでは雛とは別の機構によって骨の石灰化が促進されると考えられた。

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