Note

Inhibition of Osteoporosis Induced by Protein Deficient (PD) Food Intake by Active Vitamin D$_3$ and Vitamin K$_2$ in Rats

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Independent use of K$_2$ and D$_3$ and simultaneous application of K$_2$ and D$_3$ inhibited the development of osteoporosis caused by PD food intake. The ALP activity of urine as a marker of bone formation osteoporosis did not rise in rats fed PD foods containing D$_3$, K$_2$ or both together. Body and womb weights fell in rats fed PD foods with D$_3$ K$_2$ and both D$_3$, K$_2$. Osteoporosis caused by PD food intake found to be very similar to type II osteoporosis in respects of inhibition by D$_3$ and K$_2$ and rising urinary ALP activity.

Key words: osteoporosis; protein deficient food; active vitamin D$_3$; vitamin K$_2$; alkaline phosphatase

In general, osteoporosis is classified into primary osteoporosis and secondary osteoporosis.$^1$ The former is further divided into type I and type II. Type I includes postmenopausal osteoporosis and is also called the bone resorption type. In treatment, female hormones such as estrogen,$^2$ bisphosphonates,$^3$ and so on have been used. On the other hand, type II includes evolitional osteoporosis and is called the bone formation type. In cure of osteoporosis, D$_3$,$^4$ and K$_2$$^5$ have been used. Dpd is used as a marker of type I osteoporosis,$^6$ and ALP is used as a marker of type II osteoporosis.$^7$

Rats fed PD food are reported to have osteoporosis with significant decreases of fracture force and BMD.$^8$ Urinary ALP activity as a marker of the bone formation type had been observed and the osteoporosis is supposed to be close to type II.

As mentioned above, active D$_3$ and K$_2$ have been used in the treatment of type II osteoporosis. A further resemblance of bone formation osteoporosis and osteoporosis due to PD food intake can be clarified by studying inhibition of osteoporosis by D$_3$ and K$_2$. Therefore, this study was undertaken to detect inhibition against osteoporosis caused by PD food by active D$_3$ and K$_2$.

Female rats (6 weeks old) of the Wistar strain were kept on standard feed prepared according to the AIN-93G$^{10-12}$ for 1 week before commencement the experiment. The animals were then separated into five groups of five rats per group. Group 1 was fed the standard AIN-93G feed, Group 2 was fed PD food which contained sugar instead of protein in the standard feed, Group 3 was fed PD food with 0.004 ng of active D$_3$ (1α,25-(OH)$_2$D$_3$) every day (calculated by converting 1 μg of D$_3$ per unit weight of a 50 kg to an equivalent amount of D$_3$ per unit weight (100 g) of a rat and doubled to increase its effect. Group 3 was fed PD food containing 199 ng of K$_2$ (2-methyl-3-tetraprenyl-1,4-naphthoquinone, menaquinone 4) every day (calculated in the same way as D$_3$). Group 5 was fed PD food with 0.04 ng of active D$_3$ and 0.199 ng of K$_2$ together. The body weight and food intake of each animal were measured every day. Urine was collected between six and eight o’clock in the morning before the day killed the rats. The animals were killed after 3 weeks and blood was drawn. Serum was collected by centrifuging the blood at 12,000 rpm for 20 min. The liver, kidney, adrenal gland and uterus were then extracted, and their total weight was measured. The right and left femurs were removed and stored at −60°C. The Ryukyu University guideline for the care and use of laboratory animals was observed.

Bone breaking force, BMD and density of X-ray picture images were measured as previously described.$^{12}$ ALP activity in the urine was determined with an Alkaline Phospha K test kit (Wako). Estradiol concentration in the serum was measured with an ELA kit (Funakoshi).

Data were tested by one-way analysis of variance followed by inspection of differences between means by Duncan’s new multiple range test throughout these

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Abbreviations: PD, protein deficiency; D$_3$, Active vitamin D$_3$; K$_2$, vitamin K$_2$; BMD, bone mineral density; Dpd, deoxypyridinoline
The body weight of Group 1 fed standard food was about 120 g at beginning of the experiment and gradually grew to reach 145 g, an increase of 25 g in 3 weeks. The weights of Groups 2, 3, 4, and 5 decreased gradually to about 93 g, a fall of 29 g, 91 g, a fall of about 29 g, 93 g, a fall of 26 g and 93 g, a fall of 28 g respectively. The food intake of Group 1 was 192 g in 3 weeks. Those of Groups 2, 3, 4 and 5 were 152 g, 158 g, 158 g, and 167 g in 3 weeks respectively. Rats fed PD foods had poor appetites and lost weight. The heart weights of groups 2, 3, 4, and 5 were 152 g, 158 g, 158 g, and 167 g respectively.

Spleen weights of Group 1. The liver, kidney pancreas and adrenal deceased significantly in Groups of 2–5, compared with those of Group 1. Spleen weights of Groups 2–5 rats fed PD food were significantly heavy, compared with those of Group 1. Spleen weights deceased significantly in Groups of 2–5, compared with those of Group 1. The liver, kidney pancreas and adrenal weights were same in all groups.

The values for breaking force (toughness, \(10^{-3} \) J) of the femur in Groups 1, 2, 3, 4, and 5 were 28.49, 21.32, 23.62, 22.64, and 27.45 respectively. Group 2, fed PD food, and Group 4, fed PD feed with K2 had significantly low breaking force numbers, compared with those of group 1. The figures for Groups 3 and 5 were significantly higher than those of Group 2.

The BMD for Group 2 had significantly low values in contrast with those of Group 1. The values for Groups 3, 4, and 5 were the same and showed no significant difference with those for Group 1. The BMD values for Groups 1, 2, 3, 4, and 5 were 0.057 (g/cm^2), 0.017 (g/cm^2), 0.047 (g/cm^2), 0.117 (g/cm^2), and 0.044 (g/cm^2) respectively (Fig. 1). In the X-ray image, bone rich in calcium is white because it absorbs X-rays. The values for the densities of the bone X-ray image in Groups 1, 2, 3, 4, and 5 were 0.344, 0.823, 0.523, 0.525, and 0.505. The densities for Group 2 were considerably higher than those for Group 1. Those of Groups 3, 4, and 5 were the same and significantly higher than those for Group 1, but lower than one of Group 2.

Therefore, Group 2 rat succumbed to osteoporosis. K2 and D3 were found to inhibit the development of osteoporosis caused by PD food intake.

The womb weight values for Groups 1, 2, 3, 4, and 5 were 0.30 g/100 g, 0.20 g/100 g, 0.21 g/100 g, 0.21 g/100 g, and 0.21 g/100 g respectively. The uterus values for Groups 2, 3, 4, and 5 were significantly lower than those for Group 1.

The values (pg/ml) of serum estradiol were 58.93, 28.48, 35.12, 24.24, and 26.24 for Groups 1, 2, 3, 4 and 5 respectively (Fig. 2). The values for Groups 2 rats were significantly higher than those of Groups 1, 3, 4 or 5 respectively. The results for urinary ALP corresponded well with those for bone breaking force \(r = 0.96\), and femoral BMD \(r = 0.86\) and density in the bone X-ray images \(r = 0.77\). Alkaline phosphatase has been used as a marker of type II osteoporosis and D3 and K2 have been used as treatments for type II osteoporosis. Therefore, osteoporosis caused by PD food intake is very similar to type II osteoporosis in respect of inhibition by D3 and K2 and rising urinary ALP activity.

Moreover, protein like the casein used in this study is supposed to work as an inhibitor of type II osteoporosis. Type II osteoporosis is called senile osteoporosis. Many older men appear to have it, with a lack of appetite and kidney disease and hence little of food or protein intake. It is reasonable to take regular amount of quality protein at all time.

References
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