[S1-2] [4/19/2001(Thur) 14:30-15:00/Hall A]

Pathogenesis of Osteoporosis
- In Vitro Approach using Primary Cultures of Osteoblasts -

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Osteoporosis is a bone disease in which there is both a decrease in the amount of normally mineralized bone and disturbance in bone microarchitecture so that the risk of fractures occurring in the absence of trauma or in response to trivial trauma (a fall from a standing height or even less) is increased, or such fractures have already occurred. Primary osteoporosis refers to the occurrence of the condition among the aging population where no particular predisposing condition can be found.

There are two types of primary osteoporosis, i.e. postmenopausal osteoporosis, that is found among women at the age between 50 and 60, and senile osteoporosis, that is found among older individuals over the age of 65 years. Osteoporosis has become an object of public concern in Japan because fracture due to the disease is the cause of disability, mortality and morbidity and the number of the patients of the disease is now approximately 10 million. Bone tissue is maintained by a process called "bone remodeling" in which bone is constantly replaced by new, with little or no net change in the mass or shape of bones.

In the process of remodeling, cells called osteoclasts resorb (dissolve) bone to make a small cavity at first, and subsequently, cells called osteoblasts secret collagen and calcium phosphate to form new bone in the cavity. Osteoporosis occurs by the relative imbalance between bone resorption and bone formation although precise mechanism of the bone loss or the pathogenesis of osteoporosis is not fully understood at present. The primary cause of postmenopausal osteoporosis is a loss of estrogen secretion but that of osteoporosis of aging is not known. The purpose of this study is to clarify the mechanism of age-related decrease in the bone forming ability of osteoblasts. Cell lines which have major properties of osteoblasts, such as MC3T3-E1 cells, are widely used in the studies on the function of osteoblasts. Primary cultures of osteoblasts isolated from fetal or neonatal rat calvaria are also used in some studies. It is, however, impossible to obtain information on the age-related changes in the cell functions with these types of cells.

I would like to describe here our recent findings obtained from the experiment with the primary cultures of calvarial osteoblasts from rats of various ages (5 to 100-week-old).

1. Reduction in the serum glucocorticoid may be a cause of senile osteoporosis

We found that replacement of fetal bovine serum with adult rat serum in the cultures of osteoblasts from adult rat calvaria markedly enhanced bone nodule (BN) formation and that the BN formation-stimulating activity in adult rat serum decreased with age of serum donor. We identified the BN formation-stimulating factor in rat serum as corticosterone, a major glucocorticoid in rodents, by mass spectrometry. We also found that the serum corticosterone level decreased with age in parallel with decrease in BN formation stimulating activity. These results suggest that corticosterone at physiological level plays a key role the maintenance of bone metabolism, although long term use of glucocorticoid as an anti-inflammatory drug is well known to cause a marked decrease in bone mass as a major side effect.

2. Signal transduction system in osteoblasts and its change with age

Prostaglandin E2 (PGE2) is a potent modulator of bone metabolism that modifies functions of osteoblasts as well as osteoclasts. It has been shown in MC3T3-E1 cells that stimulate differentiation markers of osteoblasts such as alkaline phosphatase (ALP) activity and collagen synthesis. It is believed that PGE2 stimulates osteoblast functions through the production of cAMP. In our studies, however, the results inconsistent with previous findings were obtained using primary cultures of adult rat osteoblasts. Although PGE2 stimulated cAMP production, a treatment of osteoblasts with forskolin decreased BN formation, ALP activity and collagen synthesis. We therefore examined the precise mechanism of signal transduction after the stimulation of osteoblasts with PGE2 and reached the following conclusions.

i) cAMP has suppressive effect on differentiation of osteoblasts.

ii) PGE2 stimulates cAMP production through the activation of adenylate cyclase via EP2/EP4 receptor for PGE2, but it simultaneously stimulates phosphodiesterase via EP1 receptor for PGE2 resulting in the rapid attenuation of cAMP.

iii) An activation of calcium-calmodulin system stimulates osteoblast differentiation.

iv) PGE2 activates the calcium-calmodulin system through an activation of phospholipase C and subsequent production of inositol 1,4,5-triphosphate.

We also found that BN formation ability of osteoblasts decreases with the age of cell donor. This can be explained by the attenuation of the signal through EP1 receptor by the reduction in the number of this receptor subtype with aging.