LONGITUDINAL BONE GROWTH EFFECTS OF PORCINE SKIN GELATINE HYDROLYSATES ON SPRAGUE-DAWLEY RATS

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***Abstract*—This study was performed to investigate the effects of dietary porcine gelatin hydrolysates on bone growth and epiphyseal plate growth via IGF-1 and BMP-2 protein expression. The gelatin hydrolysates colpep26 significantly promote osteoblast by 44% compare to control. Also, longitudinal bone growth and epiphyseal plate growth was shown up to 16% and 12%, respectively. The low molecular weight gelatin hydrolysates colpep26 increased IGF-1 and BMP-2 protein level. These results suggest that supplementation of the porcine gelatin hydrolysates promote bone growth via increasing bone growth protein in the body. Therefore it can be suggested that the low molecular weight gelatin hydrolysates might be used for health benefit food market for growth.**

***Index Terms*— Collagen, Gelatin, Hydrolysates, Bone growth, Porcine skin**

I. INTRODUCTION

Bone is an organ composed of cortical and trabecular bone, cartilage, haemopoetic and connective tissues (Ham, 1974). Growth takes place at the epiphyseal growth plate of long bones by a finely balanced cycle of cartilage growth, matrix formation and calcification of cartilage that acts as a scaffold for bone formation. Another feature of bone growth is a process of modelling, where bone is being continuously resorbed and replaced by new bone. Modelling is most active during childhood and adolescence, and enables long bones to increase in diameter, to change shape and develop a marrow cavity (Price, Oyajobi & Russell, 1993). The hormonal regulation of skeletal tissues has been extensively studied. Important local mediators are cytokines and growth factors. These are soluble peptides produced by cells that can act in an autocrine, paracrine or endocrine manner (Price, Oyajobi & Russell, 1993). *Osteoblasts* are the cells responsible for the formation and organization of the extracellular matrix of bone and its subsequent mineralisation. They are derived from mesenchymal precursor cells in marrow that have the potential to differentiate into fat cells, chondrocytes or muscle cells (Owen & Ashton, 1986; Beresford, 1989). The origin of osteoblastic cells in the developing long bones is less well defined. One hypothesis is that osteoblasts are derived from blood-borne elements. This view is supported by evidence that cells in empty lacunae express type I collagen mRNA and are morphologically similar to osteoblasts, but unlike hypertrophic chondrocytes do not express type X collagen mRNA. The skeleton is also of central importance in mineral homeostasis, bone being the principal reservoir of calcium, phosphorous, sodium, magnesium and carbonate. Microscopically, cortical bone tissue is made up of a number of cylindrical units, the osteons, at the centre of which is a Haversian canal containing blood vessels and nerves. This canal is surrounded by up to half a dozen layers of bone, described as lamellae. In cortical bone, osteons have a well defined longitudinal arrangement. Most bone in the adult is described as lamellar bone since the collagen fibres assume an ordered arrangement in thin sheets. Where bone is formed very rapidly, such as occurs prenatally, in the rapidly growing child, or during fracture repair, the collagen fibrils often assume a very irregular orientation. This is mechanically weak, non-lamellar or woven bone. Woven bone is gradually replaced by mature bone or included within its fabric. The epiphyseal growth plate is made up of three tissue types: the cartilage component divided into distinct zones, the bony tissue of the metaphysis and the fibrous tissue that surrounds the growth plate. Type II collagen is the most abundant of the collagens in the growth plate, and since it is found almost exclusively in cartilage it is a specific phenotype marker for chondrocytes. Type II collagen is composed of three identical chains that are wound into the characteristic triple helix of the collagen molecule (Burgeson & Nimni, 1991). Type XI collagen, also a class I collagen, is present in cartilage matrix and is integrated into the interior of type II collagen fibrils (Mayne, 1989). The objective of this study was to investigate the effect of porcine skin gelatin hydrolysates on longitudinal bone growth of SD-rats and its mechanism.

II. MATERIALS AND METHODS

Gelatin hydrolysates from porcine skin were obtained from commercial company (Geltec Inc., Seoul, Korea)and separated by enzyme hydrolysis for 4 hrs at 50℃. The 36 hydrolysates were separated by their molecular weight such as

<50kDa, 50kDa>sample>3kDa, and <3kDa, respectively using membrane filtration. The effect of hydrolyzed gelatin on bone cells, osteoblasts (MG63, human source), was measured. Also, the highest bone cells proliferating gelatin hydrolysates (colpep 26) was separated and supplemented to 3 week-old SD rat for 5 days and femur bone length and length at the epiphyseal plate was determined to confirm bone growth. Osteoblasts produce osteoid, which is mainly composed of Type I collagen. Osteoblasts are also responsible for the mineralization of the osteoid matrix.

III. RESULTS AND DISCUSSION

Fig. 1 shows osteoblast cell proliferation after treatment of colpep 26, less than 3kDa, 26th fraction was selected for activating cell proliferation. The fraction was prdoduced from enzyme hydrolysis for 4 hrs at 50℃. The fraction was supplemented to 3 week-old SD-rats for 5 days and the femur length was determined. Gelatin not hydrolized did not show any significant difference (Fig. 2), while the colpep 26 supplemented with high dose (0.5 ml/100 g) showed significant bone growth of 44% compare to control (Fig. 2 and 3). Also, length at the epiphyseal plate was determined to confirm bone growth (Fig. 4. and 5). A high dose of colpep 26 significantly increased the epiphyseal plate compare to the low dose of colpep 26 and control. Fig 5 shows increased size of epiphyseal plate. Insulin-like growth factor 1 (Igf1) is reputed to augment longitudinal bone growth by stimulating growth plate chondrocyte proliferation (Wang, Zhou, and Bondy, 1999). BMPs were originally described as osteoinductive cytokines that enhance bone and cartilage formation when injected into mice (Sampath et al., 1992). Many studies have demonstrated that BMPs also normally function in the regulation of embryonic development and cellular homeostasis as well, including the regulation of proliferation, differentiation, apoptosis and remodeling of the extracellularmatrix (Hogan, 1996). Fig 6 shows supplementation of colpep26 remarkably expressed on hydpertrophic and occification zone of the bone. Similar result was shown in BMP-2 protein in both zones as well. Bone tissue is framework of calcium phosphate deposits, hydroxyapatite, on a collagen matrix. Bones are always undergoing remodeling; whether for growth, repair via osteblasts or renewal of worn-out bone via osteoclasts. The bones are also involved in maintaining blood calcium homeostasis. Mature bone changes in shape, size, strength dependent on diet, exercise, age, and compressive stress. Also bone cells regulated by hormones such as vitamin D, HGH, sex hormones, parathyroid hormone (PTH). The vitamin D increases absorption of calcium in intestines. HGH stimulates epiphyseal plate, while sex hormones stimulate osteoblsts during puberty. The parathyroid hormone (PTH) removes calcium from bone to raise falling blood calcium levels by stimulating osteoclasts. Calcitonin adds calcium to bone by stimulating osteoblasts. Determinaiotn if these hormones would be done to investgate bone growth mechanisms in human body. From these results, it can be suggested that supplementation of the porcine gelatin hydrolysates may promote bone growth of SD-rat not only bone length but also epiphyseal plate via increasing IGF-1 and BMP-2 protein and could be used for health benefit food sources in the market.

IV. CONCLUSION

In conclusion, the gelatine hydrolysates promoted osteoblast up to 44% compare to control and bone growth of SD-rat in young age, this suggests that low molecular weight less than 3kDa gelatin hydrolysates from porcin skin could be applied for functional food material.

ACKNOWLEDGEMENT

This research was supported by Grant from the Korean Rural Development Administration. The authors acknowledge a graduate fellowship provided by the Rural Development Administration through the Agenda system Projects, Korea.

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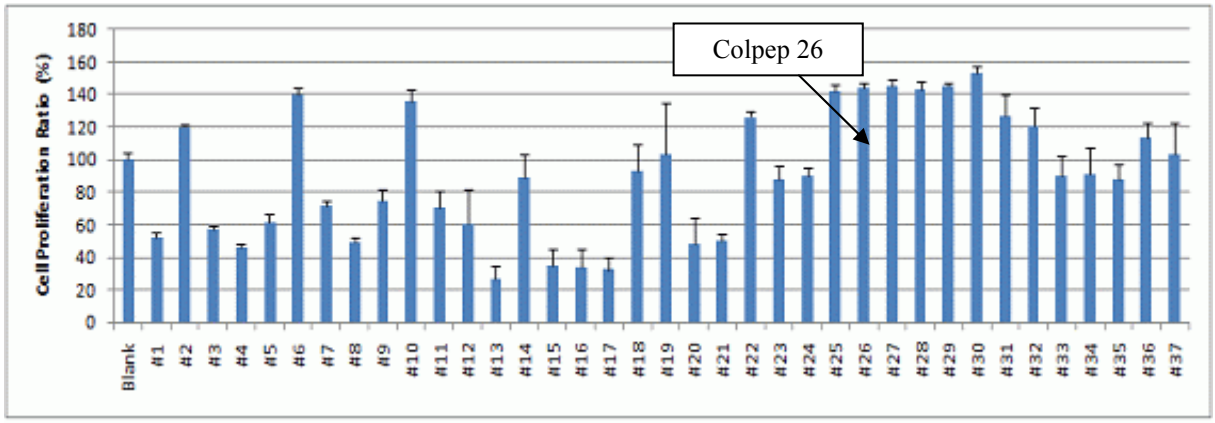

Fig. 1. Proliferation of MG 63 osteoblast after gelatine hydrolysates treatmetment whith different moclecular weight

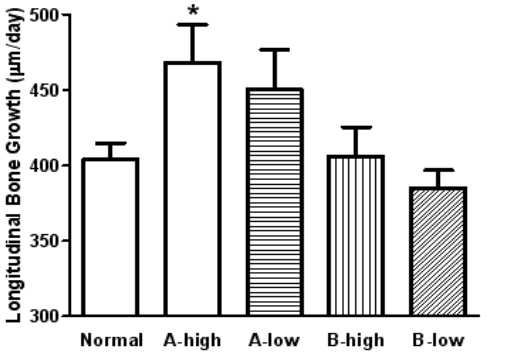

Fig. 2. Growth effect of colpep 26 on Longitudinal bone of SD rat
A high: colpep 26 0.5 ml, A-low:colpep 0.1 ml,
B-high: gelatine 37 0.5 ml, B-low: gelatine 37 0.1 ml

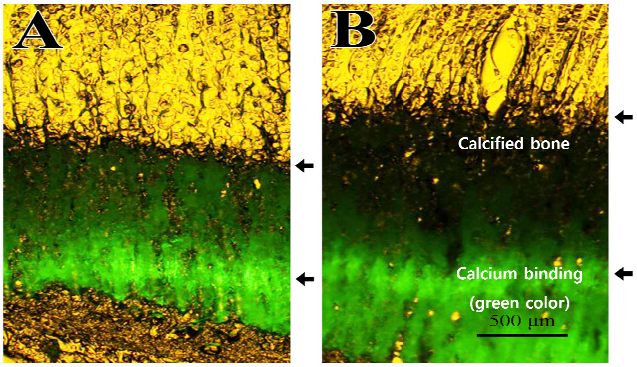

Fig. 3. Bone growth of SD-rat Femur after supplementation colpep 26
A: Without colpep 26 , B: With colpep 26 0.5ml

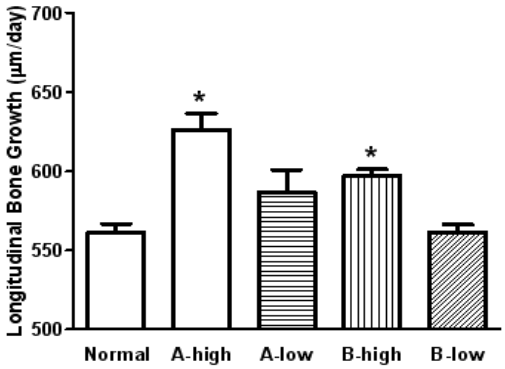

Fig. 4. Growth effect of colpep 26 on Growth plate of SD rat
A high: colpep 26 0.5 ml, A-low:colpep 0.1 ml
B-high: gelatine 37 0.5 ml, B-low: gelatine 37 0.1 ml

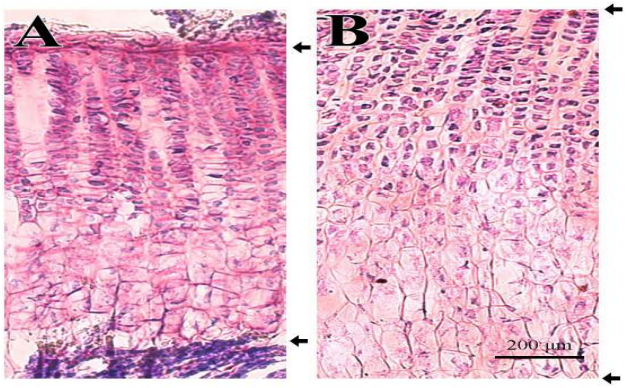

Fig. 5. Growht plate cell of SD-rat Femur with supplementation colpep 26
A: Without colpep 26 , B: With colpep 26 0.1 ml 0.5ml

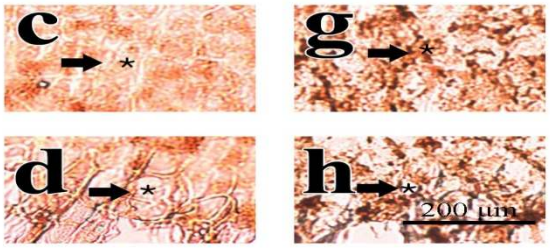

Fig. 6. IGF-1 expression on logitudinal bone of SD-rat
c: without colpep 26 on hypertrophic zone
g: with colpep 26 on hypertrophic zone
d: without colpep26 on occification zone
h: with colpep 26 on occification zone

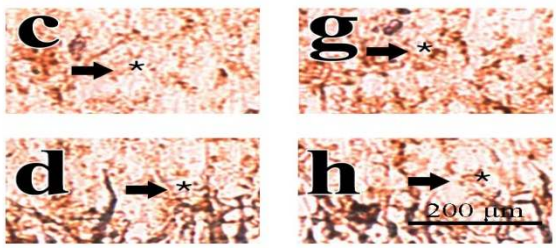

Fig. 7. BMP-2 expression on logitudinal bone of SD-rat
c: without colpep26 on hypertrophic zone
g: without colpep26 on hypertrophic zone
d: without colpep26 on occification zone
h: with colpep 26 on occification zone