Vascular Endothelial Growth Factor and Bone Morphogenetic Protein Expression after Induced by Gurami Fish Scale Collagen in Bone Regeneration

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Abstract

Periodontal tissue damage due to periodontitis is irreversible damage resulting in tooth loss. Regenerative treatments using graft materials have now developed very rapidly. Collagen, especially collagen type 1, is one of the ingredients that can stimulate the growth of bone, it is now widely developed for regeneration. Development of collagen derived from fish scales can be used as an alternative regeneration material, especially for bone regeneration. Bone morphogenetic protein 2 (BMP-2) and vascular endothelial growth factor (VEGF) are markers of bone regeneration. The aim of this study is to observe the expression of BMP-2 and VEGF in the application of collagen of gourami scales. Thirty-two experimental animals were randomly divided into 4 groups, such as 7 days control group, 7 days fish collagen group, 14 days control group and 14 days fish collagen group. The expression of BMP-2 and VEGF was analyzed under immunohistochemical analysis. Expression of BMP-2 has increased in 7 days and 14 days group and expression of VEGF has increased in 7 days group but has decreased in 14 days group after application collagen type I of gouramy scales. Application of gouramy scales collagen (Osphronemus gouramy) can increase the expression of BMP-2 and VEGF, it can accelerate angiogenesis and osteogenesis.

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Introduction

The most serious consequence of periodontal disease is loss of support of periodontal structures, which include cementum, periodontal ligaments, and alveolar bone. Periodontal tissue damage that involves bone can be repaired by the augmentation method. Bone augmentation is a regenerative therapy to restore periodontal tissue. [1–3]

Bone augmentation is a complex process involving a number of cellular functions in bone formation and mineralization followed by remodeling so that it can reach its original structure. The success of new bone formation requires and is influenced by several important components, namely progenitor cells, signaling molecules, and scaffold in damaged tissue. Scaffolds play a role in stimulating osteoblast precursors. [4,5]

Bone morphogenetic protein (BMP) is a protein that is a member of the family transforming growth factor beta, which plays an important role in osteogenesis. BMP-2 and BMP-4 expression increase at the beginning of the latent phase, possibly to help the process of differentiation of precursor cells into chondrogenic or osteogenic cells. [6–8] In the process of life, bone tissue requires adequate vascularization. One of the factors involved in the formation of new blood vessels is vascular endothelial growth factor (VEGF). [9–11]

Fish scales are an alternative source for collagen production. Gurami fish scales extract (Osphronemus gouramy) contains type I collagen. Pore size of gurami fish collagen extract ranges from 191.6 - 385.3 µm, where the optimal porosity size for bone regeneration ranges from

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100-500 µm. The size of collagen scaffold porosity plays a role in providing a place for cells to penetrate and develop in the scaffold, namely the process of cell attachment, cell migration, matrix deposition and vascularization in this collagen scaffold.[12]

Previous study found that collagen from Gurami fish scales extract has a good viability for osteoblast[13] and fibroblast cell culture[12]. The aim of this study is to observe the expression of BMP-2 and VEGF in the application of collagen of gourami scales.

Materials and methods

This study received a Code of Ethical Clearance from the Faculty of Dentistry, Airlangga University 656 / HRECC.FODM / X / 2019. This research was an experimental laboratory study using a male Wistar Rat (Rattus norvegicus), 3 months old with a body weight of 150 - 200 grams.

The sample was chosen randomly and was determined using the Lemeshow sample formula, obtained a sample of 8 for each sample group. The first group was the control group while the second group was the treatment group. The first group will be the control group, the mandibular incisor extraction socket is left filled with blood (control group). In the second group the mandibular incisor retraction socket is filled with collagen. BMP-2 and VEGF marker expressions were seen 7 and 14 days later.

Extraction of gourami fish scales collagen; washing fish scales, taken as much as 100 grams and soaked in 1 M NaOH solution for 24 hours to remove non-collagen protein. Followed by the addition of acetic acid (acid solubility collagen) 0.5 M and the addition of the enzyme pepsin (pepsin solubility collagen) 0.1 gr, then stir with an ultrasonic device at 4° C then washed with distilled water and carried out filtering to get the filtrate. Furthermore, the filtrate was added 0.5 M NaCl then centrifuged in small tubes at a speed of 4000 rpm using a 15 ml tube for 10 minutes to obtain supernatant and precipitate. The supernatant is removed, while the precipitate (collagen) is taken. Salting out and followed by lyophilization process with a freeze dryer to remove water with a condenser temperature of -76° C and ambient temperature of 23.6° C for 12 hours until the water runs out. The process of sterilization of the collagen results is carried out with Ethylene Oxide.

Data were analyzed and expressed as mean ± standard deviation. The statistical differences between the groups were evaluated using a way analysis of variance (ANOVA) and LSD post hoc test. In all the analyses, $p< 0.05$ was considered to indicate a statistically significant difference.

Results

Quantitative observation of BMP-2 and VEGF expression was carried out with immunohistochemical techniques under a light microscope with 200x and 400x magnification with 20 fields of view (figure 1 and 2), then counted the number of osteoblast cells expressing BMP-2 and VEGF markers using image tools were then recorded.

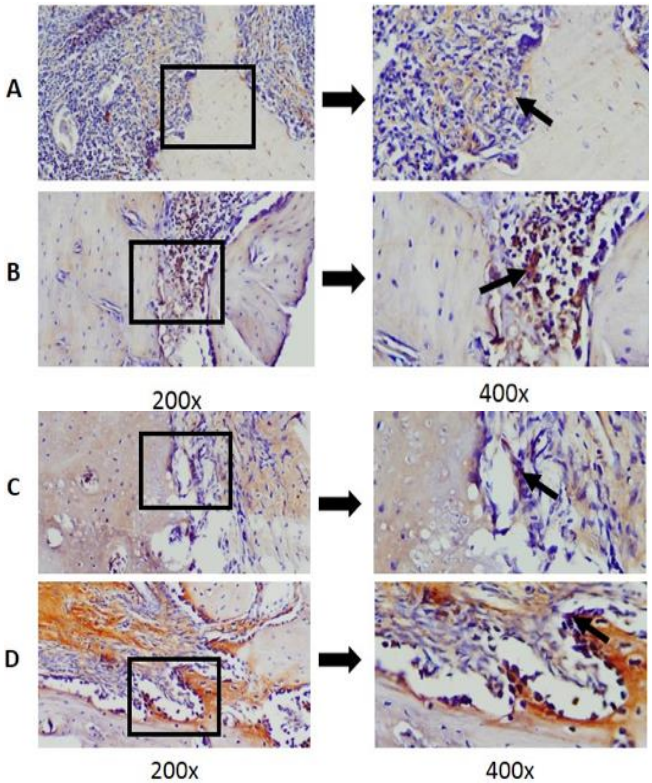

Figure 1. BMP-2 expression 7th and 14th day (black arrow). (A) C7, (B) T7, (C) C14, (D) T14. These pictures were taken at 200x and 400x magnification.

Group observation	Mean	
	7th day	14th day
C	7,75+1,66905	8,50+2,32993
T	14,75+3,24037	16,125+2,29518
p-value	0,000	0.000

Table 1. BMP-2 ANOVA test results for control group and treatment group.

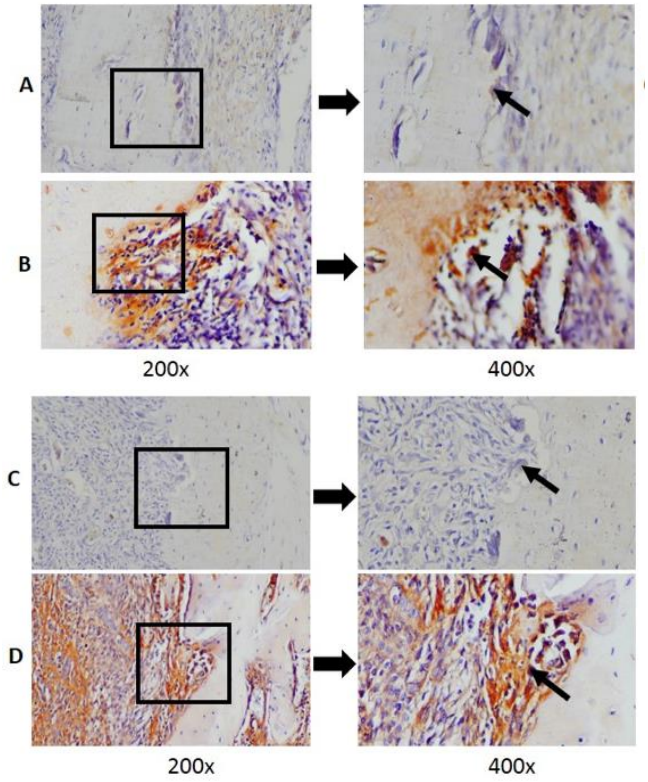

Figure 2. VEGF expression 7th and 14th day (black arrow). (A) C7, (B) T7, (C) C14, (D) T14. These pictures were taken at 200x and 400x magnification.

Group observation	Mean	
	7th day	14th day
C	4,50+1,43925	10,875+2,60151
T	10,9375+3,70750	4,375+1,64208
p-value	0,000	0.000

Table 2. VEGF ANOVA test results for control group and treatment group.

Discussion

The results of this study indicate that BMP-2 expression was significantly different, an increase in BMP-2 expression between the control and treatment groups on the 7th and 14th days. There was a significant increase in VEGF expression between the control and treatment groups on the 7th day and in the treatment group decreased significantly on the 14th day, compared to the control group.

BMP-2 is a group of growth factors that were originally discovered because of its ability to induce bone formation, so it is a biomarker that is very important to observe the activity of osteoblast cell proliferation.[6] BMP-2 expression reached its peak on day 14.[9] In this study showed an increase in BMP-2 expression on day 14 compared to expression on day 7. It can be explained that BMP-2 in rat has higher expression with the addition of collagen. Activation of the ERK signal pathway stimulates the expression of osteoblast-specific biomarkers including BMP-2 and VEGF, which shows that BMP-2 and VEGF act as bone growth regulators.[14]

The extra-cellular regulated kinase (ERK) pathway is a branch of the mitogen activated protein kinase (MAPK) pathway, which causes cell interactions with extracellular matrix and activation of runt-related transcription factor 2 (RUNX2).[14] Collagen acts as a chemical signal that can bind to the extracellular matrix of osteoprogenitor cells (integrin α2ß1). The interaction of type I collagen with integrin α2ß1 causes the activation of the extra-cellular regulated kinase (ERK) pathway, thereby causing phosphorylation and the potential for RUNX2 transcription. Then the RUNX2 bond called OSE2 (osteoblast-specific cis acting element 2) stimulates the expression of osteoblast specific genes including BMP-2, VEGF, TGF-ß, ALP, osteocalcin, osteopontin, and type I collagen so that it can be summarized that type 1 collagen from gurami scales induces osteoblast cell proliferation.[15]

Bone is a network that requires vascularization. Therefore, local reconstruction of microcirculation is an important thing for bone regeneration. Inhibited the process of angiogenesis will reduce the process of bone formation. VEGF is an important regulator of vascularization development and plays an important role in the development and repair of bones, contributes to combining the processes of osteogenesis and angiogenesis, controlling the function and differentiation of osteoblasts. During normal bone regeneration, VEGF expression increases initially and peaks on day 14.[9–11,16]

VEGF can increase vascular permeability after promoting local angiogenesis and will facilitate the recruitment of mesenchymal stem cells (MSCs) and osteoprogenitor cells thereby indirectly increasing the ability of bone regeneration.[16] VEGF can also directly attract MSC and promote osteogenic differentiation. Increased neovascularization and bone regeneration are induced by controlled VEGF expression. In addition to increasing angiogenesis and MSC recruitment, VEGF act synergistically with BMP to improve cell survival, bone formation and resorption, and bone mineralization. Studies show that the synergistic effect of VEGF and BMP is not only due to increased angiogenesis. BMP-2 itself has angiogenic activity, which leads to a decreased reaction to VEGF.[9] VEGF also stimulates the differentiation and activation of osteoclast cells. Therefore, at the remodeling stage, reduction of VEGF from osteoblast cells will reduce osteoclast recruitment.[17] In the research data it was found that VEGF expression in the treatment group day 14 decreased significantly due to BMP pathway as VEGF antagonists.

VEGF works autocrine and paracrine. Increased VEGF expression causes an increase in angiogenesis and osteogenesis through activation of β-catenin signaling in osteoblasts via VEGFR2 receptors which results in cell migration and proliferation. In addition, VEGF expression of osteoblasts also works paracrine against endothelial cells thereby stimulating vascularity and involving the secretion of an antagonist (BMP) to promote the formation of bone maturation.[17] This shows the cross regulation between BMP-2 and VEGF.

Conclusions

Increased expression of BMP-2 and VEGF after the application of type I collagen scaffold derived from extracts of gurami fish (Osphronemus gouramy) in male wistar rats. This shows an increase in osteogenesis and angiogenesis in the process of bone regeneration after the application of collagen from gurami scales.

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Declaration of Interest

The authors declared that there is no conflict of interest.

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