The MMP-2, MMP-9 Expression and Collagen Density of the Ambonese Banana Stem Sap Administration on Wound Healing

Hendrik Setia Budi[1]*, Eha Renwi Astuti[2]

1. Department of Oral Biology, Faculty of Dental Medicine Universitas Airlangga, Surabaya, Indonesia.
2. Department of Dentomaxillofacial Radiology, Faculty of Dental Medicine Universitas Airlangga, Surabaya, Indonesia.

Abstract

Matrix metalloproteinases (MMP) will degrade of the extracellular matrix and proteins, including collagen, elastin, gelatin, matrix glycoproteins and proteoglycans. It affects the BMP in the process of differentiation and new bone tissue remodeling. Ambonese banana (Musa paradisiaca var. Sapientum) have potency to accelerate wound healing throught platelet derived growth factor (PDGF-BB) signaling.

The research was conducted to prove the role of ambonese banana stem sap gel (GEGPA) on the MMP-2 and MMP-9 expression affected to collagen density of tooth extraction wound healing. This study used 24 male wistar rats were divided into 2 groups: one is a control without being treated, and the other one is a group GEGPA dose 60 mg in 4% Hydroxypropyl Methylcellulose. All groups were examined for the expression of MMP-2, MMP-9, and collagen density in alveolar socket areas on day 7 and 14.

The results showed there were significant differences in the expression of MMP-2, MMP-9, and collagen density in the alveolar socket healing between day 7 and 14 (p=0.00 and p=0.00). There was a strong correlation p=0.963 between the inhibition of MMP-2, MMP-9 and the increasing of collagen density.

It is concluded that GEGPA increases collagen density on wound healing of tooth extraction through inhibition MMP-2 and MMP-9.

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Introduction

Wound healing process is basically the same, but the infection, surgical interventions, and medications that can affect accelerate of healing. The purpose of local therapy to inhibit the excessive bleeding then the healing process is not disturbed, and minimise systemic side effect.[1,2] Normal wound healing is quite complex, involving inflammatory cells and growth factors that influence each other in every phase of the healing. It begins with formation of a blood clot consisting of fibronectin and fibrin. A blood clot is a crucial stage in the process of wound healing.

*Corresponding author:
Hendrik Setia Budi
Department of Oral Biology
Faculty of Dental Medicine, Airlangga University
Surabaya, Indonesia, Tel. +628123273272
e-mail : hendrik-s-b@fkg.unair.ac.id

The phase of inflammation is dominated by neutrophils and macrophages, followed by reepithelial and granulation cell formation. The next stage is the remodeling of granulation tissue by replacing it with connective tissue dominated by fibroblasts.[3,4] In the healing of infected wounds or in patients with diabetes mellitus, the tissue around the wound is dominated by inflammatory cells such as neutrophils and macrophages in a long term, so the formation of connective tissue is inhibited.[5,6,7]

Neutrophils and macrophages are producing Matrix metalloproteinase (MMP) cells. MMP is a proteolysis enzyme involved in extracellular matrix degradation.[8] MMP is a family of zinc-dependent endopeptidase, a large collection of enzymes responsible for tissue remodeling and degradation of various components of the extracellular matrix, including collagen, elastin, gelatin, matrix glycoproteins and proteoglycans.[9,10] Bone remodeling is formed from collagen scaffold that stimulates osteoblasts to grow through the Bone

Morphogenetic Protein (BMP). In the process of healing wounds, the high MMP level can inhibit healing in remodeling. The medical treatment to inhibit or decrease MMP around the wound are necessary. In the case of periodontal diseases such as periodontitis aggressive, MMP produced in the surrounding tissue causes damage of periodontium tissue and alveolar bone. It is also related to an increase in bacterial colonies such as Actinobacillus actinomycetemcomitans. Tetracycline is the right choice to inhibit MMP activity as anticollagenase.[11]

Ambonese banana (Musa paradisiaca var.sapientum), is a plant found in Indonesia, especially in areas with a lot of sunshine. Plants that contain lectins with high concentrations can be used for wound healing through the process of coagulation or blood clot formation.[12] Previous study of galectin-3 which is a class of lectins, showed an increase in Vascular Endothelial Growth Factor (VEGF) and Basic Fibroblast Growth Factor (bFGF) on angiogenesis response.[13]

The topical administration of banana stem sap with 30 and 60 mg doses in the socket area showed an increase of fibroblasts and osteoblasts through the expression of PDGF-BB, BMP-4 and BMP-7 in the wound healing process of rats tooth extraction.[14] This aims of study to prove the role of ambonese banana stem sap gel (GEGPA) on the MMP-2 and MMP-9 expression affected to collagen density of tooth extraction wound healing.

Materials and methods

This study was approved by the Ethical Committee of the Faculty of Dental Medicine, Universitas Airlangga, Indonesia. This study use the post-test only control group design. The sample was male rats (Rattus novergicus) Strain Wistar, 3 months of age, weighing 200-300 grams, in healthy condition, and obtained from the Biochemistry Laboratory, Faculty of Medicine, Airlangga University.

The sap can be obtained in large quantities by weighing the banana stems about 200 grams and putting them into a blender by adding 200 cc of sterile distilled water. Blend for 5 minutes until smooth, and then conduct filtration using Whatman filter paper no.1. The filtrate was dried using a freeze dry. The dry powder was obtained from different types of stocks above, and then gel form was created with hydroxypropylmethylcellulose (HPMC) so that the content of the active compound can easily be attached to the injured area and quickly absorbed, with a dose of 60 mg in 4% HPMC.

Rats were anesthesized according to the weight (30 mg / kg theo Nembutal). Tooth extraction was performed on mandibular incisive using special pull pliers. In the control group socket administrated topically of 4% HPMC. Treatment group socket were administrated 60 mg in 4% HPMC. The ginggival mucosa was sutured using a 5-0 vicryl (Ethicon; Johnson & Johnson do Brasil, São Jose dos Campos, SP, Brazil). Each group was observed for the MMP-2 and MMP-9 expression as well as collagen density on day 7 and 14 after tooth extraction.

Immunohistochemistry examination of MMP-2 and MMP-9 expression. The slide was blocked with 3% H2O2 in PBS incubation for 20 minutes and at room temperature. Slides were washed with PBS pH 7,4 and blocked with 1% BSA in PBS for 60 minutes. Slide was labeled with a primary antibody anti MMP-2 or MMP-9 in 1% BSA overnight at 4° C. Slides were washed with PBS pH 7,4 three times for 5 minutes. The slide labeled with secondary antibody goat anti rat IgG biotin for 1 hour at room temperature. The washing was done with with PBS pH 7,4 three times for 5 minutes. Slide incubation was performed with SA-HRP (Streptavidin-Horseradish Peroxidase) 1:500 for 40 minutes at room temperature. Slides were washed with PBS pH 7,4 three times for 5 minutes. The slide was dripped with substrate chromogen DAB (diamino benzidine tetrahydrochloride 3.3) for 20 minutes. Slides were washed with PBS pH 7,4 three times for 5 minutes and proceeded with dH_2O three times each for 5 minutes. Counterstain was performed with Methyl Green 1% at room temperature. The slide was soaked with tap water for 5 minutes and dried overnight at room temperature. Mounting and cover with a cover glass. The slide was viewed with a light microscope[15].

Formation of collagen in the wound healing process can be observed in histopathological tissue, using Masson Tricome staining. The area of collagen fibers is a percentage (%) of collagen density calculated by dividing the area of connective tissue collagen fibers formed at the center of healing (healing

center) with a total area of measurement[16,17].

Data were represented as Mean ± Standard deviation (SD) of each group. Statistical significance was determined by one-way ANOVA, and correlation test between variables p<0.05.

Results

Data examination of MMP-2 and MMP-9 expression on the socket healing day 7 and 14 with immunohistochemical staining were showed brown color cell (figures 1 and 2). The number of brown cells were calculated and compared between the treatment group and the control group.

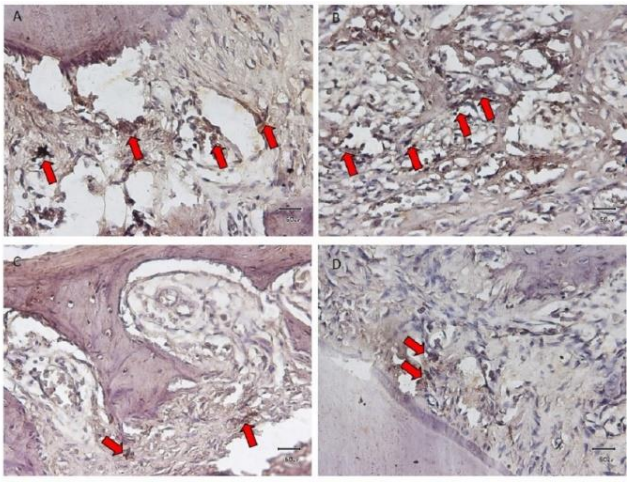

Figure 1. Expression of MMP-2 (red arrow) on the healing sockets day 7 and 14 with 400x magnification microscope. A.B. The control group day 7 and 14. C.D. The treatment group day 7 and 14.

The cells number expressing MMP-2 and MMP-9 showed a significant difference between treatment and control groups with p<0,05 (p=0,00 and p=0,00) (table 1).

The collagen fibers showed on alveolar sockets healing area (Figure 3). Extensive collagen fibers in the alveolar socket healing day 7 and 14 showed a significant difference between treatment and control groups with p <0,05 (p = 0,00 and p = 0,00) (Table 1).

GROUPS	MMP-2		MMP-9		Collagen	
	Day 7	Day 14	Day 7	Day 14	Day 7	Day 14
Control	23,33 ± 2,06[a]	31,00 ± 0,89[a]	27,83 ± 2,32[a]	31,50 ± 2,26[a]	24,26 ± 3,07[a]	75,95 ± 2.58[a]
Treatment	15,67 ± 1,96[b]	17,83 ± 2,13[b]	16,50 ± 2,35[b]	20,00 ± 1,41[b]	60,52 ± 5,40[b]	82,92 ± 4.45[b]

Description: difference on superscript showed there were significant differences.

Table 1. The cells expressing MMP-2 and MMP-9 on the alveolar sockets healing day 7 and 14.

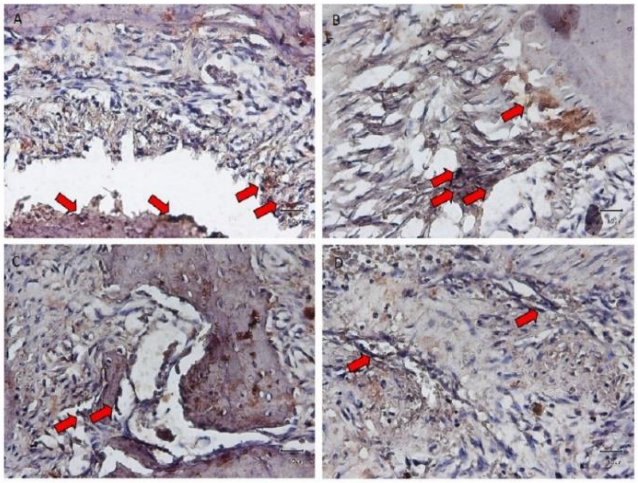

Figure 2. Expression of MMP-9 (red arrow) on the healing sockets day 7 and 14 with 400x magnification microscope A.B. The control group day 7 and 14. C, D The treatment group day 7 and 14.

Function	Eigenvalue	*% of Variance*	*Cumulative %*	*Canonical Correlation*
1	12.654[a]	*100.0*	*100.0*	.963

a. First 1 canonical discriminant functions were used in the analysis.

Table 2. Correlation test between groups.

Discussion

The alveolar healing process post tooth extraction and implant are similar in bone regeneration. Inflammation and alveolar bone resorption are response post tooth extraction. Inflammation is a response which occurs as a result of injuries, and the cause of inflammation should be immediately removed so not to interfere even inhibit the regeneration.[18,19]

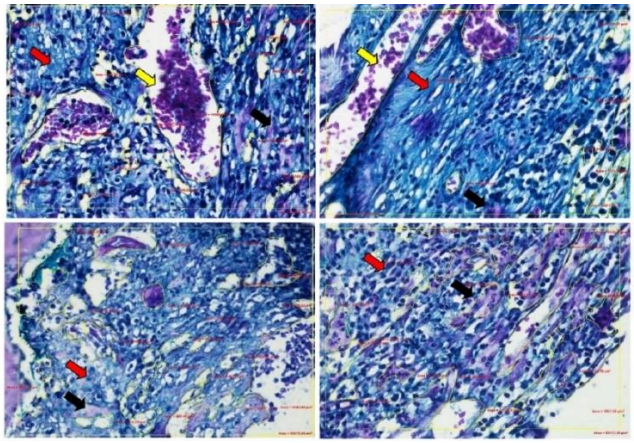

Figure 3. Collagen formation in the healing socket day 7 and 14 with 400x magnification microscope A.B. The control group day 7 and 14. C, D The treatment group day 7 and 14. Red arrow collagen, black arrow new bone, and yellow arrow blood cloth.

Inflammatory cells and fibroblasts will migrate to the socket, especially at the bottom end in day 2 after tooth extraction, and then fibroblasts will synthesize collagen fibrils gradually. Both chondroblasts and fibroblasts will form capillaries and granulation tissue, then fill the matrix extracellular in order to be stable.[20,21,22]

On day 1 socket was only filled by a blood clot containing fibrin and blood cells. Periodontal ligament cells were dominant in the socket, and began to rise rapidly on the day 2.[23,24] The process of bone resorption starts on the alveolar crest which coincides with bone apposition in the socket. Mesenchymal cells, which are an embryo of the periodontal ligament and bone cells, differentiated into osteoblasts and secretes bone matrix on the day 7 of healing socket. At the end of the healing socket, a thin layer of trabecular bone was produced at the start of day 14 and continued for several months.[25]

On the healing process at day 7 showed many areas nof filled bone matrix (Figure 1, 2 and 3). The treatment group seen fairly dense fibroblasts that indicates the material is capable to inhibit the inflammation that occurs after rats tooth extraction compared to the control group. This result similiar with Vieira et al. study,[26] which explains that the degradation of connective tissue, especially caused TGF-β1 bind to receptors of Smad2 become reduced, thereby affecting the activation of BMP in the process of differentiation and new bone tissue remodeling.

The healing socket on the 7th day showed increased expression of PDGF-BB compared to the 2nd day.[14] PDGF-BB showed activity after being attached to a receptor that is PDGFR-α and PDGFR-β as chemotaxis and differentiation of mesenchymal cells.[27] Once attached to a receptor on the cell membrane, it occurred to phosphorylate the protein signaling of Ras-Raf-MAPK pathways which led to the proliferation and the synthesis of extracellular matrix and the Jak-Stat pathway to control the synthesis of inflammatory cytokines.

As chemotaxis and strong differentiation in some cells, the fibroblasts and osteoblasts seem to accumulate at the injured area and then will form the bone matrix. The accumulation of fibroblasts and osteoblasts showed healing activity in the socket, and can be characterized by decreasing MMP around the wound.[26] This is consistent with research that shows the expression of MMP-2 and MMP-9 in the control group was higher than the group that was given GEGPA.

The content of some of the active compounds in ambonese banana stem sap can help in the healing process of wounds. Lectins are proteins that have the effect as an antitumor drug with Human Immunodeficiency Virus (HIV), antimicrobial, mitogenic, and specifically binds to specific carbohydrate part of the glycolipid or glycoprotein molecules.[28] Teh binding of lectins on bacterial cell membrane glycoprotein will form a bond mannose Binding Lectin (MBL) and then will activate the complement C1 for deposed C3b on the bacterial. The increased disposition C3b will activate C3a and C5a to attract leukocytes to destroy bacteria. Complement C3b binds to C3b-receptor on phagocytes to activate the phagocytosis process. Additionally, C3b deposition will form MAC (Membrane Attack complex) that plays a role in lyse bacteria, thus minimizing the occurrence of infection and speed the wound healing.[29]

In addition to the content of lectins, in extracts of ambonese banana stem sap there are also saponins, flavonoids, saponins and tannins that contribute to wound healing process. Saponins and lectins can cause hemostatic and antibacterial effects that contribute to the process of wound healing. Saponins and lectins on banana stem sap modulate the immune response to an increase in T cell lymphocytes with markers CD3 +, CD4 + and CD8 + T cells

and hematopoietic systems so as to minimize the occurrence of infections.[30] The role of T cells is a lymphocyte activation of B cells, NK cells and macrophages when there was antigen.[31] In healing process, the tannin content in the sap of banana trees acts as a hemostatic and antibacterial through the protein precipitation mechanism of blood cells and bacteria then causes coagulation.

Besides, tannins and anthraquinone also serve as a donor to the free radical and Reactive Oxygen Species (ROS). Free radicals cause lipid peroxidation to form with the final result of malondialdehyde (MDA), which is a compound that causes damage of cell proteins and DNA.[32,33] The content of flavonoids in the banana stems, such as leucocyanidin and anthocyanins, have the ability as antiiflammation through the barriers of the enzyme cyclooxygenase, thus inhibiting prostaglandin synthesis and as an antioxidant by oxidizing free radicals. Inhibition of free radicals in inflammatory disorders can reduce oxidative stress, and cell damage is not widespread.[34]

Conclusions

The conclusion that GEGPA increased collagen density on wound healing of tooth extraction through inhibition MMP-2 and MMP-9.

Declaration of Interest

The authors report no conflict of interest.

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