Increase of CD 34 in Bone Defect Healing Lesions of Periodontitis by Capsaicin Administration

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Abstract

The relationship between the increase of CD 34 and TRPV-1 stimulation after being given by capsaicin in bone defect healing. Twenty-one male wistar rats were disorderly divided into three categories (each category=7): the first (K0=negative control), the second (K1=positive control), and the third (K2=treatment). Both K0 and K1 were only given with serotype b Aggregatibacter actinomycetemcomintas (A.a) to the cervical part of area of the first molar. The K2 category was given by serotype b A.a and cayenne pepper extract at dose 0.0912 mg/kg/day. After a week, the rats were sacrified using thionembutal with dose 30 mg/kg in accordance with their weight and for manufacturing sample specimen from gingival tissue region by immunology testing using immunohistochemical method. The acquired data were contrasted using a one-way Anova test. Result confirmed both TRPV-1 compute and CD-34 in K2 category to be prominent divergence in both TRPV-1 and CD-34 than K1 and K0 ($p<0.05$), whereas there was no prominent divergence between K1 and K0 ($p>0.05$). Capsaisin promotes the number of CD 34, angiogenesis factors, and microbial agents that it can be confirmed as the alternative treatment for bone defect healing caused by periodontitis.

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Introduction

Bone defect healing is a serious problem in worldwide, especially in dentistry. It is defined as a disturbance of bone integrity caused by pathologic or physiologic causes and categorized from simple until complex bone defect. Complex bone defect usually disturbs epithelial integrity and causes some problems. Bone defect healing is strongly related with the haematopoietic stem cells (HSCs) which has been used in practice since past three decades. These cells to be liable in maintaining the blood system via very coherence process of self-renewal and differentiation. So that, comprehensive work have been suggested to recognize this mechanichal work of self-renewal, differentiation and homing of HSCs. One of the HSCs is Cluster of Differentiation 34 (CD 34) which is a maker factor of one of the innate immunity systems (Mast Cell/MC). Innate immunity has some important roles to prevent the invasion of foreign substances that enter to human body[1-3].

Nowadays, traditional medicine is popular in the worldwide and used to cure the diseases. One of the traditional medicines is capsaisin (trans-8-methyl-N-vanillyl-6-nonenamide).

Capsaisin is a herbaceous plant, with a lot of twigs on the trunk. It is an alcaloid crystal that has lipophilic activity, colourless, odourless. The chemical structure of capsaisin is $C_{18}H_{27}NO_3$. The previous experiment showed that 2 ml of administration capsaisin had minimum inhibitory concentration and activated TRPV-1, TNF-α, IL-6. The main cause of periodontal disease is periodontopatogen bacteria (gram negative bacteria). One of the periodontopatogen bacteria is A. Actinomycetem-comitans can provoke host immune system via secreting his inflammatory product (polysacharride). Polysacharride provoke host immune system (both innate and adaptive immunity) to secrete so many chemokines, cytokines pro inflammatory (TNF-α, IL-1).

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Cytokines and chemokines help to cure and provoke bone defect healing caused by the invasion of periodontopatheogen bacteria[4-6].

Materials and methods

This was an experimental laboratory research involving only posttest category form and all of the protocol researches have been accepted by the Ethics Committee of Faculty Dental Medicine Universitas Airlangga which number 007/HRECC.FODM/I/2018. The inclusion criteria for this research were: twenty one male Wistar rats from *Rattus novergius* species, their ages and weighing were 8-12 weeks and 150-250 grams. The number of samples used in this study was projected using Lemeshow formula. All of the rats were disorderly divided into three categories (each category=7): negative control category (K0), positive control category (K1), and treatment category (K2). The main difference among K0, K1, and K2 were all of these categories were given A. Actinomycetemcomitans bacteria serotype b on wistar's cervical section of their first maxillary first in a week until periodontitis was present, while K2 were also given cayenne pepper extract at dose 0.0912 mg/kg/day[6].

The histological examination consists of the counting of TRPV-1 and CD 34 expression. This examination was done by two mixed methodes (cell counter and manual counting). Rats were anesthetized in a week using thionembutal at dose 30 mg/kg according to their body and then being sacrificied to manufacture gingival tissue sample specimen. Specimen containing tooth sockets, fixed with 10% formalin for up to twenty four hours, decalcified with EDTA, and then followed by a histological examination stage to create block paraffin, so that the gingiva region could be longitudinally cut with a semi-serial thinnes of 4 µm to create microscopic preparation. A microcopic preparation was completed by calculating the TRPV-1 and CD 34 from expressed cells by immunohistochemical staining then the data was analysed using kolmogorov-smirnov to prove its normal distribution or not. As all the variables were normally distributed, the mean and standard deviation (SD) were analysed with SPSS 22 for Windows using ANOVA one-way test, and Tukey post hoc to obtain their significant among each category with p value of 0.05[6].

Results

Based on the results of this study on the increase of CD 34 after capsaicin administration. There were 8 experimental categories, such as: control positive (K0), control negative (K1), and other six treatments (K2). The results of the CD 34 and TRPV-1 are presented in table 1.

Category	TRPV-1 (X±SD)	CD-34 (X±SD)
K0=Control Negative	33.0150 ± 7.10[1]	7.46 ± 2.110[1]
K1=Control Positive	37.63 ± 110.60[1]	7.77 ± 2.588[1]
K2=Control Capsaicin	43.062 ± 14.98[2]	14.32 ± 4.58[2]

Table 1. The number of TRPV-1 and CD 34 in expressed cells. The superscript numbers in a column of the table show the significant different ($p<0.05$).

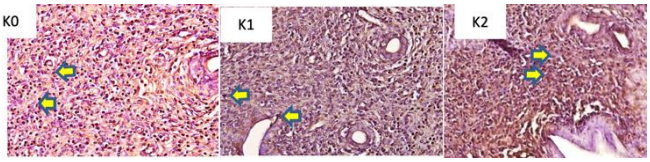

Figure 1. Immunohistochemical staining of TRPV-1 expression of K0, K1, and K2 category upon tooth socket of first maxillary molar using enlargement 400x. The yellow arrow shows the positive expressed cells.

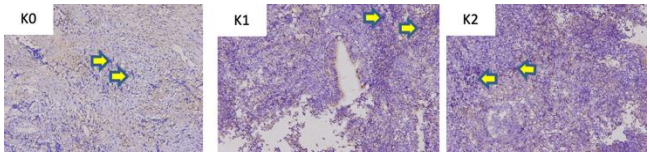

Figure 2. Immunohistochemical staining of CD 34 expression of K0, K1, and K2 category upon tooth socket of first maxillary molar using enlargement 400x. The yellow arrow shows the positive expressed cells.

Figure 1 and 2 show the immunohistochemical staining of the gingival socket of rat wistar males' gingival socket shows that the treatment of gingival portion of male wistars' rats, which was given both cayenne pepper extract and A. Actimomycetemcomitans serotype b, had more expressed cells TRPV-1 and CD 34 in the gingival socket than either the negative control and positive control. One-way Anova test confirmed that there was a prominent deviation among these three categories ($p=0.001$). The TRPV-1 count of the K0 category was extremely higher compared with those K1 ($p=0.567$) and K2 (0.00) categories. Meanwhile,

although K0 had more TRPV-1 count to those K1, but there was no prominent deviation (p=0.875)/ the observation results of expression CD 34 were a little distinct than to those relating to a TRPV-1 count. The expression of CD 34 in the K0 was higher than in the K1 categories and lower than K2 categories. The expression of each category significantly differed because the p value was 0.01.

Discussion

Bone defect healing commonly has similar and complex mechanisms, because it is related with the interaction between immunology and biology system[7,8]. The different of tissue restoration, that are trigerred by tissue damage, maybe united into a sequence of four stages: (i) coagulation and homeostasis, (ii) inflammation, (iii) proliferation, (iv) bone defect remodelling[8-11].

HSC, best known stem cells, has been used in practice since past three decades. These cells to be liable in maintaining the blood system via very coherenced process of self-renewal and differentiation. The most common extracellular signaling-regulated protein kinases 1 and 2 (ERK1 / 2) belong to the super family of mitogen-activated protein kinases (MAPK) that proceed stimulus from varied cell surface receptors to the cytosol and their target nuclei. Activation of the RAS / MEK / ERK cascade triggers increased cell proliferation and survival. ERK1 / 2 signaling is negligible for the proliferation and self-renewal of embryonic stem cells, whereas ERK is dependent on lineage commitment. In the hematopoietic system, in vivo analyse of ERK1 mice has shown the important purpose of ERK1 through maturation of thymocytes. Furthermore, based on ex vivo researches, the ERK pathway acts as an important role in regulating the differentiation of megakaryocytes, erythrocytes, macrophages, as well as granulocytes and monocytes. So that the activation of the ERK pathway can trigger HSC to leave the self-renewal program and go to the differentiation stage. In addition, there is more proof that the ERK1 / 2 signaling pathway may also be related to the control of other cellular processes of the hematopoietic system. The ending of HSC can be influenced by the periode of ERK activation and paracrine stimulation. The effect of ERK inhibition on cord blood cells was assessed after ten days in culture-free serum that contains stem cell factor (SCF), ligands such as Fms-like tyrosinekinase 3 (Flt3L) and thrombopoietin (TPO), during that the cells are under expansion. active. phase through proliferation and self-renewal. We confirm that activation of ERK1 / 2 is requisite for maintenance of HSC self-service and renewal capacity. Furthermore, inhibition of ERK by PD and consequently JUN inhibits the erythroid differentiation pathway of MNCs[12-13].

The capsicum frutescens is daily used vegetable and their substance (8-methyl–N–6–vanillylnonenamide) are liable in their hot and pungent nature of this vegetable. Capsaicin is not water-dissolved, so that alcohols and other organic solvents were always used to dissolve capsaicin in local area and sprayer. TRPV is a nonselective ligand channel and component of Transient Release Potential (TRP) that have some significant roles in our body. This channels are abundantly found in pheripheral sensory neurons helping to sense hot, warm, pH, and the others stimulies that endanger our body. Mouth are abundantly inervated by neuron afferen sensory and a specialized organ helping human to protect himself from infections (physical, chemical, stress, and temperatur changes stimulies)[14-16].

Bone defect healing is a complex process involving two different mechanisms, but it has one initial factor. The initial factor of bone defect healing is CD 34. CD 34 is a family of Hematopoietic Stem Cells. CD 34, then becomes one of the innate immunity cell, Mast Cell (MC). MC are tissue resident granulocytes derived from CD 34 myeloid progenitor cells in the bone marrow and circularize in the blood during their adolescent stages (Stem Cell Growth Factor / SCF helps the maturation of MC). MC can exist in all tissues, especially in intercommunicating with the outside circumference, such as: the intestine, skin, respiratory system. MCs also have a nociceptive ability. Nociception is an ability to transmit sensation from afferen primary neuron located on pheripheral region to brain via secondary neuron on spinal chord level. One of the ion channel that has nociceptive ability is TRPV-1. TRPV-1 is located on MCs and agonist of capsaisin. Activation the TRPV-1 on MCs with agonis of TRPV-1 (capsaisin), it will release its mediator effects, such as cytokines-chemokines. Chemokines receptors that can be activated by MCs are CCL-3 and CCR-1. 13, 14, 15 This chemokines will synthetize $G\alpha_1\beta\gamma$ amino acids

and this amino acids that break down PIP_2 into IP_3 and DAG from enzimatic reaction from PLC and G Protein mechanism. IP_3 is able to evoke the Ca^{2+} release from its depo intracelluler from Reticulum Endoplasmic (RE). This release from Ca^{2+} evokes the others biological responses, such as differentiation and angiogenesis that happened on blood vessels. The mechanism is trigerred by enzym eNOS (NO Synthase), having effect on cyclic Guanosin Monophosphat (cGMP). This enzym then phosphorilates TRPV-1 as one of the organ targets of cGMP. After this phosphorilation, the vessels becomes vasodilate and promotes the healing procces. Angiogenesis process trigerred by angiotensin and thrombospodin. There are so many kinds of angiogenesis factor, but there are only two most important angiogenesis elements, such as: VEGF (Vascular Endothelial Growth Factor) and bFGF (basic Fibroblas Growth Factor). All of the angiogenesis factors are controlled by suppresor gen of tumor (neoplasm) growth, such as gen p53, so that it means that after these factors are not needed so they will be destroyed. The disregulation of these factors will trigger neoplasm growth, because angiogenesis factors are one of the family of tumor growth promoting factor[17-19].

Another healing mechanisms from MCs after stimulation with agonist TRPV-1 (capsaisin) comes from MCs itself as an innate immunity. As we know, periodontitis is a chronic disease related with bacterial invasion and defect in bone, especially periodontopathogen bacteria. One of the most popular periodontopathogen bacterias is Aggregatibacter Actinomycetemcommitans. This bacteria possess polysacharride that recognized by MCs as an innate immunity. MCs has two kinds of scheme that will identify polysacharride, such as: Pathogen Associated Molecular Pattern/PAMPs and Danger Associated Molecular Pattern/DAMPs. One of the PAMPs is Toll-Like Receptor-4/TLR-4. TLR-4 will bind with polysacharide and then provoke MCs to secrete Calcitonin-Gene Related Protein (CGRP). CGRP has some effects, such as: suppresses IL-2 and T-cell production. After suppresing these inflamatory products, the osteoclastogenesis materials will stop and enter the healing process[20,21].

Conclusions

Capsaisin promotes the number of CD 34, angiogenesis factors, and microbial agents that it can be confirmed as the alternative treatment for periodontitis.

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Declaration of Interest

The authors declare that there are no conflict interest.

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