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Transient Receptor Potential Cation Channel Subfamily V Member-1 and *Matrix Metalloproteinase-8* Expression of Capsaicin Administration in Periodontitis

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ABSTRACT

The aim of this study is to prove the relationship between *Transient Receptor Potential Cation Channel Subfamily V Member-1* (TRPV-1) and *Matrix Metalloproteinase* (MMP-8) expression in periondotitis after capsaicin administered. Twenty-one post-weaned male Wistar rats (*Rattus novergius*) with approximatelly 8-12 week-year-old were randomly divided into three groups (n=7). The negative control group (K0) and the positive control group (K1) were administered only with Aggregatibacter actinomycetemcomitans (A.a) bacteria serotype b to the cervical portion of their maxillary first molars. The treatment group (K2) was treated with both A.a bacteria serotype b to the cervical portion of their maxillary first molars and cayenne pepper extract at a dose of 0.0912 mg/kg/day. The subjects were anesthetized with thionembutal to obtain a sample of gingival tissue. A semi-serial thinness 4 µm by microtome, and calculate the number of cells expressing TRPV-1 and MMP-8 by immunohistochemical staining. The result of which confirmed the TRPV-1 count (K0=33.0150 ± 7.10; K1=37.63 ± 110.60; K2=43.062 ± 14.98) and MMP-8 expression (K0=43.6167 ± 14.57; K1=36.75 ± 22.53; K2=28.83 ± 18.45) of the K0 group to be significantly higher than that of the K1 and K2 groups ($p<0.05$), while there was no significant difference in the TRPV-1 and MMP-8 expression between the K0 and K1 groups ($p>0.05$). The TRPV-1 stimulates the lowest level of MMP-8 in rats periodontitis with capsaicin administered.

Keywords: Cayenne pepper extract, Capsaicin, TRPV-1, MMP-8.

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INTRODUCTION

Over time, bone, the location that accommodates teeth, is subject to a continuous remodelling process occurring through internal and external processes that integrate chemical, hormonal and biomechanic stimuli. At the cellular level, a continuous cycle involving osteoblast and osteoclast results in resorption of the bone (osteoclast) followed by its renewal (osteoblast).[1] The main cause of bone disease is interrupted osteoblast and osteoclast activity resulting in osteoclast continuously resorbing the bone without osteoblast subsequently forming new bone.[2] Bone healing also occurs after a fracture but weakness can result if the defect is greater than critical in extent. Under circumstances in which bone healing is not possible, the defect will be repaired through fibrous connective tissue, with the repair being non-union in character.[3-4]

One bone-related infectious disease is periodontitis which is predominantly caused by periodontopathogen bacteria, one strain of which is *A. Actinomycetemcomitans* (A.a). Periodontopathogen bacteria are mainly gram negative and secrete polysacharride that can trigger a host immune response (innate and adaptive) consisting of the secreting of inflamatory products (chemokines, cytokines, interleukins etc). These inflamatory products directly affect the homeostasis present in *Receptor Activator of Nuclear factor κ B* (RANK)- *Receptor Activator of Nuclear factor κ B Ligand* (RANKL)- *Osteoprotegerin* (OPG) and increase *Matrix metalloproteinase-8* (MMP-8) activity with the result that they promote the remodelling process in bone and the degradation of *extracellular matrix* (ECM).[5-7]

MMP-8, or collagenase-2, is one of the central biomarkers in the connective tissue breakdown caused by periodontitis and has come to be recognised as a potential diagnostic aid. The treatment of periodontitis through *Scaling and Root Planning* (SRP) decreases the *Gingival Crevicular Fluid* (GCF) MMP-8 levels, while periodontal pockets at risk of irreversible tissue destruction indicate repeatedly elevated MMP-8 levels.[8-10]

Traditional medicine is currently popular because of its potential to cure disease and reduce the adverse effects of sinthetic drug use. One extremely popular traditional medicine is capsaicin, a colourless and odourless crystal derived from a herbaceous plant, which demonstrates alcaloid and lipophilic properties, has the chemical structure $C_{18}H_{27}NO_3$ and weighs 305.40 g/mol. The minimum inhibitory concentration of administered capsaicin is 2 mL which can activate *Transient Receptor Potential Cation Channel Subfamily V Member-1* (TRPV-1), *Tumor Necrosis Factor-α* (TNF-α), *Interleukin-6* (IL-6).[11]

MATERIALS AND METHODS

Sample preparation and Ethical clearance

The research reported here was approved by the Ethical Committee of Universitas Airlangga (certificate number 007/HRECC.FODM/I/2018). This study constituted experimental laboratory research incorporating post-test only control group design. The research population consisted of 21male, post-weaned Wistar rats (*Rattus novergius*) approximately 8-12 weeksold and weighing between 150-250 grams. The size of the sample, based on the results of the previous study, was calculated using the *Lemeshow* formula.The research subjects were randomly divided into three groups (n=7). A negative control group referred to as K0 and a positive control group entitled K1 were administered with A.a serotype b bacteria to the cervical portion of the maxillary first molar for seven days until periodontitis had been induced. A treatment group (classified as K2) was administered with both A.a serotype b bacteria to the cervical portion of the maxillary first molar for seven days until periodontitis had been induced and, subsequently, cayenne pepper extract (a dose of 0.0912 mg/kg/day).

Histological Examination

The histological examination, consisting of a TRPV-1 count and the quantifying of MMP-8 expression, was performed through a combination of cell counter calculation and manual counting. This method was adopted in order to compensate for any system error. In order to avoid any bias, the study outcome measurement was undertaken by an expert in histology who was unaware of the group allocation.

Immunohistochemical of TRPV-1 and MMP-8 Expression

The subjects were anesthetized according to their body weight (the dose of thionembutal being one of 30 mg/kg) and subsequently sacrificed to obtain gingival tissue from the induction area necessary to make preparation samples. Specimens containing tooth sockets, were fixated in formalin 10% for up to 24 hours, decalcified by EDTA, and then subjected to a histologic procedure to produce a paraffin block. These steps enabled the gingiva to be cut longitudinally to a semi-serial thinness of 4 μm, thereby facilitating the performance of a microscopic preparation which involved counting the number of cells expressing TRPV-1 and MMP-8 by means of immunohistochemical staining and, finally, analysing the resulting data. The acquired data was subjected to a statistical *Kolmogorov-Smirnov test* in order to analyse the data distribution. As all variables were normally distributed, the resulting data which was presented with mean and *Standard Deviation* (SD) was analysed by means of SPSS 16 for Windows (SPSS Inc., Chicago, USA). This statistical examination employed a one-way ANOVA test and an *Least Significant Difference* (LSD) Tukey test to identify the differences between each group with a significance value of 0.05.

RESULTS

Immunohistochemical examination of the TRPV-1 and MMP-8 expression data in subjects suffering from periodontitis showed that the administration of capcaisin had an effect on TRPV-1 expression. The increase in TRPV1 was followed by a decrease in MMP-8 in the respective groups (Figure 1).

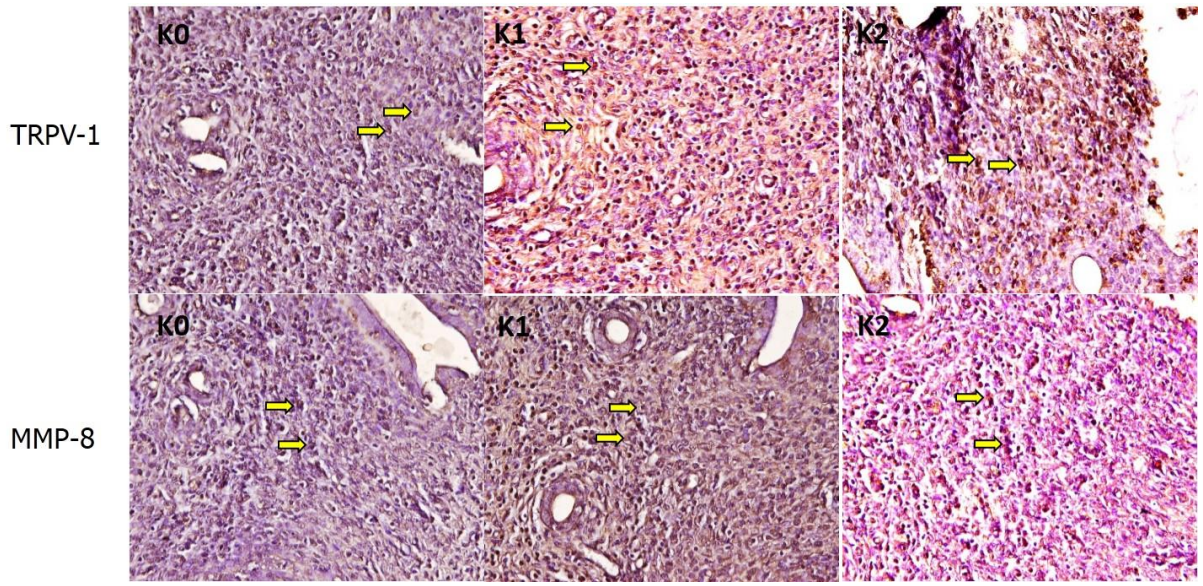

Figure 1: Immunohistochemical staining image of TRPV-1 and MMP-8 expression of control negative (K0), control positive (K1), and Capsaicin (K2) on gingival tissue magnification 400x. The yellow arrow is positive expression.

In this research, TRPV-1 constituted an independent variable, while MMP-8 represented a dependent one. Both TRPV-1 and MMP-8 required statistical examination. The Kolmogorov-Smirnov test was employed for this purpose because the TRPV-1 variable consisted of as many as fifty subjects and it was necessary to confirm normal distribution. A Levene's test was employed to establish the homogenity of this variable. Since both the TRPV-1 and MMP-8 data demonstrated normal and homogeneous distribution, both ANOVA and LSD Tukey tests were subsequently conducted. The final step involved examining the significant differences between TRPV-1 and MMP-8. Due to the post hoc test result being significant, from this statistic it can be concluded that there was a significant difference between TRPV-1 and MMP-8.

Table 1: The number of cells expressing TRPV-1 and MMP-8

Group	TRPV-1 ($\bar{X}$ ± SD)	MMP-8 ($\bar{X}$ ± SD)
Control negative (K0)	33.01±7.10[a]	43.6167±14.57[a]
Control positive (K1)	37.63±10.60[a]	36.75±22.53[a]
Capsaicin (K2)	43.06±14.98[b]	28.83±18.45[b]

The values with different superscript letters in a column are significantly different ($p<0.05$).

Theone-way Anova test revealed a significant difference between the three groups (p=0.001). The TRPV-1 count of the K0 group was considerably higher compared to those of the K1 (p=0.567) and K2 (0.00) groups. K0 had a higher TRPV-1 count than that of K1, but there was no significant difference (p=0.875).The observed results of MMP-8 expression were different than to those relating to a TRPV-1 count. The expression of MMP-8 in the K0 group was higher than that in the K1 groups but, in contrast, lower than that in the K2 groups. The respective expression within each group was significantly different because p=0.004 (Table 1). The administration of cayenne pepper extract at 0.0912 mg/kg/day dose produced more expressed TRPV-1 cells, with the result that MMP-8 decreased in the gingival tissues.

DISCUSSION

There are two mechanisms through which periodontitis negatively affects and places the teeth in jeopardy. The first effect is a disturbance in the balance of RANKL-RANK-OPG.[12] RANK is an osteoclast precursor found in dendritic cells, T cells and fibroblast. Despite this, RANKL is a precursor of the TNF family that is expressed in T cell lymphocyte and osteoblast. The binding of RANK-RANKL will be followed by *Nuclear Factor κappa-β* (NF-κβ) dan c-Fos which activates the maturing of osteoclast. RANK-RANKL has one inhibitor binding which is OPG.[13] OPG competes with RANK to prevent the binding of RANK-RANKL resulting in its impeding of the process of osteoclastogenesis. Both RANKL and OPG are present in gingival crevicular fluid (GCF).[14] Periodontitis also triggers degradation of ECM by MMPs which belong to the endopeptidase zinc family that is widely known to degrade ECM and the basal membrane. MMPs are grouped into subgroups, such as: colagenase (MMP-1, MMP-8, MMP13), gelatinase (MMP-2, MMP-9), stromelisinase (MMP-3, MMP-10, MMP-11), matrilisinase (MMP-7, MMP-26). MMP 8 plays a central role in the pathogenesis and progression of periodontitis and is of possible value in diagnostics.[15] However, it has still not been possible to show that it is predictive of disease progression, i.e. that the increased MMP-8 concentration in GCF will precede the occurrence of *Attachment Loss* (AL). Previous studies reported that MMP 8 is expressed by mononuclear phagocytes, smooth muscle cells, endothelial cells, osteoblasts and osteocytes. MMP-8 is also called neutrophil collagenase (the major source of MMP-8 in inflammatory diseases) and one of the interstitial collagenases that is expressed in large quantities by polymorphonuclear leukocytes.[16] As a member of the interstitial collagenase, MMP-8 can cleave interstitial collagen at a specific locus in the α-1 chain between Gly 775 and Ile 776. This cleavage generates fragments that are three-quarters and one-quarter the size of the original molecule. MMP-8 cleaves non matrix proteins such as serpins, casein, human 2-macroglobulin, bradykinin, angiotensin I and substance P. In the *polymorphoneclear* (PMN), it is stored in a latent pro-MMP-8 (*M*r 85 kDa) form within specific granules and released on activation by inflammatory mediators. The majority of active MMP-8 becomes membrane-bound, and in this form, is resistant to *Tissue Inhibitor Metalloproteinase* (TIMP) 1 and 2.[17] Approximately 92% of the pericellular collagenase activity associated with PMNs in vitro is attributable to membrane-bound MMP-8, yet its function in vivo remains unknown. Other cell types that express MMP-8 include rheumatoid synovial fibroblasts, endothelial cells, activated macrophages, smooth muscle cells, bronchial epithelial cells, mast cells and chondrocytes. MMP-8 expression andactivity are closely associated with chronic inflammatory and fibrotic diseases, such as cystic fibrosis, rheumatoid arthritis, periodontal disease and chronic skin conditions.The repeatedly elevated GCF MMP-8 levels indicate the sites at risk of periodontal and that testing of MMP-8 sites specifically for GCF is a valuable aid in diagnosing periodontitis.[18,19]

People frequently use capsaicin, which contains a substance commonly known as 8-methyl–N–6–vanillylnonenamide and is responsible for its hot and spicy taste, as a vegetable. Capsaicin is insoluble in water and is, therefore, usually solubilized in alcohol and other organic solvents.[20] This characteristic is usually referred to as liposolubility and explains why an excess of capsaicin in the mouth due to food consumed is not alleviated by drinking water, whereas a yogurt-based drink such as lassi is able to remove the vanilloid from the oral cavity. TRPV-1 is a capsaicin agonist receptor, a nonselective ligand channel and member of TRP that plays an important role in our body.[21] This channel helps to sense heat, warmth, pH and other stimuli that potentially endanger the human body. Pheripheral sensory neurons contain of considerable amounts of TRPV. One organ abundantly inervated by afferen sensory neuronsis the mouth whose specific role is to help humans protect themselves against infection (physical, chemical, stress and temperature-related).[22] TRP is commonly divided into six groups based on their amino acid sequences, these include: TRP C (*Canonical*/TRPC), TRP channel V (*Vanilloid*/TRPV), TRP channel M (*Melastatin*/TRPM), TRP channel A (*Ankrin*/TRPA), TRP channel P (*Polycystic*/TRPP) and TRP channel ML (*Melastatin*/TRPML).[23-25]

Periodontitis is a chronic disease resulting from bacterial invasion, especially that of periodontopathogen bacteria, one of the most common strains of which is A.a. This bacteria expresses polysacharride that known as virulence factor. Innate immunity demonstrates two patterns that recognize polysacharride: *Pathogen Associated Molecular Pattern* (PAMPs) and *Danger Associated Molecular Pattern* (DAMPs). One of the PAMPs is a Toll-Like Receptor-4/TLR-4, with the result that TLR-4 binds to polycahharide. Another type of innate immunity is macrophage. Machropage pahocytocites bacteria and communicates this to the adaptive immune system, enabling it to recognize and secrete cytokines, chemokines and interleukins to neutralize the bacteria and their products. Inflamatory products also promote the release of Ca^{2+} from its receptor on *Endoplasmic Reticulum* (ER). The mechanism comes from IP_3, a product resulting from the breaking down of $PIP_2$by phospholipase C

which is genereated by the protein $G\alpha_1\beta\gamma$. IP_3 then releases the Ca^{2+} from its ER. This Ca^{2+} release induces angiogenesis factor like as *Vascular Endothelial Growth Factor* (VEGF) and *basic Fibroblas Growth Factor* (bFGF) to form a new vessel that supports the remodeling process. Angiogenesis factors are controlled by thrombospondin and angiostatin, substances which are, in turn, controlled by tumor suppresor gen (p53). The agonist of capsaicin (TRPV-1) then induces the vascular to promote remodelling.

CONCLUSION

TRPV-1 stimulates the lowest number of MMP-8 in cases of rats periodontitis with capsaicin administered.

CONFLICT OF INTEREST

All the authors made substantial contributions to this study, manuscript, approved the final draft of the paper prior to its submission and revision. No conflict of interest associated with this work.

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