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The effectiveness of antibiotics and *Hematopoietic Stem Cell* treatment in periodontitis rat model toward TNF α expression

Retno Indrawati R[1], Indeswati Diyatri[2], and Dwi Rahmawati[3]

[1,2]Departement of Oral Biology, Faculty of Dentistry Airlangga University, Surabaya
[3]Program Magister, Faculty of Dentistry Airlangga University, Surabaya
Campus A Airlangga University, Jl. Mayjen. Prof. Dr. Moestopo No. 47 Surabaya

Abstract

Introduction: Periodontitis is a biofilm-induced chronic inflammatory. The current therapy of periodontitis is *scaling root planning* and curettage, and followed by administration of antibiotic such as combination of Amoxicillin and Clavulanic acid. *Hematopoietic Stem Cell* has ability to differentiate into blood cells which is capable of homing and regenerating itself. **Research Purpose:** The aim of this study was to prove antibiotic and *Hematopoietic Stem Cell* administration can reduce TNF α expression. **Method:** This research was divided into four different groups, P1 as negative control group, P2 as positive control group which inoculated with *P. gingivalis* 10^9 for three weeks as chronic periodontitis rat model and USP were administered, P3 as chronic periodontitis rat model received *Hematopoietic Stem Cell* injection into the tail vein of rat, P4 as chronic periodontitis rat models were given Amoxicillin and Clavulanic acid 250 mg/kg BW orally then followed by *Hematopoietic Stem Cell* 10^6 injection into the tail vein of rat. After two weeks rat were sacrified and immunohistochemically analysed for expression of TNF α. The data were analysed by using Non-Parametric Test. **Result:** TNF α expression of negative control group was different as compared to positive control group and treatment group which given *Hematopoietic Stem Cell* injection, but there is no difference between negative control group with treatment group which given Amoxicillin and Clavulanic acid 250 mg/kg BW orally and followed by *Hematopoietic Stem Cell* injection. **Clonclusion:** The administration of Amoxicillin and Clavulanic acid 250 mg/kg BW and *Hematopoietic Stem Cell* can reduce TNF α expression on periodontitis rat model.

Keywords: *Hematopoetic Stem Cell,* TNF α, *Chronic Periodontitis, Antibiotic.*

Corresponding Author:
Indeswati Diyatri
indes_diyatri@yahoo.com

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1. Introduction

Periodontitis is a biofilm-induced chronic inflammatory disease that leads to the destruction of the periodontium, the tooth-supporting structures such as the gingiva and the underlying alveolar bone (Darveau, 2010). The epidemiological study in the USA have shown the high prevalence of periodontitis (>47% of adults) (Eke *et al, 2012*). According to the survey, the prevalence and intensity of the periodontal disease in Asia and Africa is higher as compared to Europe, America and Australia. Previous study in the East Java was conducted in 1995 showed that, the periodontal disease occurs in 459 among 1000 people. The Periodontal disease in Indonesia takes second place in incidence of dental disease after dental caries. (Tampubolon dan Situmorang, 2010).

The periodontal patogen bacteria which is known as the main cause Periodontitis is *Porphyromonas gingivalis (P. gingivalis)* which has Lipopolysaccharides (LPS) as the cell wall component, is the virulence factor that induced macrophage activation through the *Toll Like Receptor 4 (TLR4) chain.* Inflammatory mechanism leads to loss of alveolar bone in periodontitis. Recruited neutrophils to the gingival crevice fail to control a dysbiotic microbiota, which can thus invade the connective tissue and interact with additional immune cell types, such as macrophages (Mw), dendritic cells (DCs), and gd T cells; a subset of innate-like lymphocytes. These cells produce proinflammatory mediators [such as the bone-resorptive cytokines tumor necrosis factor (TNF), interleukin (IL)-1b, and IL-17] and also regulate the development of T helper (Th) cell types, which also contribute to and exacerbate the inflammatory response (George, 2014).

The current therapy of periodontitis is *scaling root planning* and curretage followed by administration of the antibiotic. The administration of antibiotic can lead to bacterial resistance. If it doesn't show the sign of cure, surgery should be performed. (Saputra, 2006)

The objective of this research was to find a new innovative therapy using *stem cell,* which known has the ability to differentiate into specialized cell types, become mature cells, regenerate itself and produce more stem cells through mitosis.

In chronic periodontitis case, the administration of *Hematopoietic Stem Cell* can suppress the inflammatory responses. Recruitment of hematopoietic stem/progenitor cells (HSC) from the bone marrow into peripheral blood following treatment with chemotherapy and/or cytokines is termed mobilization. The release of HSC from the bone marrow is a physiological phenomenon for the protection of HSC from toxic injury, as circulating cells can re-engraft bone marrow, or to maintain a fixed number of HSC in the bone marrow (homeostatic mechanism). In fact, trafficking to blood is

an important death pathway to regulate the steady-state number of HSC (Abkowitz *et al,* 2003). It will cause the deppression of proinflamation cytokine. The deppression of inflammatory response will cause reduction of *fibrosis gingiva* severity, width of *attached gingiva*, *fibrogenesis* and *osteogenesis*.

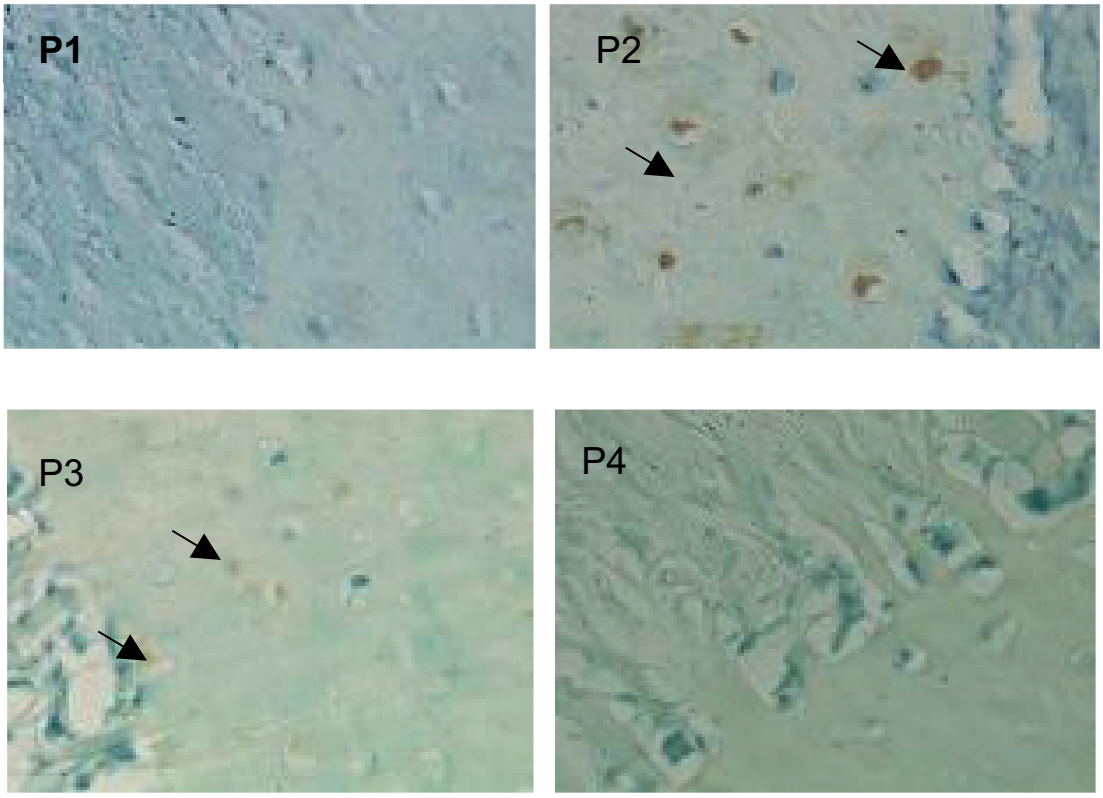
2. Material and Methods

True experimental design was used for this research. The animal used for the experiments were three month old healthy male rat (*Rattus novergicus*), which has been made rat periodontitis model. Rat periodontitis model was made by injection of the *Porphyromonas gingivalis* 10^9 CFU at the incisive sulcus gingiva. The sample of this research was part of the left periodontal incisive tissue. The research sample number was 24 rats with 6 rats replication for each group.

This research was divided into four different groups, P1 as negative control group, P2 as positive control group which inoculated with P. gingivalis 10^9 for three weeks as chronic periodontitis rat model and NaCl (USP) were administered, P3 as chronic periodontitis rat model received Hematopoietic Stem Cell injection into the tail vein of rat, P4 as chronic periodontitis rat models were given Amoxicillin and Clavulanic acid 250 mg/kg BW orally then followed by Hematopoietic Stem Cell 10^6 injection into the tail vein of rat. After two weeks rat were sacrified and immunohistochemically analysed for the expression of TNF α. The data were analysed by using Kruskal-Wallis test with significance assumed at $p< 0.05$ and then followed by Mann-Whitney test.

3. Results

The immunohistochemical staining for TNF α expression showed chocolate colour in *cementocyte*, because of TNF α antigen and antibody bond. The results are shown in Figure 1. TNF α expression of negative control group were negative. Positive control group(P2) which was innoculated by *P. gingivalis* 10^9 showed firm TNF α expression with chocolate coloured *cementocyte* as compared to P3 group, chronic periodontitis rat model received *Hematopoietic Stem Cell* 10^6 injection. The TNF α expression of P3 group *cementocyte* was fewer and light chocolate coloured. TNF α expression of P4 group as chronic periodontitis rat models were given Amoxicillin and Clavulanic acid 250 mg/kg BW orally then followed by *Hematopoietic Stem Cell* 10^6 injection into the tail vein of rat were negatively expressed.



Picture 1: TNFα expression differrence between P1, P2, P3, P4 group. P2 shows firm expression of TNFα compared to P1, P3 and P4 group. P4 were negatively expressed. *(1000x magnification; Nikon H600L microscope; DS Fi2 camera with 300 megapixel).*

Table 1: Result of TNF α expression using Mann-Whitney Test.

Group TNF α	Comparison Group	Signification
P1	P2 P3 P4	0.002 0.002 0.056
P2	P3 P4	0.020 0.003
P3	P4	0.019

Kruskal-Wallis test showed significant differences among the TNF α expression ($p<0.05$). The Mann-Whitney test was applied for analysing differences "between group" comparisons.

Tabel 1 shows the differences between groups. The difference between P1 with P2 and P3 are statistically significant ($p<0.05$), but the difference between P1 and P4 is not statistically significant ($p>0.05$).

4. Discussion

The difference between negative control group, positive control group, and chronic periodontitis rat model received *Hematopoietic Stem Cell* injection into the tail vein of

rat (P3) are statistically significant. Whereas the difference between negative control group and chronic periodontitis rat models were given Amoxicillin and Clavulanic acid 250 mg/kg BW orally then followed by *Hematopoietic Stem Cell* 10^6 injection into the tail vein of rat is not statistically significant. This is caused by *Lipopolysaccharide* (LPS), cell wall component of *P. gingivalis* bacteria which acts as an endotoxin. In periodontal dissease the *Lipopolysaccharide* causes inflamation reaction involves destruction of the periodontal tissue which consist alveolar bone, gingiva and periodontal ligament (Franklud, 2009). According to the research by Qiuping He (2015) Hematopoietic stem cells (HSCs) have the abilities of self-renewal and multilineage differentiation to maintain the supply of all mature blood cells for the lifetime. This shows that the *Hematopoietic Stem Cell* therapy reduce the TNF α expression because it can differentiate into blood cells and has several characteristics like *self renewing,* which TNF a play main role of inflammation process.

The statistic analysis for chronic periodontitis rat models were given Amoxicillin and Clavulanic acid 250 mg/kg BW orally then followed by *Hematopoietic Stem Cell* 10^6 injection into the tail vein of rat is consistent with Mart'inez *et al* (2004) that studies have revealed the presence of beta-lactamase producing species in 74–88% of patients with periodontitis. Recently, published evidence suggests that penicillin and amoxicillin are being rendered increasingly less effective because of beta-lactamase producing bacteria. More than half of the Gram-negative anaerobic bacilli (including Prevotella, Porphyromonas, Bacteroides, and Fusobacterium spp.) are capable of producing beta-lactamase leading to treatment failures in dental infections (brook *et al.,* 1991).

Beta-lactam antibiotic work by inhibit bacteria cell wall synthesis. During the process of the cell wall bulding, transpeptidase reaction has been catalyzed by the transpeptidase enzyme and led to formation of cross bond between two peptide-glucane chain. Transpeptidase enzyme which is located in bacteria cytoplasm membrane has the ability to bind beta-lactam antibiotics with the result that enzyme lose the ability to catalyze the transpeptidase reaction however the cell wall will still be formed. At the normal condition, the osmotic pressure difference in gram negative bacteria cell and the surrounding will cause cell lysis. Besides that, transpeptidase complex protein and beta-lactam antibiotic will stimulate the autolysin compounds which can digest the bacteria's cell wall (Steeve,2007). Therefore, the bacteria which losses the cell wall or lysis lead to cell death. This research shows that the Amoxicilin and Clavulanic Acid antibiotic has function to kill the *P. gingivalis bacteria* and the injection of *Hematopoietic Stem Cell* can help to repair the damage of tissue.

Recent studies shows that TNFR2 via TNFa activates the Notch and NF-kB signaling pathways to establish HSC fate, indicating a requirement for inflammatory signaling in HSC generation. Tumor necrosis factor a (TNFa) is a powerful proinflammatory cytokine that plays a pivotal role in the regulation of inflammation and immunity. HSC generates into blood cells lead to homeostasis process mark by depression of TNF α expression.

Beta-lactam antimicrobial agents exhibit the most common treatment for bacterial infections and continue to be the prominent cause of beta-lactam antibiotics among gram-negative bacteria worldwide. Beta-lactamase is an enzym that break down antibiotics belonging to penicillin and cephalosporins group and render them ineffective. Clavulanic acid is a beta lactamase inhibitor isolated from Streptomyces. It contains a beta-lactam ring and binds strongly to beta-lactamase at or near its active side, thereby hindering enzymatic activity. This protects other beta-lactam antibiotics from beta-lactamase catalysis, there by enhancing their antibacterial effect. The addition of Clavulanic Acid as beta-lactamase inhibitor has ability to inactivate beta-lactamase which inhibits the activity of the Amoxicilin. In guided tissue regeneration, systemic amoxicillin-clavulanic acid therapy has been used to suppress periodontal pathogens and increase the gain of clinical attachment. Cementocyte which has been lysis can be repaired by *Hematopoietic Stem Cell* therapy which has *self renewing* characteristic and stay in many types of cell and repair the cells by mitosis. The research conducted by Du (2013) shows that the *Hematopoietic Stem Cell* can repair the damage cause by the periodontitis, as an anti-inflamation and immunomodulatory function.

This research proves *Hematopoietic Stem Cell* can reduce TNF α expression and has the *self renewing* characteristic.

5. Conclusion

Oral Amoxicilin-Clavulanic Acid and *Hematopoietic Stem Cell* were more effective to reduce TNF α expression in periodontitis rat model compared to periodontitis rat model which were given *Hematopoietic Stem Cell* only.

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