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EFFECT OF COENZYME Q10 APPLICATION ON POST GINGIVAL CURETTAGE: TRANSFORMING GROWTH FACTOR-β1 LEVEL (TGF-β1) IN GINGIVAL CREVICULAR FLUID

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ABSTRACT
Background*: Curettage is one of the common periodontitis treatments. Mechanical cleaning of periodontal pocket requires additional materials so the wound healing in the operating area is more optimal. Coenzyme Q10 is one of the ingredients can be used to control levels of Reactive Oxygen Species (ROS). Coenzyme Q10 concentrations of 1:9 and 2:8 in previous studies have been shown to stimulate fibroblast proliferation but its impact on TGF-β1 levels is still unknown.* ***Purpose****: to compare the effect of Coenzyme Q10 concentrations 1:9 and 2:8 with PerioQ™ on TGF-β1 expression post-curettage.* ***Method****: In vivo, study was conducted on 12 periodontitis induced - Oryctolagus cuniculus. Coenzyme Q10 concentrations 1:9 (group I) and 2:8 (group II) were applied post-curettage and the results were compared with PerioQ™ (group III). The TGF-β1 was collected from gingival sulcus fluid. It was taken with paper point no. 15 on days 3, -7, and -14 followed by Enzyme-Linked Immunosorbent Assay (ELISA) examination. Data were analyzed with Two-way ANOVA and post hoc test with a significance level of p<0.05.* ***Result****: TGF-β1 levels after application of Coenzyme Q10 concentration 1:9 and PerioQ™ reached the peak on day 7 and decreased on day 14 while Coenzyme Q10 concentration of 2:8 reached the peak on day 14****.*** ***Conclusion****: The application of these three ingredients affects TGF-β1 levels of gingival sulcus fluid. The expression pattern of TGF-β1 concentration 1:9 is similar to PerioQ™, which reaches a peak at 7 days post-curettage and decreases the day after.*

Keywords*: Coenzyme Q10, Gingival Crevicular Fluid, Periodontitis-Induced, Transforming Growth Factor-β1, Ubiquinone*
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INTRODUCTION

Periodontitis occurs due to complex interaction between microorganisms, immune responses, and other factors such as oral and genetic environment. The interaction of microorganisms will trigger the body's immune response as a form of defense, but if the immune response becomes excessive it will damage the gingival connective tissue, periodontal ligaments, and alveolar bone.[1.2]

Chronic periodontitis can be treated with non-surgical or surgical therapy, provided plaque control is maintained during the supportive phase of care. Non-surgical therapy consists of oral hygiene instructions with scaling and root planing, which has consistently been selected to be one of the most effective ways to treating periodontal disease. The addition of antibiotics systemically and locally to the periodontal pocket is often done because antibiotics are more effective at accelerating healing than a single curettage therapy alone. However, frequently use of antibiotics can risk the resistant of bacteria to antibiotics.[3.4]

Patients with periodontal disease, there is an increase in excessive levels of Reactive Oxygen Species (ROS). Reactive Oxygen Species is a highly reactive oxidizing agent that functions to kill bacteria, but if chronic inflammation occurs, ROS will be produced continuously so that excessive levels cause the destruction of gingival tissue, periodontal ligaments, and alveolar bone by damaging DNA and stimulating the formation of proinflammatory cytokines. The condition of increased ROS levels and decreased antioxidant activity is known as oxidative stress. Oxidative stress accelerates the formation of lesions in periodontal tissue.[5]

Free radicals and ROS are essential in normal biological process. At low concentrations, free radicals will stimulate the growth of fibroblasts and epithelial cells in culture, but at higher concentrations can cause tissue damage.[6] Expression of Reactive Oxygen Species can be prevented by antioxidants that release an electron than it becomes a stable atom. If antioxidant levels are insufficient, periodontal tissue is unable to cope with

oxidative stress result increased severity of periodontal tissue damage.[7]

Coenzyme Q10 is a natural antioxidant in the human body with the same structure as vitamin K. This is an important substance such as vitamin found in every cell of the body and plays a role in energy production. In addition, Coenzyme Q10 plays a role in oxidation-reduction recycling. Coenzyme Q10 in reduced form gives electrons to neutralize oxidants or block free radicals thus preventing cell damage. This indicates that there is strong antioxidant activity.[7]

In nature, Coenzyme Q10 is known as ubiquinone. Its quinone structure is similar to vitamin K. Coenzyme Q10 acts as an intercellular endogenous antioxidant. The expression of Coenzyme Q10 increases in inflammatory gingiva and effectively suppress advanced periodontal tissue inflammation. Reduced levels of Coenzyme Q10 in gingival tissue can occur independently or due to periodontal disease.[8]

PerioQ™ gel is a gel produced by PerioQ INC, Manchester, USA, and is often used for periodontal pocket treatment. This gel should be used within 48 months from the date of production and stored in a dry place, away from light sources and heat sources.[9] However, PerioQ ™ gel is difficult to obtain because it is not available in Indonesia.

The raw material used in PerioQ ™ is ubiquinone. Ubiquinone is a material that is widely found in Indonesia. Based on this background, we conducted a study to make Coenzyme Q10 gel with 2 concentrations, namely 1:9 and 2:8 concentrations which have been shown to have an effect on fibroblast proliferation.[10] However, the impact of this material on TGF-β1 expression compared to PerioQ™ is unexplored. This study aims to compare the effect of the application of Coenzyme Q10 concentrations of 1: 9, 2: 8, and PerioQ™ on TGF-β1 levels after curettage.

METHOD

This study is an in vivo study on 12 males of *Oryctolagus cuniculus*, weight 1500 – 2500g, induced by periodontitis. The research procedure has received approval from the Ethics Commission of the Faculty of Dentistry, Universitas Gadjah Mada with number No.00718/KKEP/FKG-UGM/EC/2021. Experimental animals were divided into 3 groups, namely Group I applied Coenzyme Q10 concentration 1: 9, Group II applied Coenzyme Q10 concentration 2: 8, and Group III applied PerioQ™. The production of Coenzyme Q10 refers to our previous research.[10]

Before conducting the study, experimental animals were adapted for 1 week.[11] The study began with anesthesia using ketamine doses of 25mg/kgBW and xylazine doses of 3mg/kgBW intramuscular. After anesthesia, periodontitis induction was executed by combining ligation and LPS injection. The cervical part of the mandible's incisivus is wrapped in silk thread "3-0" for seven days, in which the knot resembles figure eight. The injection of 0,05 ml LPS bacteria *P.gingivalis* took place on the first day. It was injected into the interdental area of the ligated mandible's incisivus. The condition of periodontitis is confirmed by examining gingival inflammation, pocket depth, and tooth mobility.[12]

After the condition of periodontitis occurs, experimental anesthesia and curettage are conducted for pocket cleaning followed by material application. On days 3, 7, and 14, gingival sulcus fluid is taken using paper point no. 15 which is inserted for 1 minute. Paper points are stored in tubes containing 0.5 mL of 7.4% saline buffer phosphate (PBS) and stored at -20°C.[13] TGF-β1 levels were measured with an Enzyme-Linked Immunosorbent Assay (ELISA) kit. The result was measured at a wavelength of 450 nm. Data were analyzed by two-way ANOVA and post hoc test with a significance level of 0.05.

RESULT

The data were evaluated for normality with the Shapiro-Wilk test (Table 1). The results showed that the data were normally distributed (p>0.05).

Table 1. Normality Test Results

Variable		p
Experimental Group	I	p>0.05
	II	
	III	
Observation Time	Day 3	p>0.05
	Day 7	
	Day 14	

I : Coenzyme Q10 – concentration 1:9
II : Coenzyme Q10 – concentration 2:8
III : PerioQ ™

Once it is known that the data of the study results are normally distributed, the level of TGF-β1 is analyzed and presented in Table 2. In Table 2 it is known that the highest levels of TGF-β1 in groups I and III occurred on day 7 and decreased on day 14 (p>0.05). Nevertheless, a different pattern occurred in group II. In group II, TGF-β1 levels were highest on day 14.

Table 2. Level of TGF-β1 in Gingival Crevicular Fluid after Curettage and Application of Tested Material

Days	n	Level of TGF-β1 (mean ± SD)			P
		I	II	III	
3	12	9.17 ± 1.13	11.88 ± 2.17	8.55 ± 0.99	0.014*
7	12	11.97 ± 3.21	8.61 ± 0.75	10.41 ± 1.07	
14	12	9.96 ± 2.41	12.04 ± 1.99	9.40 ± 1.26	

I: Coenzyme Q10 – concentration 1:9
II: Coenzyme Q10 – concentration 2:8
III: PerioQ™
SD: standard deviation
n: sample size
*: statistically significant (p<0.05)

DISCUSSION

Chronic periodontitis affects periodontal tissue damage. One of the etiologies of this occurrence is due to the overproduction of Reactive Oxygen Species (ROS). Reactive Oxygen Species is a highly reactive oxidizing agent that function is to kill bacteria, but if chronic inflammation occurs, ROS will be produced continuously then made the levels are excessive and cause destruction of gingival tissue, periodontal ligaments, and alveolar bone. Reactive Oxygen Species will damage DNA and stimulate the formation of proinflammatory cytokines. The condition of increased ROS levels and decreased antioxidant activity is known as oxidative stress. Oxidative stress accelerates the formation of lesions in periodontal tissue.[5]

The results of this study showed that the application of Coenzyme Q10 and PerioQ^{TM} affected TGF-β1 levels in the gingival sulcus fluid of periodontitis-induced *Oryctolagus cuniculus.* The pattern of TGF-β1 expression levels after the application of Coenzyme Q10 concentration of 1:9 is the same as PerioQ^{TM} (Table 2). The highest level occurs on the 7th day and decreases on the 14th day. A different pattern occurred in the Coenzyme Q10 group with a concentration of 2:8. At a concentration of 2:8, the level of TGF-β1 peaks on day 14 (Table 2).

This study showed that Coenzyme Q10 act as an adjunct in the post-curetted healing process. At 14 days after therapy, the proinflammatory cytokine TGF-β1 was detected as response from the host due to infectious periodontitis. Another spectrum of this research supports idea that the application of CoQ10 following scaling and root planing can reduce gingival inflammation, which prevents periodontitis. Therefore, as a preliminary observation, one may indicate that PerioQ^{TM} has a potential additive effect.[14, 15, 16]

Transforming Growth Factor-β1 (TGF-β1) is one of the important cytokines in inflammatory processes and tissue regeneration.[17] This growth factor plays a role in increasing the proliferation of osteoblasts, osteoblast precursors, or the production of osteoblast matrix through chemotactic action.[18] Transforming Growth Factor-β1 is synthesized by various normal cells, osteoblasts, and platelets.[19]

Transforming Growth Factor-β can induce the proliferation of mesenchymal stem cells, preosteoblasts, osteoblasts, and chondrocytes, then stimulate the production of extracellular proteins such as collagen, proteoglycans, osteopontin, osteonectin, and alkaline phosphatase.[20] It is further explained that there are five main roles of TGF-β1 in osteoimmunity[17], namely 1) stimulating the proliferation of mesenchymal stem cells and playing a role in differentiation into chondrocytes, 2) facilitating the differentiation of osteoblast progenitors into osteoblasts, 3) high concentrations of TGF-β1 decrease the regulation of expression of Receptor Activator of Nuclear Factor Kappa-B Ligand (RANKL) in osteoblasts, 4) low concentrations of TGF-β1 play a role in osteoclast maturation. 5) nourishes hematopoietic stem cells (HSC) in the hibernation period as an antioxidant that inhibits excessive production of ROS.

The results of this study are in line with the results of our previous studies that showed Coenzyme Q10 in 1:9 and 2:8 as well as PerioQ^{TM}, effect on the proliferation of gingival fibroblast cell culture.[10] Coenzyme Q10 has a function as an intercellular antioxidant and its concentration is increased in the diseased gingiva which effectively suppresses advanced periodontal inflammation.[21]

The use of Coenzyme Q10 supplements is not only beneficial if used post-surgery. Other studies have shown that consumption of Coenzyme Q10 supplements after non-surgical procedures also affects clinical parameters, namely gingival index, plaque index, clinical attachment loss, and probing depth. In addition, Coenzyme Q10 was shown to reduce the number of pathogenic bacteria in the subgingival plaques of the gingival sulcus fluid, namely *Aggregatibacter actinomycetemcomitans (AA), Fusobacterium nucleatum (Fn), Porphyromonas gingivalis (Pg)* and *Prevotella intermedia.* Long-term consumption can be beneficial for controlling inflammation in periodontal tissue.[22] Similar results were found in another study showing that local application of Coenzyme Q10 can be used as an adjunct therapy post mechanical debridement and will improve periodontal clinical parameters.[23] This study showed that Coenzyme Q10 and PerioQ^{TM} affect the TGF-β1 levels in the gingival crevicular fluid. The expression pattern of TGF-β1 after the application of Coenzyme Q10 concentration of 1:9 is similar to PerioQ^{TM}.

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