Effect Of Coenzyme Q10 On Osteoblasts Cells Number In Male Wistar Rats Gingiva Undergoing Periodontitis

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ARTICLE INFO

ABSTRACT

Inflammatory conditions in periodontitis can lead to increased production of free radicals. Antioxidants are necessary to neutralize excess of free radicals. Coenzyme Q10 is able to protect from free radicals. This study aims to determine effect of coenzyme Q10 on osteoblast cells number in male wistar rats gingiva undergoing periodontitis. The method used in this research is true experimental laboratory with posttest only control group design. 24 male wistar rats were divided into 4 groups, negative control group and treatment group on day 7 and 14 with each group of 6 rats. Induction of periodontitis using silk ligature tied to subgingiva area and induced Porphyromonas gingivalis bacteria around the mandibular incisors teeth given every 2 days for 7 days. The treatment group was applied coenzyme Q10 0.1ml twice a day. 6 rats from each group were decapitated on day 7 and 14 then the tissue processed into histological preparations and osteoblast cells number was calculated. Data were analyzed using One-way ANOVA and LSD test. The results showed an increase in the number of osteoblasts in the treatment group given coenzyme Q10 on days 7 and 14. Based on the results of the One Way Anova parametric test, it was found that the significant value was 0.00, this value has a p-value <0.05, which means that there is a significant difference between the control group of rats that were not treated after periodontitis and the group treated with coenzyme Q10 after periodontitis on days 7 and 14. This research proved that the application of coenzyme Q10 has an effect on increasing osteoblast cells number in male wistar rats gingiva undergoing periodontitis.

Keywords: Periodontitis, Coenzyme Q10, Osteoblast

1. INTRODUCTION

Periodontitis is a chronic inflammatory disease caused by bacteria that affect the supporting structures of the teeth and the surrounding tissues, leading to the loss of periodontal ligament attachment and eventually the resorption of alveolar bone [1]. The prevalence of periodontitis in Indonesia reached 74.1% [2]. Chronic periodontitis is the sixth most common disease in the world, which is around 10.8% [3]. Poor oral hygiene, biofilm accumulation, and traumatic occlusion are the beginning of periodontitis [4].

Periodontitis therapy procedures begin with scaling and root planing. Additional drug therapy after scaling and root planing can accelerate the healing of periodontitis. The healing process of periodontitis after scaling and root planing can be given additional therapeutic drugs such as antioxidants to accelerate the healing process[5]. Antioxidants are compounds that can inhibit the development of periodontal disease by neutralizing excess free radicals due to the process of tissue inflammation in periodontitis. These compounds are normally present in our bodies, but can also be added in cases of excessive free radical production, such as the addition of coenzyme Q10 [6].

The results of literature research state that coenzyme Q10 is able to protect DNA damage and mitochondrial membrane proteins from Reactive Oxygen Species (ROS) [7]. Several studies have been conducted to prove the role of coenzyme Q10 in healing periodontitis, which can change poor periodontal health status such as reducing inflammation in patients with gingivitis and periodontitis [8]. Other studies also mention that coenzyme Q10 can reduce inflammation and can accelerate the post-extraction healing process in sprague dawley rats [9] [10]. Another study also showed that coenzyme Q10...
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Q10 significantly affected the healing process of periodontitis in the group given coenzyme Q10 as an additional therapy after scaling and root planing. It was found that coenzyme Q10 applied in intrapocket as much as 0.1ml showed an effect on periodontitis healing in terms of a decrease in Plaque index (PI), Bleeding on probing (BOP), Gingival index (GI), and Clinical Attachment Loss (CAL). It has a beneficial effect on periodontitis when used as an adjunctive therapy to scaling and root planing [11].

Several other studies have also confirmed that coenzyme Q10 can affect the bone formation process. Previous research shows that coenzyme Q10 can reduce bone resorption and increase bone formation which can affect the number of osteoblasts in osteoporotic rats [6]. Another study showed that the use of coenzyme Q10 in ovariectomized rats as an antioxidant can reduce bone resorption by inhibiting osteocyte apoptosis and reducing osteoclast activity so as to increase osteoblast activity and induce osteogenesis [12]. The focus of this study is to determine effect of coenzyme Q10 on the healing process of alveolar bone in male wistar rats undergoing periodontitis as seen from the number of osteoblast cells on days 7 and 14.

2. METHOD

The type of research is true experimental laboratory with posttest only control group design, observing both the control group and treatment group. Research on effect of coenzyme Q10 on osteoblast cells number in male wistar rats gingiva undergoing periodontitis has been completed at the Laboratory of Pharmacology and Anatomical Pathology, Faculty of Medicine, Universitas Muhammadiyah Surakarta. This study has been approved by the Ethics Committee of Health Research at Dr. Moewardi Hospital with number. 736/V/HREC/2023. The subjects of this study are 24 male wistar white rats which are divided into 4 groups, namely negative control group and coenzyme Q10 treatment group on day 7 and 14. Each group contains 6 rats with criteria of age 2-3 months, body weight 150-200 grams, and healthy condition.

Rats were anesthetized using ketamine HCl 0.2ml/KgBW intramuscularly on their thighs. Induction of periodontitis using silk ligature tied to subgingiva at cervical of mandibular incisors teeth and followed by application of P. gingivalis bacteria using a tuberculin syringe with a 30 gauge needle size in the gingival sulcus area of mandibular incisor teeth given every 2 days for 7 days. Day 7 silk ligature was removed, if there were clinical symptoms such as gingival changes, redness, gingival recession, and changes in gingival contours. This shows that the placement of silk ligature and application of P. gingivalis on the subgingiva of mandibular incisors for 7 days can induce periodontitis. Periodontitis rats were scaling and root planing to remove plaque. Furthermore, rats in the treatment group were applied coenzyme Q10 gel to the gingival sulcus using a micropipette with a dose of 0.1ml twice a day for 14 days.

Male wistar rats in each negative control group and treatment group were decapitated by 6 rats on day 7 and 14 after periodontitis. The jawbone is removed at the apical third of the tooth socket near to alveolar bone then the tissue processed into histological preparations are made with hematocycline eosin staining. Histology preparations were observed using a light microscope with a magnification of 400x for 5 visual fields to see osteoblast cells. Calculation of osteoblast cells number was seen using optilab and image raster software. Data were analyzed using parametric tests with One Way Anova with an accuracy level of 95% (p≤0.05) and LSD (Least Significance Different) test to see significant differences in each group.

3. RESULTS AND DISCUSSION

The mean and standard deviation of osteoblasts number in negative control and treatment groups increased from day 7 and 14 are presented in table 1.

<table>
<thead>
<tr>
<th>Observation Day</th>
<th>Mean ± Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative Control</td>
</tr>
<tr>
<td>Day 7</td>
<td>10,833±1,6561</td>
</tr>
<tr>
<td>Day 14</td>
<td>14,833±1,7818</td>
</tr>
</tbody>
</table>

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Based on the results from Table 1 and Figure 1 in negative control group and coenzyme Q10 treatment group, there is an increase in the average number of osteoblasts. These data indicate differences in the average between the groups. The coenzyme Q10 treatment group has a higher average number of osteoblasts compared to the negative control group. On day 7 after periodontitis, the treatment group has an average of 13.40 osteoblasts compared to the negative control group with an average of 10.833. On day 14 after periodontitis, the treatment group has an average of 17.033 osteoblasts compared to the negative control group with an average of 14.833. This indicates an increase in alveolar bone density after the application of coenzyme Q10.
Table 2 Results of the Shapiro-Wilk normality test

<table>
<thead>
<tr>
<th>Group</th>
<th>Sig. Value</th>
<th>Sig limit</th>
<th>Information</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (-) Day 7</td>
<td>0.212</td>
<td>0.05</td>
<td>Normal</td>
</tr>
<tr>
<td>Control (-) Day 14</td>
<td>0.101</td>
<td>0.05</td>
<td>Normal</td>
</tr>
<tr>
<td>Treatment Day 7</td>
<td>0.101</td>
<td>0.05</td>
<td>Normal</td>
</tr>
<tr>
<td>Treatment Day 14</td>
<td>0.093</td>
<td>0.05</td>
<td>Normal</td>
</tr>
</tbody>
</table>

Based on the Shapiro-Wilk normality test results, it shows that the p-values in each group on day 7 and day 14 have significance values > 0.05, indicating that the data is normally distributed. Therefore, the homogeneity test can be continued to determine whether the data variances are homogeneous or not using Levene's Test. The results of the homogeneity test are shown in Table 3.

Table 3 Homogeneity test results for the negative control and treatment groups on day 7 and day 14.

<table>
<thead>
<tr>
<th>Homogeneity Test</th>
<th>Sig. Value</th>
<th>Sig limit</th>
<th>Information</th>
</tr>
</thead>
<tbody>
<tr>
<td>Levene</td>
<td>0.535</td>
<td>0.05</td>
<td>Homogeneous</td>
</tr>
</tbody>
</table>

Based on the Levene's Test for homogeneity, the results show that all groups on day 7 and day 14 have p-values > 0.05, indicating that all the data is homogeneous.

Data that are normally distributed and homogeneous can be further analyzed using the parametric test, One-Way ANOVA. The results of the One-Way ANOVA parametric test are shown in Table 4.

Table 4 Results of the ANOVA test on effect of coenzyme Q10 on osteoblast cells number in male wistar rats gingiva undergoing periodontitis.

<table>
<thead>
<tr>
<th>Sel Osteoblasts</th>
<th>Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Groups</td>
<td>121.685</td>
<td>3</td>
<td>40.562</td>
<td>14.951</td>
<td>.000</td>
</tr>
<tr>
<td>Within Groups</td>
<td>54.260</td>
<td>20</td>
<td>2.713</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>175.945</td>
<td>23</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Based on the results of the One-Way ANOVA parametric test, the p-value is < 0.05, indicating a significant difference between the control group of rats without treatment after periodontitis and the group treated with coenzyme Q10 after periodontitis. This indicates that the application of coenzyme Q10 has an effect on increasing osteoblast cells number in male wistar rats gingiva undergoing periodontitis.

The statistical analysis is further continued using the LSD (Least Significant Difference) test, which is a post hoc test used to determine which groups have significant differences. The results of the post hoc test are presented in Table 5

Table 5 Summary of LSD analysis of osteoblasts between the negative control group and the treatment group.

<table>
<thead>
<tr>
<th>Group</th>
<th>Observation Day</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative Control-Treatment</td>
<td>7</td>
<td>0.014</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>0.031</td>
</tr>
</tbody>
</table>

Based on the Post hoc test results, it was found that there is a significant difference between the negative control group and the coenzyme Q10 treatment group on day 7 and day 14, with a p-value < 0.05. This indicates that coenzyme Q10 is effective in increasing osteoblast cells number in male wistar rats gingiva undergoing periodontitis.

Discussion

This study was conducted to determine the effect of coenzyme Q10 application on increase osteoblast cells number in male wistar rats gingiva undergoing periodontitis after scaling and root

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The sample consisted of 24 male wistar rats divided into four groups: the negative control group and the coenzyme Q10 treatment group on days 7 and 14. Coenzyme Q10 was administered to the treatment group on day 1 after the male wistar rats undergoing periodontitis on day 7, characterized by gingival inflammation, attachment loss, the formation of periodontal pockets, plaque accumulation both supra and subgingivally, swelling, redness, and spontaneous bleeding [13], [14].

The results of the study on the negative control group (male Wistar rats induced with periodontitis through silk ligature binding and application of P.gingivalis bacteria, followed by scaling and root planing) on day 7 and day 14 showed that the average osteoblast number was lower compared to the coenzyme Q10 treatment group. This indicates that the application of P.gingivalis bacteria can increase alveolar bone resorption, which inhibits the differentiation and mineralization of osteoblasts [15]. P.gingivalis infection can increase the production of reactive oxygen species (ROS) activated by Nuclear Factor Kβ (NF-kB). NF-kB stimulates the production of tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and interleukin 1-β (IL-1β) [16]. TNF-α, IL-6, and IL-1β accelerate the process of osteoclastogenesis and inhibit osteoblast formation [17]. Higher levels of ROS can stimulate osteoclast differentiation, which affects osteoblast formation [18].

ROS can disrupt osteoblast formation, stimulate osteoblast and osteocyte apoptosis, and promote osteoclast formation [12]. ROS acts as an intracellular mediator in osteoclast differentiation through the receptor activator of nuclear factor-kappa B ligand (RANKL) pathway [18]. RANKL involves the receptor activator of nuclear factor-kappa B (RANK) on the surface of osteoclast precursors to promote their functional differentiation. Osteoclasts attach to the bone matrix to carry out their function [19]. Osteoclasts continuously function as bone resorbing cells, while osteoblasts act as bone-forming cells. Bone mass is regulated by the balance between osteoclasts and osteoblasts. In the negative control group induced with periodontitis using P.gingivalis bacteria, osteoclasts are activated, and bone resorption exceeds the bone formation process performed by osteoblasts. Specifically, when alveolar bone damage occurs, connective tissue and gingival epithelium invade the spaces created after bone destruction by osteoclasts, making it difficult for osteoblasts to form bone [20]. The results of the study on the negative control group showed that osteoblast number was lower on day 7 compared to day 14, which is consistent with the study conducted by Fatimatuzzahro et al., stating that inducing periodontitis using P.gingivalis can reduce the number of osteoblasts. The negative control group induced with periodontitis significantly differs from the treatment group given coenzyme Q10. This indicates that the osteoblast number in the negative control group is lower compared to the coenzyme Q10 treatment group because the negative control group did not receive additional therapeutic treatment after scaling and root planing.

In the treatment group, which received coenzyme Q10, there was a significant difference in the average osteoblast number compared to the negative control group. This indicates that the treatment group, induced with periodontitis and given 0.1ml of coenzyme Q10, had an effect on osteoblast number, resulting in an increase. The increase in osteoblast number can be observed from the comparison of the average in negative control group and coenzyme Q10 treatment group on each day, with 13.40 osteoblasts in KP7, 10.833 osteoblasts in K(-)7, 17.033 osteoblasts in KP14, and 14.833 osteoblasts in K(-)14. This indicates a significant increase in the number of osteoblasts. This is because the antioxidant substances in coenzyme Q10 can enhance the osteoblastogenesis process by inhibiting osteoclast differentiation [6]. Coenzyme Q10 suppresses the production of reactive oxygen species (ROS) required as intermediaries for osteoclast formation. Coenzyme Q10 acts as an inhibitor of RANKL-induced osteoclast differentiation [21].

The antioxidant content in coenzyme Q10 can regulate osteoblast formation by inhibiting RANKL-induced osteoclastogenesis and promoting osteogenic differentiation. RANKL is a protein secreted by osteoclasts to regulate osteoclast formation. RANKL binds to the RANK receptor on osteoclast progenitors to stimulate osteoclast differentiation. OPG is a protein that binds to RANKL and functions to prevent the binding between RANKL and RANK. Coenzyme Q10 can increase the level of OPG and decrease RANKL, resulting in a balanced level of RANKL and OPG. This balance leads to a decrease in osteoclast differentiation and an increase in osteoblast proliferation and differentiation, resulting in increased bone density, which is consistent with the findings of this study [12].
4. CONCLUSION

Coenzyme Q10 application has an effect on increasing osteoblast cells number in male wistar rats gingiva undergoing periodontitis. This is evidenced by the average number of osteoblasts and the results of One-Way ANOVA test. The results showed that there was a significant difference between control group of rats that were not treated after periodontitis and group that was treated with coenzyme Q10 after periodontitis where the highest number of osteoblast cells was on day 14 followed by day 7 in the coenzyme Q10 treatment group compared to the control group that was not treated with coenzyme Q10.

REFERENCES

Effect of Coenzyme Q10 on Osteoblasts Cells Number in Male Wistar Rats Gingiva Undergoing Periodontitis; Ferren Natusya Sushera, et al