Expression of IL-1 on Mucosal Cavity’s Chronic Inflammation after Provision of Clarias Batrachus Fish Oil

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Abstract

Background: The presence of cell or tissue damage always followed by inflammatory reactions. Inflammatory process should not persist because it can interfere even inhibit regeneration. An increased circulation of IL-1, IL-6 and TNF-alpha happens in chronic inflammation. IL-1 is granulocytes chemoattractant and has function to localize inflammation. Overexpression of IL-1 can inhibit wound healing so it needs curative treatment to decrease IL-1’s expression.

Purpose: To know expression of IL-1 after provision of Clarias batrachus fish oil.

Methods: We have used the post test only design in this research. There have been 19 Rattus norvegicus as research samples and those were divided into 3 groups, group 1 as control, group 2 has given 10% fish oil and group 2 has given 5% fish oil. Chronic inflammation treated to all samples for three days. The group 1 and 2 given Clarias batrachus fish oil on the third day. We did decapitation on the fourth day to perform Immunohistochemistry staining. The data were tabulated and analyzed statistically used One-way ANOVA.

Result: Group 2 and 3 has lower IL-1 expression than Control group. There is significant difference between groups.

Conclusion: Clarias batrachus Fish oil can lowered IL-1 expression on mucosal cavity’s chronic inflammation.

Keywords: Chronic inflammation, IL-1, Fish oil Clarias batrachus

1. INTRODUCTION

Inflammation is a natural reaction of the body such as vascular and cellular responses in reaction to their chronic jejas¹. Radang involves a complex interaction of cell phagocytosis, and mediator / cytokine secreted by the cells. One of them Interleukin-1 which is a proinflammatory cytokines secreted by macrofag². In chronic inflammatory conditions of increased circulation of IL-1, IL-6 and TNF-alpha³.

Fish oil has been widely known to have anti-inflammatory effects, antioxidant, antibacterial and immunomodulator⁴. Fish oil circulating in the community are made from mackerel, tuna, salmon and of the whale blubber. The hard farmed fish and are quite expensive. Local catfish (Clarias batrachus), is easily bred catfish and many cultivated mainly in Indonesia⁵. Currently, many catfish sold in fresh form, but fish oil catfish is high selling value derivative products has not been developed ⁶.

Previous research has shown that fish oil catfish species Clarias batrachus have the highest omega-3 levels when compared with other fresh fish and shrimp with levels of 28% of...
the total fatty acid\(^7\). Additionally, in the catfish contains a number of nutrients needed by the body are protein, calcium, phosphorus and various mineral\(^8\). Omega 3 is known to have an important role in reducing the inflammatory responses that are linked to serious health problems such as heart disease, atherosclerosis, myocardial infarction and stroke because it has anti-inflammatory effects and can work as an immuno-modulator\(^9\).

Currently developing research on fish oil as an ingredient in the treatment of various diseases. Research by Kamat and Roy\(^{10}\) C. batrachus show that fish oil helps reduce elevated glucose and helps increase the activity of SOD (Super Oxide Dismutase) antioxidant that can reduce tissue damage in animals that have been induced alloxan.

Based on this, the researchers wanted to determine the effect of fish oil catfish (Clariasbatrachus) on the expression of IL-1 in the oral mucosa with chronic inflammation. Kamat and Roy (2015) have reported that fish oil catfish (Clariasbatrachus) can increase SOD at concentrations of 10% in animals induced alloxan\(^{10}\). So these results are used as a reference for determining the concentration in this study. This study will use a concentration of 10% and 5%. Hopefully this research can see the expression of IL-1 in the oral mucosa with chronic inflammation.

2. MATERIALS AND METHODS

This research has been to get ethical clearance from the ethics committee of faculty of dentistry No: 98/KKEPK.FKG/VII/2016. In this experiment, 19 samples of rats rattusnovergicus, with the healthy condition, no injuries were clinical, sex male, aged less than one year, 2-3 months of age and weight ranges between 250 grams. The samples were divided into 3 groups: control group, the treatment group 1(KP1) were given fish oil catfish concentrations of 5% and treatment groups 2(KP2) were given fish oil catfish concentration of 10%.

Mice acclimatized for seven days later, given the incision on the buccal mucosa and used as a chronic inflammation by administering H2O2 10% as much as 2 times daily 2-3 minutes for 3 consecutive days - succession until visible sign - a sign of inflammation. On the third day given fish oil in the treatment group 1 and 2. On the fourth day, the rats were decapitated and tissues were taken in the area of injury, then performed immunohistochemical staining using anti-IL-1. Macrophage cells that express IL-1 (brown) is calculated at 5 field of view with a magnification of 1000x. The data is processed by the Kolmogorov-Smirnov normality test, homogeneity test Levene, different test ANOVA and Tukey's test followed to determine the best concentration in reducing IL-1.

3. RESULTS

The results showed the expression of IL-1 in macrophages in the treatment group was lower than the control group, the treatment group 10% had a number of expressions of IL-1 at least 5% compared to the treatment group and the control group.
Figure 1. Expression of IL-1 in macrophages by immunohistochemical techniques (1000x)

Information: Expression of IL-1 was shown macrophages with brown color (designated by the direction of the arrow)
A. Control group; B. Expression of IL-1 in macrophages Treatment group 1 (K1); C. Expression of IL-1 in macrophages Treatment group 2 (KP2)

Figure 2. Graph expression of IL-1 in macrophages

In the Kolmogorov-Smirnov test results obtained throughout the study group had a significantly greater value than 0.05 (p> 0.05), which means the data on the entire study group normally distributed. Then, the Levene homogeneity test, demonstrate significant value of 0.745. Thus, it can be concluded that the variance in the data of these studies are homogeneous. Then, One Way ANOVA test to see significant differences between groups. (P <0.05)
Table 1. Test One Way ANOVA expression of IL-1 in macrophages

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>KP 1</th>
<th>KP 2</th>
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</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>.000*</td>
<td>.000*</td>
</tr>
<tr>
<td>KP 1</td>
<td>.000*</td>
<td>-</td>
<td>.003*</td>
</tr>
<tr>
<td>KP 2</td>
<td>.000*</td>
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</table>

Description: (*) = significant difference

The tables show that there were significant differences between groups. Between the control group and the treatment group 1 (p = 0.000), the control group and the treatment group 2 (p = 0.000) as well as the treatment groups 1 and 2 (p = 0.003).

After testing the One-Way ANOVA Tukey test was done to determine the concentration of the best in decreasing the expression of IL-1 in chronic inflammation of the oral cavity.

Table 2. Tukey Test

<table>
<thead>
<tr>
<th>Tukey Test</th>
<th>Mean</th>
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</thead>
<tbody>
<tr>
<td>1 (Treatment 2)</td>
<td>7.1667</td>
</tr>
<tr>
<td>2 (treatment 1)</td>
<td>15.7143</td>
</tr>
<tr>
<td>3 (Control)</td>
<td>58.6</td>
</tr>
</tbody>
</table>

From the table it can be seen Tukey test treatment group 2 had a number of expressions of IL-1 at least.

4. DISCUSSION

This study is an experimental research laboratory to find out the benefits of fish oil Clarias batrachus to decrease IL-1 in chronic inflammation. Fish oil that is currently on the market comes mackerel, tuna, salmon and of the whale blubber. The hard farmed fish and are quite expensive. According to Wang and Huang (2015), this time many marine fish contaminated with pollutants such as mercury and pesticides. Catfish is a freshwater fish that is bred in Indonesia has a chemical composition of MUFA (Mono-Unsaturated-Fatty-Acid) 36.12%, PUFA (Poly-Unsaturated-Fatty-Acid) 32.43% and SFA (Saturated-Fatty-acid) 31.45%.

In batrachus Clarias fish oil, Omega 3 PUFA which is the highest when compared with other freshwater fish and shrimp (Chedoloh et al, 2011). In this experiment, catfish are bred with organic feed so as maintained by its nutritional quality.

This study uses hydrogen peroxide to make a chronic inflammation due to its irritant. Hydrogen peroxide at low concentrations typically used in the medical field to clean the wound but this study using a higher concentration of 10%. This is in accordance with the European Commission in 2003 stated H₂O₂ with a concentration of 10% can irritate the skin and mucosa. The cut by 10% hydrogen peroxide can cause tissue damage.
Damage to the network will provide a signal to the monocytes to enter the damaged tissue and differentiate into macrophages phenotype M1 that is both pro-inflammatory. This will lead to increased expression of Interleukin-1. Interleukin-1 is a proinflammatory cytokine that can be used as indicators of the inflammatory process is prolonged. The condition, according the opinion King et al (2014)\textsuperscript{13} which states that when there is chronic inflammation will be an increase expression of Interleukin-1.

After administering the fish oil Clariasbatrachus, the results showed a decrease Interleukin-1 compared with the group not given fish oil Clariasbatrachus. The results of these studies in accordance hypothesis that fish oil catfish (Clariasbatrachus) can reduce the expression of Interleukin-1 in tissues that undergo chronic inflammation. There are significant differences between the control group and the treatment group 1 and 2. By using the Tukey test, the results obtained with the administration of the treatment group 2 fish-oil catfish (Clariasbatrachus) concentration of 10% has a number of expressions of IL-1 at least.

This is presumably because the administration of fish oil containing omega-3 PUFA have anti-inflammatory role\textsuperscript{14}. Omega 3 PUFA can suppress cell-mediated immune response, with the state of the antigen presenting cell function is inhibited. In addition, it can increase the fluidity of the membrane thus changing the expression on membrane proteins. Given the inhibition of the immune response, the expression of Interleukin-1 decreased.

In addition to having a role as an anti-inflammatory, omega-3 PUFAs may be activators of wound healing by changing the phenotype of macrophages M1 into M2\textsuperscript{15}. M2 macrophage phenotype can secrete anti-inflammatory cytokines such as IL-10 and TGF-β. TGF-β stimulates fibroblast chemotaxis, increasing the production of collagen, fibronectin and proteoglycans (Istiati, 2013)\textsuperscript{16}. This will support the tissue remodeling.

Appropriate research has been done, giving fish oil Clariasbatrachus known to decrease the expression of Interleukin-1. Fish oil Clariasbatrachus with a concentration of 10% is among the best in reducing the expression of Interleukin-1.

**REFERENCE**