Physical Exercise Does Not Improve Colon Inflammation in Mice Induced Lambda Carrageenan

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Abstract

Inflammatory Bowel Disease (IBD) is a well known reported gastrointestinal tract disease, which the prevalence continuous to increase in Southeast Asia and other developing countries. Animal model have already been widely used for gut inflammation study. Lambda (λ) carrageenan is a chemical substance which commonly used to induced inflammation in IBD animal models. Physical exercise is a non-pharmacological therapy recommended for IBD. However, further study is needed to determine the effects of this therapy. This study was aimed to determine the physical exercise effect on colon inflammation in mice induced λ-carrageenan. Thirty-eight mice were divided into 2 groups. Control group (C) which induced with λ-carrageenan and treatment group (T) which induced by λ-carrageenan and treadmill exercise. The degree of colon inflammation was obtained by histopathological examination using Hematoxylin-Eosin (HE) staining and inflammation scoring system, which include inflammatory cell infiltration and intestinal architecture. All mice colon samples in both group were inflamed with varying degrees. The treatment group had a higher Inflammatory degree score than control group (p <0.05). Physical exercise does not improved the degree of colon inflammation in mice induced λ-carrageenan due to uncontrolled stress induction and water intake containing λ-carrageenan.

Key words: IBD, inflammatory cell infiltration, intestinal architecture, λ-carrageenan, physical exercise, treadmill

Received: 29 November 2019 Revised: 9 Desember 2019 Accepted: 4 Januari 2020

INTRODUCTION

Inflammatory Bowel Disease (IBD) is intestinal inflammation whose causative factors of the disease are not fully understood yet. Among all gastroenterology cases, IBD, which consist of Ulcerative Colitis (UC) and Crohn's Disease (CD) is widely reported (Whittlem et al., 2010). IBD is associated with immune complex disorders, influenced by genetic and environmental factors (Wehkamp et al., 2016). Physical exercise is constantly associated with an increase in the immune system (Gleeson, 2007). Therefore, IBD patients are encouraged to do physical exercise as a management for the treatment of the disease. Physical exercise recommendations are mainly to reduce symptoms, complications, and extraintestinal manifestations of IBD (Narula and Fedorek, 2008; Packer et al., 2010).

In the last few decades, there have been many experimental studies for examining intestinal inflammation, which is very useful for obtaining new information about the pathogenesis of a disease and preclinical evaluation for a new therapy (Kolios, 2016). Experiments with colitis require induction of chemicals, one of which is λ-carrageenan, because it has potential inflammatory effect in the bowel. Intestinal tissue that has been induced by chemicals such λ-carrageenan, has special characteristics when evaluated through histopathological analysis HE staining using a preexisting scoring system (Ghia et al., 2006).

However, the literature which stated that physical exercise can reduce the symptoms of IBD is weak and inconsistent (Narula and Fedorek, 2008). The effectiveness of physical exercise and its mechanism on disease activity is unclear (Narula and Fedorek, 2008). So that further research is needed to strengthen the
opinion that physical exercise can be done for IBD (Narula and Fedorek, 2008) and experimental and clinical research is also needed to confirm its effectiveness as an inflammatory bowel therapy (Ko and Anyeung, 2014).

This study was aimed to determine the physical exercise effect on colon inflammation in mice (Mus musculus) induced λ-carrageenan.

**MATERIALS AND METHODS**

**Ethical Approval**

All procedures were approved ethically under supervision of the Health Research Ethic Committee, Universitas Airlangga. Letter number 232/EC/KPEK/FKUA/2019.

**Animals**

A total of 38 BALB/c mice were obtained from Animal Laboratory, Biochemistry Department in Faculty of Medicine, Universitas Airlangga. During the experiment, all mice were housed in a well-controlled environment and kept free access to mineral water and the same animal diet. After a one-week adaptation period, assigned to control and treatment groups. Both of group were induced by λ-carrageenan supplemented in drinking water at a concentration of 20 mg/L (Shang et al., 2017).

The treatment group was given treadmill exercise, 7 m/minutes for 30 minutes (include 5 minutes warming up, 20 minutes exercise, and 5 minutes cooling down) (Kim et al., 2018), and 3 times a week (Durigan et al., 2009). After six weeks of treatment (Shang et al., 2017), all mice were sacrificed with cervical dislocation technique.

**Chemical and Reagents**

λ-carrageenan, Tokyo Chemical Industry co., ltd.®

**Sample Collection and Procedure**

Colon samples were collected in the 6th week of the study. Colon samples were fixed in 10% neutral-buffered formalin for 12-24 hours and embedded in paraffin, cutted and stained with HE. Tissue samples were well-oriented with longitudinally cut crypts to precisely assess alterations in the overall intestinal tissue architecture.

**Histopathological Evaluation**

Samples were evaluated by standard light microscope with 100x and 400x magnification. The degree of inflammation was scored based on the criteria for chemically-induced colonic inflammation in the journal by Erben et al., 2014., which shown on Table 1.

**Statistical Analysis**

Data were collected and analyzed statistically for correlation between groups using statistical software (IBM SPSS Statistics version 20). All results were considered statistically significant at $p<0.05$.

**RESULTS AND DISCUSSIONS**

All of colon samples in both group showed inflammation (38/38) with varying degrees. The number of samples in each score are shown in Figure 1.

In the control group, there was one sample with score 2, twelve samples with score 3, and six samples with score 4. Score 3 is the dominant score in the control group (Figure 2). In the treatment group, there was one sample with score 3, seventeen samples with score 4, and one sample with score 5. Score 4 is the dominant score in the treatment group (Figure 3). The highest inflammation degree score in this study was 5 (Figure 4). The Inflammation degree score showed significant differences regarding control and treatment group ($p<0.05$).

Mechanism of Induction λ-carrageenan to gut inflammation is unclear and multifactorial, partly because of the stimulation of pro-inflammatory cytokines, damaging the epithelial barrier, and disrupting the innate intestinal mucosal immune response to microorganisms (Malik et al., 2009; Buffie et al., 2009), reducing the amount of glycoprotein in the colon (Al-Suhail et al., 1984), inhibiting interaction between macrophages and lymphocytes (Chong...
Table 1. Inflammation scoring system: Inflammation score is the sum of score A and score B

| Inflammatory cell infiltrate | Score A |
|-----------------------------|---------|
| Severity | Extent                        |
| Mild    | Mucosa                        |
| Moderate | Mucosa and Submucosa          |
| Marked  | Transmural                    |

| intestinal Architecture | mucosal Architecture | score B  |
|-------------------------|----------------------|---------|
| epithelial changes      | Focal Erosion        | 1       |
|                         | Erosion              |         |
|                         | + Focal Ulceration   | 2       |
|                         | extended focal ulceration | 3 |
|                         | +Granulation tissue  |         |
|                         | +pseudopolyps        |         |

Figure 1. The distribution of the number of samples for each inflammation score

Figure 2. Representing histomorphology of colon tissue with sum scores 3 referred to Table 1, submucosal infiltrates (score A:2) with focal erosion (score B:1); Original magnification x100 and x400; black arrows-inflammatory cell infiltration within submucosa; red arrows-focal erosion; HE tissue stained
and Parish, 1985), induces inflammation in the presence of microbiota because studies in guinea pigs induction carrageenan produces caecum ulceration on days 21-30 (Onderdonk et al., 1977), whereas germ-free guinea pigs do not produce any intestine lesions for up to 6 months of induction (Onderdonk et al., 1978), stimulates the inflammatory cascade in the colon epithelial cell by increasing IL-8 production (Borthakur et al., 2007) which is a key pro-inflammatory cytokines (Martino et al., 2017) and so on. Although the mechanism has not been ascertained, the findings of the effect of carrageenan on intestinal inflammation can already be proven. Increasing IL-6 and TNF-α expression and reduced IL-10 (Wei et al., 2016) and intestinal inflammation (Wu et al., 2016).

The effect of physical exercise on inflammation has been proven from previous studies, namely reducing the production of inflammatory cytokines in vitro (Flynn et al., 2003), as another example of physical exercise reducing TNF-α, IL-6, and IL-1β from skeletal muscle in adults (Gielen et al., 2003). However, this study found that physical exercise did not reduce inflammation. There are several studies that have found that physical exercise does not alleviate inflammation, especially not significantly reducing inflammatory biomarkers (Nicklas et al., 2004; Marcell et al., 2005; Hammett et al., 2006; Nassis et al., 2005).

**Figure 3.** Representing histomorphology of colon tissue with sum scores 4 referred to Table 1. Submucosal infiltrates (score A:2) with focal ulceration (score B:2); Original magnification x100 and ×400; black arrows-inflammatory cell infiltration within submucosa; red arrows-focal ulceration; HE tissue stained

**Figure 4.** Representing histomorphology of colon tissue with sum scores 5 referred to Table 1. Submucosal infiltrates (score A:2) with extensive ulceration (score B:3); Original magnification x100 and ×400; black arrows-inflammatory cell infiltration within submucosa; red arrows-extensive ulceration; HE tissue stained
However, previous research only proves that physical exercise does not provide anti-inflammatory effects, there is no research that says physical exercise exacerbate the presence of inflammation.

In the results of this study, physical exercise exacerbated the degree of inflammation, and was significantly different in the treatment group which treated with physical exercise compared to the control group without physical exercise. This can be due to several factors, which includes the inappropriate method of physical exercise and the poorly conditioned environment that give more stress on mice, thus exacerbating the presence of inflammation.

The inappropriate physical exercise may give the same appearance with forced physical exercise. Voluntarily and forcefully physical exercise would affect the intestinal microbiota in mice, as proved by differences in both diversity and structure, and could affect many sites in the intestinal tract, such as the caecum and colon (Jacob et al., 2015), where changes in the microbiota could cause IBD (Allen et al., 2015). Dysbiosis can also cause the release of endotoxins and flagellins from Gram-negative bacteria that bind TLR-5 which will induce inflammation and damage from intestinal epithelium (Vijay-Kumar et al., 2010; Cani et al., 2008).

In another study conducted by Svensson et al. in 2016, it was found that forced physical exercise can induce stress responses by increasing anxiety and corticosterone levels, ischemia causes neuronal damage in the hippocampus and results in increase cytokines in brain tissue and blood flow (Svensson et al., 2016). Thus it can be concluded that forced physical exercise will increase stress, ischemia and neuronal damage indirectly, and can induce inflammation and epithelial damage directly. And in this study, stress was not only obtained from the inappropriate method of physical exercise, the other two reasons are likely to cause mice stress, the first is the treatment group which given physical exercise and had to be moved three times a week, while the control group was almost never moved except when cage was being cleaned. Cage-switch induces stress on rats has been proven by Morimoto et al. study in 1993 (Morimoto et al., 1993), and the second in the treatment group mice, exercise was conducted during the day so that it interferes mice circadian rhythms, that resulted in mood disturbance (Vadnie et al., 2017).

Moreover, mice given physical exercise will need more water intake (Goodrick, 1978), therefore in this study, the treatment group will drink more water, and in this study induction of colon inflammation using λ-carrageenan diluted in drinking water, thus it can cause more severe inflammation in treatment group.

**CONCLUSION**

Physical exercise in mice induced λ-carrageenan resulted in worsening of inflammation both in terms of inflammatory cell infiltration and intestinal architecture.

**ACKNOWLEDGEMENTS**

We thank Dewi Ratna Sari, dr., M.Si (Histology Departement, University of Airlangga) who helped in taking samples pictures and Mr. Alfian (Biochemistry Departement, University of Airlangga) for technical support during the experiment. This study did not receive any external funding.

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