THE EFFECT OF INDOLE-3-CARBINOL (I3C) IN THE AORTA HISTOPATHOLOGY OF MICE WITH ATHEROGENIC DIET

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Abstract— Cardiovascular disease is the number one cause of death in the world 82% of deaths occur in developing countries. The process of retention, oxidation and lipid modification that occurs in atherosclerosis lead to chronic inflammation of the artery wall. Research shows that accelerated deposition of plaques in the body caused by trimethylamine oxide (TMAO). TMAO can increase the accumulation of cholesterol in macrophages that enhances the formation of foam cells in the arterial wall. Flavin Monooxygenase (FMO) can inhibit formation of TMAO. Indole-3-carbinol (I3C) is able to work as a competitive inhibitor of FMO. Treatment of I3C is expected to lower cholesterol levels and reduce the amount of foam cells in mice aorta histopathology picture. The treatment group consist of a control, negative control, group A (10 mg/kg I3C), group B (200 mg/kg I3C), and group C (500 mg/kg I3C). Mice was induced by atherogenic diet. Blood cholesterol levels were count and aortic arch mice were taken for histopathological. The blood cholesterol levels in mice induced atherosclerosis decrease and histopaology aorta of mice show decreasing amount of foam cell ($p<0,05$).

Keywords— Atherosclerosis, Indole-3-Carbinol.

I. INTRODUCTION

Cardiovascular disease is the number one cause of death in the world. In 2012, approximately 17.5 million people die from cardiovascular disease. Eighty two percent of deaths occur in developing countries like Indonesia (WHO, 2015). Atherosclerotic plaque is a major cause of cardiovascular disease, among others, may cause coronary heart disease and stroke.

Treatment options are still curative whereas the pathophysiology of atherosclerotic plaque formation showed a progressive journey. According Insull (2009), the clinical manifestations of atherosclerosis can only be reduced by about 20% -40% with the use of HMG CoA reductase inhibitor group such as statins. Antithrombotic therapy such as aspirin and clopidogrel is only capable of lysing and inhibit thrombus after rupture of atherosclerotic plaque. In addition, antithrombotic drugs can cause bleeding (Patti and Nusca, 2011). The downside of curative therapy is the drug was only given after a diagnosis is upheld. If the formationof atherosclerotic plaque can be prevented there will be millions of people who could be saved from a variety of cardiovascular diseases.The process of retention, oxidation and lipid modification that occurs in atherosclerosis lead to chronic inflammation of the artery wall. Atherosclerotic lesions can cause potentially ischemic stenosis or arterial thrombotic occlusion lead to the heart, brain, and other organs (Insull, 2009). Recent research shows that accelerated deposition of plaques in the body caused by trimethylamine oxide (TMAO) (Tang et al., 2013). TMAO can increase the accumulation of cholesterol in macrophages that enhances the formation of foam cells in the arterial wall. This compound is derived from trimethylamine (TMA) converted into TMAO by the enzyme flavin monooxygenases (FMO). TMA formed is a result of metabolism of phosphatidilcholine and l-carnitine by intestinal bacteria (Mendelsohn and Larrick, 2013). Manipulation of enzymes involved in the oxidation of TMA became TMAO in the liver, an enzyme FMO has great potential to do.

FMO enzymes in mammals consists of FMO1 until FMO5. However, the enzyme that is able to influence in the formation of TMAO is flavin monooxygenase form 1 (FMO1) and flavin monooxygenase form 3 (FMO3). FMO1 and FMO3 a dominant isoforms were in the liver rather than the other isoforms. However, by Bennett et al. (2013), FMO3 activity show a ten-fold greater than FMO1 in turning TMA into TMAO. Based on these studies overexpression of FMO3 significantly increases plasma levels of TMAO.

I3C is a compound derived from cabbage and proved to be a good anti-cancer compounds in vitro and in vivo (Aggarwal and Ichikawa, 2005). However, the use of I3C has the potential to be further developed into a compound that can prevent the formation of foam cells because these compounds are able to inhibit the enzyme activity and expression of FMO is in the liver. I3C is able to work as a competitive inhibitor of FMO(Chang, 2005). That mechanism is expected to be reduced TMAO levels. In addition, I3C is able to increase lipolysis stimulated epinephrine and lower insulin-stimulated lipogenesis (Okulicz et al. 2009). Never has there been studies and journals that discuss theeffect of indole-3-

carbinol (I3C) in the aorta histopathology of mice with atherogenic diet Therefore, the potential use of I3C as an attempt prevention of cardiovascular disease (CVD) needs to be developed further.

II. DETAILS EXPERIMENTAL

2.1. Materials and Procedures

This study uses an true experimental research design with post test only randomized control group design in the biomolecular and pharmacology laboratory of the Faculty of Medicine and the CDAST laboratory University of Jember. The inclusion criteria of the sample includes male mice 2-3 months old, 25-35 grams, healthy, active and normal moving. Exclusion criteria for the study is mice that don't want to eat and die. The number of experimental animals used in this study is 25 mice were divided into five groups with five repetitions.

2.2 Atherosclerosis Induction

Induction of atherosclerosis by damaging endothelial done through intravenous administration of adrenaline as much as 0.00084 mg/20g BB on the vena lateralis of mice on the first day of treatment and continue atherogenic diet for 14 days. Atherogenic diet used in the study is yolk per oral once a day as much as 0,2cc. Egg yolk contains cholesterol and high L-carnitine which is the precursor of TMA.

2.3 Measurement of Cholesterol Levels

Animal cholesterol measurements performed only once after 14 days of administration of atherogenic diet. Measurements were performed using an Easy Touch portable blood check up.

2.4 Treatment of indole-3-carbinol

Indole-3-carbinol was administered daily for 14 days per oral. Indole-3-carbinol is dissolved in distilled water and homogenized with mortar before gived to mice. The dose given divided into 3 groups: group A with a dose of 10mg/kg, group B with a dose of 200 mg/kg, and group C with a dose of 500 mg/kg body weight.

2.7 Histopathological Feature

Aortic arch mice were taken for histopathological. Slide was stained using Hematoxylen Eosin (HE).

2.8 Data Analysis

Data taken in the form of numerical data of the measurement results rat blood cholesterol levels, and qualitative data from rat aorta histopathologic examination. Analysis performed for numerical data is normality test and test variants that produce normal data distribution and variance of data equal ($p > 0.05$) so then used a hypothesis test One Way Anova with significant results. Histopathological changes in the aorta were analyzed by histopathology picture viewing and comparing the aorta from each group.

III. RESULTS AND DISCUSSION

3.1 Mice Blood Cholesterol Levels

Examination of blood cholesterol levels in mice conducted after the administration of atherogenic diet and indole-3-carbinole for two weeks. Cholesterol levels were measured using a strip measuring cholesterol portable Easy Touch. This tool has a range of measurement results of 100-400 mg/dL.

In this study, the data cholesterol levels in negative control is the standard used in determining normal cholesterol levels in mice. This is due to the range of the measurement gauges cholesterol portable Easy Touch is still quite high. Nevertheless, the use of these tools remains the top choice because of its advantages that it does not require much amount of blood, practical, and inexpensive.

Table 1. Total Cholesterol Levels Mice

Treatment	totalcholesterol levels(mg /dL)
control	199,4 ± 28,94
negative control	246± 8,52
A	224 ± 15,30
B	170,6 ± 12,54
C	154,8 ± 14,46

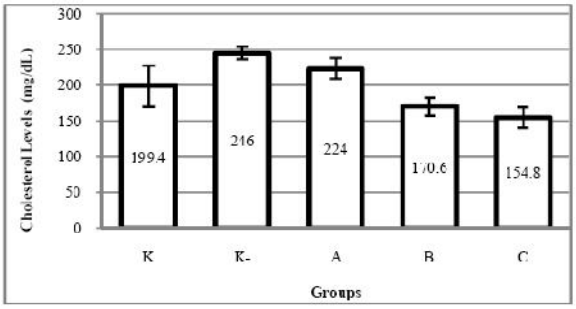


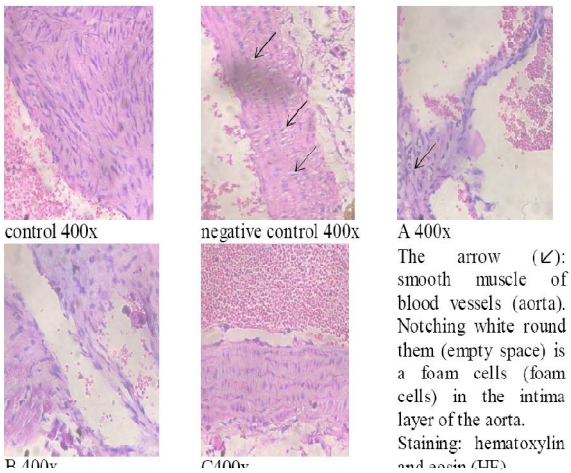
Figure 1. Graph Mice Cholesterol Levels

Based on the data above, we can see the cholesterol levels of a negative control group, A, B and Cdecrease respectively.Increased doses of I3C conducted in treatment groups A, B, and C showed an increased effect on blood cholesterol levels in mice. This is indicated by the decline in blood cholesterol levels in mice in accordance with increasing doses of the A (10 mg/kg), B (200 mg/kg), and C (500 mg/kg), respectively 224 ± 15.30 mg/dL , 170.6 ± 12.54 mg/dL and 154.8 ± 14.46 mg/dL. Even in group B and C, the data shows a lower value than the negative control for 199.4 ± 28.94 mg/dL.

The highest cholesterol levels found in negative control group. This group was given atherogenic diet that can raise blood cholesterol levels in mice. Lowest cholesterol levels is found in group c with the highest dose of I3C. In this study, we found the I3C effect are dose dependent. Supported by the statistical analysis with significancy $P<0.05$. The decreasing

cholesterol levels is same with Okulicz et al. research in 2009.

3.2 Histopathology Feature of Mice Aorta



Histopathologic features of aortic mice showed improvement with increasing dose therapy, visiblereduction in the number of foam cells in groups A and B, and not the formation of foam cells in group C. It is based on a comparison with a negative control group that did not indicate the presence of foam cell formation and the positive control group seemed full picture cell foam (black arrow). The amount of foam cell from control group, negative control, A, B and C respectively is 0, 38, 10, 6, and 0. Foam cell formation process may be affected by I3C directly. Seen from cholesterol levels is not too much difference, but the number of foam cells between negative control group with A very different.

CONCLUSIONS

From this research we can conclude that indole-3-carbinol can lower blood cholesterol levels in mice induced atherosclerosis and improves picture histopaology aorta of mice with decreasing amount of foam cell.

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