

The Comparison of Activation Nuclear Factor Kappa Beta (NFkB) at Rattus Novergicus Strain Wistar Induced by Various Duration High Fat Diet (HFD)

Titin Andri Wihastuti, Djangan Sargowo

Abstract—NFkB is a transcription factor regulating many function of the vessel wall. In the normal condition, NFkB is revealed diffuse cytoplasmic expressions suggesting that the system is inactive. The presence of activation NFkB provide a potential pathway for the rapid transcriptional of a variety of genes encoding cytokines, growth factors, adhesion molecules and procoagulatory factors. It is likely to play an important role in chronic inflammatory disease involved atherosclerosis. There are many stimuli with the potential to activate NFkB, including hyperlipidemia. We used 24 mice which was divided in 6 groups. The HFD given by *et libitum* procedure during 2, 4, and 6 months. The parameters in this study were the amount of NFkB activation, H₂O₂ as ROS and VCAM-1 as a product of NFkB activation. H₂O₂ colorimetric assay performed directly using Anti Rat H₂O₂ ELISA Kit. The NFkB and VCAM-1 detection obtained from aorta mice, measured by ELISA kit and immunohistochemistry. There was a significant difference activation of H₂O₂, NFkB and VCAM-1 level at induce HFD after 2, 4 and 6 months. It suggest that HFD induce ROS formation and increase the activation of NFkB as one of atherosclerosis marker that caused by hyperlipidemia as classical atherosclerosis risk factor.

Keywords—High Fat Diet, NFkB, H₂O₂, atherosclerosis

I. INTRODUCTION

ATHEROSCLEROSIS is a disorder in which blood vessel wall that characterized by a blockage (atheroma), so that blood flow becomes impaired.[1] For decades, deaths from coronary heart disease (CHD) atherosclerosis in emerging industrial countries increased dramatically.[2] The prevalence of atherosclerotic coronary heart disease is increasing from year to year and by the WHO declared as a global threat. An estimated 1.9 billion people or 1/3 of world population affected by this disease. In Indonesia morbidity and mortality due to CHD prevalence tends to increase and reached 50%. [3]

The cause of atherosclerosis such as dyslipidemia, free radicals, endothelial dysfunction and inflammation. [4] Dyslipidemia is a disorder in lipid metabolism and marked by increased levels of total cholesterol, LDL cholesterol and triglycerides, and decreased levels of HDL cholesterol. [4] Increasing levels of cholesterol in the blood, especially low-density lipoprotein (LDL) is harm because of peroxidation process (auto-oxidation) of lipids that exposed to oxygen.

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Lipid peroxidation is a chain reaction that continues to produce Reactive Oxygen Species (ROS) such as OH⁻, RO and H₂O₂. [5] ROS formed by creating the effect of tissue damage by causing inflammation and endothelial dysfunction. ROS also cause activation of *Nuclear Factor Kappa Beta* (NFkB), a transcription factor that plays a very important role in controlling various biological effects such as inflammation. [6].

NFkB plays an important role in atherosclerosis because of a chronic inflammatory process that occurs in atherosclerosis. Inflammation happens because activated NFkB. [7] NFkB is a heterodimer composed of p50 and p65 subunits which in normal conditions is in the cytoplasm in an inactive state. This is caused by the presence of binding protein called *Beta Kappa inhibitor* (IκB) which binds to p50 and p65 to prevent entry into the nucleus. [8] NFkB activation is a process where there is a subunit translocation of NFkB from cytoplasm into the nucleus and stimulates expression of proinflammatory genes such as VCAM-1. [9] Since the NFkB plays an important role in the process of developing the disease, the current study uses a lot of NFkB as the target parameter. Research is needed to explore the giving of high-fat diet as an in vivo model of atherosclerosis in activating NFkB.

II. MATERIAL AND METHODS

A. Experimental Design

This research is a *true experimental laboratory, in vivo, post test with control group*, using male white wistar rat, age 2 weeks, weight 150-200 grams. Number of rats in every group is according to formula $p(n-1) \leq 16$, so that gained 4 number of rats of the sample on each group. The rats are divided into 2 big groups (normal diet group and HFD group). Each of group consist of three *et libitum* diet giving for 2,4 and 6 months. Parameter of this research is the duration of HFD giving, amount of CEC and H₂O₂ level. Data taking is carried out on serial time in physiology laboratory of medical faculty Brawijaya University.

B. High Fat Diet (HFD) Rat Model

Rattus novergicus strain wistar male, age 4 weeks is given with food with energy total 104,7 Kal, energy from fat is gained from total cholesterol, Cholat acid and pig oil. Weighting rat every week, using analytic weighting with the number of carefulness is 0,01 kg, stated on gram (anthropometry measurement which describing total number of tested animal body component, including tissue and fluid in gram measurement unit). Rat food intake is the difference of food's weight consumed which stated as daily intake.

Normal Diet (ND) that consist of: confeed-pars (contents water 12%, protein 11%, fat 4%, fiber 7%, ash 8%, calsium 1,1%, phospor, antibiotic, coccidiostat 0,9%), wheat four 33,4%.

C. Measurement of H₂O₂ Level

Measuring of H₂O₂ (Reactive Oxigen Species) is carried out directly using the method of Wesson (1994). Measuring of H₂O₂ by Anti Rat H₂O₂ ELISA Kit kolorimetrically (Biossay Design, USA).

D. Detection NFKB on Circulating Endothelial Cells (CEC)

The CECs are counted in whole blood using flow cytometry. Isolation method of CEC refered to Blann et all. Peripheral blood sample take by sput. Blood incubated using anti-CD 146. Nfk B expression was observed by using ELISA.

E. Measurement of VCAM-1 expression

VCAM-1 expression was observed by using imunohistochemistry and ELISA. VCAM-1 expression was indentified on aorta endothelial cells mice with normal diet and HFD 2, 4 and 6 months.

F. Statistical Analysis

Analysis of Variance test (ANOVA) is carried out to find out the effect of duration HFD giving toward NFKB expression on various group of rats.

III. RESULT

The research was conducted by the approval of Health Research Ethics Committee Brawijaya University of Medicine 069/EC/KEPK-JK/03/2011 no. Data are obtained as follows

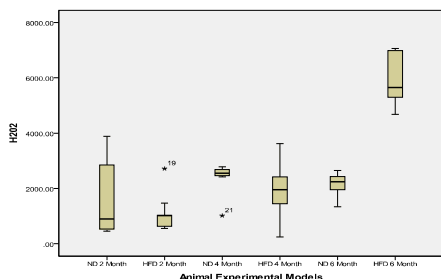


Fig. 1 The level of H₂O₂ each groups (unit/mL)

The figure shows that the highest levels of H₂O₂ is found in the group of HFD-treated for 6 months with the average rates of 5873 unit/mL. On the other hand, the lowest level of H₂O₂ is found in the group of HFD-treated for 2 months with the average rates of 1076,33 unit/mL. Statistical analysis using One-way ANOVA test with the level confidence of 99% proved that there is a significant difference of H₂O₂ levels (p values of 0,000) between the groups of HFD-treated for 2, 4 and 6 months. Similar findings is also found in the comparison of the normal diet groups and HFD-treated groups.

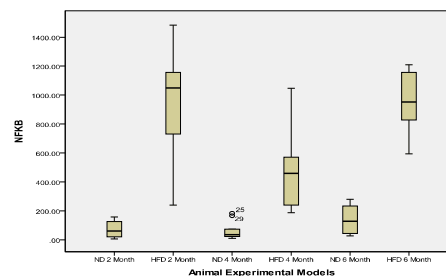


Fig. 2 The activation of NFKB each groups (ng/ml)

The figure shows that the highest expression of NFKB is found in the group of HFD-treated for 6 months with the average rates of 970,67 ng/ml. On the other hand, the lowest expression of NFKB is found in the group of normal diet treated for 4 months with the average rates of 61,334 ng/ml. Statistical analysis using One-way ANOVA test with the level confidence of 99% proved that there is a significant difference of NFKB levels (p values of 0,000) between the groups of HFD-treated for 2, 4 and 6 months. Similar findings is also found in the comparison of the normal diet groups and HFD-treated groups.

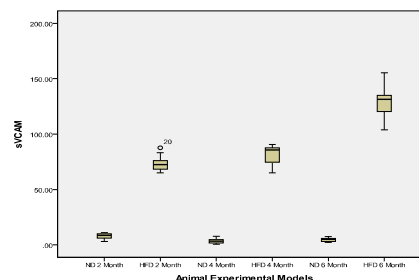


Fig. 3 The expression of VCAM-1 each groups (ng/ml)

The figure shows that the highest expression of VCAM-1 is found in the group of HFD-treated for 6 months with the average rates of 81,06 ng/ml. On the other hand, the lowest expression of VCAM is found in the group of normal diet treated for 4 months with the average rates of 3,48 ng/ml. Statistical analysis using One-way ANOVA test with the level confidence of 99% proved that there is a significant difference of VCAM-1 levels (p values of 0,000) between the groups of HFD-treated for 2, 4 and 6 months. Similar findings is also found in the comparison of the normal diet groups and HFD-treated groups.

IV. DISCUSSION

Giving of *High Fat Diet* (HFD) significantly affect toward the elevated levels of H₂O₂, and NFKB expression of ICAM-1 expression in rats. It is known from the existence of significant differences in levels of H₂O₂, and NFKB expression of VCAM-1 expression between groups of mice were given HFD and groups of rats given normal diet.

Duration of diet also significantly affect the elevated levels of H₂O₂, NFKB expression and VCAM-1 expression in rats. It is known from the existence of significant differences in levels

of H₂O₂, NFκB expression and VCAM-1 expression between the giving of HFD 2, 4 and 6 months. Elevated levels of cholesterol in the blood, especially the low density (LDL) affect the increased production of ROS such as H₂O₂ (Vogel, 2007). LDL in the plasma will be captured by the *scavenger receptor*. At high levels of LDL, down regulation will happen resulting in increased plasma LDL, that will further modified and produce H₂O₂ as one of its products. [10] Just as in this study, the giving of HFD rats increased levels of H₂O₂ at each duration. The results of this study showed elevated levels of H₂O₂ on the duration of each HFD. ANOVA analysis at the 99% confidence interval shows the levels of H₂O₂ group HFD for 2 months of giving (1076 units / ml) differed significantly better with the giving of HFD for 4 months (1879 units / ml) or with a group of 6-month HFD giving (5873 units / ml). This indicated that the duration of HFD affect the increased levels of H₂O₂. However, compared with the control group (normal diet administration) HFD given on duration of 2 months and 4 months has not shown significant differences of H₂O₂ levels. While the duration of 6 months there were significant differences in levels of H₂O₂ in the control group and the giving of HFD. Elevated levels of H₂O₂ in the giving of HFD associated with activation of NFκB. H₂O₂ as ROS will degrade the bond of kappa beta, causing translocation of NFκB from the cytoplasm to the nucleus.

NF-κB is a transcription factor that plays an important role in inducing the regulation of various genes in the inflammatory response and cell proliferation. In accordance with the results of this study which showed that the most activation of NFκB found in the giving of HFD 6 months group is 970.67 ng / mL. The lowest NFκB activation in the group giving the 4 months normal diet is 72.59 ng / ml. Giving a normal diet either 2, 4 or 6 months do not increase the activation of NFκB.

Results in this study shows there were no significant differences on the activation between groups that given normal diet. Giving of HFD for 2 months significantly increased the activation of NFκB, although the giving of HFD 4 months actually decreased activation of NFκB. This could occur because of limitations in this study that using an animal model giving HFD by *Ett libittum*, so the food is not always consumed by the rats. However, in the giving of HFD for 6 months gained the highest activation of NFκB. NFκB activation is also associated with CD40-CD40L system, a mediator whose role is to prevent the development of atherosclerotic lesions become unstable / easy to rupture (unstable advanced lesions). So that the NF-κB allegedly was the target of determining the effective terapeutik. [11] Because NF-κB is a factor that induces genes transcription and sensitive to the oxidative stress response. So NF-κB is a strong candidate as a protein that facilitates the inhibition of signaling induced by oxidative stress.

The highest level of VCAM-1 levels is in highest 6-month HFD group 130.6 ng / mL and the lowest is in the 4 months normal diet. Endothelial dysfunction that occur increase leukocyte adhesion by increasing VCAM-1. At the time endothelial cell had the inflammatory ctivation there are

increase in VCAM-1 that support the adhesion of monocytes. In the arterial intima, monocytes will turn into macrophages and begin to express scavenger receptors that will internalize modified lipoproteins and resulting the formation of foam cells. Foam cells is the characteristic of atherosclerotic lesions. [12]

IV. CONCLUSION

Giving of *High Fat Diet* (HFD) significantly affect the elevated levels of H₂O₂, NFκB expression and ICAM-1 expression in rats. Duration of diet also significantly affect the elevated levels of H₂O₂, and NFκB expression of VCAM-1 expression in rats. Increased H₂O₂ causing degradation of the bond KB resulting in translocation into the nucleus thereby increasing the expression of proinflammatory genes such as VCAM-1.

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