Conference Paper

High Fiber Diet Decreases the Level of Interleukin 1β in Hyperlipidemia Model of Rats

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Abstract

Diet containing high fat and fructose causes hyperlipidemia which is characterized by hypercholesterolemia and hypertriglyceridemia. Hyperlipidemia produces free fatty acids that induce the secretion of proinflammatory cytokines such as Interleukin Iβ (IL-Iβ). Secretion of IL-Iβ induced by NF-Kβ can be suppressed by short chain fatty acids (SCFA). This study aimed to determine the effects of high fiber diet on the level of IL-1β in hyperlipidemia model of rats. Male Wistar rats (n=25) of eight weeks old were divided into five groups: control (K), hyperlipidemia (H), hyperlipidemia with high fiber diet in a doses of 2.6 g/rat/day (P1), 5.2 g/rat/day (P2), and 7.8 g/rat/day (P3). Serum levels of IL-1β were measured in a pre- and posttest manner. The pretest was taken at 7 weeks of treatment, while the posttest was measured at the final 13 weeks of this protocol. Pretest and posttest of serum levels of IL-1β were measured with ELISA. The resulting pre- and posttest serum levels of IL-1β were analyzed with paired t-test by using SPSS 23.0 software. Serum levels of IL-1β in rats which received high fiber diet of doses of 2.6, 5.2 and 7.8 g/rat/day were significantly lower compared to the hyperlipidemia rats ($p<0.05$). We conclude that high fiber diet could reduce the level of IL-1β in rats.

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1. Introduction

Diet containing high fat and fructose increases the secretion of very low density lipoprotein (VLDL), causing hypercholesterolemia and hypertriglyceridemia, which are the symptoms of a lipid metabolism disorder called hyperlipidemia [1]. The mechanism of hyperlipidemia starts with the liver secreting VLDL which transports triglyceride to peripheral tissues [2]. Triglyceride is hydrolyzed by lipase lipoprotein, producing free fatty acids (FFA) to be stored in tissues such as healthy muscles and skeletal muscles [3]. The higher the triglyceride level, the higher the FFA level, which can induce an inflammatory response [4]. FFA bind to Toll-like receptor 2 (TLR 2) and Toll-like receptor 4 (TLR 4) which induce nuclear factor kappa β (NFkβ) to secrete proinflammatory cytokines

such as IL-1β [5]. FFA also stimulate the activation of NOD-like receptor protein 3 (NLRP3) which will activate Caspase-1 [6], then Caspase-1 will turn pro IL-1β into IL-1β [7].

Interleukin 1β is a proinflammatory cytokine which has a broad spectrum of immunological activity [8]. IL-1β production is regulated by 2 mechanisms; i.e., TLR activation and activation by NLRP3 inflammasome [9]. The first mechanism through activated TLR, especially TLR 2 and TLR 4, will trigger the activation of NFkβ, then NFkβ secretes proinflammatory cytokine such as IL-1β [10]. The second mechanism through NLRP3 inflammasome complex produces pro-Caspase 1 and is activated into Caspase-1 [11]. Caspase-1 activates pro-IL-1β into active and mature IL-1β. The secretion of IL-1β is induced by NFkβ transcription factor [12]. NFkβ and production of inflammatory cytokines such as IL-1β can be suppressed by short-chain fatty acids (SCFA) [13].

Short chain fatty acids are formed when food fiber is fermented by anaerobic bacteria in the large intestines [14]. Short chain fatty acids affect 3 signaling pathways, which are G-protein receptor (GPR) activation [15], histone deacetylase (HDAC) inhibition, and histone acetyltransferase (HAT) activation [16]. Activation of the GPR pathway, especially GPR41 and GPR43, is important for the expression of inflammatory mediators such as IL-1β [13]. The main SCFAs produced in human intestines are butyrate, propionate, and acetate [17]. Butyrate can inhibit the activation of NFkβ transcription factor [18]. Butyrate and propionate can suppress the secretion of IL-1β through HDAC inhibition mechanisms [19]. The present study investigated the effect of high fiber diet on the level and expression of IL-1β in high fat and fructose diet of Wistar rats.

2. Material and Methods

This study was conducted on eight weeks old Wistar strain male rats (*Rattusnorvegicus)* with a body weight of 150-200 grams. The rats were obtained from Biofarma, and acclimatized for one week in room temperature with access to water and food *ad libitum*. The rats (n=25) were divided into five groups. Each group consisted of 5 rats. One group of rats as a control group (K) was given standard rat food (AIN-93M), while four groups of rats were given high-fat and fructose diet. One group of high-fat and fructose diet rats received no high-fiber diet (Hyperlipidemia group [D]), while three groups received high-fiber diet in different doses of 2.6 g/rat/day (P1), 5.2 g/rat/day (P2) and 7.8 g/rat/day (P3). A high-fiber diet was formed in pellets and given orally for 6 weeks.

Pretest serum levels of IL-1β was taken at the 7th week of treatment and the posttest serum levels of IL-1β was taken at the end of 13 weeks of this protocol. Three milliliters

blood were collected from medial canthus orbital sinus to determine pretest and posttest serum levels of IL-1β (Fine test) with ELISA methods.

The protocol of this study was approved by the Medical and Health Research Ethics Committee of the Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta (Reg.00065/04/LPPT/VI/2017). Data were presented as mean ± standard deviation (SD). Statistical analysis of serum levels of IL-1β was performed using paired-t test, using a value of $p<0.05$ as statistically significant.

3. Results

Serum levels of IL-1β during pretest and posttest are shown in Figure 1. In hyperlipidemia rats (D), the IL-1β levels were significantly higher compared to control rats (K), and were also significantly higher compared to the hyperlipidemia rats which received different doses of high-fiber diet ($p<0.05$). The levels of IL-1β in hyperlipidemia rats which received the high-fiber diet were significantly lower compared to hyperlipidemia rats (D) and control rats (K), and there were no significant differences between hyperlipidemia rats which received a high-fiber diet in different doses (P1, P2, and P3).

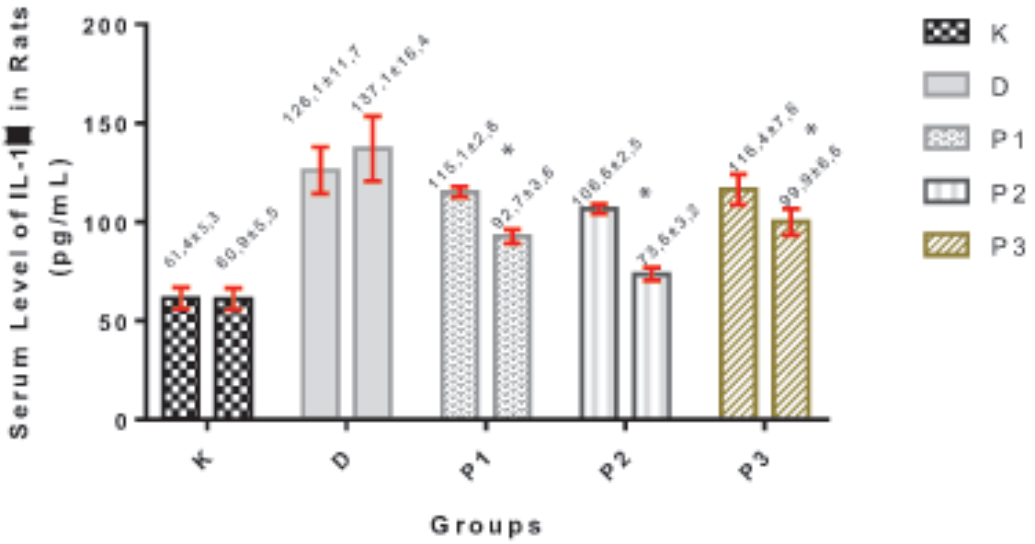

Figure 1: **Pretest and Posttest Serum Level of Interleukin 1β in Rats.** Data is displayed as Mean ± Standard Deviation (SD), * stated $p<0.05$ in paired-t test. $p<0.05$ is categorized as a significant value. K: Normal control group; D: Hyperlipidemia group; P1: Hyperlipidemia + fiber 2.6 g/rat/day; P2: Hyperlipidemia + fiber 5.2 g/rat/day; P3: Hyperlipidemia + fiber 7.8 g/rat/day.

4. Discussion

This study showed that increased levels of IL-1β occurred in rats with hyperlipidemia. This finding demonstrated that high fat and fructose diet could induce inflammation characterized by increased levels of IL-1β. It was in line with previous studies which report that high fat and fructose diet can lead to inflammation characterized by high levels of IL-1β [20]. Higher inflammatory response showed higher level of IL-1β [21], due to activation of inflammasomes, increased translocation of nNFkβ to the nucleus, and increased secretion of cytokines [22]. Increased levels of IL-1β were caused by rising FFA, which included TLR2 and TLR4. Free fatty acids bind to their receptors triggering degradation Inhibitor Kinase Kappa β (IKKβ) [23].

The research result showed that a high fiber diet could reduce the level of IL-1β. It was in line with the previous study which states that a high fiber diet can reduce inflammatory responses characterized by lower IL-1β [24]. It was suspected to be due to SCFA contents, especially butyrate, one of the results of fermentation of food fiber, suppressing cytokines which stimulate the production of inflammatory mediators such as IL-1β [25]. The main mechanism which could explain the effect was reduced HDAC activity [26]. Butyrate was the most potent HDAC inhibitor compared with acetate[26]. Histone deacetylase and HAT control the level of protein acetylation. Butyrate inhibits HDAC activity, thus increasing histone and non-histone protein acetylation, including NFκB, prevented the activation of NFκB [18]. Prevention of NFκB activation also plays a role in preventing the release of proinflammatory cytokines such as IL-1β [27].

Reduction of IL-1β level in rats given 5.2 g/rat/day fiber was higher than rats given fiber 2.6 g/rat/day, while in rats given fiber 7.8 g/rat/day it was lower than rats given fiber 2.6 g/rat/day. The result was in line with the previous study that showed relatively adequate dosage of fiber when increased to double dose increases fermentation product in the form of SCFA, including butyrate [28]. Short chain fatty acids worked with cytokines in leukocytes and endothelial cells through two mechanisms, i.e. GPR activation HDAC inhibition [25]. Short chain fatty acids regulated cytokine production, including IL-1β[13]. Microflora in the intestines and SCFA were interconnected and depend on each other. To be able to produce SCFA in the gastrointestinal tract, there should be microbes which produce enzymes for fermentation of resistant starch which could be digested by human digestion [28]. As a result, the number of microflora also could affect the amount of SCFA production [29]. As a consequence, increasing dietary fiber dosage did not always increase ability in reducing IL-1β level, depending on the number of microflora

in the colon. High fiber diet could reduce the level of triglycerides and level of IL-1β in hyperlipidemia rats, especially at 5.2 grams fiber/day.

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