

THE EFFECT OF HIGH LIPID DIET, LIPOPOLYSACCHARIDE AND LAPARATOMY SURGERY ON THE LEVEL OF C-REACTIVE PROTEIN SERUM IN *SPRAGUE DAWLEY* RATS

EFEK DIET LEMAK TINGGI, LIPOPOLISAKARIDA DAN LAPARATOMI PADA
KONSENTRASI *C-REACTIVE PROTEIN* SERUM
TIKUS *SPRAGUE DAWLEY*

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ABSTRAK

C-reactive protein (CRP) adalah protein golongan pentraksin yang mula-mula ditemukan pada serum pasien penderita sakit akut berhubungan dengan C-polisakarida pneumokokus. Konsentrasi CRP dalam serum akan meningkat sebagai bagian dari reaksi radang karena infeksi atau kerusakan jaringan akut. Tujuan penelitian adalah melihat perbedaan efek stimulus akut dan kronis pada peningkatan konsentrasi CRP serum tikus *Sprague Dawley*. Dua puluh ekor tikus *Sprague Dawley*, umur 1,5 bulan sebagai hewan coba. Tikus diadaptasikan selama 5 hari dan diberi pakan mengandung lemak normal (5%) dan air secara *ad libitum*. Tikus dibagi menjadi 4 kelompok masing-masing 5 ekor tikus. Kelompok I (kontrol), diberi diet normal selama 59 hari tanpa perlakuan yang lain. Kelompok II diberi diet lemak tinggi selama 59 hari tanpa perlakuan lain. Kelompok III, diberi diet normal selama 59 hari dan hari ke 60 tikus diinjeksi dengan LPS dari *Escherichia coli* O111:B4 dengan dosis 1 mg/kg BB secara I.P. Kelompok IV diberi diet normal lemak selama 59 hari dan hari ke 60 tikus dioperasi laparatomi. Dua puluh empat jam setelah semua perlakuan selesai, tikus diambil darahnya melalui vena orbitalis, serumnya untuk analisis konsentrasi CRP. Semua data yang diperoleh menunjukkan adanya perbedaan yang signifikan di antara perlakuan ($p < 0,05$). Rerata konsentrasi CRP serum berturut-turut dari yang tertinggi sampai yang terendah adalah: Kelompok 3, kelompok 4, kelompok 2 dan kelompok 1. Dapat disimpulkan bahwa stimulus akut meningkatkan konsentrasi CRP lebih tinggi dibanding stimulus kronis.

Kata kunci : C-reactive protein, lipopolisakarida, diet lemak tinggi, laparatomi.

ABSTRACT

C-reactive protein (CRP) is a member of the family of proteins known as pentraxins, and originally defined as a substance, observed in the serum or plasma of patient with acute infections, that reacted with the C-polysaccharide of the pneumococcus. Serum concentrations of CRP increase as part of the inflammatory response to infection or acute injury. The aim of the study was to see the differences between acute and chronic stimuli in the increasing of CRP concentration in serum of *Sprague Dawley* rat. Twenty male *Sprague Dawley* rats, 1.5 months of age were used as experimental animals. Rats were adapted for 5 days and given basal diet, containing normal fat (5% of fat) and water *ad libitum*. Rats were then divided into 4 groups of 5 each. Group 1 was used as control animal. The animals in this group were fed basal diet for 59 days without any other treatments. Group 2 was animals fed high fat diet containing 20% of fat, for 59 days without any other treatments. Group 3 was animals fed basal diet for 59 days and in the day of 60 then injected with LPS from *Escherichia coli*

O111:B4, 1 mg/kg BW intraperitoneally, and Group 4 was animals fed basal diet for 59 days and in the day of 60 animals then laparatomized. Twenty four hours after all treatments, blood sample were collected from orbitally vein, serum was separated and used to analyze CRP concentration. Statistical analyzes using one way analyzes of variance showed that there were significance differences among Group 1, 2, 3 and 4 ($p < 0.05$). Mean of CRP concentrations respectively from the highest to the lowest were: Group 3, Group 4, Group 2 and Group 1. From the result of the study it can be concluded that CRP concentration of acute stimulus was higher than chronic stimulus.

Key words : C-reactive protein, lipopolysaccharides, high fat diet, laparotomy.

INTRODUCTION

Atherosclerosis is a disease of large and medium sized arteries characterized by thickening and hardening of vascular wall. It involves a substance called plaque in the inner lining of the arteries. American Heart Association (2002) has identified several risk factors for coronary heart disease (CHD), an outcome of atherosclerosis. Both the increasing number and the increasing severity of risk factors increase the risk of developing CHD. Most of the risk factors can be modified, treated or controlled. High blood pressure, elevated serum LDL cholesterol level and tobacco smoke are considered the major classical risk factors for the development of CHD. Additional factors predisposing to CHD include age, gender (male), hereditary, race, obesity, physical inactivity, diabetes and high serum triglyceride and low HDL cholesterol levels. Other factors contributing to stress and excessive alcohol consumption (Ross, 1999). Inflammatory processes have important roles in the etiology of coronary heart disease (CHD), but the mechanisms underlying this relationship are poorly understood (Berk *et al.*, 1990).

C-reactive protein is a member of the family of proteins known as pentraxins, and originally defined as a substance, observed in the serum or plasma of patient with acute infections, that reacted with C-polysaccharide of the pneumococcus. C-reactive protein is known also as a protein that shares several functions with immunoglobulin (Ig) G including complement activation and binding to receptors on monocytes and neutrophils (Bharadwaj *et al.*, 1999). C-reactive protein largely regulated by circulating

levels of interleukin 6 (IL-6), predicts coronary heart disease incidence in healthy subjects (Yudkin *et al.*, 1999). Serum concentrations of CRP increase from less than one to hundreds of micrograms per milliliter as part of the inflammatory response to infection or acute injury (Bharadwaj *et al.*, 1999).

Several studies have shown that elevated plasma level of CRP is associated with the risk of CHD and the severity of atherosclerosis (Berk *et al.*, 1990). It is not clear whether this protein merely is marker of inflammation or whether they actually can mediate atherosclerosis. The aim of this study was to see the difference effects among high fat diet, surgery and LPS on C-reactive protein level in Sprague Dawley rat.

MATERIALS AND METHODS

Twenty male *Sprague Dawley* rats, 1.5 months of age were used as experimental animals. Rats were adapted in 20 single cages for 5 days and given basal diet containing normal fat and water ad libitum. After adaptation, rats were divided into 4 groups (Group 1, 2, 3 and 4) of 5 each. Group 1 was used as control animals, they were fed containing normal fat (4.5% of fat) for 59 days, without any other treatments. Group 2 was animals that fed containing high fat (20% of fat) for about 59 days without any other treatments. Group 3 was animals that fed containing normal fat for 59 days and injected with LPS of *E. coli* O111: B4, dose: 1 mg/kg BW intraperitoneally, and group 4 was animals that fed containing normal fat for 59 days and in the last day all animals in this group were laparatomized. Twenty four hours after treatments, blood sample were collected from

orbitally vein of all animals, serum were then separated and used for CRP level analyses.

The diet was made in Pusat Antar Universitas (PAU) Pangan dan Gizi, Gadjah Mada University. Fat concentrations were made 4.5% for basal diet and 20% for high fat diet.

Lipopolysaccharide (LPS) from *Esche-richia coli* O111:B4 was purchased from Sigma Laboratory, St. Louis, MO. Using a tuberculine disposable sput, LPS was injected intraperitoneally with a single dose of 1 mg/kg body weight.

mean \pm SD. Significance level was shown for all comparisons and the interrelationships where $p < 0.05$. The significance differences were then analyzed using Tukey HSD.

RESULTS AND DISCUSSION

The result of the study showed that CRP level was increased in several samples, especially samples from group 3 and group 4 (Figure 1).

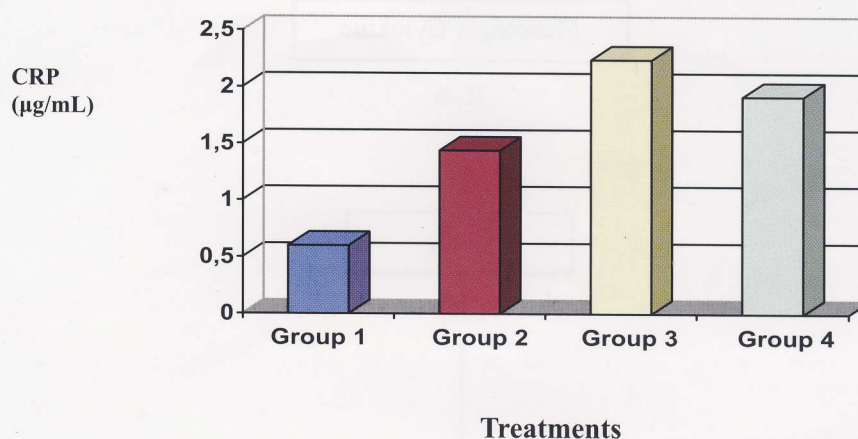


Figure 1. C-reactive protein levels of *Sprague Dawley* rats after 4 different treatment: Group 1: normal fat diet, Group 2: high fat diet, Group 3: normal fat diet and LPS 1 mg/kg BW intraperitoneally, and Group 4: normal fat diet and laparatomy.

Laparatomy was done under ketamine (90 mg/kg BW) and chlorpromazine HCl (2 mg/kg BW) combination anaesthetic. The abdomen was explored by means of a ventral midline incision. The entire abdomen, including inguinal areas, and the caudal of thorax should be prepared for aseptic surgery. The incision must extend from xyphoid process. After finished, the linea alba and the skin were closed using simple interrupted suture pattern with 0.5 cm a part.

Analysis of Variance (ANOVA) was used to analyse the effect of variables. Data was presented as

From the results we see that in group 1, although the diet was containing normal fat (control) CRP still produced and found in the serum. According to Pepys and Hirchfield (2003), in healthy young human, the median concentration of CRP is 0.8 mg/L, so normally CRP is produced in the liver. Untill the recent day, normal data of CRP in rat has not known yet. In Group 1, we see that mean of CRP value was 0.6 ± 0.22 $\mu\text{g/mL}$ and assumed that this result was the normal value. After acute stimulus (LPS and Surgery), values of CRP was increase. In this research mean of CRP values from the highest to

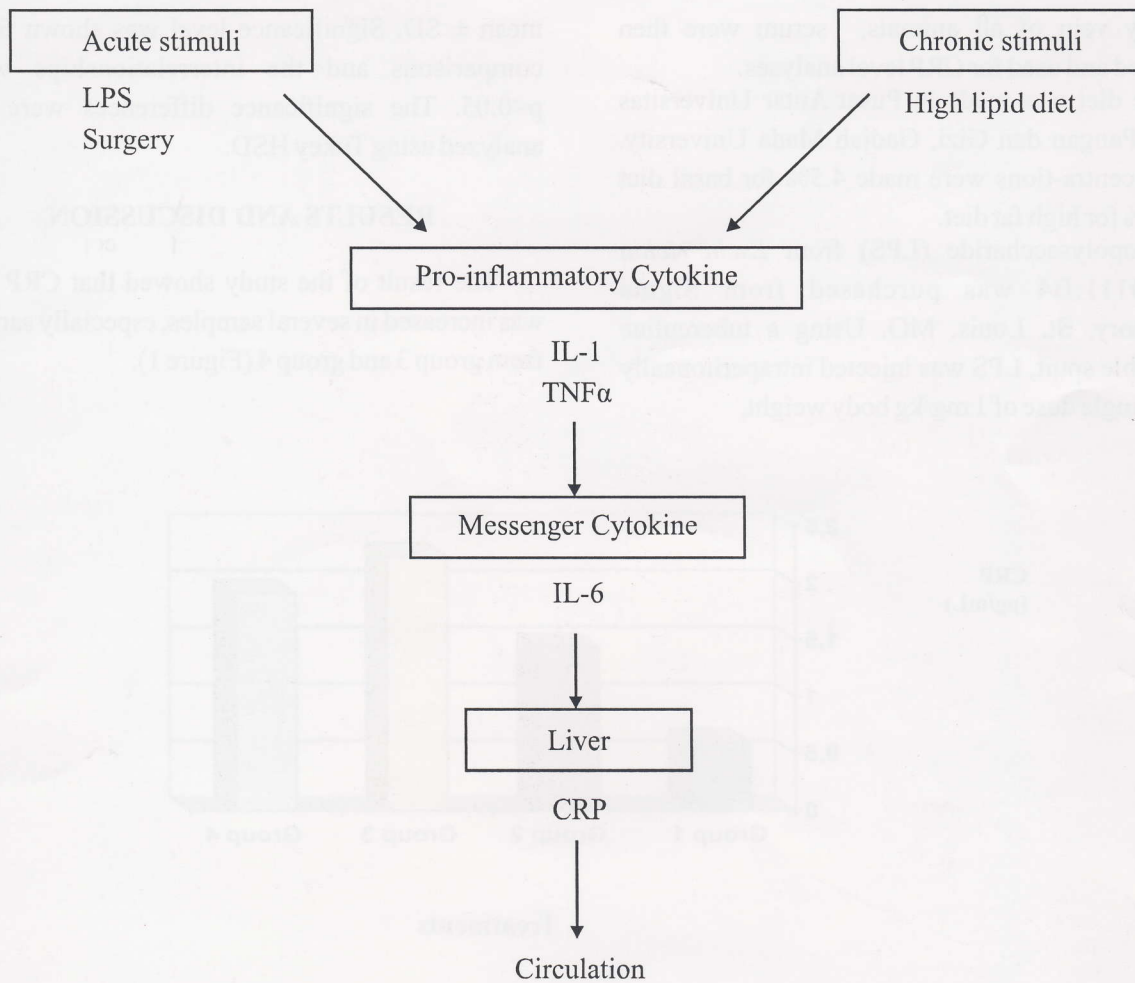


Figure 2. Assumption of elevated CRP concentration mechanisms in this research.

the lowest respectively were: (1) Group 3 (LPS) = 2.24 ± 0.87 $\mu\text{g/mL}$, (2) Group 4 (Surgery) = 1.92 ± 0.71 $\mu\text{g/mL}$, (3) Group 2 (high fat diet) = 1.44 ± 0.35 $\mu\text{g/mL}$ and (4) Group 1 (control) = 0.6 ± 0.22 $\mu\text{g/mL}$. From that results we see that CRP value after acute stimulus was higher than chronic stimulus. Statistical analysis of CRP level from Group 1,2,3 and 4 showed that there were significance differences between treatments ($p < 0.05$). After analyzed using Tukey HSD method, the differences were between Group 1 and Group 3, and Group 1 and Group 4. Although statistical analyzes of Group 2 did not increase significantly, but mean of CRP value in this group still different from Group 1, this result showed that high lipid diet

caused increase CRP value. From all of the results we assumed that the mechanisms how CRP increase was like the diagram below (Figure 2). The stimuli, both acute and chronic produces changes in gene expression within the vessel wall and alters function of blood vessels. These responses include the expression of the inducible isoform of nitric oxide (NO) synthase (iNOS) (Gunnert *et al.*, 1999). Lipopolysaccharide (LPS) increases the expressions of xanthine oxidase and superoxide levels in blood (Baggat and Vallance, 1999). Increasing the levels of reactive oxygen species will activate pro-inflammatory cytokines like interleukine-1 (IL-1) and Tumor necrotic factor (TNF α). Interleukine-1 in the circulation then activate Interleukine-6 (IL-6)

and Interleukine-6 in circulation then increase synthesis some liver protein including CRP. According to Gabay and Kushner (1999), interleukine-6 is the chief stimulator of the production of most acute phase proteins including CRP. The acute phase response (CRP) comprises the nonspecific physiological and biochemical responses of the endothermic animals to most forms of tissue damage, infection and inflammation (Pepys and Baltz, 1983).

From all of the results it can be concluded that there were significant differences between Group 1 (controle), Group 2 (LPS), Group 3 (Surgery) and Group 4 (high lipid diet) ($p < 0.05$). Means of CRP level after treatments respectively from the highest to the lowest were: Group 3, Group 4, Group 2, and Group 1. Compare with chronic stimulus, acute stimulus would be higher elevate of CRP serum concentration.

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