SOOT PARTICULATE MATTER EXPOSURE INCREASES INTERLEUKIN 6 LEVELS IN THE MECHANISM OF CARDIOVASCULAR DISORDERS

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ABSTRAK

Paparan polusi udara dihubungkan dengan meningkatnya morbiditas dan mortalitas kardiovaskular. Faktor-faktor yang memicu kejadian ini sangat menarik perhatian dan penting bagi kesehatan masyarakat, dimana saat ini bukti-bukti epidemiologis mengimplikasikan PM sebagai sebuah faktor resiko kardiovaskular. Meski demikian mekanisme dari injuri kardiovaskuler ini belum jelas. Stres oksidatif merupakan mekanisme utama yang menerangkan proses biologis akibat PM. Pada pathway inflammation akan didapatkan peningkatan Interleukin 6 untuk merespon paparan PM. Penelitian ini bertujuan menjelaskan efek paparan partikulat jelaga terhadap kadar Interleukin 6 pada mekanisme gangguan sistem penyakit kardiovaskular. Eksperimen dilakukan pada hewan coba berupa tikus betina (Rattus novergicus) yang dibagi menjadi 3 kelompok yaitu: kelompok kontrol (n=10), tanpa paparan partikulat jelaga; kelompok perlakuan 1 (n=15), dipapar dengan partikulat jelaga konsentrasi 532 mg/m^3 satu jam tiap hari selama 30 hari; kelompok perlakuan 2 (n=15), dipapar dengan partikulat jelaga konsentrasi 1064 mg/m^3 satu jam tiap hari selama 30 hari. Pengukuran kadar plasma Interleukin 6 menggunakan metode ELISA dan dilakukan setelah paparan partikulat jelaga selama 30 hari. Hasilnya, nilai rerata kadar IL-6 meningkat dibandingkan masing-masing kelompok kontrol, kelompok perlakuan 1 dan kelompok perlakuan 2 (P1= 38,78 pg/ml $\pm$ 7,50; 186,83 pg/ml $\pm$ 110,92), dan secara statistik didapatkan perbedaan yang signifikan diantara 3 grup (p=0,000). Didapatkan pula perbedaan yang signifikan antara kelompok kontrol dengan kelompok perlakuan 1, kontrol dengan kelompok perlakuan 2 serta kelompok perlakuan 1 dan kelompok perlakuan 2 (p=0,000). Paparan terhadap partikulat jelaga secara signifikan meningkatkan kadar Interleukin 6 pada hewan coba. Temuan kami menegaskan peran aktifasi jalur inflamasi sebagai respon paparan bahan partikulat pada penyakit kardiovaskular. ***(FMI 2014;50:160-163)***

Kata Kunci: *Soot particulate, IL-6, inflammatory pathways, oxidative stress*

ABSTRACT

Various mechanisms are thought to play a role in the pathophysiology of cardiovascular disorder, such as systemic and local inflammation, oxidative stress, and endothelial dysfunction. However, the definite cause to cardiovascular disorder is still not known with certainty. One of important cytokines that involves in systemic inflammation is IL-6, so this study was to determine whether IL-6 increases after exposure to particulate matter in the mechanism of cardiovascular disorders. The objective of this study was to explain the effects of exposure of soot particulate on IL-6 level in the inflammatory mechanisms of cardiovascular disorder. Experiments were performed on white female rats (Rattus norvegicus) divided into 3 groups: control group (n=10), without exposure to soot particulate; an experiment group 1 (n=15), exposed to soot particulate concentration of 532 mg/m^3 one hour/day for 30 days; and an experiment group 2 (n=15), exposed to soot particulate concentration of 1064 mg/m^3 one hour/day for 30 days. Measurement of IL-6 plasma level was done by ELISA method performed after the exposure ended. The mean IL-6 levels were increased between groups: control, treatment 1 and 2 (P1=38.78 pg/ml $\pm$ 7.50; 186.83 pg/ml $\pm$ 110.92), and statistically significant among 3 groups (p=0.000). Statistically significant differences were found between control and treatment 1, control and treatment 2, and treatment 1 and treatment 2 (p=0.000). Exposure to soot particulate significantly increased IL-6 levels in experimental animals. Our findings suggest the role of inflammatory pathways activation in response to soot particulate exposure in the mechanism of cardiovascular disorders. ***(FMI 2014;50:160-163)***

Keywords: *Soot particulate, IL-6, inflammatory pathways, oxidative stress*

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INTRODUCTION

Air pollution significantly increases morbidity and mortality in the general population. Respiratory system vulnerability is widely known as a major component of the adverse effects of air pollution. Air pollution is a mixture of several different constituents, including particulate matter (PM) or particulate matter, gases, and organic compounds.

History shows the relationship of air pollution with the incidence of mortality and morbidity. In 1930, in

Belgium, an increase in atmospheric pressure and winds cause heavy fog and barely moving. Sixty deaths were found during the incident due to the thick fog that traps emissions from smokestacks and creating a toxic cloud that is fatal. In Donora, Pennsylvania, 1948, an industrial pollutants accumulating from iron smelter in the air appeared. This incidencece caused 20 sudden deaths, an estimated 5,000-7,000 people became ill. In 1952 in London, there was a thick fog caused by local pollutants from fireplaces and factory. An increase in hospital care is as much as 48%. These events trigger countries around the world to produce rules aimed to limit toxic and lethal effects of air pollution. American Heart Association published scientific statement showing an increased risk of cardiovascular events is associated with short-term and long-term consistent exposure to a concentration of ambient particulate matter. However, the mechanism of increased cardio-vascular mortality is not yet clear.

Pollutant, such as suspended particulates, also called particulate matter (PM), is one of important components in health related-air pollution, and is a risk factor for cardiovascular ischemic events through the exacerbation of atherosclerosis, coronary heart disease, myocardial infarction and triggers. Soot is one of PM components that affect health because of cytotoxic effect.

Epidemiological studies lately show relationship between levels of PM with increased cardiovascular morbidity and mortality. Other studies link increased levels of PM with cardiovascular events, including malignant arrhythmias and myocardial infarction. Besides, PM is known to cause inflammation in the respiratory system and cardiovascular systems. In addition, it can also cause homeostatic effect on various channels, vascular dysfunction, accelerated athero-sclerosis and plaque instability.

Environmental exposure that comes from combustion products is known to affect cardiovascular health through a variety of mechanisms. One mechanism is thought to contribute to oxidative stress, which in turn increases the Reactive Oxygen Species (ROS) in the body. This can induce changes in cardiovascular system. PM exposure can also result in lung inflammation and stimulates the release of proinflammatory cytokines as well as macrophages and leukocytes. Soot stimuli are associated with elevated levels of the inflammatory cytokines interleukin 6 (IL-6), tumor necrosis factor alpha (TNF-α) and interleukin-1β are responding to the occurrence of systemic inflammatory reaction in the circulation. Increased IL-6 will serve as a trigger of acute inflammatory response, leukocyte and platelet release, stimulates the liver to form fibrinogen, as well as to improve the trigger endothelial adhesion molecules (ICAM-1, VCAM-1, E-selectin). Excessive production of inflammatory cytokines IL-6 would result in a widespread form of atherosclerosis process, so that this situation may cause cardiovascular system disorder. According the study of Tsai and colleagues showed an increase in one of the inflammatory cytokines IL-6 shortly after exposure to PM (Tsai et al 2012). Based on these facts, we studied mechanisms of the influence of exposure to air pollutant particulate matter, especially soot, on the risk of cardiovascular system disorders.

MATERIALS AND METHODS

This was an experimental study with a post-test only control group design. Experimental units were subjected to randomization, and divided into 3 groups: 1) control group, 2) the first treatment group (P1), and the second treatment group (P2). Experimental unit in this study were females white rats (*Rattus novergicus*) who met the criteria: 4 months old, 100-200 gr, healthy. Sample calculation uses type 1 error rate of 5% and type 2 error rate of 10%, and the mean differences were considered minimally significant if 1, then we obtained n1=n2=10. To anticipate the possibility of drop out or damage to the unit experiment, which resulted in loss to follow-up, the correction was done by 20% of the sample size of the original calculation.

Based on these calculations, the sample size or replication to be used in this study were 12 rats for each group. Carbon black powder was sprayed in the air box exposure. The treatment is given in a different box to monitor exposure to air temperature, flow rate of 5 to 7.5 km/h (light breeze) on local temperature and humidity with atmospheric pressure inhaled. The treatment was given in stages according to the group. Before the trial began we performed acclimation exposure on the box. The animals kept in the box were exposed to treatment for 1 month. On the 31st day, we conducted blood sampling. Blood was deposited for about 1 hour until coagulation occurred. Then, the separation of serum was carried out by cold centrifugation, 4^0C for 10 min at 3000 rpm. Serum were examined for IL-6 levels using ELISA method.

RESULTS

Descriptive data are shown as mean ± SD or median and frequency was displayed as a percentage. To test data distribution normality we used one sample Kolmogorov-Smirnov test. As the result showed no normal distribution, we carried on with Kruskal-Wallis non-parametric statistical test, then followed by Mann-

Whitney U statistic test. The results of Interleukin 6 exposure to soot particulate for 30 days revealed mean difference between groups. In control group the mean was 17.58 pg/ml ± 7.10, treatment group 1 38.78 pg/ml ± 7.50, and treatment group 2 186.83 pg/ml ± 110.92. The significance values between groups were P1 and control group (p=0.000) p <0.05, control group and P2 (p=0.000) p<0.05, and P1 and P2 (p=0.000) p <0.05.

DISCUSSION

Various mechanisms of systemic cardiovascular disorders due to the inhalation of particulate material has been described by the literature, but the exact mechanism is still unexplained. Two important mechanisms considered responsible for the effects of particulate matter on aterothrombrosis process. First, Particulate Matter (PM) induces an inflammatory process in the lung tissue and systemic circulation, while Second, PM induced oxidative stress in both local and systemic pulmonary tissues throughout the body. PM exposure can result in lung inflammation and stimulates the release of proinflammatory cytokines as well as macrophages and leukocytes. Soot stimuli associated with elevated levels of the inflammatory cytokines interleukin 6 (IL-6), tumor necrosis factor alpha (TNF-?) and interleukin 1?, that are responding to the occurrence of systemic inflammatory reaction in the circulation. Increased IL-6 will serve as a trigger of acute inflammatory response, leukocyte and platelet release, stimulates the liver to form fibrinogen, as well as to improve the trigger endothelial adhesion molecules (ICAM-1, VCAM-1, E-selectin). Excessive production of inflammatory cytokines IL-6 would result in a widespread form of atherosclerosis process, so that this situation may cause cardiovascular system.

Several studies are consistent with these studies indicate that the effect of PM were affecting the increase in interleukin 6 levels above the appropriate mechanism. Study by Rückerl et al (2007) showed an increase in IL-6 immediately after given exposure to PM, indicate that the inflammatory response occurred (2.7, 95% confidence interval (CI), 1.0-4.6). In the study also concluded that the increase in IL-6 can lead to increased cardiovascular risk of about 0.7% (95% CI,-0.06 to 1.5). The study by Tsai et al (2012) showed increased levels of IL-6 concentration of about 0.036 (95% Confidence interval; 0015-0057) pg/mL after administration of short-term exposure to PM10 in the first 24 hours and at 1-6 days later. Significant difference was also found in the study exhibited significantly IL 6 with exposure to PM10. And most recently by Nazariah et al (2013), showed an increase in IL-6 levels were significantly due to exposure to PM2, 5 (p=0.001, p<0.005) and PM10 (p=0.001, p<0.005) in a group of school in Malaysia. Soukup and Becker (2001) also reported an increase in cytokines (IL-6 and TNF-?) as a pro-inflammatory mediators after exposure to PM2.5 and PM10.

In this study we obtained an average value increased IL6 levels between the control group with group P1 (38.7793 pg/ml ± 7.49945) and P2 (186.83 pg/ml ± 110.92) who received doses greater exposure. In the present study, there were statistically significant differences in the levels of Interleukin 6, both in the experimental group 1 (P1) and treatment group 2 (P2) compared with the control group with a significance value of p=0.000. However, in this study we cannot distinguish very significant difference between each group because the value of p equal to the three groups, the control group to the treatment group 1 (p=0.000); the control group with treatment 2 (p=0.000), as well as treatment group 1 to 2 treatment groups (p=0.000).

These findings are consistent with existing data from previous studies that the PM has the ability to increase the inflammatory process in which Interleukin 6 plays a role in it. In addition it shows with increasing doses, greater exposure will lead to significant increased levels of IL-6. This is consistent with previous studies which showed that the role of Interleukin-6 in the regulation of the inflammatory response has a direct relationship with cardiovascular disease. Heart disease risk factors such as smoking and heavy exercise can cause increased levels of IL-6. Psychological stress, can also act to increase the degree of IL-6

This study only evaluate one of several factors that play a role in the pathophysiological mechanisms of particulate material to the cardiovascular system disorders, where the possibility of interactions among multiple factors will cause the difference in the end result.

CONCLUSION

Our study of soot particulates exposure for 30 days can increase the plasma levels of Interleukin 6. Increased levels of Interleukin 6 were significantly obtained in the treatment group 1 and group 2 when compared with the control group. Increased levels of Interleukin 6 is appropriate with increasing dose exposure to particulate matter are given. These findings suggest a possible role of oxidative stress and activation of proinflammatory pathways in response to soot particulate exposure. The findings in our study is important in explaining how the soot particulate matter can contribute to cardiovascular events.

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