SOOT PARTICULATE EXPOSURE INCREASES CD54/INTERCELLULAR ADHESION MOLECULE-1 (ICAM-1) EXPRESSION IN CARDIOVASCULAR DISORDER

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ABSTRAK

Paparan polusi udara menjadi satu faktor resiko penyakit kardiovaskuler. Salah satu mekanisme yang diduga berperan adalah stress oksidatif yang akan meningkatkan reaktive oxygen species (ROS), yang mengakibatkan pelepasan sitokin proinflamasi dan ekspresi molekul adhesi seperti, intercellular adhesion molecule-1 (ICAM-1). Penelitian ini bertujuan untuk menjelaskan efek paparan partikulat jelaga terhadap ekspresi ICAM-1 pada mekanisme inflamasi sistem kardiovaskular.Eksperimen dilakukan pada hewan coba berupa tikus betina (Rattus novergicus) yang dibagi menjadi 3 kelompok yaitu: kelompok kontrol (P0) (n=12), tanpa paparan partikulat jelaga; kelompok perlakuan 1 (P1) (n=12), dipapar dengan partikulat jelaga konsentrasi 532 mg/m3 satu jam tiap hari selama 30 hari; kelompok perlakuan 2 (P2) (n=12), dipapar dengan partikulat jelaga konsentrasi 1064 mg/m3 satu jam tiap hari selama 30 hari. Ekspresi ICAM-1 pada jaringan jantung dinilai dengan pewarnaan immunohistokimia. Kami menilai ekspresi ICAM-1 berdasarkan jumlah sel yang immunoreaktif dalam 5 lapangan pandang. Penghitungan ekspresi rerata ICAM-1 berbeda antara P0, P1 dan P2 (4.9, 21.47, 36.33). Ekspresi ICAM-1 secara statistic bermakna signifikan antara ketiga kelompok (p=0,001). Peningkatan ekspresi ICAM-1 P0 dengan P1 (p=0,019), P0 dengan P2 (p=0,000) dan P1 dengan P2 (p=0,045) bermakna signifikan. Paparan terhadap partikulat jelaga secara signifikan meningkatkan ekspresi ICAM-1 pada hewan coba. Temuan kami menegaskan peran aktifasi jalur inflamasi sebagai respon paparan bahan partikulat pada penyakit kardiovaskular.

Kata kunci: *partikulat jelaga, penyakit kardiovaskuler, stress oksidatif, ICAM-1*

ABSTRACT

Exposure of air pollution has become a risk factor for cardiovascular disease. One mechanism thought to contribute is oxidative stress, which will increase the reactive oxygen species resulting in release of pro-inflammatory cytokines and increased expression of adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1). The aim of this study was to describe the effects of soot particulate (carbon black powder) exposures on ICAM-1 expressions in mechanism of cardiovascular disorders. Experiments were performed on white female rats (Rattus novergicus) divided into 3 groups: control group (P0) (n=12), without exposure to soot particulate; an treatment group 1 (P1) (n=12), exposed to soot particulate concentration of 532 mg/m3 one hour/day for 30 days; and an treatment group 2 (P2) (n=12), exposed to soot particulate concentration of 1064 mg/m3 one hour/day for 30 days. Immunohistochemical staining was used to quantify the expression of ICAM-1 in cardiac tissues. We quantified the expressions of ICAM-1 with the number of immunoreactive cells in 5 fields of view. The mean ICAM-1 expressions were different between P0, P1 and P2 (4.9, 21.47, 36.33). Expression of ICAM-1 was statistically significant between three groups (p=0,001). The increase ofICAM-1 expression in P0 compared to P1 (p=0,019), P0 compared to P2(p=0,000) and P1 compared to P2 (p=0.045) were significant. Exposures to soot particulate matter increased the ICAM-1 expression in subjects significantly. Our findings suggest a key role of activation inflammatory pathway in response to soot particulate exposure in cardiovascular disease.

Keywords: *soot particulate, cardiovascular disorders, oxidative stress, ICAM-1*

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INTRODUCTION

Several epidemiological studies showing a relation between air pollution and adverse health effect has been conducted since the 1930s. In the 1990s, studies of air pollution and cardiovascular disease have been intensified, especially focused on mortality and hospitalization due to heart disease (Sun et al 2010). National Research Council (NRC) Committee on Research Priorities for Airborne Particulate Matter has explained the relationship of morbidity and mortality due to particulate matter (PM) in 1998, 1999, 2001 and 2004. National Morbidity, Mortality and Air Pollution Study (NMMAPS) showed a positive association of PM10 with cardiopulmonary mortality and hospital admission for cardiovascular disease, chronic obstructive pulmonary disease (COPD) and pneumonia in patients aged 65 years or more in 90 cities in the

United States. Morris and Naumova (1998), also found that hospital admissions for congestive heart failure associated with carbon monoxide (CO) in several cities of the United States. Zanobetti et al (2000) showed that the positive relation between hospital admission for cardiovascular disease and air pollution almost doubled in elderly patients treated concurrently with respiratory infections. Dominici et al (2003), found an increase in deaths due to cardio respiratory diseases by 0.34% on any increase by 10 μg/m3of PM10. Based on those studies, it has been showed that air pollution affects cardiovascular diseases. However, the mechanism of the increased cardiovascular risk due to air pollution still cannot be explained with certainty.

In 2004 for the first time the American Heart Association (AHA), concluded that exposure of air pollution affect cardiovascular morbidity and mortality. Whereas in 2010, the AHA concluded that PM2.5 exposure is a risk factor that can be modified towards cardiovascular morbidity and mortality (Brook et al 2010). Epidemiological and experimental data indicate exposure to air pollution causes increased incidence of ischemic heart disease and atherosclerosis, especially in patients with classic risks such as hypercholesterolemia, smoking, hypertension, older age, sex, history of families (Mills et al 2009). Pope et al (as cited in Delfino et al 2005) at the Cancer Prevention Study II examined 500,000 adults in 151 cities and concluded that the increase of PM 2.5 by 10 μg/m3is related to increased mortality rate from ischemic heart disease, dysrhythmias, heart failure and heart attack by 8-18%.

Air pollution can affect markers of coagulation (eg. fibrinogen), inflammation C-reactive protein (CRP) and pro-inflammatory cytokines (such as Tumor Necrosis Factor α (TNF- α), IL-1β, IL-6, IL-8) and endothelial function (such as intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1)) (Mills et al 2009). Elevated level of tissue plasminogen activator is an independent predictor of future cardiovascular events in patients without cardiovascular disease. In patients that suffered coronary heart disease (CHD), soluble ICAM, von Willebrand factor, tissue plasminogen activator, plasminogen activator inhibitor and endothelin-1 (ET-1) is used as a prognostic indicator (Bind et al 2012). Intercellular adhesion molecule-1 is an adhesion molecule that functions in the migration of leukocytes out of blood vessels into the tissues as well as the formation of pro-inflammatory signals. According to Witkowska and Borawska (2004), the soluble ICAM-1 exists in normal human serum and the increased level was found in the serum of patients with heart disease, cancer, autoimmune disease. Some studies have also shown a correlation of serum levels of soluble ICAM, with disease severity. The aim of this study was to describe the effects of soot particulate (carbon black powder) exposures on ICAM-1 expressions in mechanism of cardiovascular dysfunction. Exposure of soot particulates is believed to cause cardiovascular dysfunction, which would be indicated by an increased expression of ICAM-1.

MATERIALS AND METHODS

This research is an experimental study with a "post-test only control group design". Subjects of this study were female white rats (*Rattus norvegicus*) weighed 100-200 grams, aged 4 months old and healthy. The eligible subjects were then divided into 3 groups: 1) control group (P0), 2) the first treatment group (P1), and the second treatment group (P2). This study is a numerical analytic unpaired (two-sided test). Calculation of the sample by using the type 1 error rate of 5% and type 2 error rate of 10%, and the mean differences were considered significant minimum is 1 then obtained n1=n2=10. To anticipate the possibility of drop out or damage to the unit experiment which resulted in loss to follow-up, then the correction is 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.

Soot particulates was sprayed in an exposure box. The treatment is given in a different box to monitor exposure to the air temperature, flow rate of 5 to 7.5 km/h (light breeze) on local temperature and humidity with an atmospheric pressure are inhaled. The treatment is given in stages according to the group. Before the treatment, subjects were acclimatized to the exposure in the box. Subjects then enter the box and were exposed to treatment for a month. On day 31, the animals were sacrificed by dislocation of the atlas bone.

After disinfection with 70% alcohol, the subjects were immediately dissected and subject's heart was collected and fixed in formalin buffer for examination of ICAM-1 by immunohistochemistry methods. Immunohisto-chemical staining with avidin-biotin complex method was used to determine the expression of ICAM-1 in the rat cardiovascular system. Samples of subject's heart were prepared on an object glass. It was subsequently rehydrated with graded alcohol, washed with phosphate buffered saline (PBS), and then immersed in 3% hydrogen peroxide H2O2 (in DI water) for 20 minutes, 1% bovine serum albumin (BSA) in PBS for 30 minutes at room temperature. Primary Antibody (Anti- ICAM-1) 1:1000was applied and left overnight in temperature 4^{o}C.The following procedure was application of biotin-labeled secondary antibody (Anti Rat IgG biotin

labeled) and primary antibody anti- ICAM-1 for 1 hour at room temperature, the SA-HRP (Sterp Avidin-Hoseradish Peroxidase) for 60 minutes at room temperature, Chromogen DAB (3,3-diaminobenzidine tetrahydrochloride) for 20 minutes at room temperature. Samples were then counterstained (Aceto orcein) for 3 minutes at room temperature and then examined under a microscope. In each turn of the stages, slides werealways washed with PBS to clear the rest of the material attached. ICAM-1 expression reading was done with 400X magnification on 10 field view with a microscope.

RESULTS

Descriptive data are shown as mean ± SD or median and frequency is displayed as a percentage. To test the normality of the data distribution we used one sample Kolmogorov-Smirnov test. There is normal distribution of data obtained, then tested using different statistical parametric One Way ANOVA, and data distribution is not homogen, then tested the Kruskal-Wallis non-parametric statistics, and the results of the statistical tests was significant difference, then followed by Mann-Whitney test statistic U. The results of the expression of ICAM-1 due to exposure to particulate soot for 30 days obtained mean difference between groups.

In the control group mean obtained was4.9 ± 7.17, first treatment group (P1), 21.47 ± 18.63, while second treatment group (P2) mean 36.33 ± 20.00. Normal distribution of data obtained with p=0.016. Furthermore, Analysis performed using the Kruskal-Wallis non-parametric statistics which found significant differences between control group, first treatment group (P1), and the second treatment group (P2) (p=0.001). The increase of expression ICAM-1 control group compared to first treatment group (P1) (p=0,019), control group compare to first treatment group (P1) (p=0,000) and first (P1) to second (P2) treatment group. (p=0.045) were significant.

DISCUSSION

Intercellular adhesion molecule-1 (ICAM-1) is an endothelial adhesion molecules of the Ig superfamily that plays a role in embryogenesis, cell growth and differentiation and inflammatory processes such as atherogenesis. Intercellular adhesion molecule-1 is a non-traditional independent cardiovascular risk factor. Prospective data from two studies conducted in the United States, the Physicians Health Study (PHS) and the Atherosclerosis Risk in Communities (ARIC) study concluded that increase at least one of the cellular adhesion molecules, such as ICAM-1, occurred before the development of clinical coronary artery disease. Physicians Health Study (PHS) examined 15,000 healthy men at a young age were followed for 9 years and found a significant association between increased concentrations of SICAM-1 and risk of myocardial infarction. While the ARIC study examined men and women aged 45 to 64 who live in the United States in four different communities, obtained increased plasma concentrations of two adhesion molecules, SICAM-1 and E-selectin, which is associated with an increased prevalence of coronary heart disease or carotid atherosclerosis. Nakashima et al (1994) also analyzed the relationship increased expression of VCAM-1, ICAM-1 and PECAM-1 with the formation of atherosclerotic lesions in the aortic endothelium of control mice and mice homozygous apolipoprotein E-deficient (ApoE 2/2).

In our study, the expression of ICAM-1 was significantly different among the three groups (p=0.001). Increased expression of ICAM-1 was also found to be statistically significant in the control group compared to first treatment group (p=0.019), control group compared to second treatment group (p=0.000) and the first treatment group compared to second treatment group (p=0.045). This result is consistent with previous research data which reported that soot particulates have the ability to generate oxidative stress and increased expression of ICAM-1. Angelica et al.in their study in Mexico examined the effects of PM2,5 and PM10 on the expression of adhesion molecules E-selectin, P-selectin, ICAM-1, VCAM-1 and PECAM-1 in human umbilical vein endothelial cell (HUVEC) and found that PM2, 5 and PM10induce the expression of adhesion molecules on HUVEC with maximum effect at 20 ug/cm3.

It is supported by Alfaro-Moreno et al (2010), stating that exposure to PM causes increased expression of adhesion molecule; E-selectin, P-selectin, ICAM-1, PECAM-1 and VCAM-1. Bind et al (2012) also concluded that after exposure 1 hour of inhaled diesel particles in the room, found an increase in ICAM-1 and VCAM-1 in the bronchial fluid and after 4 hours to 22 hours of exposure, showed elevated levels of ICAM-1 in the blood veins. Selvi et al (2000) said that the pulmonary response stimulate human leukocyte increase in lavage fluid and increased expression of adhesion molecules such as ICAM-1 and VCAM-1 on bronchial biopsies as well as an increase in neutrophiles and platelets in peripheral blood due to particle exposure diesel. Adhesion molecule expression has been detected in several human atheroma components and cross sectional data is showing that the soluble form of the protein is increased in patients with atherosclerosis.

Yatera et al (2008) also reported female Watanabe hyperlipidemic rabbits with systemic atherosclerosis after exposure to PM10 twice a week for 4 weeks and found increased expression of ICAM-1 and VCAM-1 in plaque. Kristovich et al (2004) also concluded that exposure of gas diesel increases the expression of VCAM-1 and ICAM-1 in activated circulating monocytes in peripheral blood vessels.

CONCLUSION

Our study of soot particulates exposure for 30 days can increase the expression of tumor necrosis factor (ICAM-1) in the animal heart tissue. Increased expression of ICAM-1 was significantly obtained in the control group when compared with treatment group 1, control group with treatment group 2 and treatment group 1 with treatment group 2. These findings suggest a possible role of oxidative stress and activation of pro-inflammatory pathways in response to soot particulate exposure. The finding in our study is important in explaining how the soot particulate matter can contribute to cardiovascular events.

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