The Effect of Soot Particulate towards Vascular Cell Adhesion Molecule-1 (VCAM-1) Expression in the Mechanism of Cardiovascular System Disruption

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ABSTRACT

Background: Air pollution is associated with cardiovascular morbidity and mortality; however, the underlying mechanisms are not yet clearly understood. Several previous studies have implicated potential mechanism action including oxidative stress, systemic inflammation, autonomic dysfunction, and endothelial dysfunction. Several epidemiological studies have examined the association between ICAM-1, VCAM-1 and particulate matter.

Objective: To describe the effect of soot particulate exposure in VCAM-1 expression in the mechanism of cardiovascular dysfunctions.

Method: The experiment was conducted in laboratory female rats (Rattus novergicus) and consisted of 3 groups: Control group (n=10), without soot particulate exposure; Treatment 1 group (n=12), exposed by soot particulate with the concentration of 532 mg/m3 an hour each day for 30 days; Treatment 2 group (n=12), exposed by soot particulate with the concentration of 1064 mg/m3 an hour each day for 30 days.The expression of VCAM-1 on cardiac tissue was measured after the end of treatment by immunohistochemical examination. The differentiation of VCAM-1 expression among the groups was tested using the Kruskal-Wallis test and the Mann-Whitney test.

Results: The mean rank of VCAM-1 expression in the control group, treatment group 1 and treatment group 2 was significantly different (8.85, 17.63, 24.58, p=0.001). There was a significant difference in VCAM-1 expression by using the Mann-Whitney test among groups ($p < 0.05$).

Conclusion: The exposure to soot particles increased VCAM-1 expression significantly in laboratory animals. Our findings indicated the important role of the inflammatory activation pathway as a response to soot particulate exposure in the mechanism of cardiovascular disease.

Keywords: *Soot particulate, vascular cell adhesion molecule-1 (VCAM-1)*

Introduction

Cardiovascular disease is the leading cause of death and morbidity in the world [(1)]. Epidemiological studies showed an important association between cardiovascular morbidity and mortality after exposure to particles in air pollution, especially particulate matter (PM); however, the mechanism remains unclear.[(2,3)]. In the last 15 years, air pollution that induces cardiovascular disease has been the focus of intensive research among cardiologists and environmental medicine experts[(4)]. The comparisons of six cities in the United States with different levels of pollution found an increased risk of cardiovascular cases from atmospheric pollution with fine particles[(3)]. PM exposure as a result of air pollution has been a risk factor for cardiovascular disease including arrhythmias,

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myocardial ischemia, myocardialinfarction, and heart failure. Nearly 1 million people at risk of death from cardiovascular disease are associated with PM worldwide every year. The risk of myocardial infarction is estimated to be 1.48 times greater for a small increase in PM (25 μg / m3).Compared to the risk of myocardial infarction which was reported,it was approximately 3 times higher in smokers than non-smokers. The increased risk of cardiovascular disease associated with PM is relatively smaller compared to traditional risk factors such as smoking, diet, obesity, diabetes, metabolic syndrome, etc. However, air pollution with the PM is encountered by a largernumber of population and lasts a lifetime[1].

One of the suspected instrumental mechanisms is the occurrence of oxidative stress which will then increase the Reactive Oxygen Species (ROS) in the body that can cause cell damage through a chain reaction called lipid peroxidation. This oxidative stress further induces changes in the cardiovascular system[4]. Exposure to PM can result in lung inflammation, with the release of proinflammatory cytokines by alveolar macrophages that regulate local inflammatory responses. These cytokines also enter the circulation, resulting in systemic inflammation, in which the bone marrow is stimulated and releases leukocytes and platelets, as well as stimulate the liver to produce Creative protein (CRP) and fibrinogen[3, 5, 6].The high levels of CRP will affect endothelial function by weakening the reactivity of nitric oxide (NO) and increasing the expression of intercellular molecule adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), and E-selectin. ICAM-1 and VCAM-1 can also be stimulated by interleukin1-β (IL-1β) and tissue necrotic factor-ά (TNF-ά). Increased levels of these cytokines and adhesion molecules in the blood are associated with the widespread of coronary and carotid artery disease. ICAM-1 and VCAM-1 are the members of the Immunoglobulin superfamily and have a role in binding monocytes, lymphocytes to the endothelium, allowing them to enter the intima tunica. This is very important in the process of atherosclerosis[5,7]. The experiment on laboratory rabbits that were given PM exposure to its lung can increase the expression of adhesion molecules on the endothelium. This suggests that several stimulations that induce lung inflammation can also activate vascular endothelium. Active endothelial cells will decrease NO production and increase endothelin. Increased endothelin has been documented in patients with atherosclerosis and coronary disease. Endothelin is a vasoconstrictor and activates monocytes that affect the inflammatory response. This study suggests that pulmonary inflammation increases endothelial dysfunction markers in the circulation. It indicates a possible association between pulmonary inflammation and the occurrence of atherosclerosis [5].

In one epidemiological study, Pope et al., (2004) reported that PM exposure[2,5] was a risk factor of cardiovascular disease mortality through pulmonary and systemic inflammatory mechanisms, accelerated atherosclerosis and altered cardiac autonomic function[8]. The national study conducted in the United States (US) currently estimated each decrease is 10ug/ m3 levels of PM is associated with an increase in life expectancy of 0.61 years [9].Very few epidemiological studies examined the relationship between ICAM-1 and VCAM-1 with PM. This inflammation and endothelial dysfunction can be a process in which air pollution affects the cardiovascular system[7].

Based on the elaboration above, the researchers are encouraged to research the effect of soot particulate exposure on VCAM-1 expression in the cardiovascular system by using a laboratory experiment methodand rats as the experimental animals.

Method

Subjects: The experimental unit of this study was the hearts of female white rats (Rattus novergicus) which fulfilled the research criteria of female rats (Rattus novergicus), aged 4 months (16 weeks), weight 100-200 grams, and healthy. The research was conducted at the Biochemical Laboratory of Faculty of Medicine Universitas Airlangga and Department of Veterinary Anatomy Faculty of Veterinary Medicine, Universitas Airlangga. It was conducted for 6 months with the stages including giving particulate exposure for 30 days, laboratory animal surgery after treatment, VCAM-1 expression examination with immunohistochemical method.

This research was a laboratory experimental research conducted to examine the hypothesis through several stages of research. The study protocol was approved by the Ethical Commission to conduct basic science/clinical research in Dr. Soetomo General Hospital Surabaya. The descriptive data analysis was presented in the mean ± SD or median form and the frequency was showed in percentage. To test the normality of data distribution, the present study applied the 'one-sample Kolmogorov-Smirnov test'.One Way ANOVA parametric statistical

test was performed to examine the normal data distribution.If there was a significant difference, then it is followed by Post Hoc (Tukey HSD) statistical test. On the other hand, the abnormal data was examined by the non-parametric statistical Kruskal-Wallis test and followed by Mann-Whitney U statistical test. The results of data analysiswere displayed in graphics. The data analysis was processed by using SPSS software version 20 (SPSS,Inc., Chicago, IL)

Results

Observational Data: The results of VCAM-1 expression measurements were obtained by applying immunoreactive score scale (IRS) according to Remmele and Stegner.[32] This immunoreactive score index (IRS) or Remmele scale was the result of multiplication of the intensity of the color reaction and the percentage of cells with positive reactions (∑ = A x B) (score0-12). It was obtained the difference of VCAM-1 expression among groups (control: mean rank 8.85; T1: mean rank 17.63; T2: mean rank 24.58) (table 1, 2, 3 and figure 1, 2).

Data Analysis: There was a significant difference in VCAM-1 expression among groups(p=0.001) by using the non-parametric Kruskal-Wallis statistical test(table 4). Based on the result of the Mann-Whitney test, it was obtained a significant difference of VCAM-1 between the Control group and Treatment 1 group (p=0.015), between Control group and Treatment 2 (p=0.000), and between Treatment 1 and Treatment 2 group (p=0.048) (table 5).

Table 1. The VCAM-1 expression using IRS index (score 0-12) according to Remmele and Stegner

A-Percentage of positive cells	B-Color reaction intensity
0: no cells with positive reaction	0: no color reaction
1: < 10% cells with positive reaction	1: low color reaction intensity
2: 11-50% cells with positive reaction	2: medium color reaction intensity
3: 51-80% cells with positive reaction	3: strong color reaction intensity
4: > 80% cells with positive reaction	

Table 2. The VCAM-1 expression in Control group and Treatment group

No.	Slide number	IRS index	Slide number	IRS Index	Slide number	IRS Index
1	C 1	0	T1.1	1	T2.1	1
2	C 2	0	T1.2	1	T2.2	4
3	C 3	0	T1.3	0	T2.3	2
4	C 4	1	T1.4	2	T2.4	1
5	C 5	0	T1.5	0	T2.5	2
6	C 6	2	T1.6	3	T2.6	3
7	C 7	0	T1.7	1	T2.7	2
8	C 8	0	T1.8	0	T2.8	2
9	C 9	0	T1.9	2	T2.9	4
10	C 10	0	T1.10	2	T2.10	1
11		-	T1.11	1	T2.11	2
12		-	T1.12	2	T2.12	2
Average		0.3		1.25		2.16

Table 3. The VCAM-1 expression in Control group and Treatment group

	Group	N	Median	Mean Rank
IRS	Control	10	0.00	8.85
	Treatment 1	12	1.00	17.63
	Treatment 2	12	2.00	24.58
	Total	**34**		

Table 4. The results of Kruskal-Wallis test in VCAM-1 expression

	IRS
Chi-Square	14.809
Df	2
Aymp. Sig	0.001

Table 5. The results of VCAM-1 expression analysis using Mann-Whitney test

Group	Value P
Control-Treatment 1	0.015
Control-Treatment 2	0.000
Treatment 1-Treatment 2	0.048

Discussion

Particulate matter exposure contributes to an increased risk of cardiovascular disease by initiating and promoting the development of atherosclerosis which is the main cause of most cardiovascular diseases[3, 10]. Air pollution can induce peripheral artery, coronary atherosclerosis, and aortic atherosclerosis. PM exposure in a short period has been associated with increased mortality in acute cardiovascular disease[11].In one epidemiological study, Pope et al., (2004) reported that PM exposure [2, 5]was a risk factor of cardiovascular disease mortality through pulmonary and systemic inflammatory mechanisms, accelerated atherosclerosis and altered cardiac autonomic function[8].Once it was in PM circulation, it can interact with vascular endothelium or has a direct effect in atherosclerosis plaque which causes local oxidative stress and inflammatory effects in the lung. The endothelial dysfunction caused by PM has been examined in experimental animals in which there was increased VCAM-1 expression [10, 12].

In 2011, Jette Gjerke Hemmingsen et al. reported that the ROS production increased inthe human umbilical vein endothelial cell (HUVEC) that were exposed to PM. The smaller size of PM produces a higher ROS level. The expression of VCAM-1 increases in small size PM exposure as compared to HUVEC control ($p<0.01$)[13]. It indicates the association between PM with oxidative stress and inflammation. In 2011, Aling Dong, et al., compared the retinal vessels that had ischemia with a combination of ischemia and oxidative stress. VCAM-1 expression was higher in retinal vessels that had ischemia and oxidative stress and this increased the lacostasis and bone marrow-derived cells in the retina,which in both cases were blocked by intravenous injection of anti-VCAM-1 antibodies. Increased leukostasis will result in the neovascularization of the retina[14].

Vascular adhesion molecule -1 is a marker of the earliest lesions of atherosclerosis in experimental animals and is an adhesion molecule key that mediates the emergence of leukocytes in early lesions. Endothelial cells with VCAM-1 expression assist the monocyte cells to roll and cling tightly [15, 16].The antibody that blocks VCAM-1 or β1 or β2 integrins significantly decreases monocyte adhesion and the ICAM-1 or VCAM-1 gene mutation decreases atherosclerosis in laboratory rats[16]. Zhang Jie, et al. revealed that mast cells, neutrophils, and macrophages released the proinflammatory cytokines such as TNFά, INFγ, and IL6 that induced adhesion molecule expression of endothelial cell and recruited leukocytes that were the pathogenesis of the vascular inflammatory disease[16].Salvi et al. reported that up-regulation of bronchial adhesion molecules such as ICAM-1 and VCAM-1 occurred after exposed to PM[17].

A research conducted by Swapna Upadhyay, et al., in 2010 reported thatparticulate matter affectedthe vascular homeostasis in the lung and systemic.This has been identified based on the analysis of various biomarkers that are related to hypertension (ACE), endothelial activation (ET-1, VCAM-1), coagulation factor (TF, PAI-1) and angiogenesis (VEGF). The up-regulation of VCAM-1 in lung tissue can be due to endothelial cell activation by cytokines which were released from active macrophages or collected neutrophil cells. Various studies have proven that VCAM-1 promotes the progressiveness of atherosclerosis by the accumulation, adhesion, and migration of transcendental leukocytes[18].Furthermore, active endothelial cells can enhance the expression of PAI-1, VEGF, and VCAM-1 which are responsible for the development of atherosclerotic lesions. In the heart, all the signs measured elevated at high levels on day 3 after the exposure[18, 19].

In this study, the results indicated a significant difference in VCAM-1 expression among groups ($p=0.001$). It was also obtained a significant difference in VCAM-1 expression between the Control group and Treatment 1 group ($p=0.015$), between Control group and Treatment 2 ($p=0.000$), and between Treatment 1 and Treatment 2 group ($p=0.048$). The results of this study are following the previous studies. These findings support the hypothesis that exposure to soot particulatecan increase VCAM-1 expression in the mechanism of cardiovascular system disruption.

Conclusion

The inhalation exposure of soot particulate matter with the duration of 1 hour daily for 30 days, a dose of 532 mg/m^3 and 1064 mg/m^3 significantly increased the VCAM-1 expression in the cardiomyocyte cells of the experimental rats. The results also showed that increased VCAM-1 expression was following increased doses of soot particulate matter exposure, which plays a role in the inflammatory response underlying the occurrence of atherosclerosis. The findings of our study are important in explaining how particulate matter, especially soot, can contribute to cardiovascular causes.

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