



Cholesterol lowering effect of subchronic inhalation particulate matter 10 coal dust on rats

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Abstract

Aim of this study was have to known an effect of inhaled particulate matter 10 (PM₁₀) of coal dust on lipid profile, hematopoietic stem cells (HSC) and circulating endothelial cells (CECs) in rats. A total of 32 Wistar male rats, were randomly divided into four groups of 8 rats each, including one control group and three groups for inhaled coal dust (concentration 6.25 mg/m³; 12.5 mg/m³; and 25 mg/m³). The exposure to coal dust exposure was conducted using equipment that was designed by and available from Pharmacology Laboratory, Medical Faculty, Brawijaya University of Malang. The level of total cholesterol was lower significantly in rats inhaled to coal dust at compared with the control rats ($p < 0.05$). The level of triglycerides was higher significantly in rats inhaled to coal dust at concentration 25 mg/m³ compared with the control rats ($p < 0.05$). The level of LDL-c was lower significantly in rats inhaled to coal dust at concentration 12.5 and 25 mg/m³ compared with the control rats ($p < 0.05$), but the level of HDL-c, HSCs and CECs were not different significantly ($p > 0.05$). The level of HDL-c/LDL-c ratio was higher significantly in rats inhaled to coal dust at concentration 12.5 and 25 mg/m³ compared with the control rats ($p < 0.05$). The level of cholesterol/HDL-c ratio and atherogenic index were lower significantly in rats inhaled to coal dust at concentration 12.5 and 25 mg/m³ compared with the control rats ($p < 0.05$). The level of hematopoietic stem cells and circulating endothelial cells were not different significantly in rats inhaled to coal dust compared with the control rats ($p > 0.05$). Sub-chronic inhalation particulate matter 10 (PM₁₀) coal dust have lowering effect on atherogenic index due to decreasing cholesterol and LDL levels. Beside that, sub-chronic inhalation particulate matter 10 (PM₁₀) coal dust also have dyslipidemia which marked by increasing of triglyceride levels.

Keywords: dyslipidemia; inhaled pollution; endothelial damage; hematopoietic stem cells.

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Introduction

Unhealthy lifestyle and polluted environment were risk factors for increasing incidence of coronary heart disease (CHD) and peripheral vascular disease due to dyslipidemia. Deposition of cholesterol, triglyceride, calcium and other substances within vascular are accelerated factor of atherosclerosis progression. To date, inhalation of occupational and atmospheric coal dust has not only contributed significantly to the development of several respiratory disorders, also cardiovascular disease [1, 2]. Epidemiologic study demonstrated that the population residing near coal mine facilities suffer from a higher rate of cardiovascular disease [3], but the association of coal dust exposure and lipid profile or dyslipidemia is still unknown.

Mesoderm-derived adult stem cells, such as cardiac-derived stem cells, mesenchymal stem cells, skeletal myoblasts, hematopoietic stem cells (HSCs), and endothelial progenitor cells, represent a more suitable cell source for cell therapy intervention [4]. HSCs are multipotent stem cells that give rise to all cells of the blood lineage. In recent years, it has been proposed that these cells can circulate to the site of injury, where they contribute to myocardial repair and regeneration. As we know, there is no study to evaluate the effect of coal dust on hematopoietic stem cells.

Circulating endothelial cells (CECs) are mature cells that are not associated with vessel walls, but are detached from the endothelium and circulate within peripheral blood. The number of CECs present in the blood has been found to increase in response to cardiovascular disease, vasculitis, infectious disease, and various cancers [5]. Indeed, the level of CECs has been recognized as a useful biomarker for vascular damage. The endothelial cell damages due to dyslipidemia and proinflammatory cytokines have been demonstrated previous studies [6, 7]. Increased levels of triglycerides and lipoproteins correlate with impairment of endothelial function [8, 9]. Endothelial cell damage due to dyslipidemia plays a critical role in the development and progression of atherosclerosis [10, 11]. Several studies showed that coal dust produces proinflammatory cytokines and free radicals in vitro [12, 13] in rats [1, 14] and humans [15, 16] but for dyslipidemia and endothelial damage is still unknown.

To the best of our knowledge, no report has evaluated the effect of coal dust exposure on endothelial damage and its regeneration. Accordingly, we performed the subchronic inhalation of particulate matter 10 (PM₁₀) of coal dust on hematopoietic stem cells and

circulating endothelial cells. In addition, we also measured the change of lipid profile in subchronic inhalation of particulate matter 10 (PM₁₀) of coal dust.

Material and Methods

Animals and diets

Thirty two male Wistar rats, weighing 100–125 g, purchased from Central Animal House of Bandung were housed in an air-conditioned room at $25 \pm 1^\circ\text{C}$ and 65–70% relative humidity with a 12 h light-dark cycle. The protocol used in this study was approved by the Ethic Committee for Animal Experimentation of the University of Lambung Mangkurat. Diets were made following American Institute of Nutrition (AIN) recommendations. The animals were given food and water *ad libitum* during the experimental period.

Coal dust preparation

Coal dust was made from gross coal by pulverizing using a Ball Mill, Ring Mill and Raymond Mill in Carsurin Coal Laboratories of Banjarmasin, resulting coal dust with a diameter of $<75 \mu\text{m}$. This particle coal dust was then filtered by Mesh MicroSieve (BioDesign, USA) with size of pores less than $10 \mu\text{m}$ of diameter. This specimen of coal dust then saving in Tissue and Specimen Banking of Banjarmasin referenced as voucher code 2012-2-CD. The characteristics of coal dust were then analyzed by Scanning Electron Microscope and X-Ray Fluorescence at the Physic and Central Laboratory Faculty of Mathematic and Natural Science University of Malang.

Scanning Electron Microscope has show the aerodynamic diameter of coal dust particle is less than $10 \mu\text{m}$ in one dimension, so its call PM₁₀. In addition, the morphology of particle consisting singlet or aggregate particle. X-Ray Fluorescence has show inorganic composition (%) of coal dust, which were iron (29.3 ± 0.1), silicon (29.0 ± 0.2), calcium (12.00 ± 0.07), aluminium (10 ± 0.2), titanium (6.31 ± 0.19), phosphorus (5.90 ± 0.04), potassium (4.5 ± 0.06), and barium (1.00 ± 0.09) and several inorganic minerals is less than $<1\%$ including europium (0.70 ± 0.00), chromium (0.48 ± 0.04), nickel (0.41 ± 0.00), copper (0.34 ± 0.02), zinc (0.22 ± 0.03), vanadium (0.20 ± 0.02), and manganese (0.15 ± 0.09).

Coal dust exposure

The groups for coal dust exposure receiving coal dust inhalation at concentration 6.25 mg/m³, 12.5 mg/m³, 25 mg/m³ one hour per day for 28 days which modified from previous study [17]. Coal dust exposure was done by coal dust inhalation equipment that was designed and available in Pharmacology Laboratorium, Medical Faculty, Brawijaya University of Malang. The principal of this equipment is to provide an ambient environment which contains coal dust which inhaled by rat. The airstream of thus equipment is setting at 1.5-2 liter/minutes whic similar to environmental airstream.

Plasma lipid profile and atherogenic index analysis

At the end of the treatment, rats in all groups were anesthetized by ether; blood samples were collected by cardiac puncture and allowed to clot for 2 hours at room temperature, followed by centrifugation at 3500 g for 10 minutes to obtain the serum. The serum was stored at -80°C until analysis. The levels of total cholesterol triglycerides (TG), high-density lipoprotein cholesterol (HDL-c), and low-density lipoprotein cholesterol (LDL-c) were measured in the serum of rat using an automated enzymatic technique [18]. The atherogenic index (AI) was defined as $(TC-HDL-c)/HDL-c$ and was calculated for the experimental groups.

Measurement of hematopoietic stem cells

Hematopoietic stem cells were isolated from peripheral blood as described elsewhere [19]. Briefly, 10 mL of venous ethylenediaminetetraacetic acid (EDTA) blood was obtained by peripheral venepuncture, stored at 4°C to 10°C, and processed within 6 hours after collection. Peripheral blood mononuclear cells were isolated by density-gradient centrifugation using Ficoll-Paque Plus (Amersham Pharmacia Biotech, Uppsala, Sweden). Isolated cells were washed twice with PBS and resuspended in 20 0mL of PBS supplemented with 0.5% of bovine serum albumin and 2 mM of EDTA. CD34⁺ cells in peripheral blood were evaluated by immunostaining with PE-conjugated CD34 monoclonal antibody (Biolegend) and detected by flow cytometry (BD FACSCalibur Flow Cytometer).

Measurement of circulating endothelial cells

Circulating endothelial cells were isolated from peripheral blood as described elsewhere [18], with minor modification. Briefly, 10 mL of venous ethylenediaminetetraacetic acid (EDTA) blood was obtained by peripheral venepuncture, stored at 4°C to 10°C, and processed within 6 hours after collection. Peripheral blood mononuclear cells were isolated by density-gradient centrifugation using Ficoll-Paque Plus (Amersham Pharmacia Biotech, Uppsala, Sweden). Isolated cells were washed twice with PBS and resuspended in 20 0mL of PBS supplemented with 0.5% of bovine serum albumin and 2 mM of EDTA. CD146⁺ cells in peripheral blood were evaluated by immunostaining with FITC-conjugated CD146⁺ monoclonal antibody (Biolegend) and detected by flow cytometry (BD FACSCalibur Flow Cytometer).

Ethics

This research has been approved by research ethics committee Faculty of Medicine University of Lambung Mangkurat, Banjarmasin, Indonesia.

Statistical analysis

Data are presented as mean ± SD and differences between groups were analyzed using 1-way ANOVA with SPSS 15.0 statistical package. Post Hoc test was used if the ANOVA was significant. $p < 0.05$ was considered statistically significant.

Results

Effect PM10 coal dust on lipid profile

Table 1 showed the levels of total cholesterol, triglycerides, HDL-c, LDL-c, and atherosclerosis index, HDL-c/LDL-c ratio, and cholesterol/HDL-c ratio in serum of control and experimental groups of rats. The level of total cholesterol was lower significantly in rats inhaled to coal dust compared with the control rats ($p < 0.05$). The level of triglycerides was higher significantly in rats inhaled to coal dust at concentration 25 mg/m³ compared with the control rats ($p < 0.05$). The level of HDL-c was not different significantly in all groups ($p > 0.05$). The level of LDL-c was lower significantly in rats inhaled to coal dust at concentration 12.5 and 25 mg/m³ compared with the control rats ($p < 0.05$). The level of HDL-

c/LDL-c ratio was higher significantly in rats inhaled to coal dust at concentration 12.5 and 25 mg/m³ compared with the control rats (p<0.05). The level of cholesterol/HDL-c ratio was lower significantly in rats inhaled to coal dust at concentration 12.5 and 25 mg/m³ compared with the control rats (p<0.05). The level of atherogenic index was lower significantly in rats inhaled to coal dust at concentration 12.5 and 25 mg/m³ compared with the control rats (p<0.05).

Table 1. Lipid profile in rats inhaled to subchronic PM₁₀ of coal dust

(mg/dL)	Concentration of coal dust			
	0 mg/m ³	6.25 mg/m ³	12.5 mg/m ³	25 mg/m ³
Cholesterol	98.400 ± 9.044	80.400 ± 16.134 ^a	66.600 ± 11.610 ^a	69.000 ± 9.083 ^a
Triglyceride	73.400 ± 4.037	60.600 ± 22.300	123.600 ± 36.487 ^b	103.400 ± 22.434 ^{ab}
HDL-c	38.080 ± 4.683	33.560 ± 2.901	31.980 ± 7.982	33.140 ± 5.081
LDL-c	45.640 ± 6.975	34.720 ± 19.080	9.900 ± 8.679 ^{ab}	15.180 ± 3.697 ^a
HDL-c/LDL-c	0.851 ± 0.179	1.342 ± 0.975	5.631 ± 5.037 ^{ab}	2.270 ± 0.530 ^a
Chol/HDL-c	2.602 ± 0.257	2.396 ± 0.422	2.110 ± 0.194 ^a	2.090 ± 0.117 ^a
AI	1.602 ± 0.257	1.396 ± 0.422	1.110 ± 0.194 ^a	1.090 ± 0.117 ^a

Note: AI: atherogenic index; values are presented as mean ± SD; ^ap<0.05; in comparison with control group; ^bp<0.05; in comparison with rats inhaled to 6.25 mg/m³; ^cp<0.05; in comparison with rats inhaled to 12.5 mg/m³

There is negative correlation significantly between total cholesterol and concentration of coal dust (p=0.001; r=-0.694). There is positive correlation significantly between triglycerides and concentration of coal dust (p=0.019; r=0.520). There is negative correlation significantly between LDL-c and concentration of coal dust (p=0.000; r=-0.738). There is negative correlation significantly between atherogenic index and concentration of coal dust (p=0.003; r=-0.631).

Effect PM10 coal dust on HSC and CECs

Table 2 showed the levels of hematopoietic stem cells and circulating endothelial cells of control and experimental groups of rats. The level of hematopoietic stem cells and circulating endothelial cells were not different significantly in rats inhaled to coal dust compared with the control rats (p>0.05).

Table 2. HSCs and CECs in rats inhaled to subchronic PM₁₀ of coal dust

(% cells)	Concentration of coal dust			
	0 mg/m ³	6.25 mg/m ³	12.5 mg/m ³	25 mg/m ³
HSCs	0.118 ± 0.043	0.158 ± 0.093	0.086 ± 0.046	0.072 ± 0.051
CECs	0.060 ± 0.050	0.026 ± 0.017	0.022 ± 0.008	0.044 ± 0.025

Note: values are presented as mean ± SD; ^ap<0.05; in comparison with control group; ^bp<0.05; in comparison with rats inhaled to 6.25 mg/m³; ^cp<0.05; in comparison with rats inhaled to 12.5 mg/m³

Discussion

Lipids have been observed to play important roles in pathological changes observed in disease conditions and pre-condition diseases. Serum lipids bounding to lipoproteins can be elevated by an increase in biosynthesis and/or by a decrease in their elimination process. As we know, this is the first time a study has been designed to evaluate an effect of inhaled particulate matter 10 of coal dust on a lipid profile, hematopoetic stem cells, and circulating endothelial cells in rats.

Reverse cholesterol transport (RCT) is the mechanism by which cholesterol is transported from the peripheral tissues and cells to the liver, where it is transformed into bile acids, and then finally eliminated from the body. The main function of RCT is to prevent the formation and development of atherosclerosis, by decreasing the plasma cholesterol level [19]. The process of RCT can be divided into three stages: 1) the efflux of cellular cholesterol from peripheral cells as high-density lipoprotein, 2) conversion of cholesterol in HDL to cholesteryl esters (CE) by the enzyme lecithin-cholesterol acyltransferase (LCAT), then cholesterol is carried as CE within the core of HDL particles to the liver, and 3) delivery of CE from HDL to hepatocytes [20]. In the present study, inhalation of coal dust over four weeks resulted in 18.29% to 32.31% lower total plasma cholesterol levels, compared to the control group. Besida that, there is negative correlation significantly between total cholesterol and concentration of coal dust (p=0.001; r=-0.694). We suggest that the mechanism by which coal dust inhalation lowers cholesterol may be related to increased RCT; however, the detailed mechanisms need to be clarified in a future study. The inorganic components of coal dust that can decrease cholesterol levels are silicon, chromium and vanadium. Elevated silicon ingestion impedes cholesterol-induced atherogenesis in rabbits [21]. Chromium induced a reduction in cholesterol *in vitro* [22] and *in vivo* [23, 24]. In addition, vanadium effectively lowered hyperlipidemia in diabetic rats [25].

Increased levels of triglycerides are not as strongly associated with increased cardiovascular risk as increased plasma cholesterol levels. Nevertheless, elevated plasma triglyceride levels are clinically significant, as they are an indicator of dyslipidemia [26]. The level of triglycerides was significantly higher in rats inhaling coal dust at a concentration of 25 mg/m³ compared with the control rats (p<0.05). There is positive correlation significantly between triglycerides and concentration of coal dust (p=0.019; r=0.520). This finding indicates that coal dust inhalation at a concentration of 25 mg/m³ only lead to dyslipidemia; however, dyslipidemia is not strongly associated with increased cardiovascular risk which confirmed by negative correlation significantly between atherogenic index and concentration of coal dust (p=0.003; r=-0.631).

High-density lipoprotein has antiatherosclerotic effects, including augmentation of ability of reverse cholesterol transport [29, 30]. Cholesteryl ester transfer protein (CETP) promotes the transfer of CE from HDL to the apoB-containing lipoprotein particles, which subsequently are cleared from the circulation by the liver. Thus, CETP plays a critical role in the intravascular remodeling and recycling of HDL particles [31]. The level of HDL-c is not different significantly in the coal dust exposure group compared to the control group (p > 0.05), but the level of LDL-c was lower significantly in rats inhaling to coal dust at concentrations of 12.5 and 25 mg/m³ compared with the control rats (p<0.05). in addition, There is negative correlation significantly between LDL-c and concentration of coal dust (p=0.000; r=-0.738). The level of HDL-c/LDL-c ratio was higher significantly in rats that inhaled coal dust at concentrations of 12.5 and 25 mg/m³ compared with the control rats (p<0.05). This result indicated that coal dust maintained HDL-c at basal level and in ideal proportion to LDL-c which is important for CETP function. In addition, atheroprotective properties of HDL-c have been associated to its role in preserving endothelial function [31]. We also find that coal dust does not increase endothelial damage. This means that the basal level of HDL-c, which is found in control and coal dust inhalation groups has a beneficial effect in preventing endothelial damage.

Decrease in the level of total-cholesterol/HDL-cholesterol ratio and HDL-cholesterol/LDL-cholesterol ratio is linked to a reduction in the risk of morbidity and mortality in cardiovascular diseases [32]. The level of total-cholesterol/HDL ratio is lower significantly in coal dust exposure at concentrations of 12.5 mg/m³ and 25 mg/m³ compared to the control group. This finding indicated that subchronic coal dust exposure at concentrations of 12.5

mg/m³ and 25 mg/m³ decrease the risk of cardiovascular disease. We also find that the atherogenic index is decreased in the coal dust inhaled groups, reach different significances at coal dust concentrations of 12.5 and 25 mg/m³.

Endothelial cells appear to regulate the trafficking and release of HSCs from bone marrow [33]. Endothelial cell damage due to dyslipidemia plays a critical role in the development and progression of atherosclerosis [34]. The level of hematopoietic stem cells and circulating endothelial cells is not different in coal dust exposure groups compared to control group. This finding indicated that subchronic coal dust particle exposure does not induce cell death of hematopoietic stem cells and endothelial damage. The viability of endothelial cells determine the trafficking and release hematopoietic stem cells from bone marrow.

In conclusion, sub-chronic inhalation particulate matter 10 (PM₁₀) coal dust have dual effect on lipid profile. First effect is lowering effect on atherogenic index due to decreasing cholesterol and LDL levels. Second effect is dyslipidemia which marked by increasing of triglyceride levels.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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