Bone remodeling is a physiological process of cortical and trabecular bone reconstruction, with initial bone resorption, by osteoclasts and concurrent bone formation by osteoblasts. Oxidative stress due to coal dust exposure is not only found in the lungs, but also in the circulation or systemically. The aim of this study was to determine the effect of oxidative stress from coal dust exposure on the number of osteoblasts and osteoclasts in rats. In this experimental study, four groups were evaluated: control; coal dust exposure at 6.25 mg/m³ for 28 days; coal dust exposure at 12.5 mg/m³ for 28 days; coal dust exposure at 25 mg/m³ for 28 days (all exposures were given daily for one hour). Circulatory oxidative stress was measured by malondialdehyde level. Osteoblast and osteoclast numbers were counted by light microscopic examination of distal femoral cross-sections stained with hematoxylin eosin. This study showed that malondialdehyde levels were significantly increased in coal dust exposure groups, in comparison with the control group (p<0.05). There were also significantly decreased numbers of osteoblasts (p<0.05) and significantly increased numbers of osteoclasts (p<0.05) in coal dust exposure groups, as compared with the control group. No correlations were found between malondialdehyde levels (oxidative stress) and respective numbers of osteoblasts and osteoclasts in all coal dust exposure groups (p>0.05). Coal dust exposure increased malondialdehyde level and osteoclast numbers, and decreased osteoblast numbers, but no correlation was found between oxidative stress (caused by coal dust exposure) and osteoblast and osteoclast numbers.

Key words: Coal dust, subchronic, oxidative stress, osteoblast, osteoclast, rat

INTRODUCTION

Osteoblasts are bone-forming cells derived from multipotent mesenchymal precursor stem cells, which are also stem cells for the bone marrow stroma, chondrocytes, muscle cells, and adipocytes, with mean life span of around 3 weeks.(1,2) Osteoclasts are multinuclear cells of large size (50 to 100 µm in diameter), originating from the...
hematopoietic system,\(^{(1,3)}\) with a life span of up to \(\sim 2\) weeks.\(^{(4)}\) The precursors of osteoclasts are hematopoietic cells of monocyte/macrophage lineage,\(^{(1,2)}\) and the osteoclasts reach the bones through cell migration of progenitors from adjacent connective tissue.\(^{(1)}\)

Bone remodeling is a physiological process of cortical and trabecular bone reconstruction, with initial bone resorption by osteoclasts and concurrent bone formation by osteoblasts. This process is coordinated throughout life and is the dominant process in adult skeletons. If the resorption and absorption are quantitatively proportional, the remodeling proceeds in a balanced manner.\(^{(5,6)}\) The remodeling process may be disrupted by various toxic substances from the environment, resulting in disease. Exposure to metals, such as cadmium, aluminum, and lead is a risk factor for osteoporosis.\(^{(7)}\)

South Kalimantan is the largest coal producer in Indonesia, with mines located throughout the area (Banjar, Tanah Laut, Kotabaru, Tanah Bumbu, Hulu Sungai Tengah, Hulu Sungai Utara, Hulu Sungai Selatan, Tapin, and Tabalong). Coal dust is a complex admixture of several minerals, trace metals, and organic substances other than coal particles.\(^{(8)}\) Coal dust particles deposited in the alveolar epithelium are phagocytosed by alveolar macrophages, subsequently increasing release of \(\text{H}_2\text{O}_2\) and \(\cdot\text{O}_2\), triggering oxidative stress.\(^{(9,10)}\) Oxidative stress from coal dust exposure is not only found in the lungs, but also in the circulation or systemically.\(^{(10)}\) High levels of oxidative stress cause apoptotic necrosis.\(^{(11)}\) The numbers of osteoclasts and osteoblasts depend on two processes, i.e. osteoclastogenesis or osteoblastogenesis, and apoptosis.\(^{(4)}\) The study by Parhami et al. demonstrated that lipid oxidation inhibits osteoblast differentiation, thus disturbing bone formation.\(^{(12)}\) In vitro and in vivo it was also demonstrated that free radicals are involved in osteoclastogenesis and bone resorption.\(^{(13)}\)

Therefore the aim of our study was to evaluate the effect of oxidative stress, induced by coal dust exposure, on the numbers of osteoblasts and osteoclasts in rats.

**METHODS**

**Research design**

This was a controlled experimental study conducted in the period of October-December 2010.

**Experimental animals**

The experimental subjects were 2-3 month old male Wistar rats weighing 200-250 grams, subdivided into 4 groups: group 1 was the control group (P1) without exposure to coal dust; group 2 was exposed to coal dust at a dose of 6.25 mg/m\(^3\) for 1 hour per day for 28 days (P2); group 3 was exposed to coal dust at a dose of 12.5 mg/m\(^3\) for 1 hour per day for 28 days (P3); and group 4 was exposed to coal dust at a dose of 25 mg/m\(^3\) for 1 hour per day for 28 days (P4). The sample size was calculated from the formula of Stell et al.\(^{(14)}\) to be 6 rats per group. All male Wistar rats were obtained from the Pharmacology Laboratory, Faculty of Medicine, Brawijaya University, Malang.

**Preparation of coal dust**

Coal dust was prepared by pulverizing coal particles by means of equipment consisting of ball mills, ring mills and Raymond mills at PT. Carsurin Coal Laboratories, Banjarmasin. The resulting coal dust was \(<75\,\mu\text{m}\) in diameter, and subsequently further screened, using microSieve (BioDesign, USA), producing coal dust of \(<10\,\mu\text{m}\) diameter as respirable particulate matter.

**Exposure to coal dust**

Exposure to coal dust was performed by means of a coal dust exposure device of 0.5 m\(^3\) capacity, designed and provided by the Pharmacology Laboratory, Faculty of Medicine,
Brawijaya University. This device works on the principle of providing an ambient environment containing coal dust as particulate matter, which can be inhaled into the airways of the experimental animals. The air flow through the blower is 1.5-2 liter/minute, which corresponds to the air flow in collieries. The dose of coal dust exposure had been determined by preliminary studies to be 12.5 mg/m³ for a high dose.\(^{(13)}\)

**Parameter measurements**

After undergoing coal dust exposure for 28 days, the experimental animals were sectioned. The rats were anesthetized by means of ether on cotton wool in a plastic container. Sectioning was performed on rats with beating hearts by opening the abdomen, cutting the ribs, and opening the thoracic cavity to find the heart. Blood was obtained from the heart to be used for parameter measurements. The parameters measured in the blood were malondialdehyde (MDA) concentration, performed in the Pharmacology Laboratory, Faculty of Medicine, Brawijaya University, Malang. In addition to the blood samples, the posterior extremities were also taken for histological sections of the femoral bones with hematoxylin-eosin staining. Subsequently the numbers of osteoblasts and osteoclasts were counted in the Pathology Laboratory, Faculty of Medicine, Brawijaya University, Malang, by means of image analyzer software. For each preparation, 10 high power fields were examined at 400 x magnification.

**Ethical clearance**

Ethical clearance for work on experimental animals for this study was issued by the Research Ethics Committee, Faculty of Medicine, Lambung Mangkurat University.

**Statistical analysis**

The collected data were analyzed using ANOVA, and a p value of p<0.05 indicates a significant differences between intervention groups. Significant results of ANOVA were followed up by a post hoc test. In addition correlation tests were performed. All statistical analyses were performed using SPSS software (15.0) for Windows. Minimal significance was assumed at p<0.05.

![Figure 1. MDA levels according to coal dust exposure groups in rats.](image)
P1=control; P2=coal dust exposure at 6.25 mg/m³ for 28 days; P3=coal dust exposure at 12.5 mg/m³ for 28 days; P4= coal dust exposure at 25 mg/m³ for 28 days
RESULTS

According to ANOVA results, subchronic exposure to coal dust at various doses significantly increased malondialdehyde levels in comparison with the control group (p=0.000). Using post hoc testing, there were significant differences between intervention groups, except between group P2 and control (P1) (p=0.192) (Figure 1).

Using ANOVA, coal dust exposure significantly reduced the mean number of osteoblasts, as compared to the control group (p=0.000). On post hoc testing significant differences were found between intervention groups (p<0.05) (Figure 2).

Based on ANOVA, coal dust exposure significantly increased the mean number of osteoclasts, as compared to the control group (p=0.000). Using post hoc testing significant differences were found between intervention groups, except between P2 and P3 (p=0.064) (Figure 3).
According to the results of the Pearson correlation test, there was no significant correlation between MDA levels and osteoblast and osteoclast numbers in the intervention groups (p>0.05).

**DISCUSSION**

Inhalation of coal dust leads to formation of reactive oxygen species (ROS) through direct and indirect mechanisms. Direct mechanisms involve bioactive components contained in coal dust, while indirect mechanisms form ROS through respiratory bursts during phagocytosis by macrophages. The content of transitional metals, comprising Fe, Cr, Mn, Co, Ni, Cu, Zn, and silicon, may catalyze Fenton reactions to produce reactive oxygen compounds. During phagocytosis of inhaled particles, superoxide radicals are formed, that are subject to spontaneous dismutation to produce hydrogen peroxide. In the presence of transitional metals, the hydrogen peroxide is converted into hydroxyl radicals. In the present study, increased levels of MDA are the result of oxidative stress, due to coal dust exposure. Formation of MDA is by lipid peroxidation, which is significantly different between intervention groups. Increased lipid peroxidation is consistent with the study by Armutcu et al. In addition, according to the hypothesis of Parhami, lipid peroxidation may affect the numbers of osteoblasts and osteoclasts in the aged condition (Figure 4). In our study, the number of osteoblasts decreased concurrently with increased dose of coal dust exposure, although no significant difference was found between P2 and P3. In addition, the number of osteoclasts increased concurrently with increased doses of coal dust exposure, although no significant difference was found between P2 and P3. This indicates an imbalance between bone formation and resorption.

Interestingly, there was no correlation between MDA levels and the number of osteoblasts, or between MDA levels and the numbers of osteoclasts, which was not according to Parhami’s hypothesis. This is presumably due to the presence of other...
molecules capable of affecting the numbers of osteoblasts and osteoclasts. MDA molecules are active molecules that may undergo transformation into other molecules, e.g. via enzymatic glycosylation. The available aldehyde groups may react with amino groups of proteins, giving rise to advanced glycation end products (AGEs), which subsequently may affect osteoblasts and osteoclasts.\textsuperscript{(17-19)} Another mechanism of coal dust is through triggering of increases in nitrous oxide levels, which may trigger apoptosis of osteoblasts.\textsuperscript{(10,20)}

One limitation of this study is the failure to explore the various parameters that are increased as a result of coal dust exposure, in order to demonstrate the markers that are the cause of the reduction in the numbers of osteoblasts and the increased numbers of osteoclasts.

**CONCLUSION**

Exposure to coal dust increases MDA levels and the numbers of osteoclasts, while reducing the numbers of osteoblasts, although there was no significant correlation between oxidative stress due to coal dust exposure and the numbers of osteoblasts and osteoclasts.

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**REFERENCES**


