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Brief Report

Combined inhalation of cigarette smoke and coal dust particulate matter 10 increase bone iron level in rat

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Key Words
Bone minerals; Cigarette smoke;
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Abstract

Objective: This study aimed to elucidate whether inhalation combination of cigarette smoking and particulate matter 10 (PM_{10}) coal dust can change bone mineral elements of rats.

Methods: A total of 30 Wistar male rats were randomly divided into three groups including one non-exposure group and two groups of combined cigarette smoke and coal dust. Dose of cigarette smoking was one cigarette per day. Dose of coal dust exposure was 12.5 mg/m^3 one hour/day as PM_{10}. Cigarette smoke was exposed prior to coal dust exposure (14 and 28 days). Exposure was done by equipment available in Department of Pharmacology, Faculty of Medicine, University of Brawijaya, Malang, East Java, Indonesia. Bone mineral element was analyzed in femur using X-Ray Fluorescence in Central and Physics Laboratory, State University of Malang, Malang, East Java, Indonesia. ANOVA test was used to analyze the difference levels of bone mineral elements.

Results: There are no significant differences for phosphorus, calcium, nickel, copper, zinc and calcium/phosphorus levels in all groups ($P > 0.05$). Significant increase of iron level in combination cigarette smoke and coal dust exposure was detected when compared with the non-exposure group ($P < 0.05$).

Conclusion: Sub-chronic inhalation combination of cigarette smoke and coal dust PM_{10} increase bone iron levels in rats.

INTRODUCTION

Organic and inorganic components are responsible for toughness and rigidity of bones, respectively. These components also determine the mechanical strength of the bone. The mechanisms of trabecular bones in both rats and humans are influenced by mechanical, hormonal, biological and/or toxic processes [1, 2]. Atomic minerals are the smallest components of trabecular bones. Substitution occurs when one of these component atoms replaces a comparable atom due to similarities in their radii and charges. Ren *et al* [3] clearly showed that zinc (Zn) substitution on hydroxyl apatite crystal inhibits crystal growth due to its smaller atomic radius as compared with calcium (Ca) which was confirmed by another study [4]. Cigarette smoke contains heavy metals, particularly cadmium (Cd), chromium (Cr), plumbum (Pb) and nickel (Ni) which accumulate in tissues and fluids [5]. Noor *et al* [6] demonstrate that models of substituting several atoms in a hydroxyl apatite crystal of healthy bone would change crystal size and porosity. Previous studies indicate that deficiencies in certain bone bioelements, such as copper (Cu), magnesium (Mg) and manganese (Mn) are considered risk factors for osteoporosis [7, 8].

Smoking has been established as risk factor for cardiovascular and pulmonary disease, and also bone disease such as osteoporosis, disc disease, delayed fracture healing and nonunion [9]. Decreasing skeletal strength, increasing of fragility and fracture risk are markers of osteoporosis, and are caused by abnormalities of bone density and structure [10, 11]. Smokers who work in an industrial setting maybe exposed to combinations of toxicants. Coal mining dominates the energy supply industry and maybe potentially toxic to its worker or people in the areas surrounding coal mines. Inhalation of occupational and

atmospheric coal dust can contribute significantly to the development of several respiratory disorders, such as infection, inflammation and chronic bronchitis, and also cardiovascular disease [12, 13]. Our previous *in vivo* study demonstrated that rats exposed to sub-chronic levels of coal dust had a decreased number of osteoblasts and increased number of osteoclasts [14], indicating that exposure to coal dust is associated with change of bone mineral elements.

We hypothesize that inhaling cigarette smoke and coal dust alters bone mineral elements. Furthermore, the mechanisms referred to herein above accelerate bone degeneration. Accordingly, the aim of this study is to verify whether cigarette smoke and coal dust in combination significantly alter bone mineral elements. This study is the first time to provide more detailed information that sub-chronic combined inhalation of cigarette smoke and coal dust particulate matter 10 (PM_{10}) change bone mineral element in rats.

MATERIALS AND METHODS

Animal

Adult male Wistar albino rats weighing 170-200 g were used for the present investigation. They were housed in a clean wire cage and maintained under standard laboratory conditions (25 ± 2°C, 12/12 h dark/light cycle). They were fed a standard pellet diet and received water *ad libitum*. The animals were acclimatized to laboratory conditions for one week prior to the experiment. All experimental procedures described were reviewed and approved by the ethics committee of the Faculty of Medicine, Lambung Mangkurat University, Banjarmasin, South Kalimantan, Indonesia. All animal treatments were in accordance with the Guide for the Care and Use of Laboratory Animals.

Coal dust and cigarette content analysis

Coal dust was made from gross coal by pulverizing using a Ball Mill, Ring Mill and Raymond Mill in Carsurin Coal Laboratories of Banjarmasin. This process results with coal dust of a diameter < 75 μm. This coal dust was then filtrated by Mesh MicroSieve (BioDesign, New York, NY, USA) resulting a coal dust < 10 μm of diameter as a respirable PM_{10}. Coal dust was then characterized using X-Ray Fluorescence at the Physic and Central Laboratory Faculty of Mathematic and Natural Science University of Malang. Cigarette smoke was characterized using smoke analyzer at Technical Unit of Environmental Health, Banjarbaru, South Kalimantan, Indonesia.

Coal dust and smoking exposure

A total of 30 Wistar male rats, were randomly divided into three groups including one non-exposure (control) and two groups for 14 and 28 days combined inhalation of cigarette smoke and coal dust. Dose of coal dust exposure was 12.5 mg/m^3 for one hour/day. Dose of cigarette smoking was one cigarette per day. This dose was set as reference of smoking habitual in coal miners not exposed simultaneously. Smoking and coal dust exposure was done by coal dust equipment that was designed and available in Pharmacology Laboratory, Medical Faculty, Brawijaya University of Malang. Smoking exposure was done prior to coal dust exposure.

Tissue preparation

At the end of the treatment, the animals were anesthetized. Both femurs were collected by incision latter rinsed with physiological saline. All samples were stored in glutaraldehyde until analyzed.

Statistical analysis

Data are presented as mean ± SD and differences between groups were analyzed using ANOVA test using SPSS 16.0 statistical package. $P < 0.05$ was considered statistically significant.

RESULTS

Coal dust and cigarette smoke content

X-Ray Fluorescence has shown inorganic composition of coal dust; mainly Fe (36.9%), Si (17.9%), Mo (15%), Al (10%), Ca (8.67%), S (4.7%), and Ti (3.65%), and several inorganic minerals less than 1% including K (0.96%), Mn (0.53%), Yb (0.4%), Cr (0.34%), Ni (0.2%) and V (0.16%). The analysis of gaseous phase of cigarette smoke showed tar, nicotine and CO at the concentrations of 2.9 ppm, 44.3 ppm, and 102.3 ppm, respectively.

Effect cigarette smoke and PM_{10} coal dust on atomic mineral composition

The atomic mineral which increased in cigarette smoke plus coal dust groups significantly was iron ($P < 0.05$ compared to control). Other minerals including calcium, phosphorus, nickel, copper, zinc, and the calcium/phosphorus ratio also changed in exposure groups, however, these changes did not reach statistical significance (shown in Table 1).

DISCUSSION

The Fe content in coal dust is higher than any other mineral. In the present study, the Fe concentration in rats exposed to cigarette smoke and coal dust exposure was significantly (2.4 times) higher than the control group. Actually, the role of Fe in bone mineralization is still a controversial topic. Fe is an enzymatic co factor in collagen synthesis. It is also toxic for bone cells as it can induce osteoporosis by disturbing the bone mineralization process [15, 16].

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Table 1. Bone mineral elements of rats exposed to cigarette smoke and coal dust

	Control	CS + CD 14 days	CS + CD 28 days
Phosphorus	12.25 ± 0.173	11.232 ± 2.07	10.375 ± 2.982
Calcium	79.9 ± 8.222	77.85 ± 8.024	79.625 ± 2.876
Iron	0.857 ± 0.695	2.075 ± 0.96[a]	2.062 ± 0.094[a]
Nickel	3.6 ± 5.381	5.327 ± 3.522	4.912 ± 1.423
Copper	0.485 ± 0.624	0.762 ± 0.697	0.675 ± 0.054
Zinc	0.725 ± 0.392	1.07 ± 0.503	1.14 ± 0.777
Calcium/Phosphorus ratio	6.515± 0.058	7.23 ± 2.177	8.462 ± 3.67

Values are presented as percentages (mean ± SD); CS: cigarette smoke; CD: coal dust; [a]$P < 0.05$ in comparison with control group.

Rats having Fe deficiency were found to have decreased bone mass and bone strength [17]. Increased content of Fe indicates substitution or incorporation into the bone mineral element. Fe has a valency of 2+ and can act as a substitute for calcium which maybe one of the reasons for lower calcium level in this study.

The calcium (Ca) and phosphorus (P) content in the exposure groups was comparatively lower than the control group in the present study. This finding indicated that atomic minerals from cigarette smoke and coal dust substituted the Ca and P into hydroxyapatite crystal. Vallet-Regi and Arcos [18] proposed possibilities of substitution of several atomic minerals in the bone structure hydroxyapatite crystal. Potassium (K) and manganese are the atomic minerals in coal dust that induce substitution in the metal group of hydroxyapatite crystal [8]. Similarly, silicon (Si), sulphur (S), chromium (Cr), and vanadium (V) can induce substitution of P groups in hydroxyapatite.

Besides the above, skeletal Ca/P ratio has been shown to be a good index of the bone quality. In this study, the skeletal Ca/P ratio of rats exposed to cigarette smoke and coal dust was higher compared to the control group. This finding indicated that the bone density of rats increase when exposed to coal dust.

Scancar *et al* [19] reported that concentration of copper was 100-200 mg/kg in the bones of healthy autopsy subjects. Copper is co-factor for cross-linking and acts as an antagonist for zinc in bone, leading to reduced bone zinc level [20]. Zinc is an abundant atomic mineral in bone which acts as a co-factor for alkaline phosphatase. Zinc also supports bone metabolism and growth by promoting osteoblast activity and mineralization to inhibit bone loss. Zinc is also involved in bonding of the bone's organic structure [21, 22]. Zinc deficiency leads to reduction of osteoblast activity and collagen synthesis [7]. In this study, there is no antagonist percentage of copper with zinc. This finding suggests that cigarette smoke and coal dust exposure disturb antagonistic relationship between copper and zinc.

In summary, the present study reported that sub-chronic inhalation of cigarette smoking and coal dust PM_{10} significantly increases iron levels in the femur of rats. Cigarette smoke plus coal dust groups also change phosphorus, calcium, nickel, copper, zinc, and calcium/phosphorus ratio, but not reach statistical significance. These results maybe, in particular, useful for the orthopedic surgeon community in order to explain a possible mechanism for iron accumulation in bones.

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