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Lung Damage in Rats Following Inhalation Exposure of the Popocatepetl Volcano Ash

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Abstract: Although, at least 455 millions people word-wide live within potential exposure range of a volcano active within recorder history, surprisingly little primary epidemiological research on the health effects of volcanic emissions has been published. There may be important differences between volcanoes and between events from the same volcano in terms of eruption pattern, gaseous emissions, base composition of ash, compounds adsorbed onto ash particles, the percentage of particles small enough to be respirable and toxicological activity. Volcanic ash has been shown to activate alveolar macrophages, initiating events that cause neutrophils to infiltrate the respiratory tract. The purpose of the present study was evaluating lung damage of inhalation exposure to volcanic ash. Volcanic ash that had fallen into a polyethylene bucket at the foot of Popocatepetl volcano was collected in the locality of Atlixco-Puebla and ash samples was analyzed by scanning electron microscopy. Experimental group animals were exposed to 10 mg m^{-3} of volcanic ash for 60 days. Fixed lungs were processed to paraffin blocks, sectioned at an approximate thickness of 5 μm, placed on glass slides and stained with hematoxylin and eosin. Scanning electron microscopy analysis ash samples it showed presence of respirable fraction <10 μm. Experimental group showed decreased body weight (13%), showing significant difference ($p<0.05$) with respect to control group. Hematocrite analysis not showed significant changes, but the blood differential count showed significant difference ($p<0.05$) between the groups studied. Histopathology study on experimental samples showed neutrophils and plasmatic cells infiltration and inflammatory reaction.

Key words: Volcanic ash, popocatepetl volcano, exposure, rats, lung damage

INTRODUCTION

Popocatepetl, the most famous active volcano in Mexico, lies on the borders of the states of Mexico, Morelos and Puebla. In 1993, seismic activity intensified, as did as the emission of fumaroles, followed in December 1994 by moderate tremors and strong emissions of gases and ash. In 1996, a number of seismic events led to an unexpected explosion. A daily emission of 8,000-15,000 tonnes of sulfur dioxide has been measured. Popocatepetl is located in a densely populated region of Mexico (Zeballos *et al*., 1996).

Investigations of magma erupted in 1997 and 1998, including major element and volatile (S, Cl, F and H_2O) data from glass inclusions and matrix glasses. Magma erupted from Popocatepetl is a mixture of dacite and basaltic andesite. The pre-eruptive volatile content of the basaltic andesite is 1980 ppm S, 1060 ppm Cl, 950 ppm F and 3.3% w H_2O. The pre-eruptive volatile content of the dacite is 130±50 ppm S, 880±70 ppm Cl, 570±100 ppm F and 2.9±0.2% w H_2O (Witter *et al*., 2005).

Although, at least 455 millions people word-wide live within potential exposure range of a volcano active within recorder history, surprisingly little primary epidemiological research on the health effects of volcanic emissions has been published. There may be important differences between volcanoes and between events from the same volcano in terms of eruption pattern, gaseous emissions, base composition of ash (for example, cristobalite concentrations) compounds adsorbed onto ash particles

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(which may be volcanic in origin or derived from other pollution sources) the percentage of particles small enough to be respirable and toxicological activity. Studies of health effects of volcanic ash exposure may help elucidate mechanisms relevant to action of anthropogenic pollution (Vallyathan *et al*., 1984; Peden, 2002).

Volcanic air pollution may be responsible for this excessive mortality and it is suggested that workers employed in cleaning volcanic ash from roads are subject to particularly high levels of exposure. In response to inhaled particulate matter, inflammatory cells, such as neutrophils and monocytes are recruited to the lung to facilitate clearance of deposited particles. However, inflammatory cells can also damage the respiratory tract by releasing enzymes. Volcanic ash has been shown to activate alveolar macrophages, initiating events that cause neutrophils to infiltrate the respiratory tract (Grose *et al*., 1985). This inflammatory response is thought to be mediated by an acute reaction with production and release of pro-inflammatory cytokines, such as TNF-α produced by alveolar macrophages. In addition, volcanic ash is composed of potentially fibrogenic particles in a size range that permits access to all area of the lung and previous studies have revealed that TNF-α is upregulated in fibrosing alveolitis (Driscoll *et al*., 1993; Brambila *et al*., 1979; Martin *et al*., 1983; Sime *et al*., 1998). The purpose of the present study was evaluating lung damage of inhalation exposure to volcanic ash.

MATERIALS AND METHODS

Animals: Forty male Wistar rats, 10-12 weeks old with an average body weight of 245 g, were used for this study. Rats were divided in two groups: One control group (n = 20), one inhalation exposure to volcanic ash group (n = 20) and were housed in plastic cages on beta-chip bedding in groups of four per cage, maintained on a 12 h light-dark cycle of approximately, 22°C and 50% relative humidity. Food (Prolab RMH 3000; PMI Nutrition International, St. Louis, MO) and water were provided *ad libitum*.

Volcanic ash: Volcanic ash that had fallen into a polyethylene bucket at the foot of Popocatepetl volcano was collected in the locality of Atlixco, Puebla-Mexico, during the years from 1997-2002. The collected ash samples became sterile to 120°C/15 min.

A fraction of the ash samples was analyzed by scanning electron microscopy, the samples were chemical dehydrated (in 30→50→70→90→100% ethanol for 1 h each then 30:70, 70:50, 50:50, 70:30 ethanol: Acetone mixtures for 20 min each). The samples were placed in the critical-point drier in 100% acetone and rinsed three times in liquid CO_2, then the critical point was identified, after which the samples were secured onto racks and coated in gold in a sputter coater. The examination was carried out with a JEOL JSM 5410-LV scanning electron microscope and the pictures were digitally recorder.

Inhalation exposure to volcanic ash: Experimental group animals were exposed to 10 mg m^{-3} of volcanic ash for 60 days (4 h day^{-1}) in a 520 L, stainless steel exposure chamber which was including a dust generator with a continuous screw feeder and overflow pipe (Tanaka and Akiyama, 1984) and they weighed each third day.

Samples extraction and processing: Rats were anesthetized after 60 days by an intraperitoneal injection of sodium pentobarbital (100 mg kg^{-1}) and blood collected (10 mL) from the heart into heparin bottles in order to determine hematocrite and white blood differential count, rats were killed by cervical dislocation. Lung were removed and fixed by tracheal perfusion with ice-cold 4% paraformaldehyde at 25 cm pressure for 15 min. After 24 h the lungs were placed in Phosphate-Buffered Saline (PBS) at 4°C. Fixed lungs were processed to paraffin blocks, sectioned at an approximate thickness of 5 μm, placed on glass slides and stained with hematoxylin and eosin (Gavett *et al*., 2003). We used INSTAT version 2.0 Software and differences were considered significant at the $p<0.05$.

RESULTS AND DISCUSSION

Due to the lack of information on the effects in inhaled Popocatepetl volcanic ash, animal study was performed to determine the lung affects. Rats were exposed by inhalation to 10 mg m^{-3} Popocatepetl volcanic ash for 60 days (4 h day^{-1}) to investigate biological effects of chronic inhalation exposure to volcanic ash under controlled laboratory conditions.

Scanning electron microscopy analysis ash samples it showed presence of respirable fraction <10 μm (Fig. 1) as result of its fragmentation and conditioning abrasive capacity of the samples. The ash samples from different volcanoes varied in particle size, surface area and concentration of silica. Total crystalline silica in the respirable fraction of ashes was 1.5% (Mount St. Helens, Moses Lake); 1.36% (Galunggun, Bandung, 1.95% (Galunggun, Bandung and 1.72% (El Chichon, Tuxtla) (Vallyathan *et al*., 1984).

The August, 1991 eruption of Mt. Hudson deposited ash across southern Argentina and contributed to the deaths of thousands of grazing sheep. Early ash analysis revealed high levels of fluoride, a potential ash

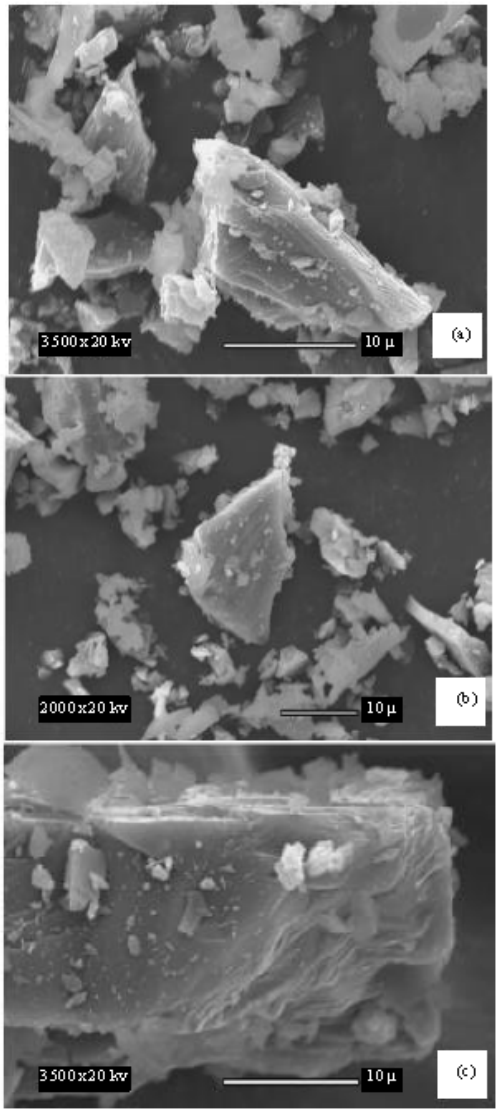

Fig. 1: Scanning electron micrograph of ashes emitted by popocatepetl volcano, particle size <10 µm. Collected ash in Atlixco-puebla during years 1997 (a), 1998 (b) and 2000 (c)

constituent toxic to humans and animals. Sheep deaths resulted from physical, rather than chemical properties of the ash (Rubin *et al*., 1994).

Mannino *et al*. (1996) support the hypothesis that volcanic air pollution affects respiratory health on the island of Hawaii, while other results do not.

High levels of cristobalite in respirable ash raise concerns about adverse health effects of long-term human exposure to ash from lava dome eruption (Baxter *et al*., 1999). Laboratory and field studies indicated that volcanic ash had mild to moderate fibrogenic potential, consisting

Table 1: Means of the variations in the analyzed parameters, weight and blood differential count showed significant difference p<0.05

Group/ analysis	Weight (g)	Hematocrite (%)	Neutrophils (%)	Lymphocytes (%)
Control	140.2	44.2	30	70
Experimental	122.3	48.5	8	92

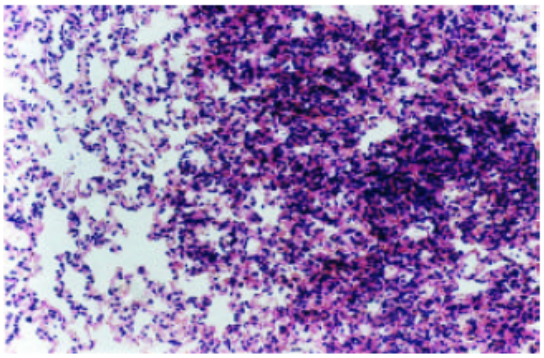

Fig. 2: Experimental lung sample showing neutrophils and plasmatic cells infiltration and focal acute bronchiolar inflammation, stained with hematoxylin and eosin. Magnification, X20

of greater than 90% (by count) respirable size particles which contained 4-7% (by weight) crystalline free silica (Bernstein *et al*., 1986; Yano *et al*., 1985).

Experimental group showed decreased body weight (13%) showing significant difference ($p<0.05$) with respect to control group (Table 1). Hematocrite analysis not showed significant changes, but the blood differential count showed significant difference ($p<0.05$) between the groups studied (Table 1). Hemolysis as an index of cytotoxicity was measured by *in vitro* test on sheep blood erythrocytes and indicated wide differences in hemolytic activity among ash samples (Vallyathan *et al*., 1984).

Histopathology study on experimental samples showed neutrophils and plasmatic cells infiltration and inflammatory reaction (Fig. 2). The control samples did not show changes in the structure (Fig. 3). Previous studies with ash samples from other volcanoes have, in general, indicated that particle size and the percentage of crystalline free silica both play a crucial part in the development of respiratory diseases such as silicosis and tuberculosis (Vallyathan *et al*., 1984; Graham *et al*., 1985; Raub *et al*., 1985; Baxter *et al*., 1983).

In vivo studies have shown that cristobalite is potentially fibrotic and may also cause silicosis and cancer. The respirable fraction is the fraction of dust that penetrates to the alveolar region and particles that deposit with about 50% in this region are commonly in the range of 3-5 µm in aerodynamic diameter, depending on which convention is used. Others results indicate differences in the mechanisms of the biological reactivity of these

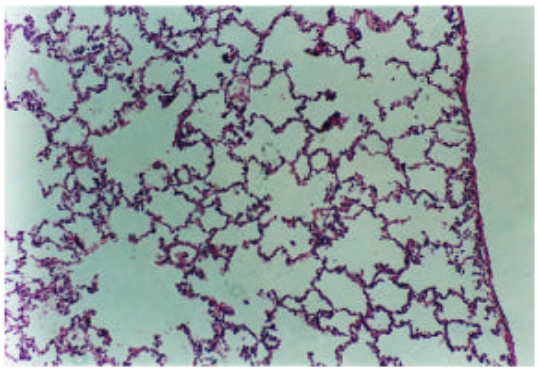

Fig. 3: Control lung sample did not show changes in their structure, stained with hematoxylin and eosin. Magnification, X20

particles in accordance with their origin (e.g., ash derived from a single explosive event and collected, sample from ash which had accumulated from the beginning of the eruptions and ash was derived from the fallout from a single pyroclastic flow, also collected) (Wilson *et al*., 2000).

Vallyathan *et al*. (1984) indicate that volcanic ash moderately cytotoxic and that exposure may lead to over reactions and the exacerbation of preexisting chronic inflammatory processes.

Shortly after Mt. St. Helens erupted in 1980, a number of laboratories began to investigate the effects of volcanic ash in a variety of experimental systems in attempts to predict effects that might occult in the lungs of humans exposed to volcanic ash. The data indicate that volcanic ash, even in high concentrations, causes little toxicity to lung cells *in vitro* and *in vivo*, as compared with effects of free crystalline silica, which known to be highly fibrogenic. The published experimental studies suggest that inhaled volcanic ash is not likely to be harmful to the lungs of healthy humans, but the potential effects of volcanic ash in patients with pre-existing lung disease are more difficult to ascertain from these studies (Martin *et al*., 1986; Yano *et al*., 1990; Rivera *et al*., 2008).

Environmental pollution is a complex issue because of the diversity of anthropogenic and natural agents, both chemical and physical, that have been detected and catalogued. The consequences to biota from exposure to genotoxic agents present an additional problem because of the potential for these agents to produce adverse change at the cellular and organismal levels. In an effort to predict effects at the population, community and ecosystem levels, current studies in genetic ecotoxicology are attempting to characterize the biologic mechanisms at the gene level that regulate and limit the response of an individual organism to genotoxic factors in their environment (Shugart and Theodorakis, 1994).

CONCLUSION

The findings provide evidence that the respiratory health of rats was affected by inhalation exposure of the Popocatepetl volcano ash, as a result of the long duration of exposure to high ambient levels of respirable ash particles. We recommend that the health sector response in volcanic eruptions should include measures to ensure that the respiratory health of population is adequately monitored and treated and appropriate advice given on limiting exposure to fine ash and we are planning an epidemiological study to compare the incidence of lung damage in areas exposed to ash from Popocatepetl volcano with that in areas of non-exposure.

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