Is There Any Effect of Volcanic Eruptions of Mount Sakurajima on Canine Lungs Exposed Naturally? — Morphometric Analysis of Intrapulmonary Particulate Deposit Amount and Histopathological Investigations

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KARIYA, M., GOTO, M., HASUI, K., YAMAMOTO, N, TASHIRO, Y. and SATO, E. Is There Any Effect of Volcanic Eruptions of Mount Sakurajima on Canine Lungs Exposed Naturally? — Morphometric Analysis of Intrapulmonary Particulate Deposit Amount and Histopathological Investigations. Tohoku J. Exp. Med., 1992, 167 (3), 197-205 — In order to see whether any effect of inhalation of volcanic ash and gases from Mt. Sakurajima on canine lungs is observed or not, we examined the amount of intrapulmonary particulate deposits (IPD) and histopathological changes. Twenty-five abandoned or stray dogs (group A) in the areas affected enormously by volcanic ash and gases were examined in comparison with 13 abandoned or stray dogs (group B) in the area scarcely influenced. The amount of IPD was measured by using an image analyzer combined with a microscope. Age-associated increase of IPD values was noted, but mean IPD values were not different between groups A and B. Incidence of goblet cell hyperplasia was not different between the two groups. In none of the cases examined, squamous metaplasia of respiratory epithelia, pulmonary fibrosis, silicotic nodules, emphysematous change, or histopathological findings, which are indicative of bronchial asthma, were observed. In conclusion, obvious effect of volcanic eruption on canine lungs was not observed through both the measurement of IPD value and the histopathological evaluation. — — volcanic eruptions; dog; lung; image analyzer; intrapulmonary particulate deposit

Mount Sakurajima in Kagoshima Prefecture has been erupting for 13,000 years. The frequency of volcanic eruption of Mt. Sakurajima has increased since 1955. Therefore, it is very important to know whether the influence of the volcanic eruption is seen on human beings, especially on their respiratory tracts.

Kariya (1992) examined whether any effect of volcanic eruptions of Mt. Sakurajima on human lungs is observed or not through both measurement of

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intrapulmonary particulate deposits (IPD) and histopathological investigation, comparing with the cases of Ohmiya City, Saitama Prefecture. According to the report, the amount of IPD was less in autopsied subjects of Kagoshima City than in those of Ohmiya City, 20-30 km northwest of Tokyo. The incidence of pulmonary emphysema was not different between both areas, and no silicotic nodules was observed in any cases. On the other hand, incidence of bronchobronchiolar squamous metaplasia and goblet cell hyperplasia was higher in smokers of Kagoshima than in those of Ohmiya. Within the Kagoshima subjects, the incidence of squamous metaplasia was higher in smokers than in nonsmokers. Consequently, it was necessary to consider synergistic effect of smoking with volcanic gases on human respiratory tracts, because residents of Kagoshima City were affected by a high concentration of sulfur dioxide (SO$_2$) spewed from Mt. Sakurajima (Environment Agency 1990).

Thus, we examined whether any effect of volcanic ash and gases of Mt. Sakurajima on the lungs of dogs in the heavy ashfall area is observed or not, through the measurement of IPD as well as by the histopathological investigation, comparing with dogs in the scarcely influenced area. Dogs habitually spend most of their lives outdoors, so that the respiratory tracts of dogs may be affected by volcanic eruptions more severely than those of human beings.

**Materials and Methods**

From the area within 10 km of Mt. Sakurajima (Sakurajima island and Tarumizu City), 25 abandoned or stray dogs were investigated (Sakurajima group). Tarumizu City, Kagoshima Prefecture, is located about 10 km southeast of the crater of Mt. Sakurajima (Fig. 1). Age of dogs was estimated chiefly by the degree of dental breakdown (Miller et al. 1964). The mean age and weight of dogs were 3.6 (range 1-10) years old and 11.5 (6.0-20.0) kg, respectively, for the Sakurajima group. For the comparison, 13 abandoned or stray dogs from Miyanojo Town were investigated. Miyanojo, Kagoshima Prefecture, is located 40 km northwest of Mt. Sakurajima (Fig. 1). Annual average amount of falling ash (1979-1988) was 50,556 g/m$^2$/year for Sakurajima (Arimura district), 5,721 g/m$^2$/year for Tarumizu, and 127 g/m$^2$/year for Miyanojo. Sakurajima, Tarumizu, and Miyanojo are rural towns and there are few manufacturing industries causing air pollution. The mean age and weight of dogs were 5.2 (2-12) years old and 11.0 (6.8-18.5) kg, respectively, for Miyanojo group. Neither significant difference of ages nor weights was found in the sampled dogs.

**Measurement of intrapulmonary particulate deposit (IPD)**

The dogs were sacrificed using deep anesthesia with barbiturates. The excised trachea and lungs were fixed in 10% formalin solution for 48 hr. From the left upper lobe, where deposition of particulate dust is more prominent than the other areas (Spencer 1985), paraffin sections were made and cut into 3 μm thick sections. Formalin pigments were removed by using alcohol and ammonium hydroxide solutions. The sections were stained by Heidenhain's aniline blue method without orange G or phosphotungstic acid solution (American Registry of Pathology 1968).

These specimens were analyzed with a self-assembled color image analyzer (MAPPS-II) (Goto et al. 1992). Briefly, a color CCD microscope camera (FCD720; Olympus, Tokyo) attached to a microscope (BH-2; Olympus) was connected to an IEEE-796 bus (multibus).
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Fig. 1. Map of study area (Kagoshima Prefecture).

Fig. 2. Deposition of dust particles in the bronchiolar wall and alveolar macrophages (Heidenhain's aniline blue, ×120).

Fig. 3. Goblet cell hyperplasia. The majority of bronchial columnar epithelia are replaced by goblet cells (HE, ×130).
video A/D converter (VDI-050; Microtechnica, Tokyo) and three frame memories (VDI-030; Microtechnica). These frame memories have 512×512×8 bits each. The IEEE-796 system was controlled by a microcomputer (PC-9801VX; NEC, Tokyo). Each image uses 768 kilobytes of memory. The programs were mainly written in C language and partly by an assembler.

One hundred consecutive fields of black colored fine granular clusters of IPD and blue-stained pulmonary interstitium (including respiratory epithelia) were measured at ×50 magnification with MAPPS-II (Fig. 2). IPD value was defined as total IPD areas divided by pulmonary interstitial areas. IPD values between the two groups were compared, and correlation between aging and IPD values was evaluated.

Histopathological investigation

From the trachea and all lobes of formalin fixed pulmonary tissue, paraffin sections were made, and then stained with hematoxylin and eosin, alcian blue, elastica-van Gieson, and Masson trichrome. Goblet cell hyperplasia and squamous metaplasia in respiratory tracts, pulmonary fibrosis, silicotic nodules and emphysematous change were investigated. Goblet cell hyperplasia was defined to be positive when at least two bronchobronchiolar luminal epithelia were replaced by goblet cells in more than 50% per one preparation (Fig. 3). Histopathological features of bronchial asthma were examined as follows according to the textbook by Cotran et al. (1989): 1) mucous plugs in respiratory tracts; 2) thickening of basement membrane of bronchial epithelium; 3) eosinophilic infiltration in the bronchial walls; 4) hypertrophy of submucosal mucous glands.

Statistical analysis

Numerical data of cases (age and IPD value) were processed by using a microcomputer (PC9801; NEC, Tokyo). The difference in age and IPD value between the two groups was examined by t-test. Probability values of histopathological changes in the two groups were calculated by Fisher’s exact probability test. Correlation between age and IPD values was evaluated by Spearman’s rank-difference correlation coefficient (Spearman’s RCC) test.

RESULTS

The results of IPD values and histopathological changes were listed in Tables 1 and 2, and Fig. 4.

IPD values

IPD values of the Sakurajima group (22.0±39.9×10⁻⁵) did not differ from those of the Miyanojo group (87.5±184.4×10⁻⁵) in mean value (t-test) (Table 1).

<table>
<thead>
<tr>
<th>Habitat</th>
<th>Number of cases</th>
<th>Age (years) mean±s.d. (Min-Max)</th>
<th>IPD (×10⁻⁵) mean±s.d. (Min-Max)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sakurajima</td>
<td>25</td>
<td>3.6±2.6 (1-10)</td>
<td>22.0±39.9</td>
</tr>
<tr>
<td>Miyanojo</td>
<td>13</td>
<td>5.2±3.0 (2-12)</td>
<td>87.5±184.4</td>
</tr>
</tbody>
</table>

Table 1. Habitat, age, and intrapulmonary particulate deposit (IPD) value
As shown in Fig. 4, IPD value increased with advancing age in all the dogs (n = 38, Spearman's rank-difference correlation coefficient = 0.562, p < 0.01). The IPD value also increased with aging in both the Sakurajima group (n = 25, Spearman's rank-difference correlation coefficient = 0.452, p < 0.05), and in Miyanojo group (n = 13, Spearman's rank-difference correlation coefficient = 0.724, p < 0.01).

As shown in Fig. 4, IPD value increased with advancing age in all the dogs examined (n = 38, Spearman's RCC = 0.562, p < 0.01). The IPD value also increased with aging in both the Sakurajima group (n = 25, Spearman's RCC = 0.452, p < 0.05) and the Miyanojo group (n = 13, Spearman's RCC = 0.724, p < 0.01).

**Histopathological changes**

Incidence of goblet cell hyperplasia in respiratory epithelia was not significantly different between the two groups. Neither squamous metaplasia in respiratory epithelia, silicotic nodules, emphysematous change, or histopathological feature of bronchial asthma was observed (Table 2). Granulation tissue associated with infection of dirofilaria was noted in some dogs, but apparent fibrosis related to dust particulate deposition was not noticed.
DISCUSSION

There are over seven hundreds active volcanoes in the world, among which about eighty are in Japan. Mount Sakurajima, Kagoshima Prefecture, 950 km southwest of Tokyo, erupts many hundreds of times a year. It spews enormous volumes of volcanic ash and gases onto the nearby areas of Mt. Sakurajima. Farming of oranges and loquats on Sakurajima is greatly affected by the ash fall. Furthermore, the influence of volcanic eruption on human and animals (especially on the respiratory tracts) is a great problem.

In this paper, the measurement of IPD values and the histopathological investigation in the lungs of abandoned or stray dogs naturally exposed to the volcanic ash and gases of Mt. Sakurajima, were performed.

The average life span of dogs is shorter than that of human beings, however, dogs habitually spend most of their lives outdoors. On the other hand, many people spend the majority of a day indoors. Total exposure duration of dogs to the volcanic ash and gases may be longer than that of human beings. As a result, volcanic eruptions may affect the respiratory tracts of dogs more severely than those of human beings. Furthermore, in dogs, we do not have to consider the influence of smoking that cause an increase of IPD values (Cohen et al. 1979; Bohning et al. 1982), changes in respiratory epithelia (goblet cell hyperplasia and squamous metaplasia) (McDowell and Beals 1986) or pulmonary emphysema (Dunnill 1987). As a result, dogs may be more appropriate subjects than human beings in order to evaluate the effects of volcanic eruptions on respiratory tracts.

The air we breathe is loaded with particulate substances which include microorganisms, pollutants and allergens, all of which may injure the lungs in a variety of ways (Clarke and Pavia 1991). IPD is the mixed, inspired materials that consist of carbon dust, soil and metals etc. floating in the air (Ohshima 1990). It is difficult to separate the volcanic ash from the other IPD components because the component of volcanic ash are similar to that of usual soil (Horai and Ohtsu 1988). In order to estimate whether heavy ash fall increases the IPD value or not, the degree of IPD was quantitatively measured by an image analyzer connected to a microscope in this study. There was no notable difference of IPD values between the samples from the area affected by heavy ash fall (Sakurajima group) and those from the area of light ash fall (Miyanojo group). This indicated that effects of volcanic eruptions on IPD value was little as well as human beings (Kariya 1992). This reason is proposed as follows. i) The majority of volcanic ash is over 10 \( \mu \)m in size (Ohba et al. 1980) and may be scarcely deposited in the respiratory tracts (Spencer 1985). ii) The atmospheric SPM (suspended particulate matter) concentration which is one of the important factors affecting IPD value (Kariya 1992), is not affected by the volcanic eruptions in the areas near Mt. Sakurajima (Horai and Ohtsu 1988). SPM is the ambient, floating particle which is smaller than 10 \( \mu \)m in diameter and may easily deposit in the lung (Environ-
The SPM concentration is being monitored in many areas of Japan. The annual average SPM concentration in 1989 was 0.027 mg/m³ in Sakurajima, 0.030 mg/m³ in Tarumizu City and 0.031 mg/m³ in Sendai City, next to Miyanojo Town, Kagoshima Prefecture. On the other hand, the average concentration level of all over Japan (40 points) was 0.039 mg/m³ (Environment Agency 1990). This indicated that the atmosphere of Sakurajima area was not so dirty from the viewpoint of SPM concentration.

Correlation between IPD values and age was noted in dogs as well as in human beings (Kariya 1992). It is probably because the periods of inhaling the pollutants in the ambient air increase with aging, but conversely pulmonary clearance decreases with aging (Spencer 1985).

Asmundsson and colleagues (1973) documented that goblet cell hyperplasia and squamous metaplasia were observed in hamsters exposed to high concentrations of sulfur dioxide (SO₂). The area most affected by the volcanic eruptions of Mt. Sakurajima (Arimura district) showed the highest annual average concentration of SO₂ in Japan in 1989 (Arimura, 0.034 ppm; Miyanojo, 0.004 ppm; average value of 11 air-polluted districts of Japan; 0.011 ppm) (Environment Agency 1990), because high concentration of SO₂ was spewed by the eruptions of Mt. Sakurajima. Therefore, if goblet cell hyperplasia or squamous metaplasia of respiratory epithelia in human beings or animals was detected, it is concerned to be occurred by the eruptions of Mt. Sakurajima. In human beings, Kariya (1992) reported that incidence of goblet cell hyperplasia and squamous metaplasia was higher in smokers of Kagoshima subjects than in those of Saitama. In Kagoshima, the incidence of squamous metaplasia of respiratory epithelia was higher in smokers than in non-smokers. Consequently, it needs to consider a synergistic effect of volcanic eruptions together with smoking on human airways. However, in this study, the incidence of goblet cell hyperplasia did not differ between the Sakurajima group and the Miyanojo group. Squamous metaplasia was not observed in any subjects. The reasons to be considered are as follows. i) The SO₂ concentration in the experiment of Asmundsson et al. (1973) (400 ppm) was prominently higher than the SO₂ concentration in the areas affected by the volcanic eruptions of Mt. Sakurajima (annual average in 1989: 0.034 ppm) (Environment Agency 1990). ii) Dogs are not obviously influenced by smoking which causes goblet cell hyperplasia and squamous metaplasia in respiratory tracts (McDowell and Beals 1986).

Occurrence of silicotic nodules or fibrosis accompanied with scar emphysema is also a worry in the areas affected by the volcanic eruptions of Mt. Sakurajima, because silicon dioxide (SiO₂) having potency of silicosis (Spencer 1985) is a main constituent of volcanic ash of Mt. Sakurajima (Ohba et al. 1980). Nevertheless, these pathological changes were not noted in any dogs as well as in human beings (Kariya 1992), probably because the main constituents of silicon dioxide in volcanic ash are amorphous silicates which are thought to be relatively inert, and

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crystalline silicates having fibrogenic potency is a minor component (Martin et al. 1983; Silicosis and Silicate Disease Committee 1988).

Nadel and colleagues (1965) reported on the exposure of SO$_2$ which causes bronchoconstriction to human beings and cats. Occurrence of bronchial asthma is also a problem in the area affected by the volcanic eruptions of Mt. Sakurajima, because SO$_2$ concentration on Sakurajima was higher than in any other areas in Japan in 1989 as already mentioned. But, no histopathological feature of bronchial asthma was noticed, probably because the minimum concentration of SO$_2$ to evoke bronchoconstriction in dogs may be prominently higher than the atmospheric SO$_2$ concentration on Sakurajima.

In conclusion, distinct effects of volcanic eruptions on canine lungs were not noted through both measurement of IPD value and histopathological findings.

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