A New Model Rat with Acute Bronchiolitis and Its Application to Research on the Toxicology of Inhaled Particulate Matter

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Abstract: The aim of the present study was to establish a useful animal model that simulates humans sensitive to inhaled particulate matter (PM). We have developed a new rat model of acute bronchiolitis (Br) by exposing animals to NiCl₂ (Ni) aerosols for five days. Three days following the Ni exposure, the animals developed signs of tachypnea, mucous hypersecretion, and bronchiolar inflammation which seemed to progress quickly during the fourth to fifth day. They recovered from lesions after four weeks in clean air. To assess the sensitivity of the Br rats to inhaled particles, two kinds of PM of respirable size were tested with doses similar to or a little higher to the recommended threshold limit values (TLVs) for the working environment in Japan. Titanium dioxide (TiO₂=Ti) was chosen as an inert and insoluble particles and vanadium pentoxide (V₂O₅=V), as a representative soluble and toxic airborne material. The Br rats exposed to either Ti or V were compared the pathological changes in the lungs and the clearance of particles to those in normal control or Br rats kept in clean air. The following significant differences were observed in Br rats: 1. delayed recovery from pre-existing lesions or exacerbated inflammation, 2. reductions in deposition and clearance rate of inhaled particles with the progress of lesions. The present results suggest that Br rats are more susceptible to inhaled particles than control rats. Therefore, concentrations of particulate matter lower than the TLVs for Japan, which have no harmful effects on normal lungs, may not always be safe in the case of pre-existing lung inflammation.

Key words: Bronchiolitic Rats, Exposure to NiCl₂, TiO₂, V₂O₅, Susceptible to Particulate Matter

Introduction

Although the degree of air pollution has been significantly reduced since the 1970s, new arguments have been raised whether inhalation of any particulate matter (PM) at concentration levels of current PM₁₀ standards in the United

States relates to the increase of daily mortality^{1,2}. In 1997, the new PM_{2.5} standards has been promulgated³, however, the recent workshop summary showed that there is still no clear linkage for any PM size or chemical constituent that extends from source of pollutant to the production of health effects⁴. Presently, not all human but some susceptible groups, such as the elderly, with chronic obstructive pulmonary disease (COPD), asthma and cardiovascular

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Table 1. Experimental design and sampling time after Ni and/or Ti exposure

Group \ Day	Exposed to		Days after Ti Exposure						
	D1–D5	D6–D10	D0	D1	D3	D7	D14	D28	D56
CC: normal	Air	Air	CC-0	CC-1	CC-3	CC-7	CC-14	CC-28	
CT: normal + Ti	Air	TiO ₂	CT-0	CT-1	CT-3	CT-7	CT-14	CT-28	CT-56
BC: Br + Air	Ni	Air	BC-0	BC-1	BC-3	BC-7	BC-14	BC-28	
BT: Br + Ti	Ni	TiO ₂	BT-0	BT-1	BT-3	BT-7	BT-14	BT-28	BT-56

Daily exposure time was 10:00 a.m. to 15:00 p.m. D0: sampled just after the last exposure. N=5/group.

disease are thought to be the target for receiving harmful effects from exposure to PM. In a broad-based approach to studying health effects of environmental particles, one of the recommended strategy to focus on the mechanistic analysis is to employ an animal model, since human exposure has a strict limitation for both ethical and technical reasons. However, only a few animal models have been established with respiratory diseases appropriate to assess the health effects of inhaled PM.

Turning this viewpoint to the working environments, the problem is more serious, since the recommended threshold limit values (TLVs) in the working places are at least one to two order of magnitude higher than in urbane air, and these values are adopted to normal healthy workers. However, all workers are not always healthy. Some elderly workers with COPD, asthma, or the increasing cardiovascular disability with age might become more susceptible to inhaling particulate matter.

We therefore, planned to develop a new animal model of acute bronchiolitis (Br) in rats, and to assess whether these rats are more susceptible to inhaled particles at the levels of TLVs in Japan than their normal controls receiving the same concentrations of particles, or Br rats kept in clean air.

Materials and Methods

Methods to develop acute broncho-bronchiolitis in rats

All animals used in these studies were male SPF rats of Wistar-Jcl strain, purchased from Clea Japan Inc., at 8-weeks of age and were kept in our animal facilities until use. Rats were housed 5 or 6 per stainless wire cage and supplied with sterilized food and water *ad libitum*. Rats at 10-weeks of age weighing 285–300 g were moved to the inhalation chambers with volume of 1.2 m³. The chambers were maintained at 24 ± 0.5°C with a relative humidity of 55 ± 5%, air was changed 20 times/h, and a 12-hour light/dark cycle starting at 8:00 a.m. Rats were exposed to aerosols

generated from 2% solution of nickel chloride (NiCl₂·6H₂O: Ni) by an ultrasonic nebulizer⁵⁾ for five hours/day starting from 10:00 a.m. to 3:00 p.m., over five days, and they did not receive food and water during five hours daily exposure time. The average concentrations of aerosols were 0.8–0.85 mg as Ni/m³ for the first day, and 0.23–0.25 mg as Ni/m³ for the second to fifth day. The mass median aerodynamic diameter (MMAD) of the particles was 1.8 µm and geometric particle size distribution (σ_g) was 1.6. These procedures resulted in a stable production of broncho-bronchiolitis (Br) in the shortest time course ever reported^{6–9)}.

Exposure methods of Br rats to particulate matter

1. Exposure of Br rats to titanium dioxide (TiO₂: Ti) aerosols

Aerosols of Ti were generated from an aqueous suspension of ultrafine TiO₂ powder (Idemitsu Titania, Idemitsu Kosan Co, Japan) by an ultrasonic nebulizer⁵⁾. The aerosols consisted of globular aggregates of primary particles (20 nm in diameter, amorphous); and the secondary particles with MMAD of 1.2 µm and σ_g was 1.58. Animals were divided into four experimental groups: (1) Br rat + Ti (BT), (2) normal control rat + Ti (CT), (3) Br control (BC), and (4) normal control (CC). Table 1 shows the experimental design. Inhalation of Ti aerosols started from the following day after 5 days exposure to Ni (from Day 6–10), at average concentration of 0.95 ± 0.06 mg/m³ for 5 hours/day over 5 days. Five rats/groups were sacrificed at 0 hr and 1, 3, 7, 14, and 28 days after the last exposure to Ti. All the left lobes of the lung were weighed, dried and analyzed for the tissue concentrations of Ti, and the right lobes were instilled with fixatives and processed for histopathology.

2. Exposure of Br rats to vanadium pentoxide (V₂O₅: V) aerosols

Aerosols of V₂O₅ generated from saturated aqueous solution of V₂O₅ using an ultrasonic nebulizer. The MMAD of V particles was 1.1 µm, and σ_g was 1.6. A single inhalation of V at an average concentration of 2.2 mg/m³ was performed

Table 2. Experimental design and sampling time after Ni and/or V exposure

Group \ Day	D1	D2	D3	D4	D5	D6	D7	D8
CC: normal control	Air	Air	Air	CC				
CV: normal + V	Air	Air	Air	V	CV-D5			
	Air	Air	Air	Air	V	CV-D6	Air	CV-D8
BC: Br + Air	Ni	Ni	Ni	Ni	Ni	Air	Air	BC-D8
			3dN	4dM, 4dN	5dM	6dM		
BV: Br + V	Ni	Ni	Ni	V	BV-D5			
	Ni	Ni	Ni	Ni	V	BV-D6	Air	BV-D8

Daily exposure time was 10:00 a.m. to 15:00 p.m. N: sampled at 3 hs after the last exposure, at night.

M: sampled in the morning, 18 hs after the last exposure. N=5 for BALF, and 2 for histopathology.

for only 5 hours at either Day 4 or Day 5 from the start of Ni exposure. Seven rats/group were sacrificed; 5 rats for bronchoalveolar lavage fluid (BALF) analysis and 2 rats for V concentrations in the lungs and for histopathology. Table 2 shows the experimental design. For BALF collection, rats were anesthetized by i.p. injection of pentobarbital sodium. After insertion of a catheter in the trachea, 2 ml of blood was collected from the right ventricle and the hilum of the left lung was ligated. Immediately after bleeding through the cut abdominal aorta and vein, BALF was collected after repeated washings of the lung *via* an inserted tube with sterile saline up and down for 3 times at 25 cm hydrostatic pressure. Cells in BALF were prepared for differential counting by cytocentrifuge, and stained with May-Grünwald Giemsa.

Histopathology

The lungs were instilled through the trachea with 10% neutral formalin and were fixed for 2 days. The right lobes were embedded in paraffin, and serial sections under 2 μ m thickness were cut and stained with hematoxylin and eosin (HE), alcian blue (pH 2.5) and periodic acid Schiff (AB/PAS) with or without α -amylase digestion.

Statistic analysis

ANOVA (two-way, one-way) were adopted except for the number of polymorphs in the tissue, in which Kruskal-Wallis test was applied. The criterion for statistical significance was set at $P < 0.05$.

Results

Profiles of the Br rats and the effects of inhaled PM

Figure 1 shows the changes in the lung weight and the lung/body weight (L/B) ratios with time after the start of Ni

Fig. 1. Changes in the Lung Weight ratios, and Wet Weight of Left-lobe after Ni Exposure.

The lung weight ratios {L/B expressed as (wet left lung weight/body weight) $\times$ 1000}, and wet weight of left-lobe (g) after exposure to NiCl_2 (Ni). Values are mean $\pm$ standard deviation (SD); $n=5$. Days are from the start of exposure to Ni. N: sacrificed at night (3 hours after the last exposure) and M: sacrificed in the following morning (18 hours from the last exposure). * $P < 0.05$ vs control, ** $p < 0.01$ vs control. a=significant difference ($p < 0.05$) between two points connected with each lines.

exposure. Both the wet weight of the left lungs and the L/B ratios increased significantly in the rats sacrificed at Day 4 morning after 3 days exposure to Ni aerosols. Pathogenesis coincident with those changes showing the submucosal and peri-vascular edema started on Day 3 night accompanied with an increased influx of inflammatory cells in the same areas. Prominent transmigration of both macrophages and neutrophils from the submucosa into the bronchiolar lumen occurred mainly through the enlarged intercellular space of the airway epithelium. In contrast to drastic acute changes in the whole bronchiolar tree such as epithelial hypertrophy,

Fig. 2. Light photomicrograph of the lung of a rat exposed to Ni for 5 days and sacrificed just after the last exposure.

This is a representative morphology of the lung of a rat with Ni-induced bronchiolitis. A small bronchiole and the terminal airways are covered with acidic mucus intermingled with inflammatory cells. Edema and inflammatory cell influx into the submucosal and perivascular interstitium are noted. A slightly enlarged alveoli appeared with their normal structure. S=small bronchiole; T=terminal airways. Stained with alcian blue and PAS.

proliferation, and an increase in the number of goblet cells, the present exposure conditions caused little or a very slight, if any, insult in the alveolar region (Fig. 2). After 5 days exposure to Ni, rats developed acute bronchiolitis judging from the following histopathological criteria: (1) increase in number of secretory cells in the airway epithelium including the terminal bronchioles, (2) abundant mucus production and a shift in the type of glycoprotein present in secretory goblet cells or lumen from a neutral to acidic form, (3) increase in peri-bronchiolar muscle constriction resulting in frequent folding of bronchioli, and (4) increase in submucosal infiltration of inflammatory cells¹⁰.

Figure 3 shows the changes in the L/B ratios in control

Fig. 3. Changes in the L/B Ratios in Control and Bronchiolitic Rats after Exposure to TiO_2 (Ti) particles.

Values are mean $\pm$ SD; $n=5$. The group total values during the first 7 days, and 14 days after the last exposure to Ti in BT vs BC are significantly different (** $p<0.01$ and * $p<0.05$, respectively), and returned to normal levels at Day 28. No difference was found between CC and CT groups.

and Br rats after exposure to Ti. There was no change in the L/B ratios for both CC and CT groups during the experimental periods. However, in Br groups, BT rats maintained significantly higher ratios than those of BC rats during 2 weeks after the termination of Ti exposure. Histopathological observations supported that there was no morphological change in CT groups except the appearance of intact alveolar macrophages (AM) with phagocytosed Ti particles⁹. However, in the BT groups, prominent hypertrophy and hyperplasia of the airway epithelium and abundant infiltration of inflammatory cells in the submucosal interstitia remained while the BC groups showed less lesions indicating faster recovery than that in BT groups.

Figure 4 shows the changes in the number of AM and neutrophils in BALF after exposure to Ni and/or V aerosols. The number of AM in BC groups decreased significantly on Day 5 and 6 compared to normal control or Day 3 and 4 values, then slightly recovered on Day 8 after 3 days in clean air. The number of AM in BV groups on Day 5 and 6 were the same as those of BC groups, and significantly lower than those of CC and CV groups. No change was found in the number of AM in the CV groups compared to CC value during the experimental periods. The number of neutrophils increased significantly with time after exposure to Ni and the maximum value was obtained on Day 4, then decreased gradually with time. No difference was found in the number of neutrophils between BC and BV groups, and no increase

Fig. 4. Changes in the Number of Intact Alveolar Macrophages (AM) and of Neutrophils (Neu) Recovered in Brochoalveolar Lavage Fluid (BALF) after Exposure to V_2O_5 (V) aerosols.

Values are mean $\pm$ SD; n=5. ##, #: Number of Neu at Day (D) 4 in BC groups is higher than those in BC-D3, 6, and 8 ($p<0.01$), and BC-D5 ($p<0.05$). a: Number of AM in BV groups at D5, 6, and 8 is much less than those in BC groups at D5, and 6 ($p<0.05$). b: Number of AM in BC-D6 and BV-D5 is less than those in normal control, CC ($p<0.05$). No difference was found between CC and CV groups during through the experimental periods. No difference was observed in the number of neutrophils between BC and BV groups at D5, 6 and 8 (bars not shown).

Fig. 5. Number of PMN in the Peri-vascular and Submucosal Interstitium. Number of polymorphonuclear leukocytes (PMN) infiltrated in the peri-vascular and submucosal interstitium after exposure to V_2O_5 (V) aerosols.

Values are mean $\pm$ SD; n=2. Total number of cells in the ten equivalent areas from the medium-sized bronchioles were counted and expressed as the number of cells/mm² of tissue. Both BC and BV groups show prominent influx of PMN in the interstitium compared to CC and CV groups.

Fig. 6. Light photomicrograph of the bronchioles of a Br rat exposed to Ni for 4 days, then received a single exposure to V and sacrificed 3 days after recovery in clean air at Day 8.

There are abundant goblet cells (arrow heads) remained in the airway. Prominent infiltration of inflammatory cells in the submucosal and perivascular interstitium (arrows) is noted. L=Large (axial) airway lumen; ML=medium-sized bronchiolar lumen. Stained with hematoxylin and eosin.

observed in the CV groups as compared to CC (data not shown). On the contrary, significantly greater numbers of polymorphonuclear leukocytes (PMN) infiltrated into the submucosa and perivascular interstitia in the BV groups at Day 6 and 8 as compared to BC groups, while the values of CV groups were unchanged and remained at CC levels (Fig. 5). Figure 6 shows the overall delayed recovery or more progressed tissue lesions in a BV rat sacrificed on Day 8 even after 3 days recovery period in clean air. A prominent goblet cell metaplasia was observed in BV rats as compared to the BC groups¹⁰, and a marked influx of PMN was observed predominantly in the interstitia surrounding the juxtapulmonary vein and venule.

Fig. 7. Concentration of Ti in the lung.

The lung contents of Ti of rats exposed to Ti for 5 days. Values are mean $\pm$ SD; $n=5$. Significantly lower Ti concentration in the BT groups vs CT groups was found only in the rats sacrificed just after the last exposure (30 min. to 1 hr).

Deposition and clearance of PM in the lung of Br rats

Figures 7 and 8 show the concentrations of Ti and V in the lungs. In both the BT and BV groups, the concentrations of Ti and V were significantly lower than their normal controls, CT or CV, at 0 hr or 1 day after exposure of particles. However, the initial clearance of the inhaled particles appeared faster in the normal groups for a few days after the termination of exposure, the lung contents of inhaled particles in the Br and normal groups became almost the same after several days. No difference in Ti contents in the lung was observed in the rats during 2 to 8 weeks after the last exposure to Ti aerosols (Data shown in Fig. 7 was obtained from the comparable, but different experimental groups). The results of BALF cell counts from a repeated experiment showed a significant decrease in the number of AM, but significant increases in neutrophils and lymphocyte in BT groups as compared to those values in CT groups¹¹).

A significant difference of V concentrations was noticed after one day exposure to V aerosols between the BV rats exposed to Ni for 3 days and those exposed for 4 days. Vanadium content in the CV groups at Day 8 was 20% of the value of that on Day 6, on the other hand, almost 80% remained in the BV groups on Day 8 as compared to that of Day 6. The only significant effect of V in CV groups found in this experiment was an increase in the number of leukocytes in the peripheral blood on Day 8 as compared to non-exposed groups CC (data not shown).

Fig. 8. Concentration of V in the lung.

The lung contents of V of rats after a single exposure to V and sacrificed the following day. Values are mean $\pm$ SD; $n=2$. Asterisk denotes a significant difference from the groups shown by the respective lines illustrated.

Discussions

There are a number of animal models of bronchitis or chronic bronchitis (CB). The most widely used is the Reid's model of CB which is developed by exposing the SO_2 gas to animals from a few weeks to a several months^{6-8, 12-14}). However, the duration of CB in this SO_2 model is too short to be called chronic because the recovery of the bronchiolar epithelium can occur in a month, without the continuous exposure. Since the present Br rats induced by Ni inhalation showed the same morphological changes with those induced by SO_2 in that the lesions spread in the whole intrapulmonary airways within a few days, and their recovery time is also one month after the last exposure to Ni for only five days, we thought that the Ni-induced Br rats are quick and useful as an acute phase model of bronchiolitis.

The results of exposure of Br rats to Ti or V particles strongly indicate different responses of Br rats to the inhaled PM as compared with those of the normal healthy rats that have received the same exposure. Noteworthy are significantly lower concentrations of Ti and V in the lungs of both in the BT and BV groups than their normal controls, CT or CV, at 0 hr or 1 day after exposure of particles. Maruyama *et al.*¹⁵) showed that the rats exposed to Ni started higher pulmonary ventilation per minutes from Day 3 to Day 5, and this state continued until 2 days post exposure, but returned to control level after 3 days post exposure (Day 8 from the start of Ni exposure). The above result indicates that Br rats inhaled much more total amounts of Ti or V,

nevertheless, the final concentration of either Ti or V in the lungs of Br rats at 0 hr or 1 day after the last exposure to Ti, or to V was lower than that of normal control. At the same time, initial clearance was faster in the CT or CV groups than in the Br groups as shown in the Figs. 7 and 8. The above results suggested disabled clearance even for the inhaled insoluble and rather innocuous particles like Ti in the Br rats during the periods of acute inflammation.

A significant difference of V concentrations in the BV rats exposed to V aerosols at Day 4 or Day 5 that have received exposure of Ni for 3 or 4 days, may indicate that the degree of airway lesions alters the deposition and clearance pattern of inhaled PM. Since the airway inflammation, constriction of airways, and hypersecretion of mucus was advanced in the rats that had been exposed to V at Day 5 and sacrificed on Day 6. It is plausible that much more inhaled V was deposited in the narrowed airways and the total deposition of V in the alveolar region was reduced. It is also supported that Br rats are more susceptible to a single exposure to V_2O_5 which caused adverse effects on them, while normal rat showed no detectable changes with the same dose, which was about 28 times ($2.2 \text{ mg/m}^3 \times 5\text{h}$ vs $0.05 \text{ mg/m}^3 \times 8\text{h}$) higher than the recommended TLV for V_2O_5 in the work place in Japan. Vanadium pentoxide is soluble and absorbed in the lung after several hours, then it is excreted via blood circulation from the kidney. A transient acute toxicity caused by inhaled V has been well documented¹⁶⁾. Knecht *et al.* reported that exposure of monkeys to V_2O_5 at 5 mg/m^3 caused air-flow limitation but not at $0.5 \text{ mg } V_2O_5 / \text{m}^3$, under conditions that do not produce detectable changes in parenchymal function. Their results of the BAL performed just after the lung function test at 1 day after inhalation showed about a two-fold increase in total cells due mainly to a dramatic increase in the absolute number of polymorphonuclear leukocytes (PMN), despite a slight decrease in the number of AM recovered from BALF¹⁷⁾. Although the concentration of V_2O_5 / m^3 in the present study was less than half of their dose, and BAL data showed no difference in the number of both AM and PMN between CC and CV groups. However, the number of AM significantly decreased at Day 5 and Day 6 in the BV groups as compared to CC or CV values. The delayed clearance of V_2O_5 in the BV groups might be attributable to both the reduced number of AM and the disability of AM following exposure to soluble Ni. Vanadium pentoxide is only slightly soluble in water, and the present data suggest that inhaled V in the present conditions caused no adverse effect both on AM and parenchymal tissue during 24 hours post exposure, since there was no significant

difference observed in the number of AM and PMN between BV and BC groups. On the other hand, the significant accumulation of PMN in the submucosa or perivascular interstitium in BV groups at Day 8 than that of BC groups suggests that the adverse effect of V_2O_5 appeared after solubilization of V particles in the alveoli and then a transport *via* blood stream, because the number of peripheral blood leukocytes in the CV groups also increased on Day 8 without any extra accumulation of PMN in the interstitia.

No direct evidence is available regarding the possibility that the residual Ni in the lungs of Br rats may interact with the inhaled PM and interfere with the clearance of inhaled PM. The residual concentration of highly soluble $NiCl_2$ in the lung after 5 days exposure to develop bronchiolitis was about $3 \mu\text{g}$, and $1 \mu\text{g/dry g lung}$, at Day 1 and 4 post exposure, respectively (unpublished data). Because of the highly hygroscopic nature of inhaled $NiCl_2$ aerosols, it is plausible that most of the inhaled Ni was deposited on the surface of mucous layer of the airways and directly absorbed by the airway epithelium and only a minor part was deposited in the alveoli and being absorbed by alveolar epithelium or phagocytosed by AM as Ni-surfactant complex. As we had chosen carefully the doses of exposing Ni that cause minimal injury to the alveoli according to the histopathological observations and also there was no exfoliation of the airway epithelium found on Day 5 when bronchiolitis has been developed, the AM may be subjected to the primary target of the residual Ni. Both the reduced number and damage of AM may result in decrease of initial clearance of the inhaled PM. Disturbance of clearance at Day 8 (Fig. 8) as reflected in 80% of the deposited V after 72 hours post inhalation supports the above hypothesis. It has been well known that any kinds of Ni compounds either highly soluble or insoluble cause injury to AM^{18, 19)}. Bingham *et al.*²⁰⁾ reported that an increase of AM after inhalation exposure to $NiCl_2$ (0.1 mg/m^3) 8 hr/day, 5 days/week for 18 days was very low as compared to the prominent increase after the inhalation of the same dose of an insoluble Ni compound, nickel oxide (NiO). However, as Benson *et al.* has reported¹⁸⁾, we should keep in mind that the species differences exist in the sensitivity of canine and rat AM to Ni. For example, the dog AM was at least 10 times more sensitive than was the rat AM.

The above results suggest that Br rats are more susceptible to inhaled particles than normal rats. Therefore, our Ni-induced Br model is useful to assess the differences in toxicity of any PM in a pre-existing inflammation of the lung. Finally, the concentrations of PM lower than the recommended

threshold limit values in Japan, which have no harmful effects on normal lungs, may not always be safe in the case of pre-existing lung disease.

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References

- 1) Utell MJ, Frampton W (1995) Particles and mortality: a clinical perspective. *Inh Toxicol* **7**, 645–55.
- 2) Samet JM, Zeger SL, Berhane K (1995) The association of mortality and particle air pollution. The phase I report of the particle epidemiology evaluation project.
- 3) McClellan RO, Miller FJ (1997) An overview of EPA's proposed revision of the particulate matter standard. *CIIT Activities* **17** (4), 1–21.
- 4) Utell MJ, Drew R (1998) Summary of the workshop on clinical studies and particulate matter. *Inhal Tox* **10**, 625–38.
- 5) Serita F (1997) Development of a new aerosol generating cell with an ultrasonic nebulizer. *Ind Health* **35**, 433–9.
- 6) Lamb D, Reid L (1968) Mitotic rates, goblet cell increase and histochemical changes in mucus in rat bronchial epithelium during exposure to sulphur dioxide. *J Pathol Bacteriol* **96**, 97–111.
- 7) Jones R, Reid L (1978) Secretory cell hyperplasia and modification of intracellular glycoprotein in rat airways induced by short periods of exposure to tobacco smoke, and the effect of the anti-inflammatory agent phenylmethyloxadiazole. *Lab Invest* **39**, 41–9.
- 8) Reid L, Jones R (1983) Experimental Chronic Bronchitis. *Int Rev Exp Pathol* **24**, 335–82.
- 9) Kyono H, Serita F, Maruyama R, Kubota H, Toya T (1995) Cell dynamic analysis in a course of development for bronchiolitis in rats exposed to NiCl_2 . *Int Cong Tox-VII Abstracts* 20-p-8.
- 10) Kyono H, Serita F, Arito H, Maruyama R, Takahashi M, Kubota H, Toya T, Homma K (1994) Development of a new method to assess the biological effects of exposure to environmental pollutants using model animals with respiratory disease. *Env Res II, Japan* (74) 1-22 (in Japanese).
- 11) Kyono H, Serita F, Kubota H, Toya T, Nakakita M, Yamuchi Y, Ohta H, Seki Y, Ishihara Y, Kagawa J, Maruyama R (1996) Development of a new method to assess the biological effects of exposure to environmental pollutants using model animals with respiratory disease. *Env Res II, Japan* (76) 1-44 (in Japanese).
- 12) Chakrin LW, Saunders LZ (1974) Experimental chronic bronchitis. *Pathology in the dog. Lab Invest* **30**, 145–54.
- 13) Drazen JM, O'Cain CF, Ingram RH. Jr. (1982) Experimental induction of chronic bronchitis in dogs. Effects on airway obstruction and responsiveness. *Am Rev Respir Dis* **126**, 75–9.
- 14) Seltzer J, Scanlon PD, Drazen JM, Ingram RH Jr., Reid L (1984) Morphologic correlation of physiologic changes caused by SO_2 -induced bronchitis in dogs. *Am Rev Respir Dis* **129**, 790–7.
- 15) Maruyama R, Kyono H, Serita F, Toya T, Fukuda Y (1995) A pathogenesis of broncho-bronchiolitis model rats. *Proc. 36th annual meet Jap Soc Air Pollution, Tokyo* p 311 (Abstract in Japanese).
- 16) KiViluoto M (1980) Observations on the lungs of vanadium workers. *Br J Ind Med* **37**, 363–6.
- 17) Kneck EA, Moorman WJ, Clark JC, Lynch DW, Lewis TR (1985) Pulmonary effects of acute vanadium pentoxide inhalation in monkeys. *Am Rev Respir Dis* **132**, 1181–5.
- 18) Benson JM, Henderson RF, McClellan RO, Hanson RL, Rebar AH (1986) Comparative acute toxicity of four nickel compounds to F344 rat lung. *Fund Appl Toxicol* **7**, 340–7.
- 19) Adkins B. Jr., Richards JH, Gardner DE (1979) Enhancement of experimental respiratory infection following nickel inhalation. *Env Res* **20**, 33–42.
- 20) Bingham E, Barkley W, Zerwas M, Stemmer K, Taylor P (1972) Response of alveolar macrophages to metals. I. Inhalation of lead and nickel. *Arch Environ Health* **25**, 406–14.