Supplementary

A cluster of lung injury associated with use of home humidifiers: clinical, radiological and pathological description of a new syndrome

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We added methods, risk factor exposure rate and odds ratio for lung injury associated with humidifier use, further discussion about pathology and animal study data on here.

Radiological examinations

All patients underwent CT around their initial visit to the hospital (mean interval from the initial visit to the CT exam, 8.3 days; median, 3 days). In most patients (13/17), a follow-up CT exam was performed within one month of the first CT (Table 2): the mean interval between these two CT exams for the 13 patients was 23.8 days. The CT protocol that was used varied because the patients underwent their radiological examinations in various outside hospitals prior to referral. However, thin-slice 1–3 mm images were included in all CT exams. The CT images were evaluated by two thoracic radiologists (K.H.D .and E.J.C., who had 16 and 13 years of experience, respectively) until a consensus was reached. The CT features of the disease were a consolidation or ground-glass opacity pattern, a diffuse, multifocal patchy, or centrilobular distribution, and the presence of pneumomediastinum or pneumothorax.

Laboratory studies

Sputum, bronchoalveolar lavage (BAL) fluid, and blood samples were tested for a panel of bacteria, virus and fungi. The microbiological studies included (i) three sets of blood culture; (ii) gram staining and cultures of sputum, endotracheal aspirates, and BAL fluid; (iii) BinaxNOW urinary antigen tests for *Streptococcus pneumonia* (Binax Inc., Portland, Maine, USA) and *Legionella pneumophila* serogroup 1 (Binax Inc.); (iv) multiplex reverse-transcription polymerase chain reaction (RT-PCR) analysis on nasopharyngeal aspirate or BAL fluid for influenza virus A and B, respiratory syncytial virus A and B, adenovirus, human metapneumovirus, parainfluenza virus types 1–4, enterovirus, rhinovirus, human coronavirus 229E/NL63, human coronavirus OC43, human coronavirus HKU1, and bocavirus (Seegene Inc., Seoul, Korea); (v) shell vial culture for influenza virus, respiratory syncytial virus, parainfluenza virus, adenovirus, and cytomegalovirus (when indicated); (vi) PCR for *Mycoplasma pneumonia*, *Chlamydia pneumonia*, and *Legionella pneumophila* (BD Diagnostics, Sparks); and (vii) direct fluorescent assay for *Pneumocystis jirovecii* (when indicated). Flocked swabs with nylon fibres were used for nasopharyngeal sampling (Copan Diagnostics, Corona, CA, USA). Galactomannan (GM) antigen was measured in all patients who were admitted to the ICU by using an enzyme-linked immunosorbent assay (Platelia *Aspergillus*; Bio-Rad). A serum or BAL fluid sample was considered to be positive for GM if an optical density (OD) of $\geq$0.5 was achieved.

e-Table 1. Risk factor exposure rate and odds ratio for lung injury associated with humidifier use

Variable	Case group		Control group		Univariate analysis*	
	N	(%)	N	(%)	Odds ratio	(95% Confidence interval)
Marriage	11	(100)	16	(72.7)	6.6	(0.8-∞)
Have children†	10	(90.9)	11	(68.8)	5.3	(0.4-331)
Air cleaner use	7	(63.6)	16	(72.7)	0.8	(0.1-5.2)
Air freshener use	5	(45.5)	4	(18.2)	3.6	(0.6-26.1)
Humidifier use	11	(100)	6	(27.3)	33.7	(4.5-∞)
Humidifier disinfectant use	11	(100)	3	(13.6)	47.8	(6.9-∞)
House fungus	9	(81.8)	11	(50.0)	4.9	(0.7-∞)
House-hold insecticide use	7	(63.6)	11	(50.0)	2.6	(0.4–20.7)
Sodium hypochlorite use‡	8	(88.9)	15	(88.2)	0.95	(0.04–65.2)
Sauna	5	(45.5)	6	(27.3)	2.5	(0.4–16.1)
Chemical use in job§	1	(11.1)	1	(4.6)	2.3	(0.03–174)
Chemical use in hobby§	5	(45.5)	5	(23.8)	3.4	(0.5–27.1)
Hair spray	4	(36.4)	6	(27.3)	1.6	(0.2–9.8)

* An exact logistic regression model was used to calculate the risk of disease development because the study population may be too small, skewed or sparse for use of the usual asymptotic methods.

† Six participants were excluded because of missing data regarding children.

‡ Seven participants were excluded because of missing data regarding the use of sodium hypochlorite.

§ Two and one participants were excluded because of missing data about chemical use in the job or hobby, respectively.

Discussion about pathology

AFOP is more recently described histologic pattern associated with acute lung injury in which the alveolar spaces are filled with organizing fibrin balls and differs from classic histologic feature of DAD in terms of absence of hyaline membrane. Beasley et al suggested that the fibrin present in the AFOP pattern was typically patchy, with an average of 50% airspace involvement, as opposed to our cases. Some of the cases showed focal fibrin balls, but dominant histologic feature was not that of AFOP. Two of four patients having AFOP like histologic feature were dead in our series. Generally, it is known that overall mortality rate of AFOP is similar to DAD and therefore may represent a histologic variant (Beasley et al, Arch Pathol Lab Med 2002;126:1064).

The BOOP pattern is characterized by patchy accumulation of intra-alveolar fibroblastic plugs around bronchioles. Presence of intra-bronchiolar fibroblastic tissue and intra-alveolar fibroblastic plugs shares the histology of our cases. While the alveolar septa in involved areas exhibit mild chronic inflammation and significant fibrosis should not be present in the BOOP pattern, the interstitial expansion and myxoid fibrosis with marked pneumocyte hyperplasia are present in the cases.

In addition, differential diagnosis includes acute exacerbation of usual interstitial pneumonia (UIP). Although DAD pattern or OP pattern could be accompanied with underlying UIP in acute exacerbation, the cases are readily distinguished from acute exacerbation of UIP, because the latter showed temporal and spatial heterogeneity of fibro-inflammatory process with subpleural involvement.

The few patients who had high eosinophil numbers in their BAL fluid did not have any radiological or pathological features that were compatible with acute eosinophilic pneumonia; they also did not respond to steroid treatment. The possibility of a bacterial, viral, or fungal infection was excluded by extensive laboratory investigations, including PCR and cultures. The involvement of an infectious agent was also discredited by the universal ineffectiveness of antiviral, anti-fungal, or broad spectrum antibacterial agents.

Animal study

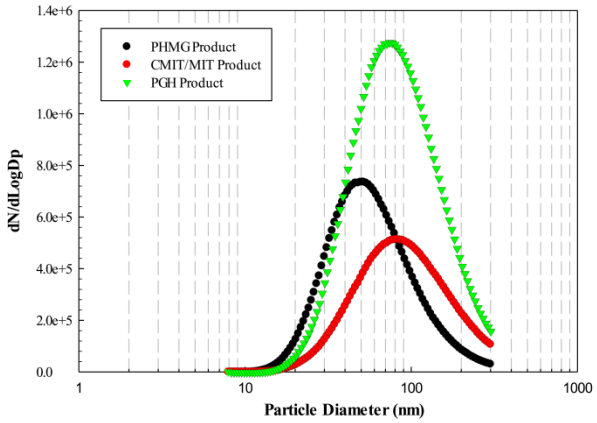
The team visited the houses of two patients and measured the room size and the volume of the humidifier and the disinfectant. In the laboratory, humidifiers with one of three disinfectants (PHMG-ph, PGH, and CMIT/MIT) were turned on in 1500 litre chambers and the aerosol distribution and concentration were measured. Target concentrations were determined on the basis of aerosolised disinfectant peak sizes, which were below 100 nm (e-Fig. 1).

Rats were exposed to these conditions for 4 weeks. Subsequent histological analysis of their lungs revealed no significant histological changes in the lungs of the control or CMIT/MIT-exposed animals (e-Fig. 2A). However, after PHMG and PGH inhalation, minimal inflammation in bronchioles (e-Fig. 2B) and inflammatory changes in the bronchioles and terminal bronchioles (e-Fig. 2C and D) were observed. These pathological features are similar to those seen in the human cases. Fibrotic changes were also observed in the lungs after disinfectant inhalation. Moreover, the PHMG and PGH groups exhibited significant loss of body weight relative to the control group after the 4 week exposure period (e-Figure 3). The PHG group also exhibited an increased respiratory rate.

e-**Figure 1. Humidifier disinfectant aerosol size distribution**

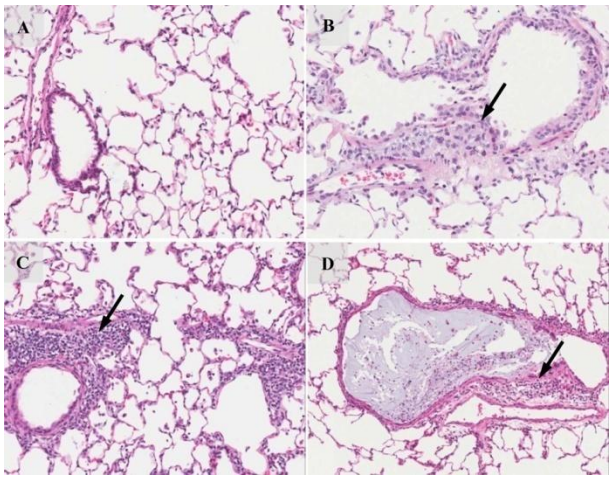
The three most commonly used humidifier disinfectant ingredients were polyhexamethyleneguanidine (PHMG), 5-chloro-2-methylisothiazol-3(2H)-one/2-methylisothiazol-3(2H)-one (CMIT/MIT), and oligo(2-(2-ethoxy)ethoxyethyle guanidine chloride (PGH). The distribution of the humidifier-induced particles was measured by using a scanning nanoparticle spectrometer (SNPS, HCT, Korea). Mass concentrations of exposed particles were also measured every 2 hours (i.e., three times during the exposure) by using 1 L/min sampling (25 mm ø, Pallflex filter, Japan and XR5000 pump, SKC, Korea). After sampling, the mass concentrations were determined by a gravitational method using a microbalance (ME5, Sartorius, Germany).

The measured particle sizes (x-axis, range between 8 and 300 nm) and the normalised particle number concentrations (y-axis) are shown below. The PHMG-, CMIT/MIT-, and PGH-exposed rat groups were subjected for 4 weeks to mean exposed mass concentrations of aerosol of 0.44±0.10 mg/m^3, 1.84±0.39 mg/m^3, and 1.76±0.46 mg/m^3, respectively. These concentrations are similar to the exposure concentrations of the patients for each disinfectant.



e-Figure 2. Lung histology in rats exposed to aerosolised disinfectant.

After 4 weeks of aerosol inhalation, the lungs of the rats were harvested and fixed with 10% neutral phosphate-buffered formalin, embedded in paraffin blocks, prepared as microtome slices, and placed onto glass slides. Pathological changes were analysed after hematoxylin & eosin (H&E) staining. The animal facilities and their management have been accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International. While significant histological changes were not observed in the lungs of the control and CMIT/MIT groups (panel A), inflammatory changes were observed in the lungs of the PHMG- and PGH-exposed animals. In the PHMG group, minimal infiltration of inflammatory cells, mainly consisting of macrophage and lymphocytes, was seen in the bronchioles and terminal and respiratory bronchioles. Intra-alveolar aggregation of macrophages containing foamy cytoplasm was also observed. Epithelial hyperplasia was occasionally seen in the alveolar ducts (panel B, arrow). In the PGH group, inflammatory infiltration was observed in the bronchioles, terminal and respiratory bronchioles, and alveoli. The bronchioles showed severe epithelial detachment and accumulation of mucus in the lumen. Fibrosis with collagen deposition, fibroblast proliferation, and inflammatory cell infiltration was seen in the alveolar ducts and alveoli. Aggregates of alveolar macrophages containing foamy cytoplasm were present in the alveoli. Squamous metaplasia occurred, with hyperplastic bronchiole-alveolar lining cells in the alveoli showing alveolar bronchiolarisation at the alveolar duct in some animals. Minimal bronchiole-alveolar hyperplasia was also observed in the alveolar duct (panels C and D, arrows).



e-Figure 3. Body weight change in rats exposed to aerosolised disinfectant.

Seven-week-old male or female Sprague-Dawley rats were housed under a 12 hour light/dark cycle. Temperature and relative humidity were maintained at 22±3 ℃ and 50±20%, respectively. HEPA-filtered clean air was supplied to the animal room. The rats were fed a standard diet (PMI Nutrition International) and were housed in steel wire cages (255 W × 465 L × 200 H mm).

Four groups of 10 rats (five female and five male) were exposed for 4 weeks to polyhexamethyleneguanidine phosphate (PHMG-ph), 5-chloro-2-methylisothiazol-3(2H)-one/2-methylisothiazol-3(2H)-one (CMIT/MIT), oligo(2-(2-ethoxy)ethoxyethyle guanidine chloride (PGH), or water. Based on estimations of exposure concentrations, the target PHMG, CMIT/MIT, and PGH exposure concentrations were 0.4, 1.8, and 1.75 mg/m^3, respectively. The body weights of the rats were measured over the 4 week exposure, as shown below. For both the male and female subgroups, PHMG and PGH elicited significant weight loss relative to the vehicle control (V.C.) group (+ $p<0.05$, *$p<0.01$).