수의학석사 학위논문

Evaluation of 6 week-inhalation exposure to benzalkonium chloride in rats

랫드를 이용한 벤잘코늄염화물의 6주 흡입 노출 평가

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최 혜 연

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Abstract

Evaluation of 6 week-inhalation exposure to benzalkonium chloride in rats

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Benzalkonium chloride (BKC) is commonly used as an antimicrobial biocide and is likely to be exposed by inhalation; however, studies on the effects of its repeated inhalation are insufficient. In this study, I have investigated the time-dependent and concentration-dependent effects of BKC inhalation on the respiratory tracts of rats. Bronchoalveolar lavage fluid was collected from these rats to estimate the number of immune cells, to determine cytokine levels, and to perform histopathological analysis in response to BKC inhalation. The rats were divided into four groups and were exposed to BKC aerosol

at a concentration of 0 (control group), 0.8 mg/m^3 (T1 group), 4.0 mg/m^3 (T2 group), and 20 mg/m^3 (T3 group) for up to 6 weeks and sacrificed on days 14 and 42. After 2 weeks of inhalation exposure, histopathological changes were observed in the lung and nasal tissues of the inhaled animals at concentration of 0.8, 4, and 20 mg/m^3 and the levels of inflammation related cytokines, IL-1β, IL-6, and MIP-2, decreased in the exposed groups as compared to the control group. After 4 weeks of additional exposure, these changes recovered. Although the changes were diminished after additional inhalation exposure, the changes by BKC aerosol inhalation were observed in animals exposed to concentrations above 0.8 mg/m^3. Therefore, the no-observed-adverse-effect level by inhalation exposure of BKC aerosol is suggested to be less than 0.8 mg/m^3.

keywords : Benzalkonium chloride, Cytokines, Inhalation, Rats, Respiratory tract, No-observed-adverse-effect level

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INTRODUCTION

Benzalkonium chloride (BKC; CAS Registry Number 8001-54-5) structurally consists of a quaternary ammonium group and is composed of a mixture of alkyldimethyl ammonium chlorides. BKC can inhibit bacterial enzymatic processes with these structural features (Adelson & Sunshine, 1952) and is thus an active ingredient in sterilizers, preservatives, eye drops, floor cleaners, and skin disinfectants. BKC is also widely used in nasal and bronchial drug formulations (Storaas et al., 2000; Larsen, Verder, & Nielsen, 2012).

Many previous studies have performed intranasal instillation experiments on BKC solution and some studies have conducted inhalation exposure experiments on BKC aerosol. Histological examination following an injection of BKC into the nasal cavity of rats showed an intraepithelial gland formation and inflammatory cell infiltration in the septal mucosa (Kuboyama, Suzuki, & Hara, 1997; Cho et al., 2000; Lebe et al., 2004). Acute inhalation exposure of BKC aerosol in mice resulted in pulmonary inflammation (Larsen et al., 2012). After 5 days of BKC inhalation exposure, and 6 hours of exposure per day after a 2-week interval, the lung tissue revealed inflammation and damage to the blood-air barrier (Świercz et al., 2013). However, safety data are still insufficient to indicate the level at which clinically adverse effects of BKC inhalation exposure

are observed compared to the intranasal administration of BKC solution (Johnson, 2018).

BKC is mainly used in a spray form, and it is likely to be inhaled as an aerosol while being used as a biocide (Larsen et al., 2012; Mc cay, Ocampo-Sosa, &Fleming, 2010). The need for research on repeated inhalation exposure of such biocides has been enhanced by the adverse effects reported to be caused by biocides in domestic humidifiers. Additionally, in a report on the use of biocide aerosols in workplaces, BKC was selected as a biocide ingredient in need of an occupational exposure assessment, because it was frequently used, and health risks have been reported; however, inhalation exposure data were insufficient (Occupational safety and health research institute, 2012). In addition, the globally harmonized system of classification and labelling of chemicals (GHS) has classified BKC as category 2 (>0.05 ~ ≤0.5 mg/L of LC50), based on the acute toxicity due to inhalation and the 50% lethal concentration (LC50) values obtained from 4-hour tests in animals. This classification is considered to represent relatively severe toxicity, but a classification was not possible for repeated inhalation exposure due to a lack of data.

Therefore, I conducted this study to evaluate the effects of repeated inhalation exposure of BKC aerosol. I investigated the concentration-dependent effects over 6 weeks of exposure, and the time-dependent effects in the 2-week exposure group and the

additional 4-week exposure group to obtain additional information such as recovery, persistence, and delayed occurrence of toxicity. I intend to obtain further toxicological information on the inhalation exposure of BKC aerosol and to accumulate safety data for a risk assessment of occupational exposure through these results.

MATERIALS AND METHODS

Animals and Chemicals

Forty-six-week-old male Fischer 344 (F344) rats were supplied from Japan SLC Inc. (Shizuoka, Japan) and were used for the experiments after 8 days of an acclimation period. Rats were individually housed in a 6-wire mesh cage (W240 * L1200 * H200 mm) during the exposure period. The environment of the animal rooms was set at a temperature of 22 ± 3 ° C, relative humidity at 30 to 70%, a light / dark cycle of 12 hours each, with the light intensity of 150-300 Lux, and air ventilation of 10-15 times/hour. Animals were fed pelleted food (ENVIGO RMS Inc., Indianapolis, IN, USA) sterilized by gamma rays and were given filtered and sterilized tap water ad libitum. All experiments were approved by the Institute Animal Care and Use Committee of Occupational Safety and Health Research Institute (AEC-200806230002).

BKC was purchased from Samchun Chemicals (Pyeongtaek, Korea) with a purity of 50.5% and 50.3%. In order to generate the aerosol form in the chamber, BKC was diluted to 1-2% (v/v) with drinking water used as the excipient.

Experimental design

Based on the body weight of 40 male rats, grouping was performed

randomly, using PRISTIMA 7.1.0 software (Xybion Medical Systems Corporation, Morris Plains, NJ, USA). The rats were divided into four groups; control group and the 3 test groups that were exposed to a whole-body inhalation exposure for duration of 2-weeks and an additional 4-week period. The experimental concentrations were selected based on the results of my previous acute inhalation toxicity experiments. Considering the classification and safety of GHS, I selected the concentration of 0.8, 4.0, and 20 mg/m^3 to T1, T2 and T3 group, respectively, using a scaling factor of 5. The control group was exposed to clean air filtered through HEPA filters. The BKC solution was injected into a mist generator (NB-2N, Sibata Co. Ltd., Soka, Japan), and then clean air was injected from air handling unit. The sprayed BKC-generating material and clean air were mixed and supplied to the inhalation chambers to achieve the desired concentrations. To confirm the concentrations, samples were collected from the breathing area of the experimental animals in the chambers 3 times using a personal sample collector (Model No. Airchek XR 5000, SKC Inc., Eighty Four, PA, USA) connected to a filter holder equipped with a 25 mm micro glass fiber filter. The experimental concentrations were measured by calculating filter weight before and after sample collection. Also, the number of aerosol particles and particle size distribution in the chamber were checked using a real time portable aerosol spectrometer (Model 1.109, GRIMM Aerosol Technik GmbH

& Co. KG, Ainring, Germany) and cascade impactor (Model 135, MiniMOUDI Impactor, MSP Co. Ltd., Shoreview, MN, USA). Clinical symptoms were monitored during the entire experimental period and body weight was measured on the first day of exposure, twice a week during the 2-week inhalation exposure period, once a week during the additional 4-week period and on the necropsy day. Food consumption per animal was measured once a week during the entire exposure period. Rats were euthanized using isoflurane at day 14 and day 42, and then necropsy was performed. Right lung lobes were removed after BAL collection and the left lobes were collected, weighed, and fixed in 10% neutral-buffered formalin. Nasal tissues were also collected and fixed.

Histopathological analysis

The left lung lobe and nasal tissue were removed and fixed in 10% neutral-buffered formalin; the fixed tissues were embedded in paraffin. The embedded tissue blocks were cut into 3 μm thickness and hematoxylin and eosin (H&E) staining was performed. The stained slides were examined by a light microscope.

BAL & cell count analysis

To perform the BAL isolation, the upper end of the trachea was incised, and a polypropylene tube attached to a syringe was inserted, and the trachea was washed thrice with 4 mL of

phosphate buffered saline (PBS). The collected samples were centrifuged at 450 × g for 10 minutes, and the supernatant was frozen at -80 °C. The cell pellet was re-suspended in fresh PBS and the total immune cell count was determined using a hematology analyzer (ADVIA2120i, Siemens, Munich, Germany). The re-suspended cell pellet was centrifuged at 270 × g for 10 min using cytospin centrifuge (Cellspin; Hanil, Gimpo, Korea) and stained with Diff-Quick staining solution. The differential cell counts were determined with light microscope at 200 × magnification.

Measurement of cytokine concentration in the BAL fluid

The supernatant isolated from the BAL fluids was thawed around 20°C just before the cytokine analysis. To evaluate the concentrations of IL-1β, TNF-α, IL-6, IL-4, and MIP-2 in BAL fluids, a commercially available cytokine multiplex magnetic bead array kit (Rat Magnetic Luminex assay; R&D systems, Minneapolis, MN, USA) was used. Magnetic Bead Single Plex Kit (MILLIPLEX MAP, Merck Millipore, Darmstadt, Germany) was used to measure the concentration of TGF-β and the assays were performed per manufacturer's instructions. The median fluorescence intensity (MFI) of samples was calculated using a Luminex 100 instrument (Luminex, Austin, TX, USA), and a standard curve was

obtained using MasterPlex software (MasterPlex QT 2010; Miraibio, Hitachi, CA, USA). The cytokine concentration per sample was calculated using standard curve.

Statistical analysis

Statistical analysis was performed using PASW Statistics 18 (SPSS Inc., Chicago, IL, USA) or PRISTIMA 7.1.0 (Xybion Medical Systems Corporation, Morris Plains, NJ, USA). Bartlett's test or Levene test was used for the analysis of variance and in case of an equal distribution. A one-way analysis of variance (ANOVA) test was performed, followed by Dunnett's Test. In case of an unequal distribution, Kruskal-Wallis test was performed and the Dunn Rank Sum test was performed afterwards. Data were expressed as mean ± standard deviation (SD) and were considered statistically significant when $p < 0.05$.

RESULTS

Particle size distribution, body and organ weight, food consumption

The mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD) of BKC aerosols were calculated to be 1.614 μm, ± 2.00, 1.090 μm ± 1.86, 1.215 μm ± 1.51 at T1, T2, and T3, respectively. The MMAD and GSD values of BKC aerosol were confirmed to be within the proper range recommended by the Organization for Economic Cooperation and Development (OECD, 2018). The size distribution of the BKC aerosol particles is shown in Table 1. The body weight of the rats in the control group exposed to clean air for 2 weeks increased normally, but the weight of rats in the T3 group exposed to BKC aerosol at 20 mg/m^3 decreased significantly at 6, 9, and 13 days by 13.3 %, 23.0% and 33.9%, respectively, as compared to the control group. The body weight of rats showed a continuous increase in the T1 group (0.8 mg/m^3 BKC aerosol) at the aforementioned days, however, the T2 group (4 mg/m^3 BKC aerosol) exhibited a slight reduction in body weight on the 9th day, and an increase on the 13th day (Fig. 1A).

In the additional 4-week exposure period, the control group, in which the rats were only exposed to clean air, also showed a

sustained increase in body weight. During this period, the T1 and T2 groups exhibited body weight values lower than those in the control group, but the body weight increased steadily over time. The body weight of rats in the T3 group increased continuously after the 13th day, but the body weight was still significantly lower than any of the other groups until the last day of exposure (Fig. 1A). The differences in measurements of the lung weights of rats between the control and treated groups, on the 14th and 42nd days are shown in Fig. 1B. On day 14, the relative weight of the lungs in groups T2 and T3 exhibited a marked increase (126.5% and 145.3%, respectively). At 42 days, however, there was no significant difference in the relative lung weights between control group and all inhalation exposure groups.

The food consumption of rats was measured for 6 weeks and the food intake was monitored on the 8th and 14th day. On day 8, the food consumption of groups T1 and T2 decreased by 13.5% and 11.9%, respectively. The reduction in food intake was consistent on day 14 with a 30.2% and 29.1% reduction in food intake in groups T1 and T2, respectively. The food consumption of the T3 group was significantly reduced by 42.7% and 73.0% on days 8 and 14, respectively, suggesting an inverse correlation between the aerosol concentrations of BKC and the food intake in exposed rats. However, on the 22nd, 27th, and 35th days, the food consumption did not show any significant difference between the

control and exposed groups (Table 2).

Histopathological analysis upon BKC inhalation

The histological examination of the lung and nasal tissues was performed to discern the morphological differences post BKC inhalation exposure. Representative micrographs of the lung and nasal tissues for the animal groups are shown in Fig. 2 and Fig. 3. The rats from the control group showed a normal parenchyma at all time points (Fig. 2A, 2E and Fig. 3A, 3E). At day 14, however, degeneration and regeneration of the terminal bronchiolar epithelium and smooth muscle hypertrophy at the bronchioloalveolar junction were observed in the lung tissue of the T3 groups, and hypertrophy and hyperplasia of mucous cells in bronchi or bronchiole ware observed in all the exposed groups (Fig. 2B-D). In the nasal cavity, ulceration with suppurative inflammation, squamous metaplasia, and erosion with necrosis were observed in respiratory epithelium and transitional epithelium in the T3 group. In addition, hypertrophy and hyperplasia of mucous cells in respiratory epithelium and metaplasia of mucous cell in transitional epithelium were observed in all the BKC exposed groups (Fig. 3B-D). After 42 days, the lung tissue of all the groups demonstrated no significant findings (Fig. 2E-H). However, the nasal cavity of the T3 group showed hyperplasia in the transitional epithelium and hypertrophy and hyperplasia of the mucous cells in

the respiratory epithelium (Fig. 3E-H).

Analysis of total and differential cell counts in BAL fluid

Total cell counts and differential cell counts were obtained from the BAL fluid of rats exposed to clean air at the mentioned BKC concentrations and are shown in Fig. 4. At day 14, a total cell count of the control group was marginally higher than the other groups but the difference was not statistically significant. A similar trend was observed at day 42, wherein, no significant difference was observed between cell counts from the control and exposed groups (Fig. 4A). The differential cell count analysis suggested that the macrophage ratios between all groups did not show any significant differences at the 14th day, but the lymphocyte ratio of the control group was higher, compared to the all the other exposed groups. The level of polymorphonuclear leukocytes (PMNs) was slightly higher in the control group; however, the differences were not significant (Fig. 4B). At day 42, no significant changes in differential cell counts were observed between groups (Fig. 4C).

Cytokine analysis of BAL fluid

To analyze the immune response to BKC inhalation exposure, the concentrations of cytokines from the BAL supernatant were

determined. The concentrations of IL-1β, IL-6, and MIP-2 showed marked changes in the rats with BKC exposure as compared to the rats from the control group. IL-1β and IL-6 concentrations were significantly reduced in the T3 group after 14 days of BKC exposure (Fig. 5A). In addition, the concentration of IL-6 showed a marked reduction in the T3 group after 42 days (Fig. 5B). The concentrations of MIP-2 were also lower in the T2 and T3 groups when compared with the control group on the 14th day, but there was no significant difference in all the groups on the 42nd day (Fig. 5C). Examination of IL-4, TNF-α, and TGF-β levels showed no significant differences between groups on both the 14th and 42nd days (Fig. 5D-F).

DISCUSSION

Inhalation exposure of BKC had an effect on the lung and nasal tissues of the 2-week BKC-inhaled animals however; these effects were diminished after an additional 4 weeks of exposure. Although these effects were reduced in the additional 4-week exposure group, the no-observed-adverse-effect level (NOAEL) for the BKC inhalation exposure is considered to be less than 0.8 mg/m^3.

BKC aerosol affected the experimental animals in all inhalation groups after 2 weeks exposure. Previous studies have shown that quaternary ammonium compounds (QACs), including BKC, are associated with oxidative stress (Debbasch et al., 1997; Debbasch et al., 2001). In addition, there is some evidence that oxidative stress, which occurs by an imbalance between oxidants and antioxidants, plays a central role in the inflammatory response through the signal transduction or gene expression of proinflammatory mediators (MacNee, 2001). Body weight and food consumption were decreased, and histologic findings were observed, which is consistent with the results of the previous studies on the robust inflammation after inhalation exposure to BKC aerosol (Świercz et al., 2013). Therefore, I suggested that the effects observed in the present study were significantly related to oxidative stress and the inflammatory response caused by BKC inhalation exposure.

After 4 weeks of additional exposure, some of negative effects

were improved in the BKC-inhaled rats. This improvement may be related to the repair mechanism of epithelial cells or the clearance process of deposited particles in the respiratory tract. Pulmonary injuries induce the secretion of factors associated with repair mechanisms, such as epidermal growth factors or fibroblast growth factors, chemokines, and interleukins and the self-recovery of epithelial cells through the secretion of anti-inflammatory molecules. Factors associated with this mechanism include IL-4, TGF-α, TGF-β, and prostaglandins (Crosby and Waters, 2010; Brirukov and Karki, 2018). However, it is not clear whether the improvement after 4 weeks of additional exposure is related to the repair mechanism, because the concentrations of IL-4 and TGF-β showed no significant changes in this study.

Because there were no differences in factors related to the repair mechanism, I considered other possibilities. Particle clearance occurs in three main regions of the respiratory tract, the nasopharynx, the tracheobronchial, or the conducting airways and pulmonary or gas exchange regions. In the nasopharyngeal or tracheobronchial regions, the deposited particles are removed through the mucociliary transport system or phagocytosis by pulmonary alveolar macrophages. In the conducting airways and pulmonary or gas exchange region, particles migrate to other regions of the lungs through the blood stream or lymphoid fluid (Stuart, 1984). In the previous studies, after the intranasal

administration of the BKC solution, the prominent changes were found in the anterior part of the nasal cavity, suggesting that BKC particles were diffused or inactivated by respiratory secretions in the posterior regions or removed by the mucociliary system (Cho et al., 2000; Berg, Lie, & Steinsvag, 1997). Likewise, in the current study, the change occurred more prominently in the anterior region than in the posterior region of the nasal tissue. Based on these results, I suggested that the changes observed in the 2-week exposure group were due to the migration and deposition of the inhaled BKC aerosol particles within the deep respiratory tracts; these changes were not observed in the additional 4-week exposure group, because BKC aerosol was removed by the clearance processes.

In addition, the results of the cytokine concentration analysis seem to be related to these changes. In a previous study, increased levels of inflammation-related cytokines were observed in single and 3-day exposures (Świercz et al., 2008) and were also seen in single-exposure trials after a 2-week interval of 5-day repeated exposure (Świercz et al., 2013). However, in the present study, the concentrations of inflammation-associated cytokines, IL-6, MIP-2, and IL-1β, were decreased. This may be related to the anti-inflammatory response, which regulates the excessive inflammatory response and the response of proinflammatory cytokines in the immune system (Opal & DePalo, 2000). Cytokines

such as IL-10 and IL-13 are known to be anti-inflammatory cytokines (Arai, et al., 2000; Chapoval, Dasgupta, Dorsey, & Keegan, 2010). However, there is a large standard deviation, and because of a lack of sample availability, I could not subsequently analyze the relative concentrations of the anti-inflammatory cytokines; therefore, additional studies are needed.

In summary, I suggested that the observed changes in the inhaled animals after 2 weeks of inhalation exposure were due to the inflammatory reaction. However, excessive inflammation initiated an anti-inflammatory response, and the inhaled particles were removed by the clearance process, suggesting that the changes were reduced after 4 weeks of additional exposure.

The occupational exposure standards for BKC are unknown to date. The NOAEL is the primary indicator used in threshold-based risk assessments and an important factor in identifying the highest concentration that is unaffected when exposed to substances in toxicity studies (Alexeeff, Broadwin, Liaw, & Dawson, 2002; Lewis et al., 2002). In the present study, the negative effects were recovered after 4 weeks of additional inhalation exposure; however, changes by BKC aerosol inhalation were observed in animals exposed to concentrations above 0.8 mg/m^3. Therefore, the NOAEL is suggested to be below 0.8 mg/ m^3.

CONCLUSION

This study is the first to confirm the effects of BKC inhalation exposure for 6 hours per day over 6 weeks in a time-dependent and concentration-dependent manner. After 2 weeks of exposure, body weight, food consumption, and relative lung weight were significantly changed in 4 and 20 mg/m^3 exposed groups. Histopathological changes were observed in the lung and nasal tissues of 0.8, 4, and 20 mg/m^3 exposed groups. However, the concentrations of inflammation-associated cytokines, IL-6, MIP-2, and IL-1β, were decreased. After 4 weeks of additional exposure, some of the negative effects were improved. I suggest that the observed changes in the inhaled rats of the 2-week exposure group were associated with the inflammatory response found in the previous studies. The reduced concentration of inflammatory cytokines is likely due to the anti-inflammatory response that controls the excessive inflammation. Additionally, the results from the additional 4 weeks exposure group may be related to the clearance process of deposited particles in the respiratory tract. Although the negative effects improved after additional inhalation exposure, changes by BKC aerosol inhalation were observed in animals exposed to concentrations above 0.8 mg/m^3. Therefore, the NOAEL is likely below 0.8 mg/m^3.

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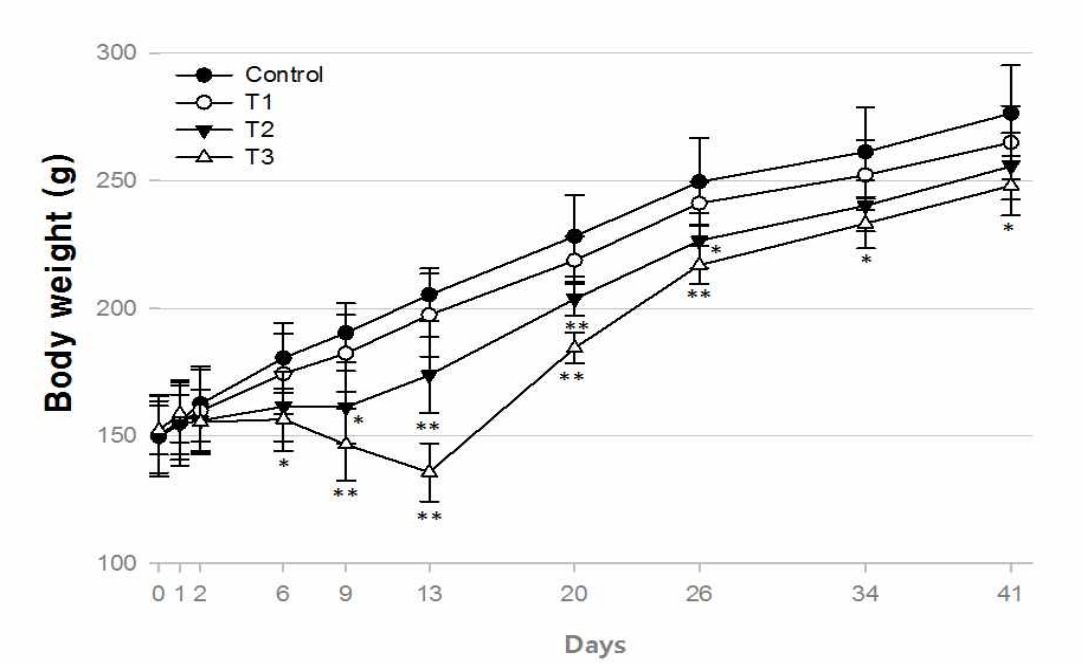
(A)


Control
T1
T2
T3
Body weight (g)
300
250
200
150
100
0 1 2
6
9
13
20
26
34
41
Days

(B)

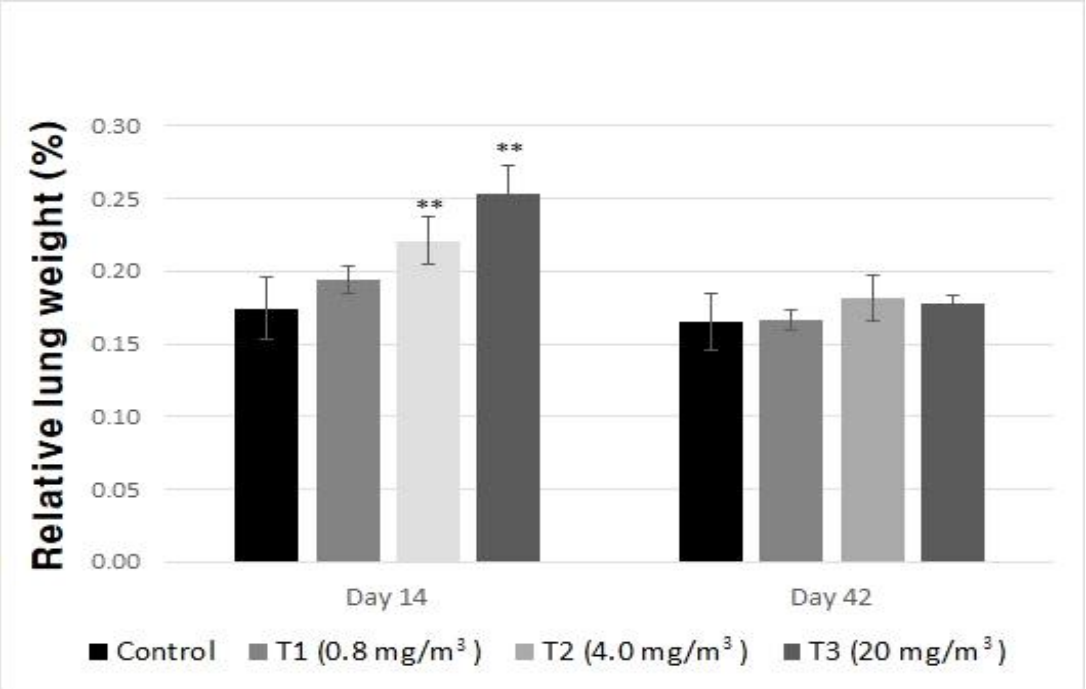

Relative lung weight (%)
0.30
0.25
0.20
0.15
0.10
0.05
0.00
Day 14
Day 42
Control
T1 (0.8 mg/m³)
T2 (4.0 mg/m³)
T3 (20 mg/m³)

Figure 1. Changes in body and lung weights of rats (A-B). Rats were exposed to benzalkonium chloride (BKC) aerosol or clean air for 2 or 6 weeks, T1 = 0.8 mg/m^3, T2 = 4 mg/m^3, and T3 = 20 mg/m^3. Rats were sacrificed at 14 or 42 days after the initial exposure. Changes in body weight over time were calculated in rats belonging to different groups (A). The relative weight of the left lung was calculated as the ratio of the lung weight (mg) to the terminal body weight (g) of each rat (B). The values were expressed as mean ± SD (n = 5 per group). * represents statistical significance as compared to the control group, $p < 0.05$. ** represents statistical significance as compared to the control group, $p < 0.01$.

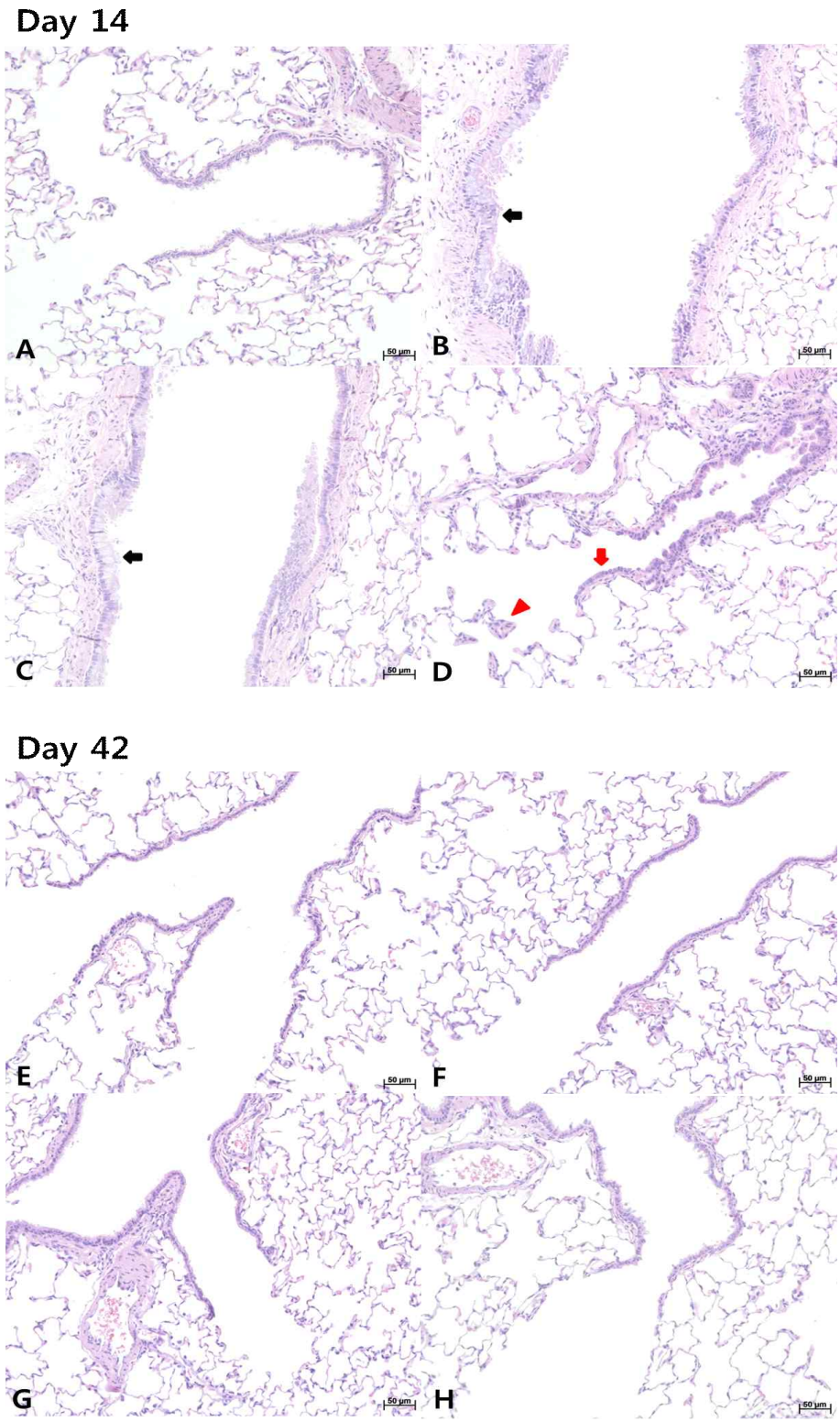

Day 14
A
B
C
D
Day 42
E
F
G
H

Figure 2. Histopathological changes in hematoxylin and eosin (H&E) staining in lung tissue (A-H). Paraffin sections from left lung of rats exposed to benzalkonium chloride (BKC) aerosol inhalation were stained with H&E. Black arrows indicate hypertrophy and hyperplasia; red arrows indicate degeneration and regeneration; red filled triangles indicate smooth muscle hypertrophy. Scale bar = 50μm (200X magnification).

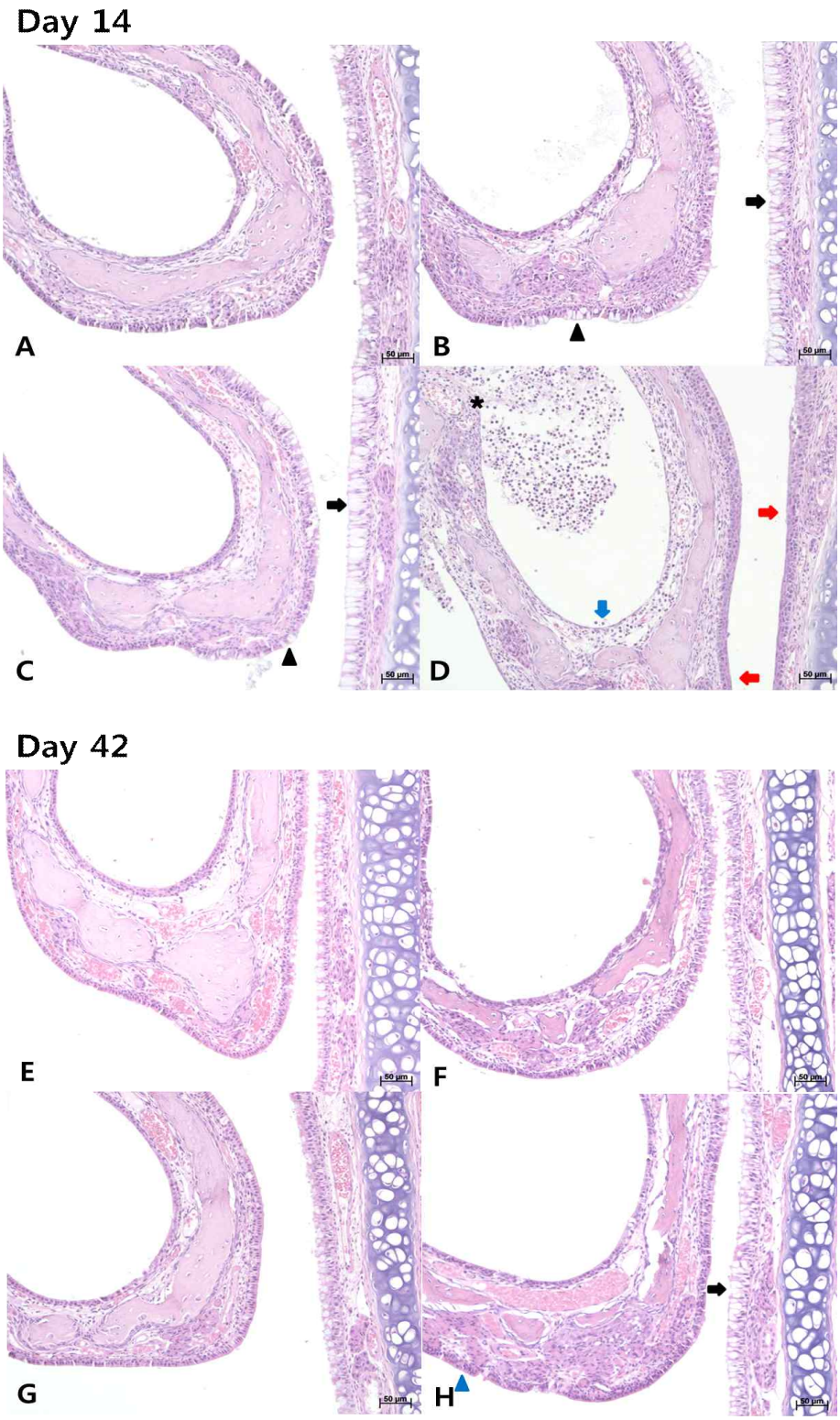
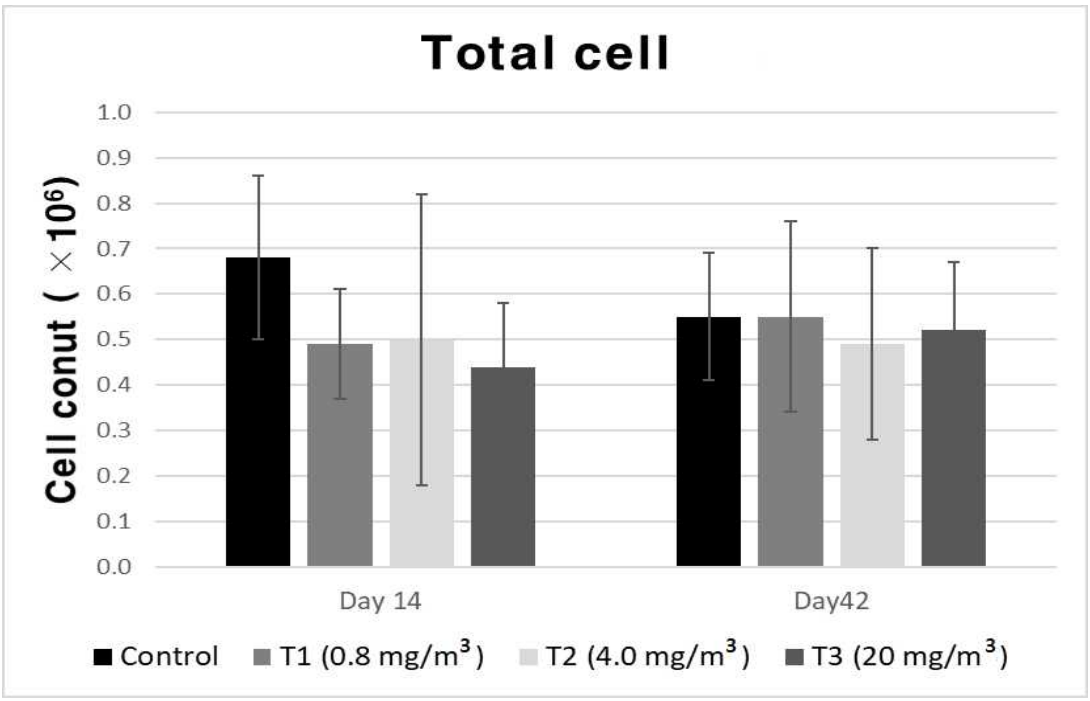
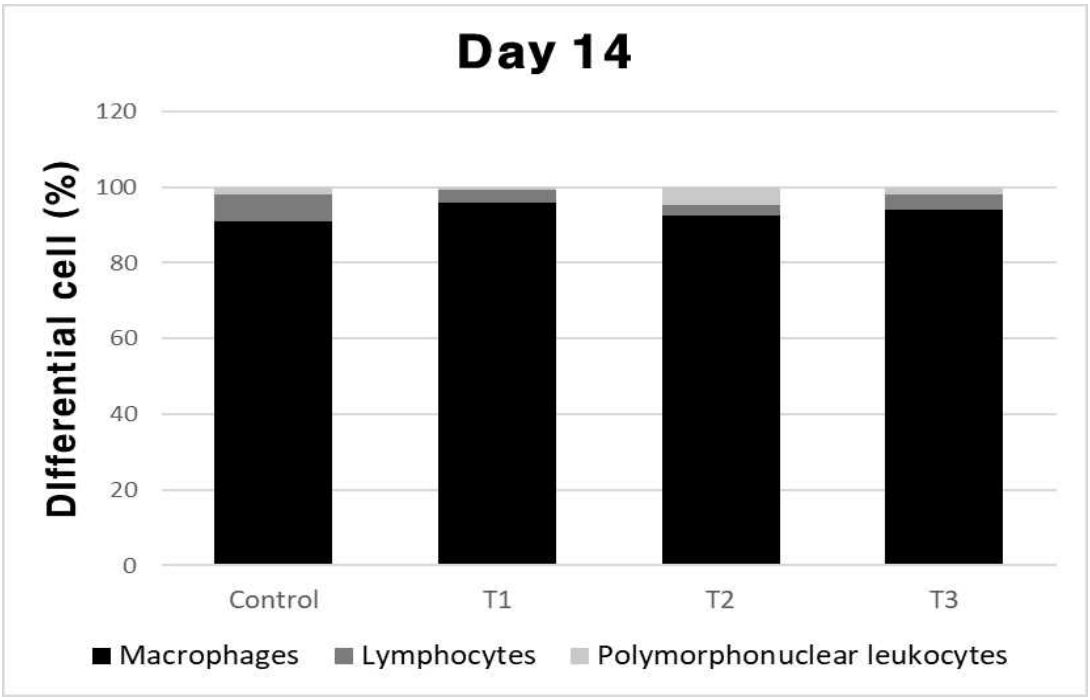

Day 14
A
B
C
D
Day 42
E
F
G
H

Figure 3. Histopathological changes in hematoxylin and eosin (H&E) staining in nasal tissue (A-H). Paraffin sections from nasal tissues of rats exposed to benzalkonium chloride (BKC) aerosol inhalation were stained with H&E. Black arrows indicate hypertrophy and hyperplasia; black filled triangles indicate metaplasia; red arrows indicate squamous metaplasia; blue arrow indicates erosion with necrosis; asterisk indicates ulceration with suppurative inflammation; blue filled triangle indicates hyperplasia. Scale bar = 50μm (200X magnification).

(A)


Total cell
Cell conut (×10⁶)
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Day 14
Day42
Control
T1 (0.8 mg/m³)
T2 (4.0 mg/m³)
T3 (20 mg/m³)

(B)


Day 14
DIfferential cell (%)
120
100
80
60
40
20
0
Control
T1
T2
T3
Macrophages
Lymphocytes
Polymorphonuclear leukocytes

(C)

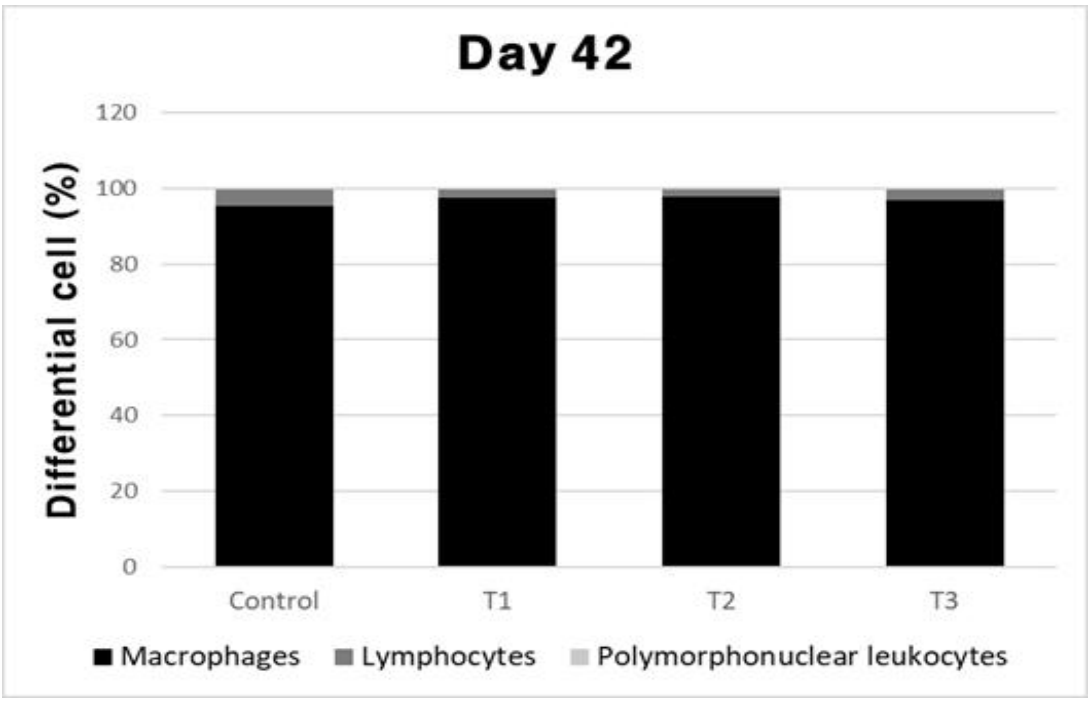
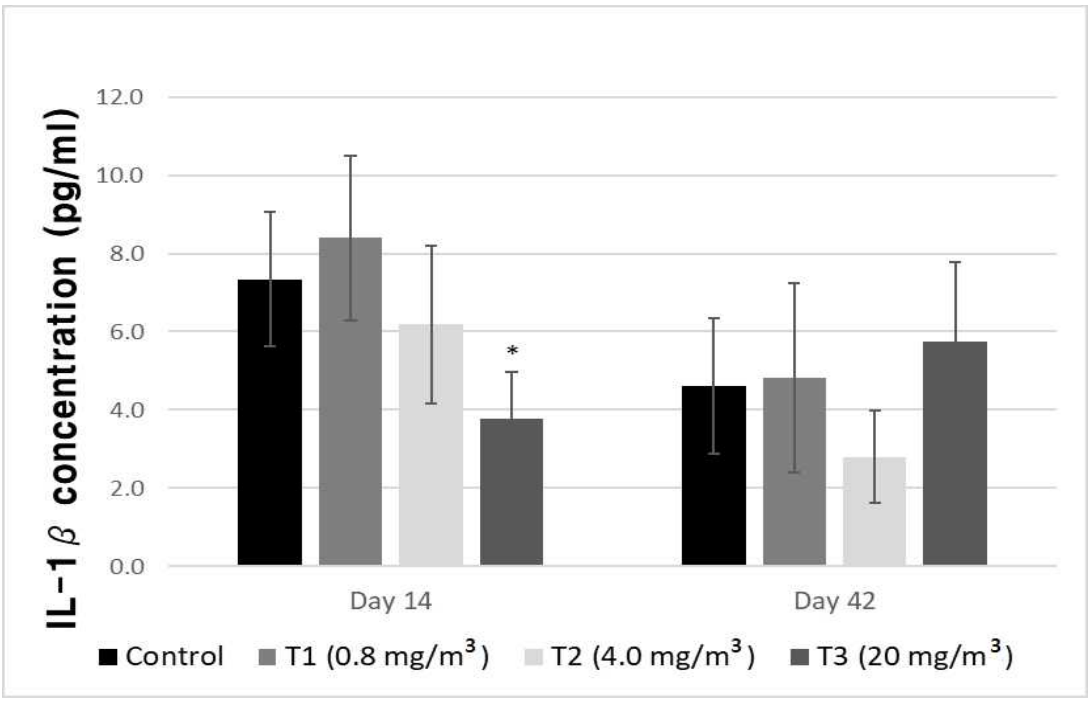
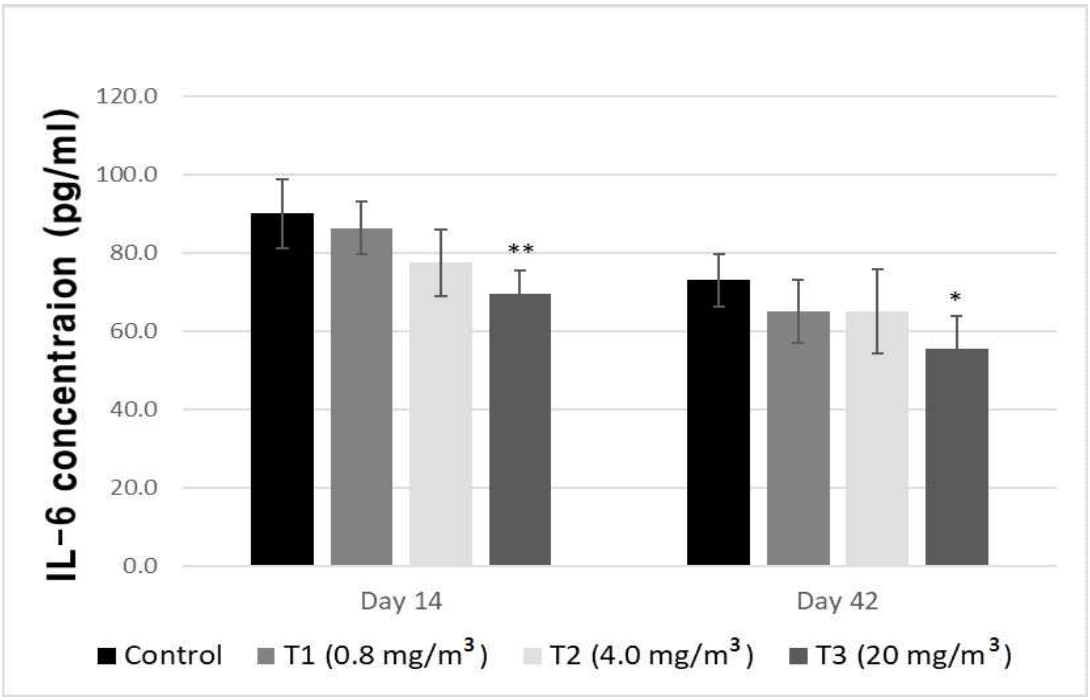

Day 42
Differential cell (%)
120
100
80
60
40
20
0
Control
T1
T2
T3
Macrophages
Lymphocytes
Polymorphonuclear leukocytes

Figure 4. Total cell counts from bronchoalveolar lavage (BAL) and composition of cell population as a percentage of total cells after benzalkonium chloride (BKC) exposure (A-C). The number of total cells in the bronchioalveolar lavage fluid and the composition of cell populations as a percentage of total cells, in control, T1 (0.8 mg/m^3), T2 (4 mg/m^3), and T3 (20 mg/m^3) groups are shown. The values were expressed as mean ± SD (n = 5 per group). No statistically significant differences were found between rats in BKC exposed groups as compared to control group.

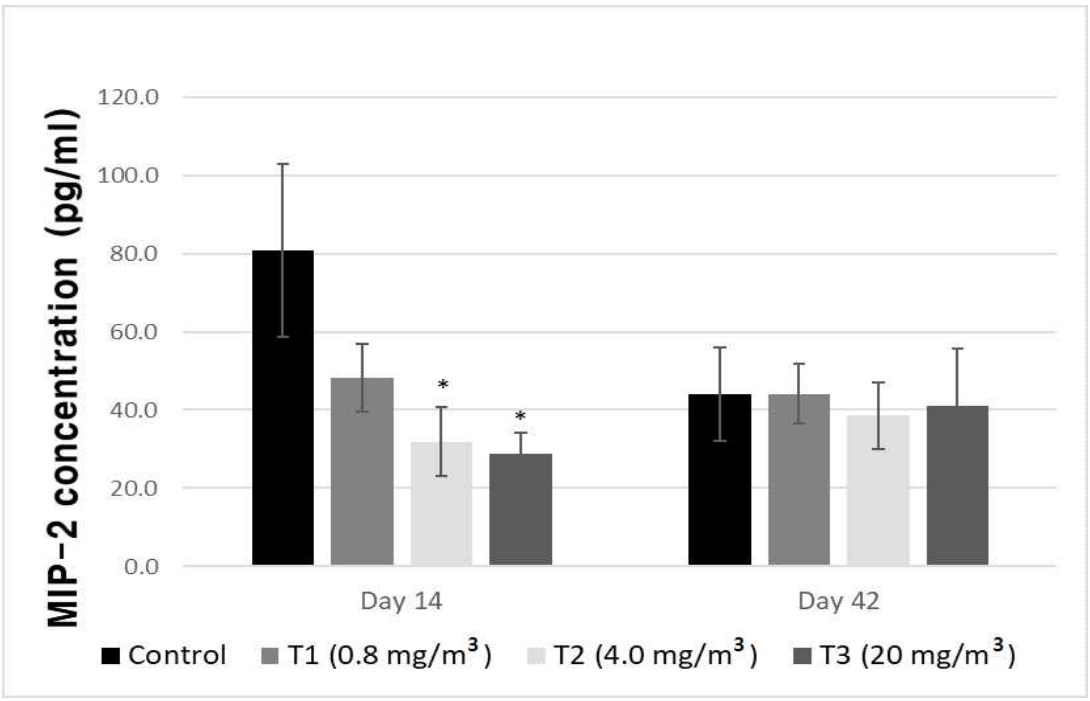
(A)


IL-1β concentration (pg/ml)
12.0
10.0
8.0
6.0
4.0
2.0
0.0
*
Day 14
Day 42
Control
T1 (0.8 mg/m3)
T2 (4.0 mg/m3)
T3 (20 mg/m3)

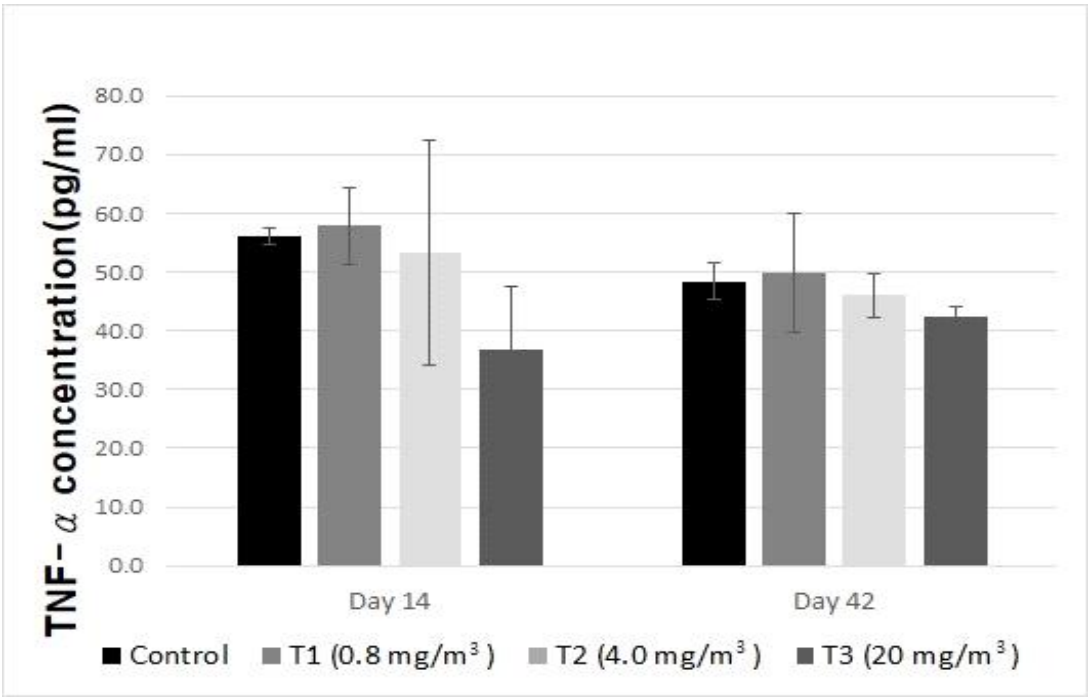
(B)


IL-6 concentraion (pg/ml)
120.0
100.0
80.0
60.0
40.0
20.0
0.0
**
*
Day 14
Day 42
Control
T1 (0.8 mg/m3)
T2 (4.0 mg/m3)
T3 (20 mg/m3)

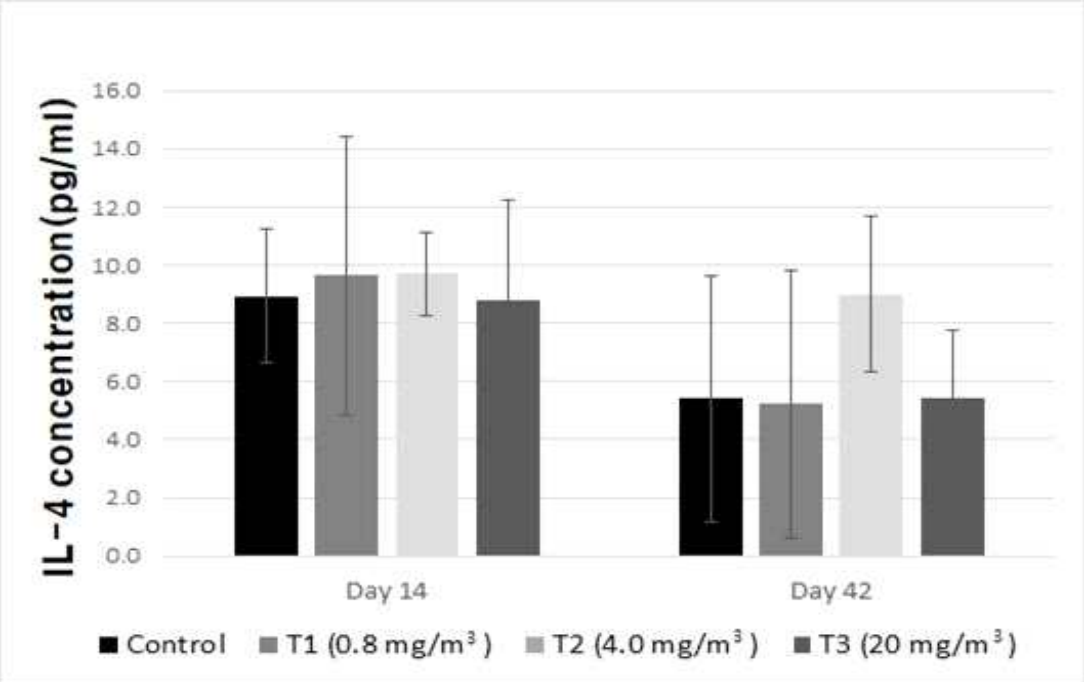
(C)


MIP-2 concentration (pg/ml)
120.0
100.0
80.0
60.0
40.0
20.0
0.0
*
*
Day 14
Day 42
Control
T1 (0.8 mg/m3)
T2 (4.0 mg/m3)
T3 (20 mg/m3)

(D)


TNF-α concentration(pg/ml)
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
Day 14
Day 42
Control
T1 (0.8 mg/m3)
T2 (4.0 mg/m3)
T3 (20 mg/m3)

(E)


IL-4 concentration(pg/ml)
16.0
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
Day 14
Day 42
Control
T1 (0.8 mg/m³)
T2 (4.0 mg/m³)
T3 (20 mg/m³)

(F)

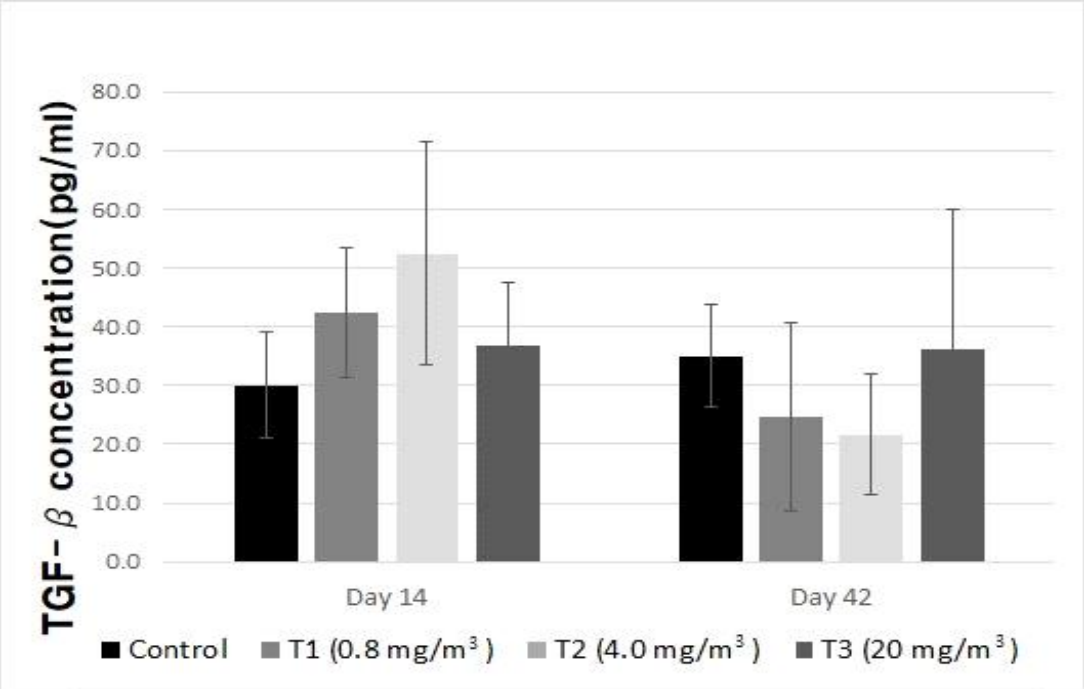

TGF-β concentration(pg/ml)
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
Day 14
Day 42
Control
T1 (0.8 mg/m³)
T2 (4.0 mg/m³)
T3 (20 mg/m³)

Figure 5. Changes in concentrations of cytokines in bronchoalveolar lavage fluid (A-F). Levels of IL-1β, IL-6, MIP-2, TNF-α, IL-4, and TGF-β in the bronchioalveolar lavage fluid of rats were measured and were detected by magnetic bead array. The values were expressed as mean ± SD (n = 5 per group). * represents statistical significance as compared to the control group, $p < 0.05$. ** represents statistical significance as compared to the control group, $p < 0.01$.

Table 1. Particle size distribution of Benzalkonium chloride aerosol.

	Control	T1 (0.8 mg/m^3)	T2 (4 mg/m^3)	T3 (20 mg/m^3)
Mass Median Aerodynamic Diameter (MMAD) (㎛)	ND	1.614	1.090	1.215
Geometric Standard Deviation (GSD)	ND	2.00	1.86	1.51

ND, Non-detectable.

Particle size distributions of benzalkonium chloride (BKC) aerosols in the inhalation chambers. The number of aerosol particles in the chamber was checked using a real time portable aerosol spectrometer.

Table 2. Changes in food consumption of rats after benzalkonium chloride (BKC) inhalation exposure.

(Unit, g)

Days after inhalation exposure	Control	T1 (0.8 mg/m^3)	T2 (4 mg/m^3)	T3 (20 mg/m^3)
8	20.33 ±0.58	17.58 ±1.05	14.19 ±1.07*	11.65 ±1.11**
14	21.22 ±1.36	18.69 ±1.13	15.05 ±0.84*	5.74 ±1.42**
22	19.95 ±3.40	16.50 ±4.88	18.81 ±1.98	16.14 ±1.28
27	20.73 ±3.39	22.19 ±1.31	19.43 ±1.63	21.57 ±0.78
35	17.19 ±1.40	18.64 ±4.42	18.08 ±1.54	19.19 ±0.97
42	19.66 ±2.04	20.63 ±3.10	18.63 ±1.89	19.77 ±1.47

The values are expressed as mean ± SD (n = 5 per group). * represents statistical significance as compared to the control group, $p < 0.05$. ** represents statistical significance as compared to the control group, $p < 0.01$

국문초록

랫드를 이용한 벤잘코늄염화물의 6주 흡입 노출 평가

벤잘코늄염화물은 살생물제로 흔히 사용되고 있어 지속적인 흡입노출이 될 가능성이 높지만 반복적인 흡입노출에 대한 영향 연구는 부족하다. 본 연구는 벤잘코늄염화물을 흡입노출 시킨 랫드를 이용하여 벤잘코늄염화물 에어로졸이 호흡기관에 미치는 영향을 시간 그리고 농도 의존적인 방식으로 확인하기 위해서 조직병리학적 검사와 함께 기관지폐포세척술을 실시하여 면역세포수와 사이토카인의 농도를 확인하였다. 랫드는 2주 그리고 추가로 4주(총 6주) 동안 0 (대조군), 0.8mg/m^3 (T1군), 4mg/m^3 (T2군), 20mg/m^3 (T3군)의 농도로 하루 6시간동안 벤잘코늄염화물 에어로졸에 전신흡입 노출되었고 14일과 42일에 부검을 실시하였다. 2주 동안의 흡입노출 후 조직병리학적 검경을 실시한 결과 0.8, 4, 20mg/m^3 노출군의 폐조직과 비강조직에서 변화가 관찰되었고 IL-1β, IL-6, MIP-2의 농도가 대조군에 비해 노출군에서 감소하였다. 그러나 4주의 추가적인 노출 후 이러한 변화들은 회복되었다. 4주의 추가적인 흡입노출 후 변화들이 감소하였으나, 2주의 벤잘코늄염화물 흡입노출 후 0.8mg/m^3 농도 이상으로 흡입 노출된 실험동물의 폐와 비강에서 벤잘코늄염화물 에어로졸과 관련된 변화들이 관찰되었으므로 벤잘코늄염화물 에어로졸의 흡입노출에 대한 최대무독성용량을 0.8mg/m^3 이하로 제안한다.

주요어 : 벤잘코늄염화물, 사이토카인, 흡입노출, 랫드, 호흡기관, 최대무독성용량
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