Bull Environ Contam Toxicol (2012) 89:718–722
DOI 10.1007/s00128-012-0786-5

Sodium Sulfite Enhances Rhinovirus-Induced Chemokine Production in Airway Epithelial Cells

Yoon Hong Chun · Hyun Sook Kim · Huisu Lee · Sulmui Won · Jong-seo Yoon · Hyun Hee Kim · Jin Tack Kim · Joon Sung Lee

Received: 27 June 2012 / Accepted: 7 August 2012 / Published online: 18 August 2012
© Springer Science+Business Media, LLC 2012

Abstract We investigated the effects of sodium sulfite (Na_2SO_3) on rhinovirus (RV)-induced chemokine production in A549 airway epithelial cells. Our results demonstrated that the treatment of A549 cells with 2,500 μM Na_2SO_3 enhanced the mRNA expression of RV-induced interleukin (IL)-8 1.8 fold ($p = 0.025$); and regulated on activation, normal T cell expressed and secreted (RANTES), 2.9 fold ($p = 0.025$). Moreover, the secretion of IL-8, RANTES, and interferon-γ-inducible protein (IP)-10 was increased in a statistically significant manner without affecting cell viability and RV replication. Our results suggest that Na_2SO_3 may potentiate RV infection by enhancing chemokine production.

Keywords Sodium sulfite · Rhinovirus · Chemokines · Epithelial cells

Rhinoviruses (RVs) are the most common cause of the common cold (Arruda et al. 1997). They are also associated with acute exacerbations of asthma (Corn et al. 2002) and chronic obstructive pulmonary disease (Papi et al. 2006). RVs are believed to directly infect the airway epithelium and to induce proinflammatory cytokine production (Edwards et al. 2007), leading to the recruitment and activation of inflammatory cells and thereby resulting in airway inflammation (Jackson and Johnston 2010).

Y. H. Chun · H. S. Kim · H. Lee · S. Won · J. Yoon (✉) · H. H. Kim · J. T. Kim · J. S. Lee
Department of Pediatrics, School of Medicine, The Catholic University of Korea, 505 Banpo-dong, Seocho-gu, Seoul 137-701, Republic of Korea
e-mail: pedjsyoon@catholic.ac.kr

Inhaled sulfur dioxide (SO_2) can easily be hydrated to yield sodium sulfite (Na_2SO_3) in the respiratory tract. Further, inhaling SO_2 has been reported to cause tissue damage and bronchoconstriction (Balmes et al. 1989) and may impair the immunity and defense function of the respiratory system (Basbaum et al. 1990). Moreover, Na_2SO_3 has been shown to possess pro-inflammatory properties and to enhance the release of IL-8 from airway epithelial cells (Yang et al. 2009). Several studies have indicated that elevated ambient SO_2 levels are associated with an increased risk of respiratory tract infection (Barnett et al. 2005; Love et al. 1981; Luginaah et al. 2005; Wilson et al. 2005), suggesting that Na_2SO_3 may influence the susceptibility to respiratory tract infection. However, little is known about the underlying mechanism. Therefore, we investigated the effects of Na_2SO_3 on the production of RV-induced chemokines in airway epithelial cells.

Materials and Methods

A549 alveolar epithelial type II-like cells were purchased from the American Type Culture Collection (ATCC, Rockville, MD, USA) and were grown in F-12 K Nutrient Mixture (Gibco, Grand Island, NY, USA) supplemented with 10 % fetal bovine serum (FBS, PAA, Pasching, Austria), 100 U/mL of penicillin, and 100 U/mL of streptomycin at 37°C in 5 % CO_2. Tests for detecting mycoplasmal contamination of the cells were performed routinely when new stocks were thawed by using a mycoplasma detection kit for conventional PCR (Minerva Biolabs, Berlin, Germany). RV-7 (ATCC) was purified and prepared as a stock, as previously described (Bartlett et al. 2008).

A549 cells were seeded into each well of a 96-well plate (Nunc, Roskilde, Denmark) at 1×10^5 cells/well in 200 μL

of media with 10 % FBS. Cells were cultured at 37°C in a humidified 5 % CO_2 incubator for 24 h. After the culture medium was aspirated, the cells were rinsed with phosphate buffered saline (PBS, Welgene, Daegu, Republic of Korea). Next, we performed experiments by exposing the cells to 3 different conditions, using media with 2 % FBS. In the Na_2SO_3 group, the cells were treated with Na_2SO_3 at 2,500 μmol/well (Sigma, St. Louis, MO, USA) for 6 h. Afterwards, the medium was replaced with Na_2SO_3-free medium, and the cells were cultured at 33°C for 18 h. In the RV group, the cells were cultured with medium alone at 37°C for 6 h and then infected with RV-7 at 1×10^4 $TCID_{50}$/mL at 33°C for 2 h. Thereafter, the viral solution was removed, and the cells were rinsed with PBS and cultured at 33°C for 16 h. In the Na_2SO_3 plus RV group, the cells were treated with Na_2SO_3 at 2,500 μmol/well for 6 h at 37°C, infected with RV-7 for 2 h at 33°C, and then cultured at 33°C for 16 h. Moreover, we conducted experiments using inactivated RV-7 as a negative control. RV-7 was inactivated by placing viruses thawed in PBS at a distance of 4 cm from a 30 W UV light source for 24 h. After cell culture, the supernatants were removed and stored at −70°C for later assaying interleukin (IL)-8; regulated on activation, normal T cell expressed and secreted (RANTES); and interferon-γ-inducible protein (IP)-10. The cells were rinsed with PBS and harvested for later assaying the mRNA levels of each chemokine. All experiments were performed 3 times.

The harvested cells were lysed, and total RNA was extracted using TRIzol reagent (Life Technologies, Rockville, MD, USA) according to the manufacturer's protocol. One microgram of total RNA was converted to cDNA using the High Capacity RNA-to-cDNA Kit (Applied Biosystems, Foster city, CA, USA). The cDNA products were stored in aliquots at −80°C until needed. Real-time quantitative PCR was performed in triplicate in 96-well plates; each 20-μL reaction mixture consisted of 10 μL of 2× SYBR I Mix (Roche, Basel, Switzerland), 0.8 μL of 10 pmol forward and reverse primers, and 0.5 μL of cDNA. Oligonucleotide PCR primer pairs were designed as follows: IL-8, 5′-AAGA AACCACCGGAAGGAAC-3′ (forward) and 5′-AGCTG CAGAAATCAGGAAGG-3′ (reverse); and RANTES, 5′-GGTTCTGAGCTCTGGCTTTG-3′ (forward) and 5′-GC CAGTAAGCTCCTGTGAGG-3′ (reverse). After reverse transcription, the PCR reaction was performed in an ABI7900HT real-time sequence detection system programmed for 40 cycles of denaturation for 30 s at 95°C, annealment for 30 s at 60°C, and extension for 30 s at 72°C. The expression of the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) also was assayed using reagents obtained from Applied Biosystems. The mRNA levels of IL-8 and RANTES were calculated by using a comparative parameter threshold cycle and normalized to GAPDH.

The level of IL-8, RANTES, and IP-10 were determined by ELISA using the Bio-Plex Pro Human Cytokine assay 8-plex (Bio-Rad, Hercules, CA, USA), according to the manufacturer's protocol. The assay dynamic ranges were 1.9–26,403 pg/mL for IL-8, 2.2–8,617 pg/mL for RANTES, and 18.8–26,867 pg/mL for IP-10. Data were expressed in pg/mL and were derived by extrapolation from standard curves generated in parallel with each experiment.

We measured the cell viability for all the above experimental conditions by performing the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay (Bayram et al. 2006). Briefly, after each experiment, A549 cells were washed with PBS. Next, we suspended the cells in 100 μL of serum-free F-12 K Nutrient Mixture and added 10 μL of MTT solution (5 mg/mL; Amresco, Olon, OH, USA). The cells were then incubated in a humidified 5 % CO_2 incubator at 37°C for 3 h. The MTT solution was removed and replaced with dimethyl sulfoxide (DMSO, Sigma-Aldrich, St. Louis, MO, USA). The change in color was read at 540 nm using a colorimetric plate reader (PowerWave XS, BIO-TEK, Winooski, VT, USA).

We determined whether Na_2SO_3 has an effect on RV replication by performing the same experiment as above for the RV group and Na_2SO_3 plus RV group. After the experiment, we assayed the infectivity of RV-7 in the supernatants using $TCID_{50}$.

Differences between groups were analyzed using the Mann–Whitney U test. All data are expressed as mean values ±SD. A value of $p < 0.05$ was considered statistically significant.

Results and Discussion

IL-8 mRNA levels showed a 1.8-, 1.3-, and 2.3-fold increase in the Na_2SO_3, RV, and Na_2SO_3 plus RV groups, respectively, relative to those of the control group ($p = 0.019$, 0.019, 0.025, respectively). Moreover, the Na_2SO_3 plus RV treatment showed a 1.3-fold increase relative to the Na_2SO_3 treatment ($p = 0.025$) and a 1.8-fold increase compared with RV infection ($p = 0.025$). IL-8 mRNA expression was decreased in the UV-irradiated RV group ($p = 0.025$) and the Na_2SO_3 plus UV-irradiated RV group ($p = 0.025$) compared with the non-UV-irradiated RV group (Fig. 1a).

Na_2SO_3 significantly increased the mean concentration of IL-8 from 201 pg/mL in the control group to 477, 945, and 1,488 pg/mL in the Na_2SO_3, RV, and Na_2SO_3 plus RV groups ($p = 0.025$). Moreover, IL-8 secretion was decreased to 532 pg/mL in the UV-irradiated RV group ($p = 0.025$) and to 1,004 pg/mL in the Na_2SO_3 plus UV-irradiated RV group ($p = 0.025$) relative to the non-UV-irradiated RV group (Fig. 1b).

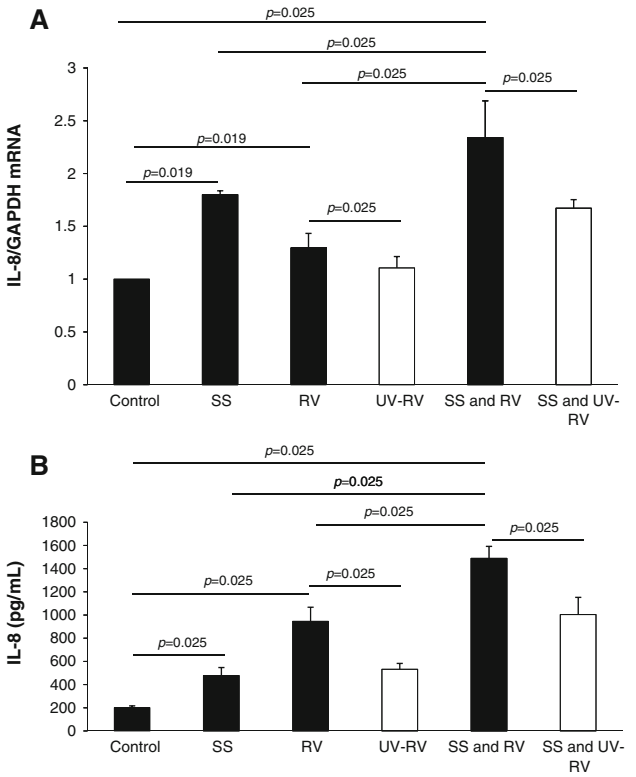

Fig. 1 The effect of Na_2SO_3 on interleukin (IL)-8 production in rhinovirus (RV)-7-infected A549 cells. Cells were treated with 2,500 μmol Na_2SO_3 for 6 h (SS), 1×10^4 $TCID_{50}$/mL RV for 2 h (RV), or both (SS and RV). The effect of UV-irradiated RV (UV-RV) was also examined. **a** IL-8 mRNA expression. The total RNAs from cells were analyzed by qRT-PCR. The amount of IL-8 mRNA was normalized to the amount of GAPDH mRNA and its induction relative to the control. **b** IL-8 secretion. The supernatants were assayed for IL-8 by ELISA. Results represent the mean ± SD of 3 independent experiments. Significant differences ($p < 0.05$) are indicated by the p values

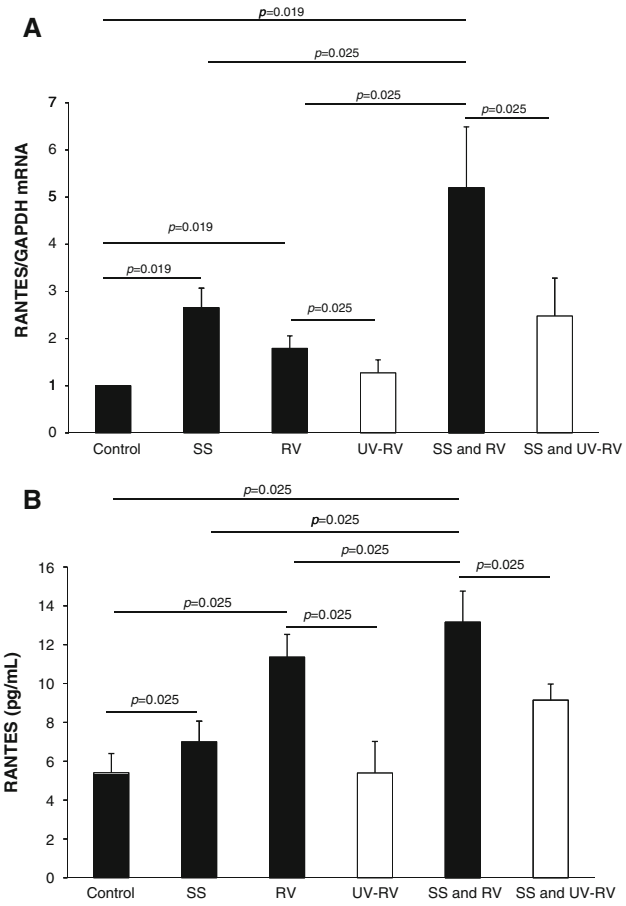

Fig. 2 The effect of Na_2SO_3 on RANTES production in RV-7-infected A549 cells. Cells were treated with 2,500 μmol Na_2SO_3 for 6 h (SS), 1×10^4 $TCID_{50}$/mL RV for 2 h (RV), or both (SS and RV). The effect of UV-irradiated RV (UV–RV) was also examined. **a** RANTES mRNA expression. The total RNAs from cells were analyzed by qRT-PCR. RANTES mRNA levels were normalized to GAPDH and its induction relative to the control. **b** RANTES secretion. The supernatants were assayed for RANTES by ELISA. Results represent the mean ± SD of 3 independent experiments. Significant differences ($p < 0.05$) are indicated by the p values

The RANTES mRNA levels were increased 2.9, 1.8, and 5.2 fold in the Na_2SO_3, RV, and Na_2SO_3 plus RV groups, respectively, compared with those of the control ($p = 0.019$). In addition, the Na_2SO_3 plus RV treatment resulted in a 1.9- and 2.9-fold increase in RANTES mRNA levels compared with Na_2SO_3 treatment ($p = 0.025$) and RV infection ($p = 0.025$), respectively. RANTES mRNA expression was decreased in the UV-irradiated RV group ($p = 0.025$) and the Na_2SO_3 plus UV-irradiated RV group relative to those of the non-UV-irradiated RV ($p = 0.025$) (Fig. 2a).

Na_2SO_3 significantly increased the mean secretion of RANTES from 5.4 pg/mL in the control cells to 7.0, 11.4, and 13.2 pg/mL in the cells of the Na_2SO_3, RV, Na_2SO_3 plus RV groups, respectively ($p = 0.025$). RANTES secretion was decreased to 5.4 pg/mL in the UV-irradiated RV cells ($p = 0.025$) and to 9.1 pg/mL in the Na_2SO_3 plus UV-irradiated RV cells ($p = 0.025$) compared with that of the non-UV-irradiated RV (Fig. 2b).

RV infection significantly increased IP-10 secretion to 5.9 pg/mL from 0 pg/mL in the control cells and the Na_2SO_3 treatment cells ($p = 0.017$). The combined treatment of Na_2SO_3 and RV increased IP-10 secretion to 16.0 pg/mL, which was significantly higher than the value observed for the RV group ($p = 0.023$). IP-10 secretion was decreased to 0 pg/mL in the UV-irradiated RV cells and the Na_2SO_3 plus UV-irradiated RV cells (Fig. 3). The additive experiment of real-time quantitative PCR was not performed because IP-10 mRNA was not detected in the negative control group.

An MTT colorimetric assay was used to determine the survival of A549 cells exposed to Na_2SO_3 and RV-7. Na_2SO_3 at 2,500 μM/well and RV-7 at 1×10^4 $TCID_{50}$/mL had no effect on cell viability up to 48 h (Fig. 4).

We determined the viral titer in the supernatants of RV-infected A549 cells. In the RV plus Na_2SO_3 group, the

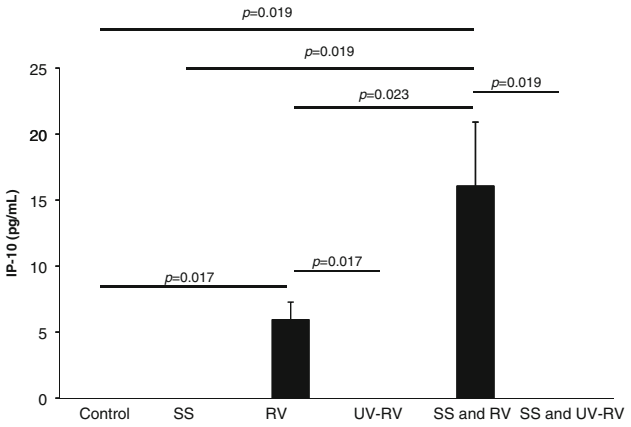

Fig. 3 The effect of Na_2SO_3 on IP-10 production in RV-7-infected A549 cells. Cells were treated with 2,500 μmol Na_2SO_3 for 6 h (SS), 1×10^4 $TCID_{50}$/mL RV for 2 h (RV), or both (SS and RV). The effect of UV-irradiated RV (UV-RV) was also evaluated. The supernatants were assayed for IP-10 by ELISA. Results represent the mean ± SD of 3 independent experiments. Significant differences ($p < 0.05$) are indicated by the p values

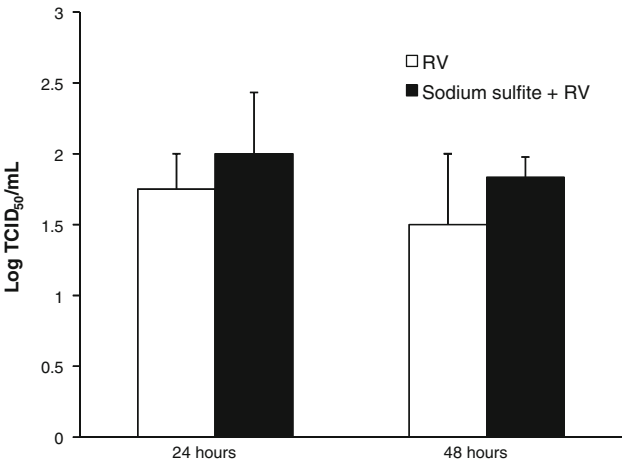

Fig. 5 The effect of Na_2SO_3 on RV-7 replication in A549 cells. Cells were treated with RV-7 (1×10^4 $TCID_{50}$/mL) or Na_2SO_3 (2,500 μmol) plus RV-7. The infectious RV-7 in the supernatants was assayed by viral titration. Results represent the mean ± SD of 3 independent experiments. Significant differences ($p < 0.05$) are indicated by the p values. $TCID_{50}$, 50 % tissue culture infection dose

viral titers were not significantly different from those of the RV group, indicating that Na_2SO_3 treatment did not significantly affect RV replication (Fig. 5).

We have shown here that Na_2SO_3 treatment enhanced RV-induced mRNA expression and secretion of IL-8, RANTES, and IP-10 without affecting cell viability and RV-7 replication in A549 cells. Metropolitan New York reports an increased risk for the development of upper and lower respiratory tract infections in children and adults who reside in areas of the city with the highest ambient air levels of SO_2 (Love et al. 1981). Barnett et al. (2005) found that ambient 1-h SO_2 levels were associated with increases in hospital admissions for respiratory disease and for pneumonia and acute bronchitis in children in 5 Australian and 2 New Zealand cities. Luginaah et al. (2005) also found a small increase in respiratory-related hospital admissions among female children less than 15 years old with a 19.25-ppb interquartile increase in SO_2 at a 1-day lag. Wilson et al. (2005) noted an increase in the relative risk for all respiratory-related and for asthma-related emergency room visits in 2 New England cities with a 10 $\mu g/m^3$ increase in ambient SO_2 concentrations.

However, we were unable to find a study investigating the mechanism for the effect of SO_2 on respiratory tract

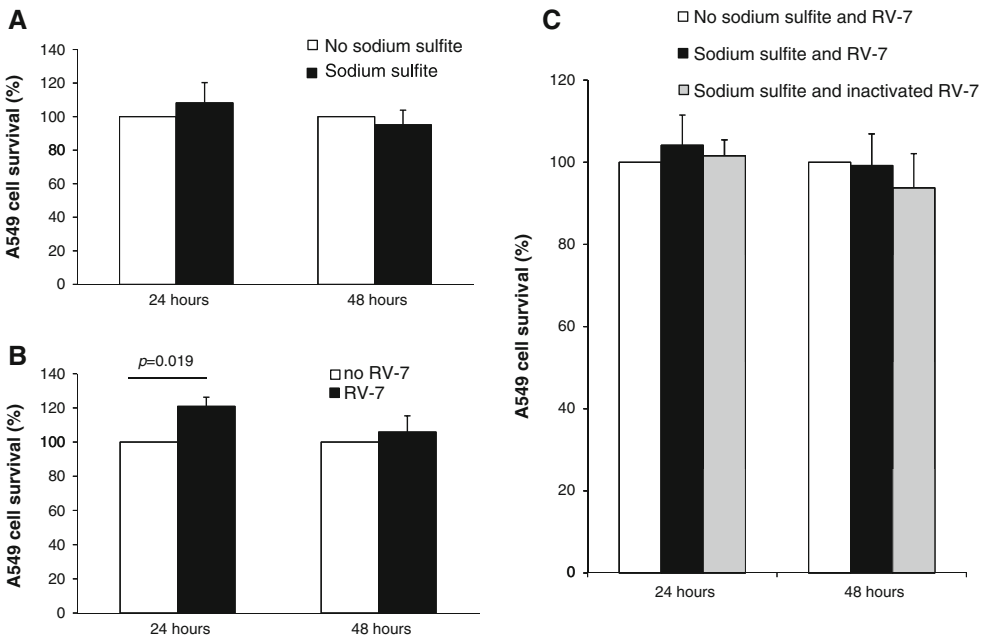

Fig. 4 The effect of Na_2SO_3 (2,500 μmol), RV-7 (1×10^4 $TCID_{50}$/mL), or both on the viability of A549 cells, as assessed by the MTT assay. **a** sodium sulfite **b** RV **c** both. Results represent the mean ± SD of 3 independent experiments

infection, but Spannhake et al. (2002) have examined the effects of other air pollutants. In these studies, primary human nasal epithelial cells and BEAS-2B cells were grown at the air–liquid interface with RV-16 and exposed to NO_2 (2.0 ppm) or O_3 (0.2 ppm) for 3 h. RV16, NO_2, and O_3 independently and rapidly increased the release of the IL-8 through oxidant-dependent mechanisms. The combined effect of RV-16 and the oxidant was 42 %–250 % and 41 %–67 % greater than that of NO_2 and O_3, respectively. The surface expression of intercellular adhesion molecule 1 underwent additive enhancement in response to combined stimulation. These data indicate that oxidant pollutants can amplify the generation of proinflammatory cytokines by RV16-infected cells and that virus-induced inflammation in the upper and lower airways may be exacerbated by concurrent exposure to ambient levels of oxidants commonly encountered in indoor and outdoor environments. Cigarette smoke extract increased RV-induced Toll-like receptor 3 expression and RV-induced IL-8 secretion at lower concentrations in A549 cells, suggesting that cigarette smoke may potentiate viral common cold symptoms by enhancing IL-8 secretion but not by increasing viral replication (Wang et al. 2009). In our study, we showed that Na_2SO_3, a derivative of the air pollutant SO_2, can enhance RV-induced IL-8, RANTES, and IP-10 production. Interestingly, Na_2SO_3 was unable to induce IP-10 production; however, it was able to enhance IP-10 production by RV-7-infected cells. We suspect this phenomenon of an air pollutant enhancing RV-induced chemokine production in airway epithelial cells may be a general effect of air pollutants and may summarize our results and those of others. This type of experiment may help in understanding how air pollutants, including Na_2SO_3, can affect respiratory tract infection.

In the future, we need to investigate the mechanism of air pollutants on cytokine production in terms of the intracellular signaling pathway using in vitro cell lines and animal models and to examine the effect of drugs on the chemokine production. In conclusion, our study demonstrated that Na_2SO_3 enhances RV-induced chemokine production by airway epithelial cells in vitro. Our results suggest that Na_2SO_3 may potentiate RV infection by enhancing chemokine production.

Acknowledgments This work was supported by a Korea Research Foundation (KRF) grant, funded by the Korean Government (MEST) (No. 2009-0066649) and by Seoul St. Mary's Clinical Medicine Research Program (2009) through the Catholic University of Korea.

References

Arruda E, Pitkäranta A, Witek TJ Jr, Doyle CA, Hayden FG (1997) Frequency and natural history of rhinovirus infections in adults during autumn. J Clin Microbiol 35:2864–2868

Balmes JR, Fine JM, Gordon T, Sheppard D (1989) Potential bronchoconstrictor stimuli in acid fog. Environ Health Perspect 79:163–166

Barnett AG, Williams GM, Schwartz J, Neller AH, Best TL, Petroeschevsky AL, Simpson RW (2005) Air pollution and child respiratory health: a case-crossover study in Australia and New Zealand. Am J Respir Crit Care Med 171:1272–1278

Bartlett NW, Walton RP, Edwards MR, Aniscenko J, Caramori G, Zhu J, Glanville N, Choy KJ, Jourdan P, Burnet J, Tuthill TJ, Pedrick MS, Hurle MJ, Plumpton C, Sharp NA, Bussell JN, Swallow DM, Schwarze J, Guy B, Almond JW, Jeffery PK, Lloyd CM, Papi A, Killington RA, Rowlands DJ, Blair ED, Clarke NJ, Johnston SL (2008) Mouse models of rhinovirus-induced disease and exacerbation of allergic airway inflammation. Nat Med 14:199–204

Basbaum C, Gallup M, Gum J, Kim Y, Jany B (1990) Modification of mucin gene expression in the airways of rats exposed to sulfur dioxide. Biorheology 27:485–489

Bayram H, Ito K, Issa R, Ito M, Sukkar M, Chung KF (2006) Regulation of human lung epithelial cell numbers by diesel exhaust particles. Eur Respir J 27:705–713

Corne JM, Marshall C, Smith S, Schreiber J, Sanderson G, Holgate ST, Johnston SL (2002) Frequency, severity, and duration of rhinovirus infections in asthmatic and non-asthmatic individuals: a longitudinal cohort study. Lancet 359:831–834

Edwards MR, Hewson CA, Laza-Stanca V, Lau HTH, Mukaida N, Hershenson MB, Johnston SL (2007) Protein kinase R, IkappaB kinase-beta and NF-kappaB are required for human rhinovirus induced pro-inflammatory cytokine production in bronchial epithelial cells. Mol Immunol 44:1587–1597

Jackson DJ, Johnston SL (2010) The role of viruses in acute exacerbations of asthma. J Allergy Clin Immunol 125:1178–1187

Love GT, Lan SP, Shy CM, Struba RJ (1981) The incidence and severity of acute respiratory illness in families exposed to different levels of air pollution, New York metropolitan area 1971–1972. Arch Environ Health 36:66–74

Luginaah IN, Fung KY, Gorey KM, Webster G, Wills C (2005) Association of ambient air pollution with respiratory hospitalization in a government-designated 'area of concern': the case of Windsor, Ontario. Environ Health Perspect 113:290–296

Papi A, Bellettato CM, Braccioni F, Romagnoli M, Casolari P, Caramori G, Fabbri LM, Johnston SL (2006) Infections and airway inflammation in chronic obstructive pulmonary disease severe exacerbations. Am J Respir Crit Care Med 173:1114–1121

Spannhake EW, Reddy SPM, Jacoby DB, Yu XY, Saatian B, Tian J (2002) Synergism between rhinovirus infection and oxidant pollutant exposure enhances airway epithelial cell cytokine production. Environ Health Perspect 110:665–670

Wang JH, Kim H, Jang YJ (2009) Cigarette smoke extract enhances rhinovirus-induced toll-like receptor 3 expression and interleukin-8 secretion in A549 cells. Am J Rhinol Allergy 23:e5–e9

Wilson AM, Wake CP, Kelly T, Salloway JC (2005) Air pollution, weather, and respiratory emergency room visits in two northern New England cities: an ecological time-series study. Environ Res 97:312–321

Yang YF, Hsu JY, Fu LS, Weng YS, Chu JJ (2009) Asthma drugs counter-regulate interleukin-8 release stimulated by sodium sulfite in an A549 cell line. J Asthma 46:238–243