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The Effects of Disodium Cromoglycate on Enhanced Adherence of *Haemophilus influenzae* to A549 Cells Infected With Respiratory Syncytial Virus

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ABSTRACT: Nontypeable *Haemophilus influenzae* (NTHi) secondary infection often complicates respiratory syncytial virus (RSV) infections. Previous studies have revealed that RSV infections enhance NTHi adherence to airway epithelial cells. In this study, we investigated the effects of disodium cromoglycate (DSCG) and corticosteroids, which are frequently used for the treatment of wheezing often related to RSV infections, on the adherence of NTHi to RSV-infected A549 cells. DSCG inhibited enhanced adherence of NTHi to RSV-infected A549 cells, whereas dexamethasone (Dex) and fluticasone propionate (Fp) did not. DSCG suppressed the expression of ICAM-1, which is one of the NTHi receptors. Furthermore, DSCG exhibited an inhibitory effect on RSV infections. It is suggested that DSCG exerts an anti-RSV effect, and consequently attenuates the expression of NTHi receptors. **(*Pediatr Res* 66: 168–173, 2009)**

Respiratory syncytial virus (RSV) is one of the major pathogens of upper and lower respiratory tract infections in children. RSV infection at a younger age often involves the lower respiratory tract and is frequently associated with expiratory wheezing, which is referred to as bronchiolitis or wheezy bronchitis, asthma, and pneumonia (1). It is known that RSV infections can be complicated by bacterial superinfections (2–5). Nontypeable *Haemophilus influenzae* (NTHi) is one of the most common bacteria involved in mixed RSV-bacterial bronchopulmonary infections in pediatric patients (2,4). It has long been recognized that a preceding local respiratory viral infection seems to play an important role in the pathogenesis of infections by bacteria, including NTHi. The mechanisms underlying bacterial superinfections include virus-induced local destruction of the epithelium, which compromises the host's physiologic barrier, and virus-induced modulation of the immune response (6). In addition, enhanced bacterial adherence to virus-infected cells is considered an important factor increasing the risk of bacterial superinfections (7).

Recent studies demonstrated that some respiratory viruses including RSV lead to both expression of viral glycoproteins and up-regulation of cellular molecules including ICAM-1 (CD54), carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1), and platelet activating factor receptor (PAFr) on the host-cell membrane. Both could serve as bacterial receptors and promote bacterial adhesion to the cells (8–11). Strategies for preventing interaction between RSV and bacteria may reduce the incidence of secondary bacterial complications of RSV infection.

Disodium cromoglycate (DSCG) and corticosteroids, which are recognized as inhalation drugs for the management of bronchial asthma, are also used for the treatment of acute infantile wheezing and exacerbation of asthma that are often related to RSV infections. The effects of these medicines against RSV infections and secondary bacterial complications are not clear. In this study, we investigated the effects of DSCG and corticosteroids on the *in vitro* interaction between RSV and NTHi.

MATERIALS AND METHODS

Epithelial cell culture. A549 human pneumocyte type II carcinoma cells (RIKEN Cell Bank, Tsukuba, Japan: RCB0098) were used for the RSV infection experiments. A549 cells were grown at 37°C in 5% CO_2 in DMEM (GIBCO, Grand Island, NY) supplemented with 10% heat-inactivated fetal bovine serum (FBS, GIBCO). HEp-2 cells (human laryngeal epithelial carcinoma, RIKEN Cell Bank: RCB1889) were used for RSV growth and plaque assays. HEp-2 cells were grown at 37°C in 5% CO_2 in Minimum Essential Medium Eagle (Sigma Chemical Co., St. Louis, MO) supplemented with 10% heat-inactivated FBS.[1]

Virus. Human RSV serotype A (A2 strain) (provided by Dr. Tsutsumi, Graduate School of Medicine, Sapporo Medical University, Sapporo, Japan) was grown in HEp-2 cells. Supernatant fluids were clarified and titrated for infectivity testing by plaque assay as described previously (12). The viral growth medium comprised Minimum Essential Medium Eagle with 1% heat-inactivated FBS.

Bacteria. NTHi attachment assay was performed with NTHi strain 03H113, 05H11, and 06H18, clinical isolates obtained from the pediatric patients' airways. All the other assays were performed with NTHi strain 03H113. Strain 03H113, 05H11, and 06H118 express high–molecular weight 1 and 2 (HMW1/HMW2) adhesins and P5 fimbriae. Strain 03H113 also expresses Hap and lacks Hia and pilli (Hif A), 05H11 expresses Hap and Hia and lacks Hif A, and 06H18 lacks Hap, Hia, and Hif A. The gene expression of these adhesins was examined by PCR. NTHi were grown on chocolate agar plates at 37°C in 5% CO_2 overnight. One or two colonies were propagated in brain-heart infusion (BHI) broth (Becton Dickinson, MD) supplemented with nicotinamide adenine dinucleotide and haemin (both at 10 mg/L) at 35°C overnight. A portion of this culture was inoculated as a preculture into a fresh sample of BHI broth, at the final concentration of 5%. The new culture was then incubated for 3 h at 35°C. Before assays, bacteria were washed three

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Abbreviations: Dex, dexamethasone; **DSCG,** disodium cromoglycate; **FBS,** fetal bovine serum; **Fp,** fluticasone propionate; **MOI,** multiplicity of infection; **NTHi,** nontypeable *Haemophilus influenzae*; **RSV,** respiratory syncytial virus

times with PBS (Nikken, Kyoto, Japan) and then diluted with tissue culture medium to 1×10^6 CFU/mL. Bacterial numbers were determined by OD at 600 nm and confirmed by plating dilutions on agar plates.

Viral infection. A549 cells were grown as confluent monolayers and then incubated with RSV at a multiplicity of infection (MOI) of 1–7.5 virus/cell for 1 h at 37°C in 5% CO_2. The cells were then washed with PBS, followed by replacement of the medium and incubation for 48 h at 37°C in 5% CO_2.

NTHi attachment assay. The NTHi attachment assay was performed using a modified method described previously (13). The A549 cells were grown as confluent monolayers in 24-well tissue culture plates (IWAKI, ATG, Chiba, Japan). The cells were then inoculated with RSV and incubated for 48 h at 37°C in 5% CO_2. The monolayers were then washed twice, and 1 mL of DMEM-containing NTHi was inoculated into each well (MOI = 1). After incubation for 1 h at 37°C in 5% CO_2, the monolayers were washed gently three times with PBS to remove loosely adherent bacteria. The A549 cells were then detached using 0.05% trypsin-EDTA (GIBCO), and serial dilutions were plated on chocolate agar for the quantitative colony counts. For each assay, triplicate wells for each condition were examined, and the numbers of adherent bacteria were normalized as to the numbers of epithelial cells.

Drugs. DSCG is clinically used as a solvent inhalation for prophylactic and acute treatment for asthma and infantile wheezing. The concentration of the clinically used solvent of DSCG is 10 mg/dL (20 mM). The experiments on DSCG were examined below the concentration of 20 mM, which is thought as clinically relevant, and no visible cytotoxicity was observed morphologically, though the concentration at pulmonary alveolous is unknown. DSCG [Intal; disodium 5,5′-(2-hydroxytrimethlenedioxy) bis 4-oxo-4*H*-1-benzopyran-2-carboxylate] was kindly provided by Astellas Pharmaceutical Co., Ltd., Tokyo, Japan. Dexamethasone crystalline (Dex) and fluticasone propionate (Fp) were examined at the concentrations below 10^{-6}M, which have been reported to exhibit anti-inflammatory effect *in vitro*. Dex and Fp were from Sigma Chemical Co.

Cell surface receptor expression. It has been reported that ICAM-1 expressed by airway epithelial cells is one of the major NTHi receptors (8,14), and RSV infection up-regulates ICAM-1 expression by A549 cells (8). Previous studies have revealed that Dex- or Fp-attenuated cytokine-induced ICAM-1 expression in human airway epithelial cells *in vitro* (15,16) and ICAM-1 expression in the bronchial epithelium was inhibited after treatment with inhaled DSCG in patients with bronchial asthma *in vivo* (17).

Therefore, first we determined the effects of DSCG, Dex, and Fp on the cytokine-induced ICAM-1 expression in A549 cells. As reported previously (16), A549 cells were incubated with IL-4 (Sigma Chemical Co., 20 ng/mL) plus TNF-α (Sigma Chemical Co., 20 ng/mL) for 24 h to induce ICAM-1 expression. ICAM-1 expression in A549 cells was assayed by fluorescence-activated cell sorting (FACS). A549 cells were grown as confluent monolayers in 6-well tissue culture plates and stimulated with cytokines or inoculated with RSV, and then incubated with or without each drug. At 24- to 48-h intervals, A549 cells were detached from the plates using Cell Dissociation Solution (Sigma Chemical Co.) and washed with PBS, and 10^6 cells were resuspended in 100 μL of FACS buffer (1% FBS and 0.1% sodium azide in PBS). Cells were incubated with 20 μL of phycoerythrin (PE)-conjugated mouse anti-human ICAM-1 (CD54) MAb (IgG1; Becton Dickinson Biosciences, Cockeysville, MD) or an isotype-matched control antibody (IgG1; Becton Dickinson Biosciences) for 30 min at 4°C. Cells were then washed extensively and fixed with Cell FIX (Becton Dickinson Biosciences) and analyzed on a flow cytometer (FACS Calibur, Becton Dickinson Biosciences). The mean fluorescence intensity of cells was estimated after subtracting the background produced by the isotype control Ab.

Inhibition of bacterial adhesion. The ability of NTHi to adhere to RSV-infected A549 cells was assessed by blocking ICAM-1 on the cell surface. A549 cells at 48 h after RSV-infection (MOI = 2.5) were incubated with different concentrations (5–50 μg/mL) of purified mouse anti-human ICAM-1 MAb (Calbiochem, Darmstadt, Germany; clone 8.4A6; IgG_1, Alexis Biochemicals, Lausen, Switzerland; clone RR1/1; IgG_1) or the isotype control (IgG1; BD Biosciences, San Jose, CA) for 1 h at 37°C in 5% CO_2 and then washed with PBS, followed by NTHi adhesion assay.

Effects of drugs on RSV infection. A549 cells were grown as confluent monolayers in 6-well tissue culture plates and then inoculated with RSV at MOI of 2.5. After viral adsorption, A549 cells were incubated with or without each drug. Furthermore, to determine the inhibitory effect of DSCG on RSV infection, treatment with DSCG was evaluated using two other protocols involving treatment of DSCG at different points in viral infection as described previously (18). For treatment of cells before viral adsorption, cells were cultured for 24 h at 37°C in 5% CO_2 in the presence or absence of DSCG. For treatment of cells during viral adsorption, viral solutions were first preincubated for 30 min at room temperature with the indicated concentrations of DSCG, and then inoculated with the virus-DSCG mixtures. To determine cell-associated viral contents, the cells were washed with PBS extensively and replaced with new medium at 48 h after viral inoculation, after which the cells were harvested with cell scrapers (IWAKI), homogenized by secure vortexing for 1 min and spun down, and then the supernatants were stored at −80°C. Cell-associated viral contents were quantitated by plaque assay using HEp-2 cells as described previously (12).

Viral syncytium assay. Monolayer cultures of A549 cells in 6-well culture plates were infected with RSV at MOI of 2.5 at 37°C in 5% CO_2. After a 1-h adsorption period, the monolayers were washed with PBS and then overlaid with fresh medium with indicated concentrations of drugs. At 48-h post infection, the cell monolayers were examined microscopically for syncytium formation.

Statistical analysis. Each NTHi adherent assay and RSV plaque assay was performed in triplicate of wells, and the results are expressed as the means ± SD. Between-group comparisons were tested using Mann-Whitney's *U* test. $p < 0.05$ was considered significant.

This study has been approved by the Institutional Review Board of Chiba University.

RESULTS

NTHi adherence to RSV-infected/noninfected A549 cells. The number of any NTHi strain of 03H113, 05H11, or 06H18 attached to RSV-exposed A549 cells was significantly higher than for noninfected A549 cells ($p < 0.05$; Fig. 1*A*). Because the attachment assay of these three strains exhibited similar results, the additional analyses were performed using the strain 03H113. The adhesion of NTHi (strain 03H113) to A549 cells at 48 h after inoculation with RSV at MOI of 2.5 and 7.5 increased by 12.7- and 16.9-fold, respectively (Fig. 1*B*).

Effects of drugs on NTHi adherence to RSV-infected A549 cells. To determine the effects of DSCG, Dex, and Fp on the RSV-induced increase in NTHi adherence to A549 cells, A549 cells inoculated with RSV at MOI of 2.5 were incubated with medium containing the indicated concentra-

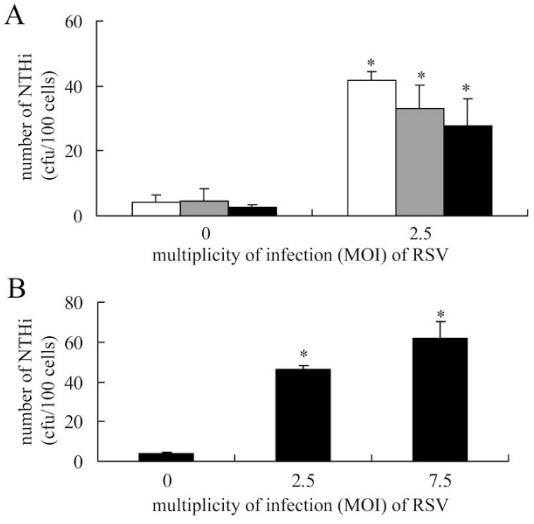

Figure 1. Adhesion of NTHi to A549 cells increased after infection with RSV. A549 cells were incubated with NTHi at 48 h after RSV inoculation. The numbers of adherent bacteria were normalized as to those of epithelial cells. *Panel A* shows that antecedent infection with RSV resulted in a statistically significant increase in adhesion of NTHi strain 03H113 (*white bar*), 05H11 (*gray bar*), or 06H18 (*black bar*) compared with noninfected cells (*$p < 0.05$). *Panel B* shows that adherence of NTHi (strain 03H113) increased with the MOI of RSV. Data are expressed as the means ± SD for three samples. Between-group comparisons were performed using Mann-Whitney's *U* test. *$p < 0.05$ *versus* noninfected control.

tions of the drugs for 48 h at 37°C in 5% CO_2. DSCG reduced the number of NTHi attached to RSV-infected A549 cells, significantly ($p < 0.05$; Fig. 2*A*). At the concentration of 20 mM, DSCG reduced NTHi adherence to 30% of that in the vehicle control. Meanwhile, Dex and Fp did not reduce NTHi adherence to RSV-infected cells significantly (Fig. 2*B*).

Effects of drugs on ICAM-1 expression by A549 cells. The increase of ICAM-1 expression caused by A549 cells at 48 h after RSV infection was investigated by FACS analysis. The increase in ICAM-1 expression was RSV dose dependent (Fig. 3*A*).

To determine the contribution of ICAM-1 to NTHi adherence to RSV-infected cells, we performed an inhibition study on ICAM-1. Blocking of ICAM-1 by preincubating RSV-infected A549 cells with anti-ICAM-1 MAb reduced the number of adherent bacteria. Anti-ICAM-1 MAb (25 μg/mL) inhibited NTHi adhesion by 49% compared with the isotype control ($p < 0.05$; Fig. 3*B*).

DSCG, Dex, and Fp reduced the cytokine-induced and RSV infection-induced ICAM-1 expression. The reducing effects of each drug were dependent on the concentration of the drug, and the maximum reducing concentrations of each drug are shown in Figure 3*C* and *D*. DSCG, Dex, and Fp reduced the cytokine-induced ICAM-1 expression significantly compared with the control, whereas no significant differences between the drugs were observed (Fig. 3*C*). Meanwhile, a reducing effect on RSV infection-induced ICAM-1 expression by

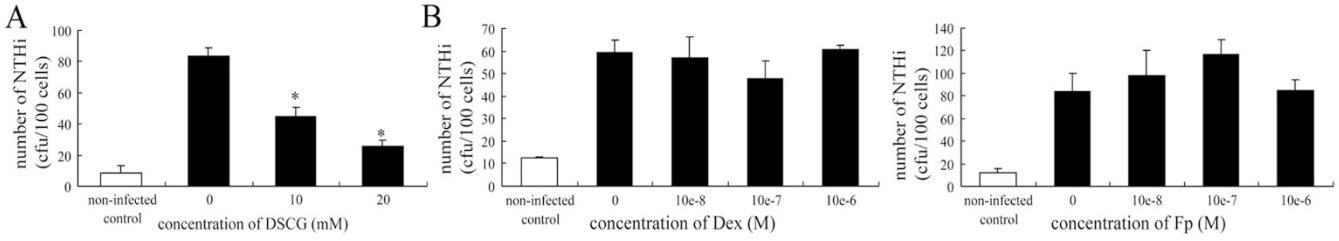

Figure 2. Effects of drugs on NTHi adherence to A549 cells infected with RSV (MOI = 2.5, 48 h) are shown by *black bars*. The results for RSV noninfected controls are shown by *white bars* (*A*, *B*). Between-group comparisons were performed using Mann-Whitney's *U* test. Incubation with DSCG for 48 h after RSV adsorption reduced the number of NTHi attached to RSV infected-A549 cells compared to that without DSCG significantly (*$p < 0.05$; *A*). Dex and Fp did not reduce NTHi adherence to RSV-infected A549 cells significantly (*B*). Data are expressed as the means ± SD for three samples.

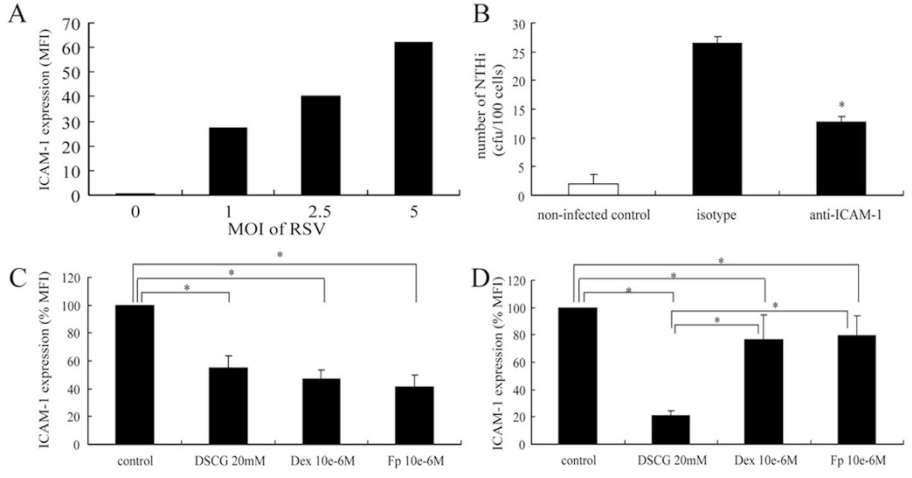

Figure 3. *A*, ICAM-1 expression on A549 cells post-RSV infection (48 h) was analyzed by FACS. ICAM-1 expression on A549 cells infected with RSV increased with the MOI of RSV. ICAM-1 expression is expressed as the mean fluorescence intensity (MFI) of cells. These experiments were repeated more than twice with similar results. *B*, Adhesion of NTHi to A549 cells infected with RSV was blocked by anti-ICAM-1 MAb or isotype control. The data shown are for representative experiments with the anti-ICAM-1 MAb (clone 8.4A6) or isotype control (25 μg/mL). The result for the RSV noninfected control is shown by a *white bar*. Preincubation of A549 cells with anti-ICAM-1 MAb significantly reduced the adhesion of NTHi compared with preincubation with the isotype control Ab (*$p < 0.05$). Data are expressed as the means ± SD for three samples. Between-group comparisons were performed using Mann-Whitney's *U*-test. *C*, Cytokine-induced ICAM-1 expression on A549 cells. A549 cells were stimulated with IL-4 plus TNF-α (20 ng/mL) and then incubated with or without the indicated drugs for 24 h. The data shown are for the maximum reducing concentration of each drug. ICAM-1 expression was reduced significantly by DSCG, Dex, or Fp compared with the control without a drug (*$p < 0.05$), whereas no significant differences between the drugs were observed. ICAM-1 expression is expressed as the mean fluorescence intensity of cells. Data are expressed as the means ± SD for three samples. Between-group comparisons were performed using Mann-Whitney's *U*-test. *D*, ICAM-1 expression by A549 cells at 48 h after RSV infection (MOI = 2.5). A549 cells were incubated with or without drugs. ICAM-1 expression was reduced significantly by DSCG, Dex, or Fp compared with the control without a drug (*$p < 0.05$). RSV infection-induced ICAM-1 expression on A549 cells incubated with DSCG (21.1 ± 3.6%) was significantly weak compared with those incubated with Dex or Fp. (*$p < 0.05$). ICAM-1 expression is expressed as the relative MFI of cells. Data are expressed as the means ± SD for three samples. Between-group comparisons were performed using Mann-Whitney's *U*-test.

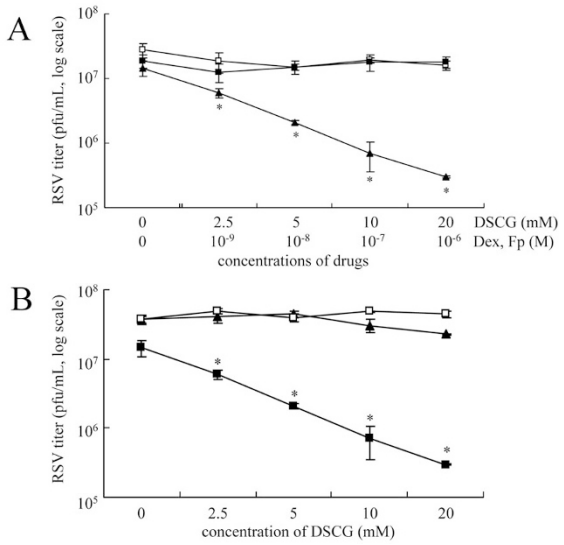

Figure 4. *A*, Effect of DSCG (*closed triangles*), Dex (*open squares*), or Fp (*closed squares*) on RSV infectivity for A549 cells. A549 cells were incubated with DSCG, Dex, or Fp at each concentration for 48 h after viral adsorption (MOI = 2.5). Only DSCG reduced the RSV titer significantly ($*p < 0.05$). The viral titer was determined by plaque assay and is shown as a log scale. Data are expressed as the means ± SD for three samples. Between-group comparisons were performed using Mann-Whitney's *U* test. *B*, Inhibitory effect of DSCG on RSV infection was examined by treating A549 cells with DSCG at different stages of viral infection. For treatment of cells before adsorption (*closed triangles*), the cells were cultured for 24 h at 37°C in 5% CO_2 in the presence or absence of DSCG. For treatment of cells during viral adsorption (*open squares*), viral solutions were first preincubated for 30 min at room temperature with the indicated concentrations of DSCG. The cells were then inoculated with the virus-DSCG mixtures. For treatment of cells after viral adsorption (*closed squares*), the cells were first inoculated with the virus, and then treated with or without the indicated concentrations of DSCG. The viral titer was determined by plaque assay and is shown as a log scale. Only the treatment after viral adsorption significantly suppressed the viral titer in a dose-dependent manner. Between-group comparisons were performed using Mann-Whitney's *U* test. $*p < 0.05$, *vs* control, 0 mM DSCG. Data are expressed as the means ± SD for three samples.

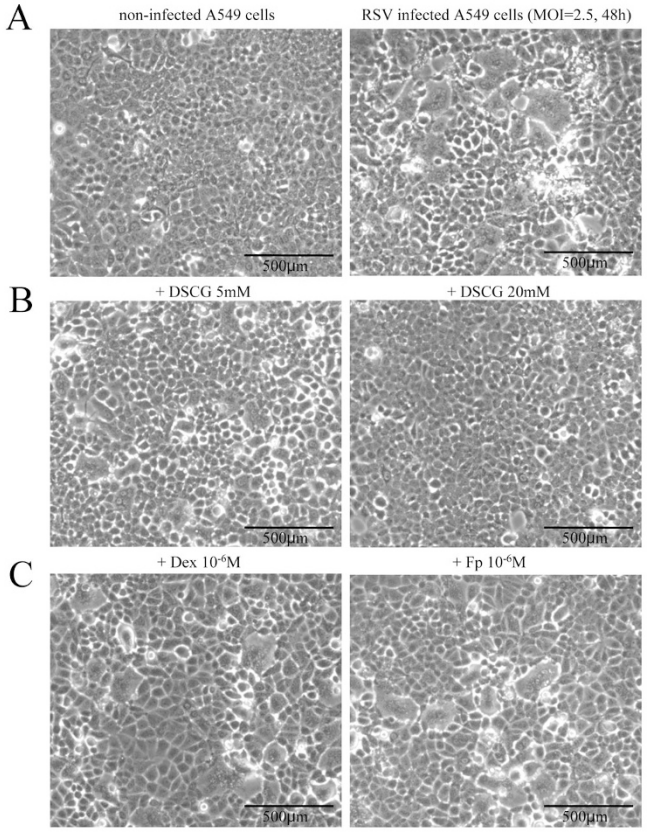

Figure 5. Effect of DSCG, Dex, or Fp on the morphologic changes of A549 cells infected with RSV. The monolayers of A549 cells were examined microscopically at ×100 magnification. Syncytium formations appeared on A549 cells infected with RSV (MOI = 2.5) for 48 h (*A*). Inhibition of RSV-induced sycytium formation was observed with increasing concentrations of DSCG (*B*). RSV-induced syncytium formation was not inhibited by Dex or Fp (*C*).

DSCG was significantly stronger than Dex and Fp ($p < 0.05$; Fig. 3*D*).

Effects of drugs against RSV infection. To determine whether DSCG, Dex, and Fp have inhibitory ability as to RSV infection, titration of RSV treated with each drug after viral adsorption was performed by plaque assay. The RSV titer decreased significantly with increasing concentration of DSCG ($p < 0.05$; Fig. 4A). Dex and Fp did not inhibit RSV infection significantly (Fig. 4B).

To determine the inhibitory effect of DSCG on RSV infection, we treated A549 cells with DSCG at different points of viral infection. Only the treatment after viral adsorption significantly suppressed the viral titer in a dose-dependent manner ($p < 0.05$; Fig. 4*C*).

A characteristic of RSV infection *in vitro* is that infected cells fuse with adjacent infected or uninfected cells to form giant syncytia (Fig. 5*A*). When DSCG was added to infected cells, inhibition of RSV-induced syncytium formation was observed (Fig. 5*B*). Meanwhile, Dex and Fp did not cause inhibition of syncytium formation (Fig. 5*C*).

DISCUSSION

It is known that RSV infections are often associated with secondary bacterial infections and that bacterial superinfections increase the severity of RSV infections. Preventing secondary bacterial infections could be a key for the management of lower respiratory infections with RSV.

In this study, we showed that RSV infection enhanced NTHi adherence to A549 cells, as demonstrated by previous studies (8,13). The effects of DSCG, Dex, and Fp, which are often used as therapies for wheezing and bronchial asthma, on NTHi adherence to RSV-infected A549 cells were investigated at clinically relevant concentrations. Only DSCG, *i.e.* not Dex or Fp, reduced the number of adherent NTHi.

It has been reported that ICAM-1 acts as a major receptor for NTHi on RSV-infected A549 cells (8). In our experiments, RSV infection up-regulated ICAM-1 expression on A549 cells, and NTHi adherence to RSV-infected cells was inhibited by blocking of ICAM-1. These results indicate that ICAM-1 contributes to the enhanced adherence of NTHi to RSV-infected A549 cells.

DSCG, Dex, and Fp have been reported to attenuate ICAM-1 *in vivo* or *in vitro* (15–17). In this study, DSCG attenuated ICAM-1 expression induced by RSV infection more strongly than corticosteroids. It is suggested that the

reduction of NTHi adherence caused by DSCG is associated with attenuation of ICAM-1 expression on A549 cells. Meanwhile, cytokine-induced ICAM-1 expression was reduced more strongly by the corticosteroids than DSCG. The difference in the extent of ICAM-1 inhibition between RSV-induced and cytokine-induced indicated that DSCG might affect RSV infection.

As seen in plaque assay and viral syncytium assay, DSCG treatment after RSV adsorption significantly reduced the viral infectivity of A549 cells. DSCG is a safe and widely used drug for the prevention of bronchial asthma (19–21). DSCG is known to have effects such as mast cell stabilization and suppression of various inflammatory cells (22–24). Previous studies have also demonstrated that DSCG has antiviral effects, and there has been a recent report about inhibitory effects on influenza virus *in vitro* and *in vivo* (18). However, the molecular mechanisms underlying DSCG-induced signaling and anti-viral effects have remained unclear. In this study, to determine the inhibitory effect of DSCG on RSV infection, treatment with DSCG at different stages of viral infection was investigated. DSCG administered after, but not before or during, RSV adsorption effectively inhibited viral infection. These results suggest that DSCG predominantly inhibits the late stages of viral infection, such as the budding of progenitor viruses. Hidari *et al.* have supposed that the anti-influenza viral effect of DSCG is a combination including an inhibition of viral neuraminidaze activities and inhibition of membrane fusion. We speculate that the inhibition of membrane fusion is one of the mechanisms of anti-RSV effect of DSCG. Further elucidation of the mechanism underlying the anti-RSV effect of DSCG is needed.

We showed that DSCG inhibited RSV infection of A549 cells and attenuated the cell surface expression of ICAM-1. It is indicated that ICAM-1 down-regulation is one of the mechanisms that modulate NTHi adhesion to A549 cells. Not only ICAM-1 but also CEACAM1 and PAFr have been reported to be NTHi adhering receptors and up-regulated by RSV infection (8). This is why blocking of NTHi adhesion to RSV-infected cells with anti-ICAM-1 MAb did not completely prevent excess NTHi adhesion. It is speculated that inhibition of RSV infection by DSCG might also down-regulate these receptors, *i.e.* not only ICAM-1.

Differences in the magnitude of bacterial adhesion and receptor expression have been reported for different cell types (8). Avadhanula *et al.* (8) asserted that the differences in the responses of distinct cell types must be taken into consideration when interpreting the findings of *in vitro* studies. In this study, we used A549 cells as lower airway epithelial cells, because of the characteristic higher increase in adhesion molecules expression and bacterial adhesion when they are infected with RSV.

There has been a report that DSCG treatment in hospitalized infants with RSV bronchiolitis has no clinical effect (25). This clinical study was for the hospitalized infants who probably have already received considerable airway injury and represented respiratory dysfunction by RSV infection. It suggests that the effect of DSCG on the RSV-induced airway inflammation including scavenging oxygen radicals is not clinically sufficient. We demonstrated that DSCG inhibits RSV infection and NTHi adhesion to the RSV-infected epithelial cells *in vitro* in this study. DSCG treatment on the earlier stage of RSV infection might have a clinical effect by inhibiting RSV infection and secondary NTHi infection. Further examinations including an *in vivo* study will clarify the effects of DSCG on RSV infection and NTHi adhesion to RSV-infected cells.

In conclusion, we demonstrated that DSCG inhibits enhanced adherence of NTHi to A549 cells infected with RSV, whereas Dex and Fp do not. It is suggested that DSCG exerts an anti-RSV effect, and consequently attenuates the expression of NTHi receptors.

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