

Effect of formaldehyde on the expression of adhesion molecules in nasal microvascular endothelial cells: the role of formaldehyde in the pathogenesis of sick building syndrome

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Summary

Background Formaldehyde is associated with sick building syndrome (SBS), a set of diffuse and irritative symptoms predominantly involving the eyes and the respiratory tract. However, its pathophysiological mechanism in SBS has not yet been clarified.

Objective In this study we investigated the effect of formaldehyde on the expression of adhesion molecules on human mucosal microvascular endothelial cells (HMMECs). Furthermore, we investigated the effect of formaldehyde on adhesiveness of HMMECs to eosinophils.

Materials and methods HMMECs were incubated with various concentrations of formaldehyde (1 ng/mL–1 µg/mL) for 24 h, and the expressions of intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule (VCAM-1) on HMMECs were evaluated by flow cytometry. The change in the expression of ICAM-1 and VCAM-1 mRNA was then evaluated by reverse transcriptase polymerase chain reaction. To understand the role of formaldehyde in eosinophilic inflammation of the nasal mucosa, we examined the effects of formaldehyde on the adhesiveness between HMMECs and eosinophils by eosinophil adhesion assay.

Results Formaldehyde increased the surface expressions of ICAM-1 and VCAM-1 on HMMECs. Formaldehyde also induced ICAM-1 and VCAM-1 mRNA. In addition, the adhesiveness between HMMECs and eosinophils was also increased by formaldehyde.

Conclusion These *in vitro* studies suggest that formaldehyde may play a role as the irritant of the nasal mucosa by increasing the expressions of adhesion molecules on HMMECs and by enhancing the adhesiveness between HMMECs and eosinophils.

Keywords eosinophils, formaldehyde, human nasal microvascular endothelial cells, ICAM-1, sick building syndrome, VCAM-1

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Introduction

The condition of indoor air is an important issue because generally people spend a lot of their time indoors. In general, indoor exposures to noxious chemical, physical and biological hazards occur at low levels. Unlike industrial or accidental exposures, however, such low-level indoor exposures are very common [1]. Recently, there has been an increase in sick building syndrome (SBS), whereby non-specific complaints, including upper respiratory irritative symptoms, headaches, fatigue and rashes, occur in occupants of new buildings.

It is now well known that the main causes of SBS are poor indoor air quality due to a inadequate ventilation or fresh air intake, biological contamination of the indoor environment, and an accumulation of noxious compounds such as formaldehyde and volatile organic compounds (VOC), NO, NO₂, CO [2].

Many studies have examined the effect and mechanism of combustion (e.g. NO, NO₂, CO) and biological sources (e.g. infectious sources), especially the relationship between humid/mouldy buildings and symptoms, among indoor air pollutants implicated in SBS, but those of the miscellaneous sources, such as formaldehyde and VOC, have been studied only by some research groups [3,4].

Formaldehyde is a low molecular weight organic compound that is frequently used in occupational environments (hospitals, textiles, paper, resins, wood composites) and household indoor environments (insulating materials, chipboard and plywood, fabrics, and heating and cooking emissions). Some decades ago, formaldehyde was a big problem in Scandinavia when it was evaporating from building materials in newly built houses, because acid curing paint was commonly used for spray painting of kitchen wood details [5], although nowadays it is limited and encountered in places where there are fresh resin-based glues or evaporation of it. Some epidemiological studies suggest that the increasing incidence of allergic diseases such as asthma and allergic rhinitis is due to exposure to environmental formaldehyde [6,7], and a positive relationship exists between

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exposure to formaldehyde and allergic diseases of the respiratory tract [8–10]. However, many studies had limitations in regard to the correlation between symptoms and formaldehyde, because it is difficult to expose humans to formaldehyde experimentally. Moreover, it is difficult to discuss the biological significance and pathogenesis of these effects of formaldehyde *in vivo*, because it is possible that formaldehyde acts in combination with other stimuli, such as another chemical or physical stress under certain conditions.

To our knowledge, there are few *in vitro* studies available on the pathogenesis of asthma and inflammation in relation to indoor exposure to emissions from formaldehyde. In this regard, in order to know how formaldehyde acts as an allergenic inflammatory material, we hypothesized that formaldehyde may up-regulate the expression of adhesion molecules on HMMECs, which may contribute to the pathophysiology of allergic rhinitis. Furthermore, the adhesiveness between HMMECs and eosinophils was also investigated.

Materials and methods

mAbs and reagents

IL-4 was purchased from Genzyme (Boston, MA, USA). Tumour necrosis factor- α (TNF- α) was purchased from R & D Systems (Minneapolis, MN, USA). Monoclonal mouse anti-human intercellular adhesion molecule-1 (anti-human ICAM-1) and vascular cell adhesion molecule-1 (anti-human VCAM-1) were both purchased from Genzyme (Cambridge, MA, USA). CD16 microbeads were purchased from Miltenyi Biotec (Sunnyvale, CA, USA). Formaldehyde was prepared from paraformaldehyde (TAAB Laboratories, Berkshire, UK) as described previously by Saneyoshi et al. [6]. Volatile organic compounds, 1,2 benzene, 1,3 benzene, 1,4 benzene, *o*-xylene, *m*-xylene, *p*-xylene, and toluene were purchased from Sigma Chemicals (St Louis, MO, USA).

Collection of mucosa from the inferior turbinate

Turbinal mucosa samples were collected from 10 perennial allergy patients sensitized to house dust and/or mites. Inferior nasal mucosa samples were obtained at the time of surgery for submucosal resection.

Purification and determination on HMMECs

Human mucosal microvascular endothelial cells (HMMECs) were isolated from human nasal mucosa specimens, as previously reported [11].

According to the morphology and positive reactions for anti-factor VIII antibody (Dakopatts, Glostrup, Denmark) in immunofluorescence staining tests, more than 95% of the HMMECs were shown to be endothelial cells. After two or three passages, we used the purified endothelial cells for the following experiments. Materials contaminated with fibroblasts were not used.

Eosinophil purification

Eosinophils were isolated from the peripheral blood of patients with allergic rhinitis. The diagnosis of allergic rhinitis was based on a typical history of perennial symptoms of allergic rhinitis, eosinophilia in nasal smears, and a positive radioallergosorbent test (RAST) to house dust and/or mites. The leucocyte fraction

was obtained by dextran sedimentation. Eosinophils were then isolated by negative immunomagnetic selection with CD16 microbeads and the MACS System (Miltenyi, Biotec München, Germany) [12]. The purity of the resulting eosinophils was $96.4 \pm 3.2\%$ as determined by microscopic examination with Hinkelman staining. For this study we used eosinophils in which the viability (based on the trypan blue exclusion test) was $93.4 \pm 4.9\%$.

Flow cytometry

HMMECs were cultured in type I collagen-coated, 12-well tissue culture plates (Sumitomo Bakelite, Tokyo, Japan). After reaching confluence, the cells were incubated in the presence or absence of the indicated concentration of 1 ng/mL to 1 μ g/mL of formaldehyde and 0.1 μ g/mL of volatile organic compounds for 24 h at 37°C in 5% CO₂. Formaldehyde less than 10 μ g/mL had no effect on HMMECs' viability or proliferation. However, formaldehyde at 10 μ g/mL did elicit a change in cellular morphology and decreased the viability of HMMECs (data not shown). Then the cells were washed with fluorescence-activated cell sorter (FACS) buffer (PBS containing 0.1% NaN₃ and 1.0% BSA). The cell suspensions were incubated with saturating concentrations of IgG mouse mAb-recognizing ICAM-1, VCAM-1, or an irrelevant control mAb for 30 min at 4°C. Then they were washed free of unbound antibodies and incubated with a 1:50 dilution of fluorescein isothiocyanate (FITC) conjugated anti-mouse IgG (Southern Biotechnology Associates, Birmingham, AL, USA) in PBS for 30 min at 4°C. After washing, the cells were suspended in 0.5 mL of FACS buffer. Labelled cells were analysed by using flow cytometry (FACScan, Becton-Dickinson, San Jose, CA, USA).

Eosinophil adhesion assay

The procedure for eosinophils adhesion assay has been described previously [9]. Briefly, HMMECs monolayers were seeded in collagen type-I coated 96-well culture plates (Sumitomo Bakelite Co, Tokyo, Japan). At confluence, HMMECs were pre-incubated in the presence or absence of the indicated concentration of formaldehyde for 24 h at 37°C. The viabilities of these cells were determined by trypan blue exclusion test after the pre-incubation, and were noted to be more than 95%. Eosinophils were labelled with ³⁵S by incubating them with [³⁵S] Protein Labelling Mix (Du Pont, Biotechnology Systems, Wilmington, DE, USA) in RPMI without methionin and cysteine (Gibco BRL, Rockville, MD, USA) for 3 h at 37°C. Labelled eosinophils in an enriched medium (RPMI 1640 with methionin and cysteine, Gibco BRL) were then added to HMMECs. Following this, they were allowed to adhere to the monolayers of HMMECs for 60 min at 37°C. Monolayers with eosinophils were rinsed by PBS and then lysed by the addition of 0.5% Triton X-100 (Sigma). After 30 min had elapsed, a liquid scintillator (SuperMixTM, Wallac, Finland) was added and ³⁵S radioactivity was determined by Micro BetaTM (Pharmacia Biotech, Uppsala, Sweden). Previous studies have shown that eosinophils take up ³⁵S at different rates, depending on the stimulation. Therefore, the numbers of adherent eosinophils were calculated with each standard curve.

In some experiments, neutralizing antibodies of ICAM-1 mAb and VCAM-1 mAb were added to HMMECs 30 min before the addition of labelled eosinophils, and then washed.

Extraction of total RNA and RT-PCR

The total RNA extracting procedure was performed using RNeasy kit (Qiagen, Hilden, Germany). The complementary DNA synthesizing procedure was performed using Omniscript RT (Qiagen, Hilden, Germany), reverse transcriptase inhibitor (Takara shuzo Co., Otsu, Japan), and Oligo dT primer (Life Technology Science, Rockville, MD, USA). ICAM-1 and VCAM-1 PCR reaction mixtures contained the LightCycler FastStart DNA Master Hybridization Probe buffer from Roche Molecular Biochemicals (10-fold stock solution containing nucleotides, Thermus aquaticus DNA polymerase, and 10 mmol/L Mg^{2+}), 2mmol/L of $MgCl_2$, 0.5 μ mol/L of each primer, 0.2 μ mol/L of anchor probe and 0.4 μ mol/L of detection probe. The reaction mixture for β -actin PCR contained 3mmol/L $MgCl_2$ and the other reagents were of the same concentration. Reactions on the LightCycler were performed in glass capillaries of 20 μ L volumes.

The temperature profile for ICAM-1, VCAM-1 and β -actin included an initial denaturation for 10min at 95°C, followed by denaturation at 95°C for 15s, annealing with fluorescence monitoring at 60°C for 15s, and extension at 72°C for 15s. The PCR was performed for 40 cycles.

Fluorescence monitoring using hybridization probes is based on the concept that a fluorescence signal is generated if fluorescence resonance energy transfer (FRET) occurs between two adjacent fluorophores [13]. The primers and LightCycler hybridization probes were designed as shown in Table 1.

Statistical assessment

The results of the study were expressed as the mean value $\pm$ SE. The statistical significance of within-group differences was determined by the paired *t*-test for normally distributed data and the Wilcoxon signed rank test for data without a normal distribution. Differences were considered significant only when the *P*-value was less than 0.05.

Results

Effect of formaldehyde on ICAM-1 and VCAM-1 expression on HMMECs

To evaluate whether the expression of ICAM-1 and VCAM-1 on HMMECs could be up-regulated by formaldehyde, we incubated HMMECs with various concentrations of formaldehyde (1ng/mL to 1 μ g/mL). ICAM-1 was constitutively expressed on cultured HMMECs at a high level and was up-regulated in the formaldehyde treated HMMECs. Expression rate of ICAM-1 following an unstimulated 24-h incubation period was $51.0 \pm 11.6\%$. The expression rate increased to $71.9 \pm 11.35\%$ following a stimulation with IL-4 10ng/mL + TNF- α 10-ng/mL, used as a positive control. Formaldehyde at 0.1 μ g/mL and 1.0 μ g/mL also increased the expression rate to $73.2 \pm 8.0\%$, $68.3 \pm 11.9\%$, respectively (Fig. 1). These differences were statistically significant ($P < 0.05$).

The intensity of VCAM-1 expression on HMMECs was significantly higher with formaldehyde stimulation than with the PBS control ($P < 0.05$). VCAM-1 expression rate on PBS-treated HMMECs was $14.5 \pm 3.7\%$. It was remarkably up-regulated in the formaldehyde-treated HMMECs and its expression rate showed $30.5 \pm 7.2\%$ and $31.3 \pm 6.8\%$ at 0.1 μ g/mL and 1.0 μ g/mL, respectively.

Table 1. Oligonucleotides used in this study

	Sequence 5'–3'
β -actin	
Forward primer	CCAACCGCGAGAAGATGAC
Reverse primer	GGAAGGAAGGCTGGAAGAGT
Anchor probe	CCTCCCCCATGCCATCCTGCGTC-FL
Detection probe	LC-GGACCTGGCTGGCCGGGACCTGA
ICAM-1	
Forward primer	CTCCAATGTGCCAGGCTT
Reverse primer	TATGACTGCGGCTGCTACC
Anchor probe	AGGGCACCTACCTCTGTCTGGG-FL
Detection probe	LC-CAGGAGCACTCAAGGGGAGGTC
VCAM-1	
Forward primer	GGAAATGACCTTCATCCC
Reverse primer	CTGGCTCAAGCATGTCATA
Anchor probe	CCAAACAAAGGCAGAGTACGCAAA-FL
Detection probe	LC-ACTTTATGTCAATGTTGCCCCAG

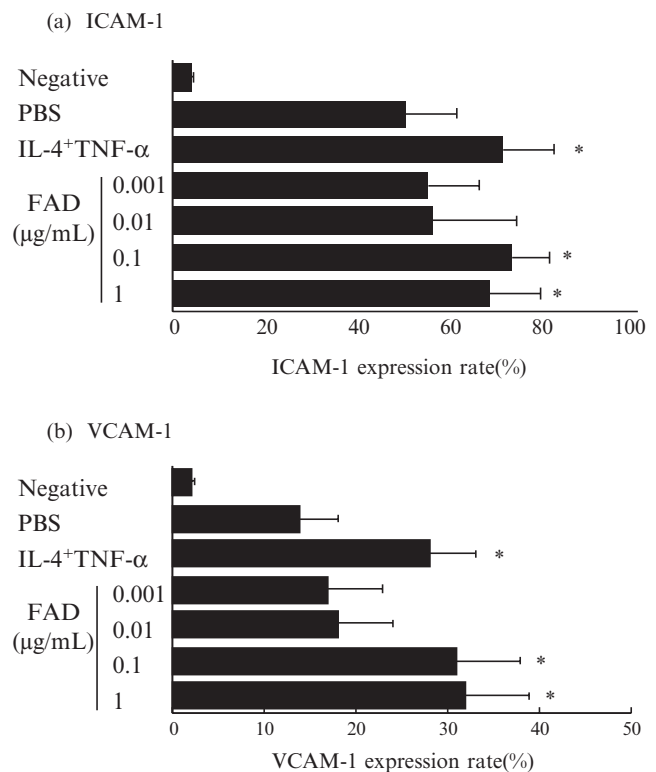


Fig. 1. Effect of formaldehyde on ICAM-1 and VCAM-1 surface expression on HMMECs. Expression of surface ICAM-1 and VCAM-1 was evaluated by flow cytometry on HMMECs with medium alone, PBS, IL-4 (10ng/mL) + TNF α (10ng/mL), or various concentrations of formaldehyde (1ng/mL to 1 μ g/mL) for 24h. ICAM-1 and VCAM-1 induction by formaldehyde is expressed as the expression ratio compared with the negative control. Data are means $\pm$ SE of four separate experiments. * $P < 0.05$ compared with PBS-treated HMMECs. Formaldehyde induced a marked and significant up-regulation of VCAM-1 surface expression on HMMECs at concentrations of 0.1 and 1.0 μ g/mL.

The kinetics of formaldehyde-induced ICAM-1 and VCAM-1 expression on HMMECs

Next, to determine the temporal kinetics of ICAM-1 and VCAM-1 induction by formaldehyde, surface ICAM-1

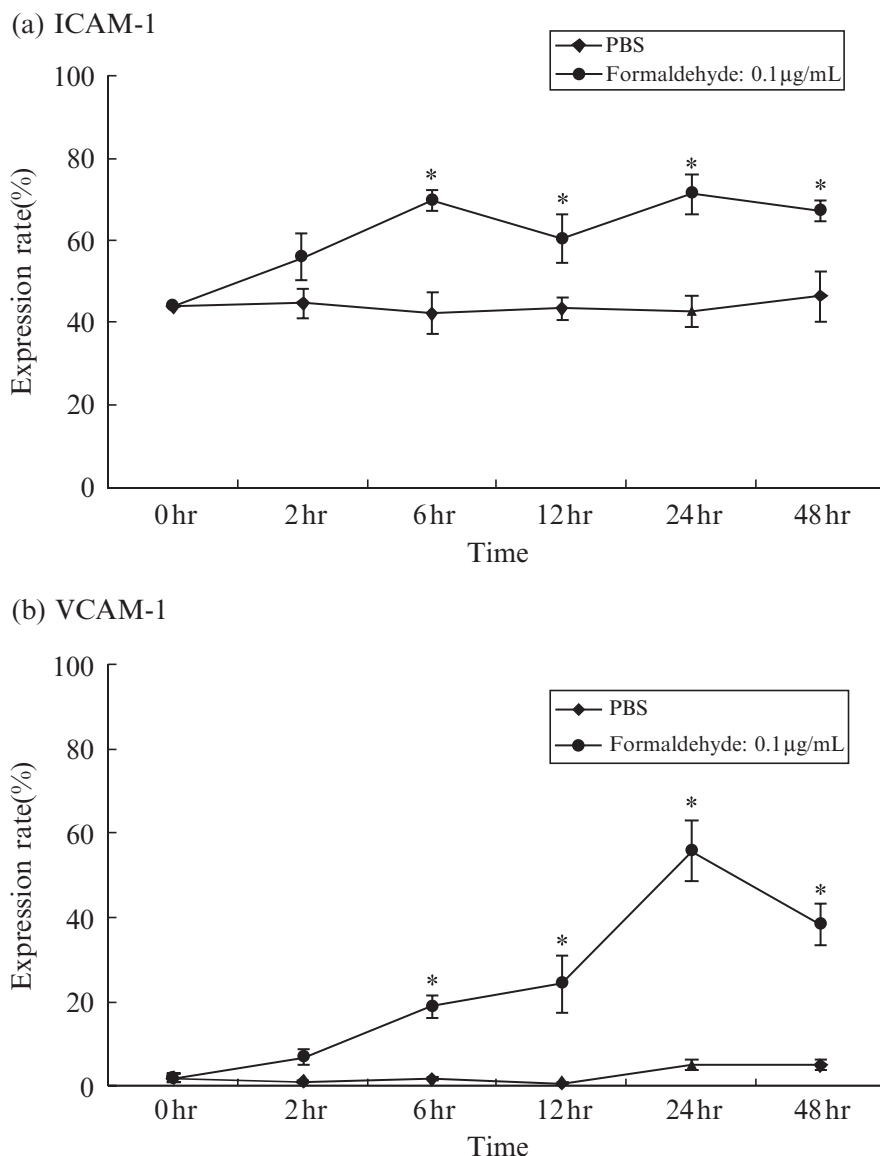


Fig. 2. Time course of ICAM-1 (a) and VCAM-1 (b) expression on HMMECs induced by formaldehyde. Expression of surface ICAM-1 and VCAM-1 was evaluated by flow cytometry on HMMECs cultured with PBS alone (control) or formaldehyde at 0.1 µg/mL for 2, 6, 12, 24 and 48 h. ICAM-1 and VCAM-1 induction by formaldehyde is expressed as expression ratio. Data are means $\pm$ SE of four separate experiments. * $P < 0.05$ compared with PBS-treated HMMECs. Formaldehyde induced a significant up-regulation of ICAM-1 and VCAM-1 surface expression on HMMECs within 6 h after incubation, which peaked at 24 h after incubation and was still evident up to 48 h within our experimental range.

and VCAM-1 were studied at 2, 6, 12, 24 and 48 h after incubation with 0.1 µg/mL of formaldehyde. In ICAM-1 and VCAM-1, significant up-regulation was apparent within 6 h, was maximal at 24 h ($P < 0.05$ compared with PBS control) and was still significantly increased at 48 h after stimulation ($P < 0.05$ compared with PBS control) (Fig. 2). HMMECs incubated with PBS alone showed no remarkable change.

Effect of volatile organic compounds on ICAM-1 and VCAM-1 expression on HMMECs

To evaluate whether the expression of ICAM-1 and VCAM-1 on HMMECs could be up-regulated by volatile organic compounds, we incubated HMMECs with 1,2 benzene, 1,3 benzene, 1,4 benzene, *o*-xylene, *m*-xylene, *p*-xylene and toluene (0.1 µg/mL) for 24 h at 37°C. ICAM-1 and VCAM-1 were not up-regulated on cultured HMMECs by volatile organic compounds compared with that by PBS (Fig. 3).

Effect of formaldehyde on expression of ICAM-1 mRNA and VCAM-1 mRNA in HMMECs

Then, we wished to study the effects of formaldehyde on the expression of ICAM-1 and VCAM-1 mRNA in HMMECs by using RT-PCR. Figure 4 shows that PBS-treated HMMECs expressed VCAM-1 mRNA at low levels. After stimulation with formaldehyde, however, HMMECs showed significantly increased VCAM-1 mRNA levels compared with those for PBS-treated HMMECs (3.3 ± 0.8 fold increased at 0.1 µg/mL of formaldehyde, $P < 0.01$). Expression of ICAM-1 mRNA showed a similar pattern (2.2 ± 0.2 -fold increase at 0.1 µg/mL of formaldehyde, $P < 0.01$). In addition, actinomycin D strongly suppressed formaldehyde inducing ICAM-1 and VCAM-1 mRNA ($P < 0.01$).

Eosinophil adhesion assay

To know whether the induction of the expression of ICAM-1 and VCAM-1 by formaldehyde was associated with an

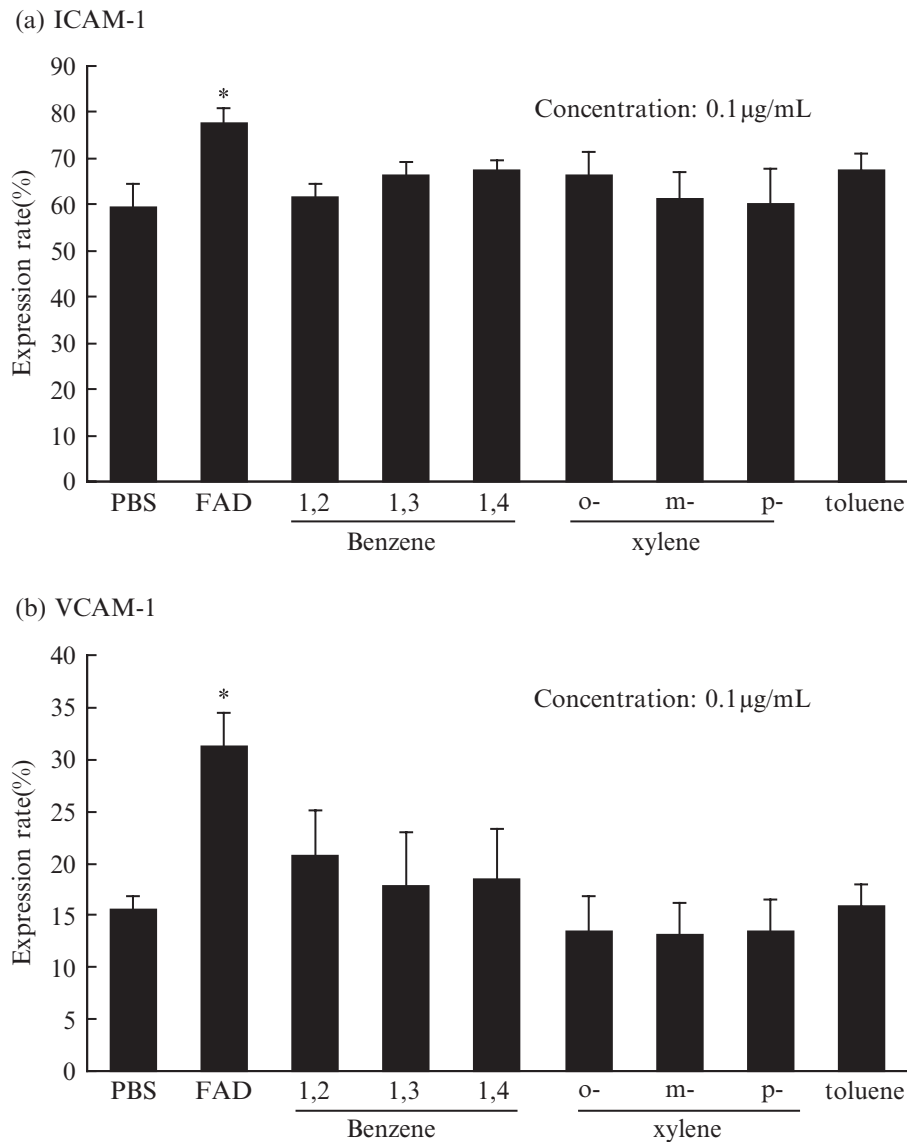


Fig. 3. Effect of volatile organic compounds on ICAM-1 (a) and VCAM-1 (b) expression on HMMECs. Surface ICAM-1 and VCAM-1 expression was evaluated by flow cytometry on HMMECs with PBS, 1,2 benzene, 1,3 benzene, 1,4 benzene, *o*-xylene, *m*-xylene, *p*-xylene, toluene or formaldehyde (0.1 µg/mL) for 24 h. ICAM-1 and VCAM-1 induction was expressed as the expression ratio compared with a negative control. Data are means $\pm$ SE of four separate experiments. * $P < 0.05$ compared with PBS-treated HMMECs. Volatile organic compounds did not induce an up-regulation of VCAM-1 surface expression on HMMECs at a concentration of 0.1 µg/mL.

increased adhesiveness of HMMECs, we performed adhesion assay using eosinophils purified from peripheral blood. As shown in Fig. 5(a), formaldehyde increased the adhesiveness of HMMECs to eosinophils in a dose-dependent manner. More than 0.1 µg/mL of formaldehyde significantly increased the adhesiveness of HMMECs to eosinophils. Moreover, the increase in the adhesiveness of HMMECs to eosinophils induced by 1 µg/mL of formaldehyde was significantly diminished by anti-ICAM-1 and anti-VCAM-1, alone or in combination as indicated (Fig. 5b).

Discussion

In this study we have demonstrated that formaldehyde induced the expression of ICAM-1 and VCAM-1 on HMMECs. We

also found that formaldehyde induced the expression of ICAM-1 mRNA and VCAM-1 mRNA. Volatile organic compounds, however, did not increase the expression of ICAM-1 and VCAM-1 on HMMECs. Furthermore, the adhesiveness between HMMECs and eosinophils was also increased by formaldehyde.

It is still controversial whether formaldehyde acts as an allergen or not, and the mechanisms by which formaldehyde triggers the exacerbation of allergic symptoms are poorly understood, although it is certain that formaldehyde causes the various mucosal irritative symptoms.

Some articles have reported that formaldehyde acts as a modulating factor of inflammation by affecting the ability to release pro-inflammatory cytokines such as IL-1 and IL-8 from dermal keratocytes [14], as well as IL-4 and IL-6 from mast cells [6], which leads to clinical deterioration of atopic eczema [15].

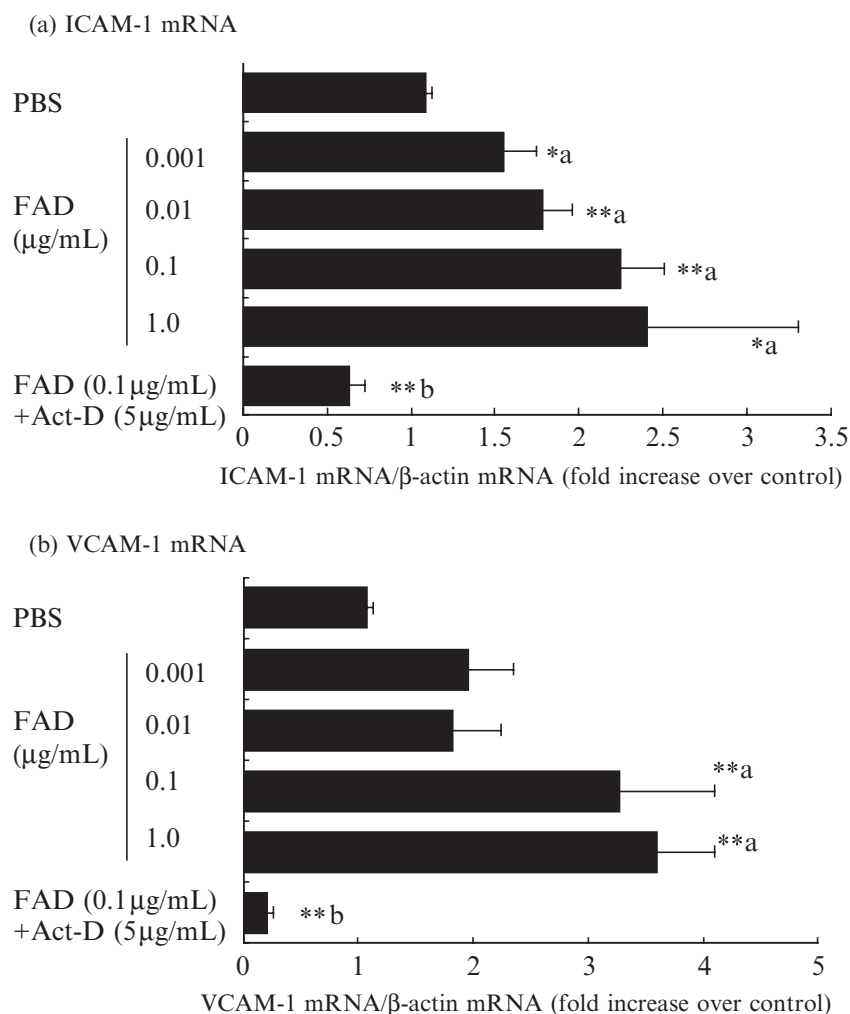


Fig. 4. Effect of formaldehyde on ICAM-1 mRNA and VCAM-1 mRNA in HMMECs. ICAM-1 mRNA and VCAM-1 mRNA expression were evaluated by LightCyclerTM in HMMECs cultured with PBS control, IL-4 (10 ng/mL) + TNF- α (10 ng/mL), formaldehyde (1 ng/mL, 10 ng/mL, 0.1 µg/mL, 1.0 µg/mL) and formaldehyde (0.1 µg/mL) + actinomycin D (5 µg/mL) for 6 h. ICAM-1 mRNA and VCAM-1 mRNA induction by formaldehyde is expressed as -fold increase over the PBS control. Data are means $\pm$ SE of at least four separate experiments. *a, $P < 0.05$; **a, $P < 0.01$ compared with PBS treated HMMECs, **b, $P < 0.01$ compared with 0.1 µg/mL of formaldehyde-stimulated HMMECs.

Wieslander et al. found that low-level exposure to formaldehyde causes an allergic inflammation, with significantly increased concentration of blood eosinophils [9] and eosinophil cationic protein in nasal lavage [16]. Moreover, Pazdrak et al. reported that formaldehyde increased the number and proportion of eosinophils in nasal lavage fluid [17]. In contrast, some other studies reported that formaldehyde acts as only an inflammatory toxic material, because it did not cause significant sensitization of the respiratory tract, or increased eosinophils [18,19].

Endothelial cells of the nasal mucosa play a significant role in the pathogenesis of allergic rhinitis by releasing cytokines or by expressing membrane-associated adhesion molecules that are related to eosinophils. Therefore, in this study, we examined the effect of formaldehyde as an environmental air pollutant on the expression of adhesion molecules by human nasal mucosal microvascular endothelial cells to determine its possible role in allergic rhinitis or allergic symptomatic inflammation.

ICAM-1 is expressed on a variety of cells, including endothelial cells, epithelial cells, fibroblasts and leucocytes, and is deeply involved in the immune reactions requiring cell-to-cell

contact [20,21]. The expression of ICAM-1 on endothelial cells may participate in allergic inflammation by promoting the interaction between inflammatory cells and a target organ [20,22], which acts as a marker of allergic inflammation [23,24]. Our data show that the expression of ICAM-1 on HMMECs was increased following exposure to formaldehyde in a time- and dose-dependent pattern.

In our opinion, the most interesting result of these studies was the increased VCAM-1 expression in response to the stimulation of formaldehyde. VCAM-1 has some functional differences compared with ICAM-1. Although ICAM-1 is normally expressed on the endothelial surface, VCAM-1 is not [25,26]. Furthermore, antibodies to ICAM-1 inhibited the adherence of all leucocyte types, whereas anti-VCAM-1 antibody was extremely effective at inhibiting eosinophil adherence but had no effect on neutrophil adherence, suggesting that eosinophils, unlike neutrophils, recognize VCAM-1 [27]. Jahnsen et al. reported that selective VCAM-1 up-regulation has been shown to support selective adhesion or transmigration of eosinophils without affecting neutrophils [28,29]. Our data show that

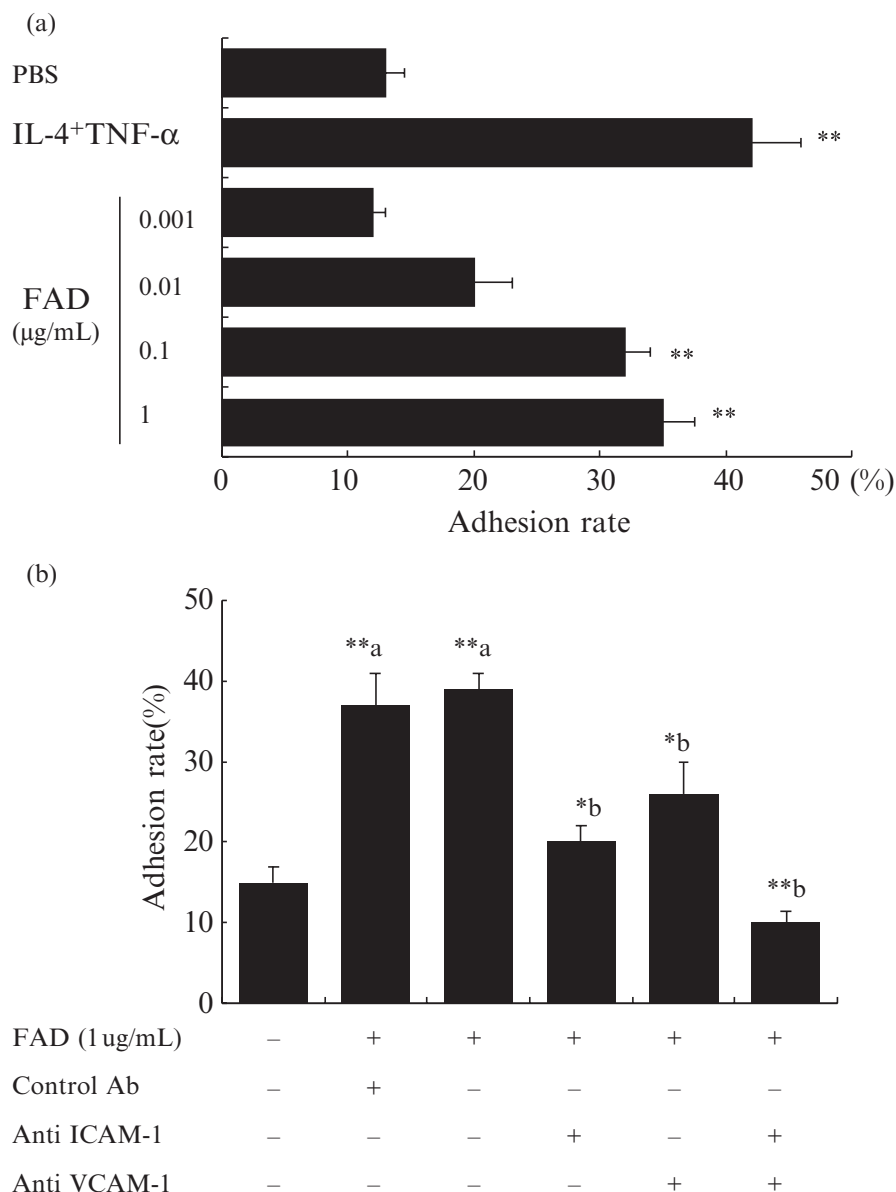


Fig. 5. Eosinophil adhesion assay. **(a)** Effects of formaldehyde on the adhesiveness of HMMECs to eosinophils. HMMECs were incubated for 24 h in medium containing PBS alone, or with the addition of the combination of IL-4 and TNF- α , or with various concentrations of formaldehyde as indicated. Labels were then added to HMMECs. After 30 min, the numbers of adherent eosinophils were evaluated as described in the text. Six separate experiments were analysed. * $P < 0.05$, ** $P < 0.01$ vs. unstimulated HMMECs (PBS). **(b)** Effect of mAb to ICAM-1 and VCAM-1 on formaldehyde induced increases in the adhesiveness of HMMECs to eosinophils. Neutralizing antibodies of ICAM-1 mAb and VCAM-1 mAb, alone or in combination as indicated, were added to HMMECs 30 min before the addition of formaldehyde, and then washed. Six separate experiments were analysed. **a, $P < 0.01$, vs. unstimulated HMMECs (PBS); *b, $P < 0.05$, vs. HMMECs stimulated with formaldehyde; **b, $P < 0.01$, vs. HMMECs incubated with isotype matched control antibodies.

VCAM-1 expression on HMMECs with formaldehyde was time- and dose-dependent, and was significantly increased at 24 h after stimulation.

To examine whether ICAM-1 and VCAM-1 mRNA were associated with adhesion molecule expressions, we performed RT-PCR by LightCyclerTM. Our study showed that ICAM-1 and VCAM-1 mRNA were induced by formaldehyde and significantly suppressed by actinomycin D co-treatment. Although further detailed molecular bases for these findings remain to be determined, these results strongly suggest that

the increase of ICAM-1 and VCAM-1 induced by formaldehyde is accompanied by *de novo* protein synthesis.

It is well known that allergic rhinitis is characterized by inflammatory cell infiltration, especially including eosinophils. Eosinophils are the principal inflammatory cells of allergic reaction, mainly mediating mucosal damage, because they release basic proteins able to induce typical inflammatory cytokines [30–32]. Therefore, we were interested to find that formaldehyde induces not only the expression of adhesion molecules morphologically, but also the enhancement of adhesion

activity functionally, and that increases the adhesiveness of HMMECs to eosinophils. These findings were further confirmed by eosinophil adhesion assay, where formaldehyde increased the adhesiveness of HMMECs to eosinophils in a dose-dependent manner. Any volatile organic compounds did not increase the adhesiveness of HMMECs to eosinophils (data not shown). Moreover, we have observed that the increase in the adhesiveness of HMMECs to eosinophils induced by 1 µg/mL of formaldehyde was significantly diminished by anti-ICAM-1 mAb and anti-VCAM-1 mAb, alone or in combination. Taken together, these findings strongly suggest that formaldehyde increases ICAM-1 and VCAM-1 expression and functionally promotes eosinophil accumulation in nasal mucosa.

As previously described, some articles have suggested that formaldehyde exposure in SBS caused the activation and infiltration of eosinophils in respiratory tract mucosa [9,16,17]. Our results were consistent with these reports of predominance of eosinophils in nasal mucosa by formaldehyde exposure. In addition, we found that formaldehyde also increased the expression of adhesion molecules on eosinophils (data not shown). On the basis of our data, it seemed likely that the increase of adhesiveness between HMMECs and eosinophils by formaldehyde depended on not only HMMECs, but also eosinophils.

The mechanism of formaldehyde in SBS is not clear. According to our study, it seems possible that 'irritants' such as formaldehyde may promote an inflammatory cell infiltration by an up-regulation of the expression of adhesion molecules such as atopic disease. However, it is not quite as clear that formaldehyde led to its characteristics in SBS like atopic eczema, because generally SBS is not an atopic disease. Moreover, cell adhesion is associated with some factors such as the adhesion molecules, the extracellular matrix proteins, and cytoplasmic plaque proteins [33–35], and a variety of cell adhesion mechanisms can exist in multiple functional states. Unfortunately, the exact mechanism by which formaldehyde increases the adhesiveness of eosinophils and whether the involvement of another as yet unidentified adhesion factors exists is still unclear, and it seems that further studies about the details and subtleties of these interactions are needed. However, it is a certain that the expression of ICAM-1 and VCAM-1 by formaldehyde is one mechanism by which eosinophils adhere to HMMECs in SBS and subsequently produce an allergic reaction.

Some articles reported the concentration of formaldehyde in a real environment, such as during industrial spray painting, when average concentrations of 480 µg/m³ and peak exposures up to 3000 µg/m³ of formaldehyde have been measured [5], and during indoor water based painting, when 30–400 µg/m³ of formaldehyde has been measured in the breathing zone of house painters [36,37]. In regard to the relation to level of formaldehyde between environment and *in vitro*, Hidekazu et al. reported that exposure to 5 and 10 p.p.m. formaldehyde gas for 30 min *in vitro* produced concentration of solubilized formaldehyde in medium of 2.7 and 5.7 µg/mL, respectively [38]. However, it is still difficult to know what kind of relation exists between the concentration of formaldehyde between the real environment and *in vitro* and how it acts *in vivo*. It seems that further studies are needed, although it is certain that formaldehyde may play a role in SBS.

In conclusion, we have shown that formaldehyde up-regulated the expression of ICAM-1 and VCAM-1 on HMMECs and the adhesiveness of HMMECs to eosinophils.

Although the mechanisms by which formaldehyde could stimulate allergic inflammation and induce the preferential accumulation of eosinophils in the nasal mucosa are not completely clarified, the induction of ICAM-1 and VCAM-1 on HMMECs by formaldehyde may play an important role in allergic inflammation of SBS.

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