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Monochloramine Inhibits the Expression of E-selectin and Intercellular Adhesion Molecule-1 Induced by TNF-α Through the Suppression of NF-κB Activation in Human Endothelial Cells

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Reactive oxygen species have various effects on the expression of cell adhesion molecules induced by pro-inflammatory cytokines, such as tumor necrosis factor α (TNF-α). We studied the effects of monochloramine (NH_2Cl), a physiological oxidant derived from activated neutrophils, on the TNF-α-induced expression of e-selectin and intercellular adhesion molecule-1 (ICAM-1) in human umbilical vein endothelial cells (HUVEC). HUVEC were pretreated with or without NH_2Cl (20–90 μM for 20 min), then stimulated with TNF-α (10 ng/ml), and the expression of e-selectin and ICAM-1 was measured. Without NH_2Cl, TNF-α induced marked expression of e-selectin and ICAM-1. Pretreatment with NH_2Cl resulted in a significant, but transient inhibition of the expression of adhesion molecules. Higher dose of NH_2Cl showed more pronounced inhibition, and the inhibitory effect lasted for 8 h when 70 μM of NH_2Cl was added. TNF-α stimulation also induced marked activation of nuclear factor κB (NF-κB). Notably, NH_2Cl also inhibited this NF-κB activation in a dose- and time-dependent manner, which was similar to the inhibition of e-selectin and ICAM-1 expression. In addition, IκB-α phosphorylation and degradation were also inhibited by NH_2Cl pretreatment. These observations indicated that NH_2Cl inhibited TNF-α-induced expression of e-selectin and ICAM-1 through the inhibition of NF-κB activation. We speculate that neutrophil-derived chloramines may have a regulatory role in the recruitment of leukocytes.

Keywords: Reactive oxygen species; e-selectin; ICAM-1; NF-κB; Endothelium

INTRODUCTION

Inflammation is a common host response against various invading stimuli, and involves a series of reaction such as hemodynamic change, cell growth and proliferation, leukocyte migration and activation, and tissue remodeling. Various cytokines are working as messenger molecules to organize this inflammatory reaction. Recently, it is recognized that reactive oxygen species may also work as signaling molecules.[1,2] Activated neutrophils and macrophages are potent source of oxidants, such as superoxide anion, hydrogen peroxide, hypochlorous acid and chloramines.[3] Monochloramine (NH_2Cl) is a membrane-permeable chloramine derivative, and modulates various responses related to inflammation. NH_2Cl at a concentration below 100 μM has been shown to inhibit protein kinase C-mediated activation of nuclear factor κB,[4] stimulate Fas-induced apoptosis,[5] affects cell-cycle status[6] and induce calcium influx.[7] In addition, neutrophil suspension at a physiologically relevant concentration produces about 100 μM of chloramine in a short-term culture.[8] These findings suggest that NH_2Cl may modulate inflammatory reactions.

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Leukocyte recruitment to the site of inflammation involves extravasation through the vascular endothelium, and cell adhesion molecules, such as e-selectin (CD62E, endothelial leukocyte adhesion molecule-1) and intercellular adhesion molecule-1 (ICAM-1, also called CD 54) play an essential role.[9] E-selectin and ICAM-1 are expressed on the endothelial cell surface upon stimulation with inflammatory cytokines, such as TNF-α and interleukin-1β[10]. Their expression is transcriptionally regulated through the action of transcription factors such as nuclear factor-κB (NF-κB).[11–13] The NF-κB family is composed of at least five members including p65 (RelA), p50 (NF-κB1), p52 (NF-κB2), RelB and c-Rel, which form homo- or heterodimers. In endothelial cells, p50–p65 heterodimer is the predominant species, and is present in the cytosol as an inactive form, which is bound to the inhibitory protein, IκB.[14] Upon stimulation, IκB-α is rapidly phosphorylated by IκB kinases, and degraded by proteasome to liberate active NF-κB.[15–18] The liberated NF-κB dimers subsequently translocate into the nucleus, where they activate gene transcription.

It has been reported that reactive oxygen species are essential for the expression of adhesion molecules.[19,20] Exogenous H_2O_2 at low dose induced ICAM-1 expression.[21] TNF-α-induced expression of adhesion molecules is also reported to be dependent on the intracellular production of reactive oxygen species.[22] On the other hand, our previous study suggested that NH_2Cl inhibited NF-κB activation,[4] and NH_2Cl might have inhibitory effects on the expression of adhesion molecules. Thus, we explored the effects of NH_2Cl on TNF-α-induced expression of e-selectin and ICAM-1 and its mechanism. We observed that NH_2Cl suppressed the expression of both e-selectin and ICAM-1 through the inhibition of NF-κB activation in human endothelial cells. We also showed that NH_2Cl inhibited the TNF-α-induced phosphorylation and subsequent degradation of IκB-α protein.

MATERIAL AND METHODS

Materials

Anti-e-selectin antibody (Clone #68-5H11) was obtained from Pharmingen (San Diego, CA), FITC-conjugated anti-mouse antibody and FITC-conjugated ICAM-1 antibody (Clone #84H10) were from Immunotech (Westbrook, ME). Human TNF-α was from Peprotech. Inc. (London, England). [γ^{32}P]ATP was from Amersham (Buckinghamshire, England). Anti-IκB-α polyclonal antibody (#sc-847), NF-κB p50 (#sc-7178) and p65 (#sc-109G) rabbit polyclonal antibodies, and NF-κB mutant oligonucleotides (#sc-2511) were from Santa Cruz Biotechnology (Santa Cruz, CA). NF-κB oligonucleotide (#E3292) was from Promega (Madison, WI). Anti-phospho (Ser32)-IκB-α polyclonal antibody (#9241s) was from New England Biolabs (Beverly, MA). Proteasome inhibitor (Z-Leu-Leu-Leu-H) was from Peptide Institute, Inc. (Osaka, Japan). Aprotinin was from Loche Diagnostic GmbH (Mannheim, Germany). Other chemicals were of analytical grade or better. Monochloramine (NH_2Cl, approximately 5 mM) was prepared fresh just before experiments and the concentration was determined by the UV absorption spectrum as described previously.[23]

Cell Culture and NH_2Cl Pretreatment

Human umbilical vein endothelial cells (HUVEC) were obtained from KURABO (Osaka, Japan), and maintained in a 5% CO_2 incubator at 37°C. The growth medium was Humedia EB2 (KURABO) supplemented with 2% fetal bovine serum (FBS), 5 ng/ml human fibroblast growth factor-β, 1 μg/ml hydrocortisone, 10 ng/ml human epidermal growth factor, 10 μg/ml heparin, 50 μg/ml gentamicin, and 50 ng/ml amphotericin B. All experiments were performed using passage 3 cells. HUVEC were grown on 100 mm dishes to 80% confluence (approximately 2×10^6 cells/dish). Just before NH_2Cl treatment, the medium was replaced with Humedia EB2 (5 ml/dish) without FBS and growth factors. Then NH_2Cl was added to a final concentration of 20–90 μM and immediately mixed with the medium. It was noted that NH_2Cl reacted with medium constituents with apparent half-life on 2.5 min, even without any cells (data not shown). After incubation for 20 min at 37°C, the medium was replaced with the growth medium, and the cells were stimulated with TNF-α (10 ng/ml). Cells were then cultured for the indicated times before each analysis.

Measurements of E-selectin and ICAM-1 Expression

E-selectin and ICAM-1 expression were measured at various time points after TNF-α stimulation.[9,24,25] After the incubation, cells were rinsed twice with ice-cold phosphate-buffered saline (PBS), then scraped off and collected by centrifugation. Collected cells were incubated with either anti-e-selectin antibody or FITC-labeled anti-ICAM-1 antibody for 1 h on ice, and washed three times with PBS. In the case of e-selectin, the cells were further labeled with an FITC-conjugated secondary antibody. Immunostained cells were washed and resuspended in PBS, and immediately analyzed by a flow cytometer (FACSCalibur, Becton Dickinson, Franklin Lakes, NJ). Mean fluorescence intensity and percentage of positive cells were analyzed. Controls included HUVEC stimulated with TNF-α only. The cells that have the

fluorescent intensity of more than 6.2 arbitrary unit for e-selectin, and 5.6 arbitrary unit for ICAM-1, were considered positive, because these values best separated the positive control (TNF-α-stimulated) and negative control (non-stimulated) cells.

Electrophoretic Mobility Shift Assay (EMSA) for NF-κB

Nuclear protein extraction and EMSA were performed as described previously.[26,27] Two micrograms of nuclear protein was incubated with ^{32}P-end-labeled NF-κB consensus oligonucleotide for 30 min at 37°C. The DNA–protein complex formed was separated from free oligonucleotide on 6% native polyacrylamide gels. NF-κB mutant oligonucleotide was employed to examine the specificity of NF-κB binding. Binding specificity was also confirmed by the competition with excess amount of unlabeled consensus oligonucleotide. For supershift assays, nuclear extracts prepared from TNF-α treated cells were incubated with antibodies to either the p50 or p65 NF-κB subunits for 45 min at room temperature before the complex was analyzed by EMSA.

Western Blot Analysis for IκB-α

It is reported that TNF-α induces phosphorylation and degradation of IκB-α within 15 min in endothelial cells.[28,29] Thus, HUVEC were collected 15 min after the addition of TNF-α, rinsed twice in ice-cold PBS, and cellular proteins were extracted in RIPA lysis buffer (10 mM Tris HCl, pH 7.4, 0.1% SDS, 1% NP-40, 0.15 M NaCl, 1 mM EDTA 10 μg/ml aprotinin, 0.1% sodium deoxychorate). For the detection of phosphorylated form of IκB-α, the cells were preincubated with 5 μg/ml of proteasome inhibitor (Z-Leu-Leu-Leu-H) for 30 min before TNF-α stimulation. The samples (50 μg protein/well) were electrophoresed on 12.5% SDS-polyacrylamide gels and transferred to a nitrocellulose membrane. Anti-IκB-α rabbit antisera and anti-phospho-IκB-α rabbit antisera were used at 1:200 dilution. Immunoreactive proteins were detected using the enhanced chemiluminescence system (NEN, Boston, MA).

Glutathione Analysis

After the NH_2Cl treatment, cells were rinsed three times with ice-cold PBS, scraped off and collected by centrifugation. The cells were resuspended in ice-cold PBS, and aliquots were kept for protein determination. The rest of the cell suspension was added with an equal volume of 10% (w/v) trichloroacetic acid + 2 mM EDTA, sonicated and centrifuged to obtain acid extract. Total and oxidized glutathione in the acid extract were measured as described previously.[30]

Statistical Analysis

Results were tabulated for the indicated number of experimental samples. Group means were compared using Student's *t*-test for unpaired and paired samples with a two-tailed distribution.

RESULTS

Monochloramine Treatment of HUVEC Suppressed E-selectin Expression

Monochloramine pretreatment suppressed the TNFα-induced e-selectin expression. Non-stimulated HUVEC were slightly positive for e-selectin, in which 14% of the cells were positive with mean fluorescence intensity of 6 arbitrary units (data not shown). Stimulation with TNF-α for 4 h resulted in marked enhancement of e-selectin expression. About 70% of the cells became positive with mean fluorescence intensity of 35 arbitrary units (Fig. 1). Pretreatment of the cells with 20–90 μM of NH_2Cl resulted in a significant decrease in the expression. The suppression was evident by as low as 20 μM of NH_2Cl and higher doses of NH_2Cl showed more pronounced effects. Mean fluorescence intensity was affected more than the percentage of positive cells, which suggested that each cell showed lower e-selectin expression.

Monochloramine Induced Transient Cell Shrinkage Without Loss of Viability

When HUVEC were treated with NH_2Cl, the cells glittered and shrank, showing thin fiber-like structures within 5 min, especially among the cells located

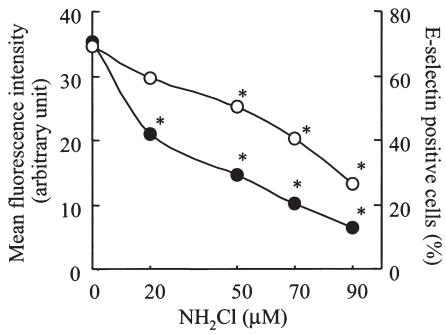

FIGURE 1 NH_2Cl pretreatment of HUVEC resulted in the suppression of TNF-α-induced e-selectin expression: Cells were pretreated for 20 min with 20–90 μM of NH_2Cl, then stimulated with 10 ng/ml of TNF-α, and incubated for 4 h. Cells were collected, immunostained using mono-specific antibodies, and analyzed by flow cytometry. Mean fluorescence intensity and percentage of positive cells for e-selectin were measured. Filled circle, mean fluorescence intensity; open circle, percentage of positive cells. *Significant inhibition ($P < 0.05$) *vs* "0 μM samples". The results shown are representative of more than three similar experiments.

TABLE I Cell viability after NH_2Cl treatment

NH_2Cl concentration (μM)	NH_2Cl only (%)	NH_2Cl + TNF-α (10 ng/ml) (%)
0	96.4 ± 1.9	92.5 ± 1.3
20	95.3 ± 0.9	94.6 ± 1.2
50	95.6 ± 0.9	94.0 ± 1.5
70	96.4 ± 1.1	95.0 ± 1.4
80	96.1 ± 1.5	95.1 ± 1.0

HUVEC were treated with the indicated concentrations of NH_2Cl for 20 min at 37°C in Humedia EB2, then stimulated with TNF-α. Cell viability was studied by the trypan blue exclusion test 16 h after the treatment. Mean ± SD for three determinations are shown.

at the periphery of the sheets. This cell shrinkage was maximal at 10 min, and after the medium was changed to the growth medium, the cells gradually spread again and returned to the original morphology within 3 h. The cells still proliferated thereafter. Complete detachment was not observed. The cell viability was evaluated by trypan blue exclusion test 16 h after the treatment, which showed no loss of viability (Table I).

The Suppression of TNF-α Induced Expression of E-selectin and ICAM-1 by NH_2Cl was Transient

In addition to e-selectin expression, NH_2Cl also suppressed TNF-α-induced ICAM-1 expression and these inhibitory effects were transient in both cases. When HUVEC without NH_2Cl pretreatment was stimulated with TNF-α, e-selectin expression increased rapidly, reached a maximum at 4–8 h and declined thereafter (Fig. 2A). ICAM-1 expression showed more gradual and steady increase during the 16 h period of observation (Fig. 2B). Notably, NH_2Cl pretreatment (70 μM) resulted in a significant inhibition of e-selectin and ICAM-1 expression, especially at 4–8 h (Fig. 2A, B). E-selectin expression of the NH_2Cl + TNF-α treated cells increased slightly from 8 to 16 h, whereas that of the positive control (TNF-α stimulated cells) decreased, and these values were no longer different at 16 h (Fig. 2A). ICAM-1 expression increased from 8 to 16 h in both groups, but NH_2Cl-pretreated cells showed slightly lower value at 16 h (Fig. 2B).

The transient nature of the e-selectin and ICAM-1 inhibition was more obvious when the positive cell population was compared. In both cases, more than 80% of the TNF-α-stimulated HUVECs became positive for the adhesion molecules in 4 h, which persisted for at least 16 h (Fig. 2C, D). In contrast, NH_2Cl pretreated cells showed apparently fewer cells being positive for e-selectin and ICAM-1 at 4 and 8 h. At 16 h, however, comparable proportion of the cells became positive for the adhesion molecules. Without TNF-α stimulation, NH_2Cl treatment alone did not induce either of the adhesion molecules (data not shown).

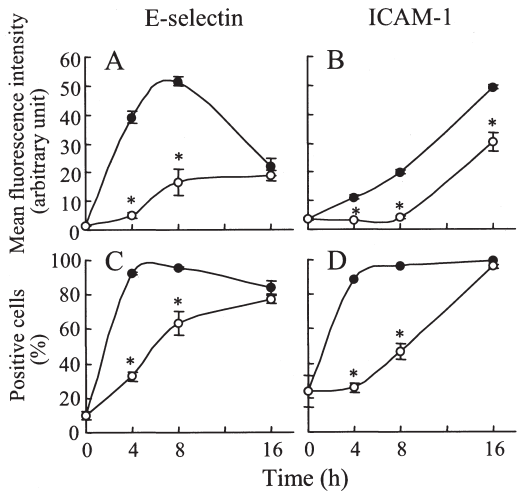

FIGURE 2 Time course of e-selectin and ICAM-1 expression after treatment with NH_2Cl and/or TNF-α: HUVEC were pretreated for 20 min with or without 70 μM of NH_2Cl and then stimulated with 10 ng/ml of TNF-α for the indicated time periods (*Panel A*: mean fluorescence intensity of e-selectin, *Panel B*: mean fluorescence intensity of ICAM-1, *Panel C*: percentage of e-selectin positive cells, *Panel D*: percentage of ICAM-1 positive cells). Cells were collected, immunostained with mono-specific antibodies, and analyzed by flow cytometry. Filled circle, TNF-α stimulation only; open circle, NH_2Cl pretreatment followed by TNF-α stimulation. *Significant inhibition ($P < 0.05$) *vs* cells stimulated with TNF-α only. The results shown are means ± SD of triplicate samples in a single experiment, and representative of two similar experiments.

Inhibition of TNF-α-induced NF-κB Activation by NH_2Cl

TNF-α (10 ng/ml) induced marked activation of NF-κB, which is one of the essential transcription factors for the expression of e-selectin and ICAM-1, and this activation was inhibited by the NH_2Cl pretreatment (Fig. 3). The inhibitory effect was apparent at 20 μM of NH_2Cl, and increasing the concentration up to 70 μM resulted in a further inhibition in NF-κB activation. This NF-κB inhibition correlates well with the inhibition of e-selectin expression as shown in Fig. 1. Antibodies against either the p50 or p65 subunits of NF-κB supershifted the bands in the EMSA assay, indicating that the lower NF-κB complex was composed of p50/p65 heterodimers and the upper complex of p65/p65 homodimers, as reported previously.[14] NH_2Cl treatment resulted in a decrease in both bands. These bands disappeared by excess of unlabeled consensus oligonucleotide

but not by mutated oligonucleotide, which further confirmed the specificity.

Inhibition of NF-κB Activation by NH_2Cl was Transient

The inhibitory effects of NH_2Cl on the TNF-α-induced NF-κB activation were also transient. When NF-κB activation was compared between NH_2Cl pretreated and non-treated HUVEC at various time points, the pretreated cells showed significantly lower NF-κB bands at 4 and 8 h (Fig. 4). Interestingly, at 16 h, the NF-κB bands became comparable between the two groups, and these bands were substantially stronger than that of non-stimulated control. The result was consistent with the time-course of e-selectin and ICAM-1 expression as shown in Fig. 2.

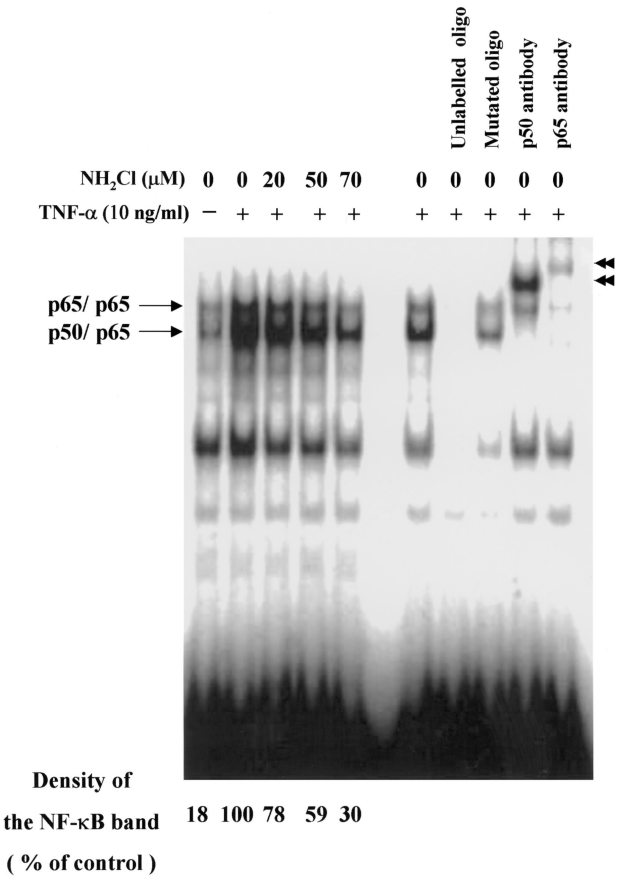

FIGURE 3 Pretreatment of HUVEC with NH_2Cl resulted in the inhibition of TNF-α-induced NF-κB activation: HUVEC were treated with various concentrations of NH_2Cl for 20 min at 37°C. Cells were then stimulated with TNF-α (10 ng/ml) and incubated for 1 h prior to the extraction of nuclear proteins. NF-κB activation was analyzed by EMSA. NF-κB bands (p50/p65 heterodimers and p65/p65 homodimers) are indicated. The specificity of binding was examined by competition with the unlabeled consensus oligonucleotide and mutated oligonucleotide. Double arrowheads identify antibody supershifts, produced by binding of the antibody to the NF-κB protein–DNA complex. Results are representative data from three independent experiments.

NH_2Cl Inhibited IκB-α Phosphorylation and Degradation

To study the mechanism how NH_2Cl abrogated TNF-α induced NF-κB activation, we examined the phosphorylation and degradation of IκB-α. As reported previously, stimulation with TNF-α resulted in marked phosphorylation of the IκB-α protein, which was detected in the presence of proteasome inhibitor.[29,31] This phosphorylation was partially inhibited by the pretreatment of NH_2Cl (70 μM), in which the optical density of the phospho-IκB-α band was about 60% of the non-pretreated sample (Fig. 5). The degradation of IκB-α was more strongly affected by the NH_2Cl pretreatment. With TNF-α stimulation, most of the IκB-α protein in the non-pretreated cells disappeared, whereas 77% of the protein was still preserved in NH_2Cl pretreated cells (Fig. 5).

Total and oxidized glutathione were measured just after NH_2Cl treatment, which showed no significant change for up to 70 μM NH_2Cl (Table II).

DISCUSSION

In this study, we showed that NH_2Cl inhibited TNFα-induced expression of e-selectin and ICAM-1.

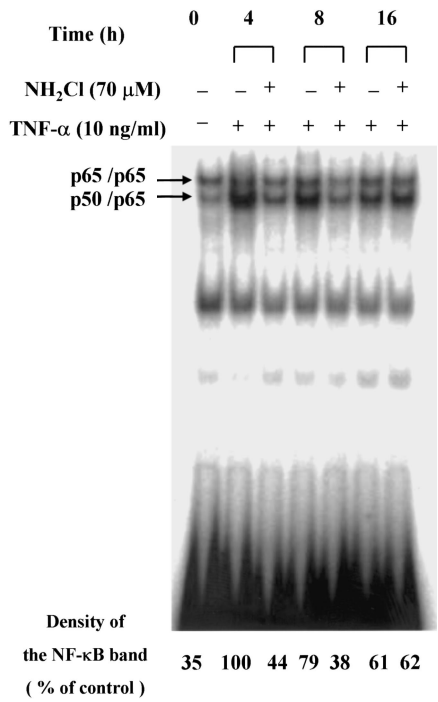

FIGURE 4 NH_2Cl transiently inhibited TNF-α-induced NF-κB activation: HUVEC were pretreated for 20 min with 70 μM of NH_2Cl at 37°C. Cells were then stimulated with TNF-α (10 ng/ml) for the indicated time period (4, 8, and 16 h) prior to nuclear protein extraction. NF-κB activation was analyzed by EMSA. Results are representative data from three independent experiments.

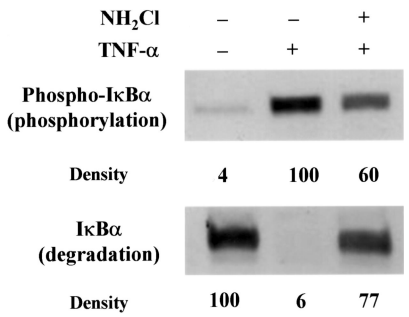

FIGURE 5 NH_2Cl inhibited TNF-α-induced IκB-α phosphorylation and degradation: HUVEC were pretreated for 20 min with 70 μM of NH_2Cl at 37°C. Cells were then stimulated with TNF-α (10 ng/ml) for 15 min. Cell extracts were separated by electrophoresis through a 12.5% SDS polyacrylamide gel. Phosphorylated IκB-α and IκB-α were detected by the specific antibodies using Western blot analysis. For the detection of phosphorylated IκB-α, the cells were preincubated with a proteasome inhibitor for 30 min prior to the addition of TNF-α. Results are representative data from three independent experiments.

Recent reports showed that reactive oxygen species have various effects on the expression of adhesion molecules of leukocytes[32] and endothelial cells.[20,21] Particularly, H_2O_2 is reported to have an interesting dual-effect on the ICAM-1 expression in endothelial cells. When administered in a low dose (0.1 mM), exogenous H_2O_2 induced ICAM-1 expression by enhancing ICAM-1 transcription, probably through the transcription factors AP-1 and Ets.[21] However, exogenous H_2O_2 in a relatively high dose (1 mM, 5 min) inhibited the TNF-α-induced expression of ICAM-1 and e-selectin, which accompanied by the inhibition of NF-κB activation.[33] In contrast to this dual-effect of H_2O_2, NH_2Cl at a physiologically attainable dose (<0.1 mM) strongly inhibited both e-selectin and ICAM-1 expression. In this respect, NH_2Cl was effective at lower concentration than H_2O_2 in the inhibition of adhesion molecule expression. Moreover, NH_2Cl alone did not stimulate the ICAM-I expression at any dose tested (data not shown). Thus, NH_2Cl may work as a feedback regulator for the leukocyte accumulation.

The expression of e-selectin and ICAM-1 is transcriptionally regulated, and NF-κB is one of the essential transcription factors. As reported previously, TNF-α stimulation showed a marked activation of NF-κB in HUVEC.[15,34,35] Our study showed that NH_2Cl pretreatment inhibited this TNF-α-induced NF-κB activation. Notably, the inhibition of NF-κB activation and adhesion molecule expression occurred at similar concentration of NH_2Cl. Moreover, the time course of the inhibitory effect was also similar in both cases. These observations showed that NF-κB activation was tightly linked to the adhesion molecule expression and suggested that inhibition of adhesion molecule expression was due to the inhibition of NF-κB activation. As NF-κB regulates a variety of genes involved in inflammation including cytokines, receptors and host cell responses to viruses such as HIV,[12,13,36] NH_2Cl may also affect the expression of these genes and modulate the inflammatory response.

We further studied how NF-κB activation was inhibited, and found that IκB phosphorylation and degradation were inhibited by NH_2Cl pretreatment. NF-κB activation usually involves phosphorylation and degradation of IκB-α[37,38] and the inhibition of IκB-α degradation would result in the decrease in liberation of active NF-κB. IκB phosphorylation is catalyzed by IκB kinase.[18,39] The phospho-IκB-α-specific antibody that we used in this experiment recognizes phosphorylation at Ser32, which is essential for TNF-α-induced NF-κB activation.[37] It is interesting to note that NH_2Cl also inhibited serine phosphorylation in neutrophils. We previously reported that NH_2Cl pretreatment of neutrophils resulted in the inhibition of phorbol ester-inducible protein kinase C activation, and inhibition of the phosphorylation of a 47 kDa protein, most likely $p47^{phox}$, a cytosolic component of NADPH oxidase.[4] Protein kinase C is a Ser/Thr kinase[40] and $p47^{phox}$ is phosphorylated at multiple serine residues upon phorbol ester stimulation.[41] As NH_2Cl pretreatment inhibited serine phosphorylation of IκB in HUVEC, Ser/Thr kinases, such as IκB kinase, may be possible targets of NH_2Cl.

It has been reported that thiol-modifying agents modulate NF-κB activation and cell adhesion molecule expression.[33,42] We studied total and oxidized glutathione after NH_2Cl treatment, but no significant change was detected. The result is not surprising, because glutathione can be quickly replenished by *de novo* synthesis, and the cells have

TABLE II Total and oxidized glutathione in HUVEC after NH_2Cl treatment

NH_2Cl concentration (μM)	GSH + GSSG (nmol/mg protein)	GSSG/GSH + GSSG (%)
0	23.7 ± 2.3	11.1 ± 1.9
20	26.6 ± 2.5	11.6 ± 1.1
50	25.0 ± 2.6	11.6 ± 1.9
70	26.7 ± 3.1	10.9 ± 1.5

HUVEC were treated with the indicated concentrations of NH_2Cl for 20 min at 37°C. Just after the treatment, cellular glutathione was extracted and measured as described in "Material and methods" section. Mean ± SD for three determinations are shown. GSH, glutathione; GSSG, glutathione disulfide.

glutathione reductase that effectively reduces oxidized glutathione. Our previous report on neutrophils also showed that NH_2Cl oxidized glutathione, which was rapidly replenished by the new synthesis.[43] Further studies on the cellular redox status and kinase activity will be needed.

In summary, this study demonstrated that NH_2Cl inhibited TNF-α-induced e-selectin and ICAM-1 expression in HUVEC. This inhibition appeared to be due to the inhibition of IκB-α phosphorylation, which resulted in the inhibition of NF-κB activation that normally occurs upon TNF-α stimulation of endothelial cells. The data suggest that there is an oxidant-mediated feedback mechanism that prevents inappropriate activation of the inflammatory response.

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