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The Effect of Hypochlorous Acid on the Expression of Adhesion Molecules and Activation of NF-κB in Cultured Human Endothelial Cells

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ABSTRACT

Exposure to oxidants can up-regulate the expression of adhesion molecules in endothelial cells with a consequent increase in neutrophil attachment. Similarly, the transcription factor nuclear factor-κB (NF-κB), which controls the expression of the intercellular adhesion molecules (ICAMs), can also be activated by oxidants in some cells. We have investigated whether hypochlorous acid (HOCl), the major strong oxidant produced by neutrophils, can affect the expression of adhesion molecules on human umbilical vein endothelial cells (HUVEC) and promote neutrophil adhesion. We found that HOCl could induce an increase in neutrophil adhesion to the endothelial cells after 60 min of treatment. Activation of NF-κB could be detected under similar conditions. However, the dose of HOCl required for this effect resulted in considerable longer-term toxicity to the cells. Treatment of HUVEC with sublethal doses of HOCl had no effect on NF-κB activation, neutrophil adhesion, or the surface expression of E-selectin, ICAM-1, or P-selectin. However, pretreatment with low concentrations of HOCl prevented phorbol myristate acetate-induced von Willebrand factor expression (a marker for P-selectin). These results show that, unlike H_2O_2, HOCl does not significantly enhance neutrophil attachment to the endothelium. Rather it may be able to inhibit the expression of adhesion molecules with important consequences for endothelial function and inflammatory vascular disease. Antioxid. Redox Signal. 4, 5–15.

INTRODUCTION

THE ENDOTHELIUM has a central role in regulating the inflammatory response. In particular, the recruitment and trafficking of leukocytes to the site of inflammation are controlled by the expression of adhesion molecules on the endothelial cell surface following activation by various inflammatory stimuli, including tumor necrosis factor-α (TNF-α), interleukin-1, histamine, or lipopolysaccharide (for review, see 47). The adhesion molecules involved are under varied control. Of the selectins, which are responsible for the initial attachment of leukocytes to the endothelium, P-selectin is rapidly released upon cell activation, whereas E-selectin requires *de novo* synthesis (3, 10). Intercellular adhesion molecule-1 (ICAM-1), which is involved in firm adhesion, also requires *de novo* synthesis. Both ICAM-1 and E-selectin gene expression rely upon the activation of the transcription factor nuclear factor-κB (NF-κB) (6).

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Recent studies have demonstrated that neutrophil adhesion to the endothelium can be stimulated by oxidants, via the altered expression of particular adhesion molecules (13, 17, 24, 32). In a study by Patel and co-workers (25), treatment of endothelial cells with hydrogen peroxide (H_2O_2) resulted in increased levels of neutrophil adhesion, which was attributed to increased surface expression of P-selectin. H_2O_2 has also been demonstrated to increase expression of ICAM-1, and this correlated with enhanced neutrophil-endothelial cell adhesion (18). E-selectin expression could potentially be induced by oxidants, with a study by Rahman *et al.* attributing TNF-α-induced E-selectin expression to oxidant generation (28). In addition, several studies have shown that oxidants can induce the activation of NF-κB in some cell types, suggesting a potential for the oxidative regulation of pro-inflammatory gene expression (2, 21, 29, 31).

One oxidant that has not been investigated previously in this regard is hypochlorous acid (HOCl), the major strong oxidant produced by neutrophils (9, 44). HOCl is generated during the respiratory burst of neutrophils and monocytes by the enzyme myeloperoxidase (MPO) according to the following reaction:

$$H_2O_2 + Cl^- + H^+ \rightarrow HOCl + H_2O$$

It is a reactive oxidant with potent microbicidal and cytotoxic properties, and its production at sites of inflammation is thought to contribute to the tissue injury associated with a range of pathologies (1, 19, 33, 37, 38). Whereas the toxicity of HOCl has been well documented, more recent studies have provided evidence for its potential to react selectively with cell thiols and to modulate cell signaling (26, 27, 39, 42, 43). The endothelium is likely to be exposed to HOCl *in vivo*, with an immunological study of atherosclerotic lesions demonstrating the presence of HOCl-modified proteins in the endothelial cells lining the vasculature (12). The effect of low-level exposure to HOCl on endothelial cell function has not been investigated previously. Cultured human umbilical vein endothelial cells (HUVEC) have commonly been used as a model of endothelial cell-neutrophil adhesion (16, 34, 35). In this study, we have used these cells to determine whether this exposure could modulate NF-κB activation or affect neutrophil-endothelial cell adhesion via the surface expression of the endothelial cell adhesion molecules P-selectin, E-selectin, and ICAM-1.

MATERIALS AND METHODS

Materials

Sodium hypochlorite was purchased from Reckitt and Colman (Auckland, New Zealand). The concentration of HOCl was established by reaction with 5-thio-2-nitrobenzoic acid and measurement of A_{412} (ε = 28,200 $M^{-1}cm^{-1}$) (41). Cell culture materials, including medium 199, fetal bovine serum, trypsin, penicillin, and streptomycin, were from Gibco-BRL supplied by Life Technologies Inc. (Rockville, MD, U.S.A.). Type I collagenase was purchased from Worthington Biochemical Corp. (Freehold, NJ, U.S.A.) and heparin from Fisons Pty., Ltd. (Sydney, Australia). Fibronectin was purified from human plasma by affinity chromatography on gelatin-Sepharose as described (26).

Hexadecyltrimethylammonium chloride (CETAC) was obtained from Acros Organics (Ceel, Belgium), and 3,3′,5,5′-tetramethylbenzidine (TMB) was purchased from Fluka AG (Buchs, Switzerland). Monoclonal anti-human E-selectin (clone 1.2B6), monoclonal anti-human ICAM-1 (clone 8.4A6), goat anti-mouse IgG fluorescein isothiocyanate (FITC) conjugate, and goat anti-rabbit horseradish peroxidase conjugate were all purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). The rabbit anti-human von Willebrand factor was obtained from Dako A/S (Glostrup, Denmark). Rabbit anti-human p65 antibody was from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, U.S.A.), and goat anti-rabbit IgG-Cy3 conjugate was from Amersham Pharmacia Biotech UK Ltd. (Buckinghamshire, U.K.). Human recombinant TNF-α was a gift from Dr. Jurgen Michaelis.

All other chemicals were purchased from Sigma Chemical Co. or BDH Laboratory Supplies (Poole, U.K.).

Cell culture

HUVEC were isolated from umbilical cords by collagenase digestion (14). Cells were cultured in fibronectin-coated flasks in medium 199 supplemented with fetal calf serum, endothelial cell growth factors, heparin, penicillin, and streptomycin (26), and maintained at 37°C in a humidified atmosphere with 5% CO_2. For experimental purposes, cells were transferred to 24- or 96-well culture dishes.

HOCl treatment

At the pH used, the HOCl solutions contained about equimolar concentrations of HOCl and OCl^- (pK_a = 7.5); we have subsequently referred to it as HOCl. The standardized HOCl solution was diluted in Hanks' balanced salt solution [HBSS; phosphate-buffered saline (PBS), pH 7.4, containing 5 m*M* glucose, 0.5 m*M* magnesium, and 1 m*M* calcium] to give final working concentrations. Confluent HUVEC were washed several times in HBSS to remove media, the HOCl was added, and the cells were incubated at 37°C for the times indicated. For extended incubation times of up to 24 h, HBSS was replaced with fresh medium.

Preparation of neutrophils

Neutrophils were isolated from the blood of healthy donors by density gradient separation and dextran sedimentation, with removal of any contaminating red cells by hypotonic lysis (4). Neutrophils were resuspended in HBSS at 1×10^6 cells/ml.

Neutrophil adhesion assay

The percentage of neutrophils adhered to endothelial cells was determined by measuring MPO activity based on its ability to oxidize TMB (36). Endothelial cells grown in 96-well plates were treated with HOCl and then washed, and 80,000 neutrophils per well were added in 100 μl of HBSS. Each well contained 20,000 endothelial cells, and this number was sufficient to allow unimpeded adhesion of neutrophils (8, 13, 16, 17). After 30 minutes at 37°C, the wells were washed gently two to four times in HBSS to remove nonadherent neutrophils. Washes were transferred to fresh wells and assayed for neutrophils. CETAC (1%) was added to each well to lyse the cells. After 30 minutes at room temperature, 100 μl of TMB solution (2.4 m*M* TMB in 300 m*M* sodium acetate buffer, pH 4.5, with 450 μ*M* H_2O_2 added immediately prior to addition to the plate) was added to the wells. After a further 5 min in the dark, the absorbance was read at 630 nm on a microplate reader.

The total MPO activity from the adherent and nonadherent neutrophils was measured in each sample and then averaged to give the total absorbance for 80,000 neutrophils. The adherent population was calculated as a percentage of this and the results expressed as fold increase above control adherence.

ICAM-1 and E-selectin surface expression

ICAM-1 and E-selectin expression was analyzed by indirect immunofluorescence using monoclonal antibodies to each. After treatment, cells were harvested with trypsin and incubated with anti-ICAM-1 or anti-E-selectin antibody for 20 min at 4°C. Cells were washed twice in PBS and then incubated with the secondary antibody, an anti-mouse IgG FITC-conjugate, for 20 min in the dark at 4°C. Following washing, 2 μg of propidium iodide was added and the cell fluorescence was determined by a bivariate flow cytometer (FACS vantage, Becton–Dickinson, Mountain View, CA, U.S.A.).

Immunofluorescent localization of NF-κB

The activation of NF-κB was monitored by immunofluorescence using a primary antibody to p65, one of the subunits of NF-κB (15). HUVEC, cultured on glass chamber slides, were washed free of medium and exposed to varying concentrations of HOCl or to TNF-α for 1 h. The cells were then fixed in 100% methanol at room temperature for 30 min and permeabilized with 0.1% (vol/vol) Triton X-100 for 20 min. After three washes with PBS containing 1% (wt/vol) bovine serum albumin (BSA), the slides were covered with anti-p65 antibody (200 μl of 2.5 μl/ml in PBS/BSA) and left overnight at 4°C. After further washes

with PBS/BSA (3 × 20 min), the slides were immersed in goat anti-rabbit IgG-Cy3 (0.2 μg/ml in PBS/BSA) for 2 h at room temperature, washed with PBS to remove excess antibody, and examined by fluorescence microscopy.

Von Willebrand factor release assay

An ELISA measuring release of von Willebrand factor was used to estimate surface expression of P-selectin. Endothelial cells grown in 96-well plates were treated with HOCl or phorbol myristate acetate (PMA), and 20 μl of supernatant was removed from each well and transferred to a 96-well Nunc-Immuno plate (Nunc A/S, Roskilde, Denmark). Standard ELISA procedure was followed using rabbit anti-human von Willebrand factor as the primary antibody and a horseradish peroxidase-conjugated goat anti-rabbit IgG as the detecting antibody.

RESULTS

Neutrophil adhesion

MPO activity was used to determine the percentage of neutrophils adherent to endothelial cells (36). The experimental system was validated with TNF-α as a positive control. When HUVEC were stimulated with TNF-α for 12 h, and then neutrophils added, a dose-dependent increase in neutrophil adherence was observed (Fig. 1). Neutrophil adhesion to endothelial cells increased from 7% of total cells in control HUVEC to ~35% after 12 h of exposure to TNF-α.

When endothelial cells were exposed to HOCl for 1 h in HBSS, then neutrophils added, a small increase in neutrophil adhesion was observed at the higher HOCl concentrations, although this was quite variable and did not reach statistical significance (Fig. 2). Similar results were seen after HOCl treatment of HUVEC for 30 min in HBSS (data not shown), with adhesion for both increased ~50% above control levels at the higher HOCl concentrations. The increased neutrophil adhesion was abrogated when the cells were reincubated in complete medium

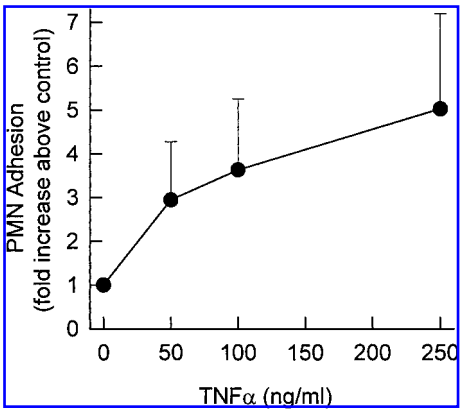

FIG. 1. Neutrophil adherence to HUVEC treated with TNF-α for 12 h. Confluent monolayers were exposed to TNF-α in complete medium for 12 h and washed, 80,000 neutrophils were added per well, and the cells were incubated for 30 min at 37°C. Adherence was measured by monitoring MPO activity. Results are expressed as fold increase above control adherence and are the means ± SD of five experiments performed in duplicate.

(Fig. 2). At HOCl concentrations of 30 μ*M* or lower, endothelial cell morphology did not differ from that of control monolayers. Above these concentrations, the cells were contracted and rounded up with large gaps ob-

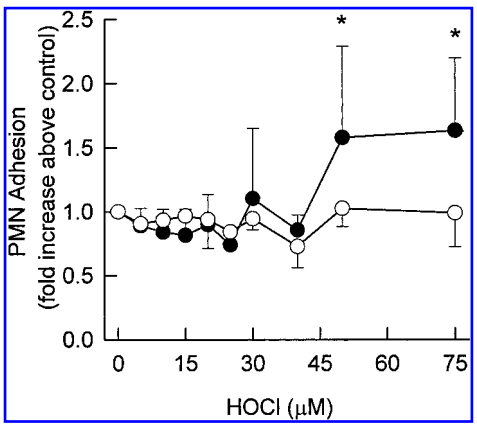

FIG. 2. Neutrophil adherence to HUVEC treated with HOCl. HUVEC were exposed to HOCl for 60 min in HBSS (●) or 30 min in HBSS, followed by 30 min in conditioned media (○). After washing, neutrophils were added and the cells incubated for 30 min at 37°C. Adherence was measured by MPO assay. Results are expressed as fold increase above control adherence and are the means ± SD of three to five experiments performed in duplicate. There were no significant differences between control and any HOCl treatment. At 50 and 75 μ*M* HOCl, samples treated in HBSS for 60 min were significantly different from cells reincubated in media after 30 min ($^*p < 0.05$).

served in the monolayers. It was impossible to determine whether neutrophils were adherent to the cells or to the substratum. When endothelial cells were assayed 4, 12, or 24 h after HOCl exposure, no change in the level of neutrophil adherence was observed (data not shown).

ICAM-1 and E-selectin expression

To determine whether HOCl could affect the expression of adhesion molecules on endothelial cells, ICAM-1 surface expression was monitored by flow cytometry. Treatment of HUVEC with TNF-α for 1 h induced a slight shift in fluorescence, indicating a small increase in ICAM-1 expression (Fig. 3A). This shift was significantly increased after 24 h (Fig. 3B). One hour of treatment with either 20 or 50 μ*M* HOCl did not alter ICAM-1 expression (Fig. 3C and E). When endothelial cells were treated with HOCl for 24 h, there was no change in ICAM-1 expression at lower HOCl concentrations (Fig. 3D and data not shown). With 50 μ*M* HOCl, a definite shift in fluorescence was apparent in two out of three experiments (Fig. 3F). This was not due to nonspecific binding of the secondary antibody at the higher HOCl concentrations. However, it should be noted that there was considerable toxicity at these doses of HOCl, and the significance of this shift in fluorescence is unclear.

Exposure of endothelial cells to TNF-α for 1 h induced increased expression of endothelial E-selectin, as observed by a small shift in fluorescence compared with control cells (Fig. 4A). There was no change in the fluorescence intensity 1 h after exposure to HOCl at any of the concentrations tested (Fig. 4B and C and data not shown). Similar results were obtained with 24-h exposures apart from a low nonspecific antibody binding observed at 50 μ*M* HOCl.

Activation of NF-κB by HOCl exposure

The synthesis and expression of ICAM-1 and E-selectin are under the control of NF-κB, a transcription factor that plays a major role in inflammation and has been shown to be activated by oxidants in several cell types (2, 6, 31). NF-κB exists in the cell cytoplasm as an inactive complex and translocates into the nucleus on activation (15). We used an antibody to the p65 subunit of NF-κB to determine its location in the cell, and hence its state of activation. In unstimulated HUVEC, NF-κB was localized completely in the cell cytoplasm (Fig. 5A). Upon stimulation with TNF-α, most of the fluorescence was found consistently in the cell nucleus of >90% of the cells, indicating generation of the active NF-κB complex (Fig. 5B). When the cells were exposed to HOCl, various patterns were obtained. With sublethal concentrations of HOCl of 20 μ*M* or less, no translocation of NF-κB was observed (Fig. 5C). With HOCl concentrations of 30–50 μ*M,* some cells showed NF-κB staining in the nucleus (Fig. 5D). However, in contrast to the results with TNF-α, only some regions of the slide were positive in this way, although positive cells were observed consistently. The number of cells with nuclear staining for NF-κB never exceeded 20%. It was notable that, at these concentrations, there were substantial changes in the cell morphology (rounding and some detachment), and longer-term incubation resulted in loss of cell viability.

Von Willebrand factor release

Surface expression of P-selectin was not measured directly. Both P-selectin and von Willebrand factor are present in the Weibel–Palade bodies of endothelial cells, and are expressed/released concurrently with fusion of these bodies to the plasma membrane following endothelial stimulation (10, 11, 45). Release of von Willebrand factor was measured to estimate surface expression of P-selectin. Cells stimulated with PMA as a positive control (10) showed increased levels of von Willebrand factor in the medium (Fig. 6). This increase was apparent 45–60 min after the addition of the PMA (data not shown). When endothelial cells were exposed to HOCl at various concentrations, there was no change in the levels of von Willebrand factor in the cell supernatant either at 90 min (Fig. 6) or after 120 min (data not shown).

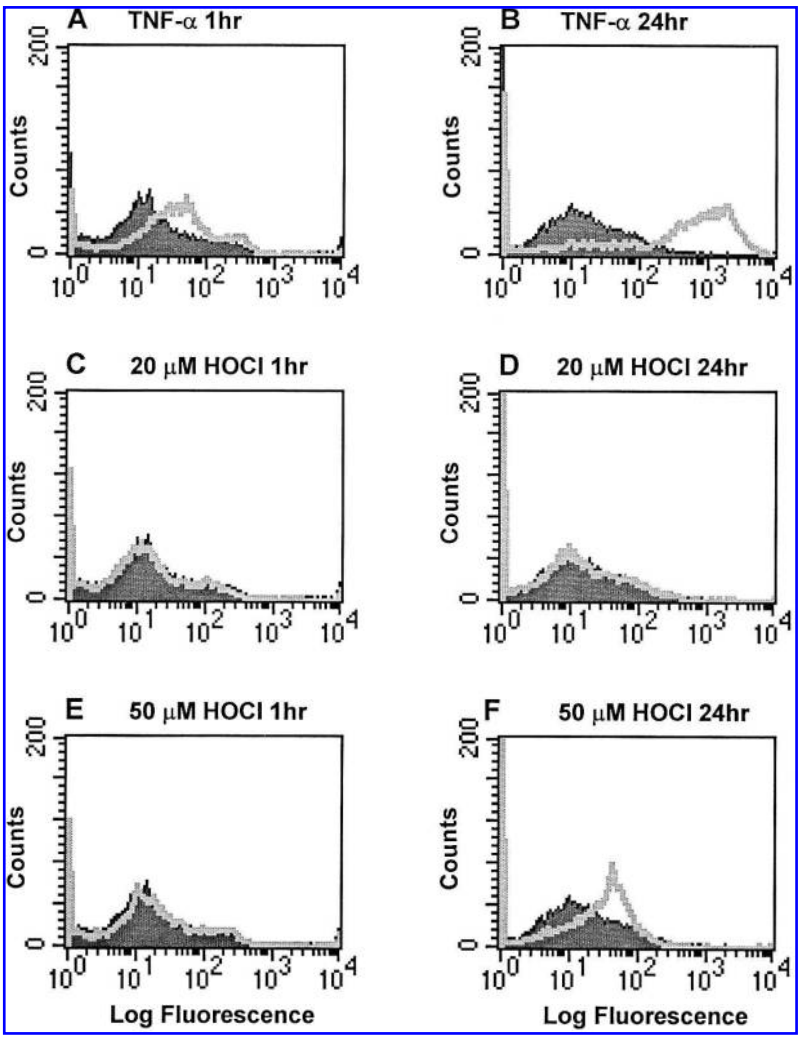

FIG. 3. Effect of HOCl on ICAM-1 expression on the endothelial cell surface. Confluent HUVEC monolayers were exposed to TNF-α (250 ng/ml) or HOCl for either 1 or 24 h. ICAM-1 expression was quantified by flow cytometry using a monoclonal antibody against ICAM-1 (clone 8.4A6) and a FITC-conjugated secondary antibody. Comparative FACS profiles are shown of control untreated HUVEC (filled histograms) or treated HUVEC (gray line). (**A**) TNF-α, 1 h; (**B**) TNF-α, 24 h; (**C**) 20 μ*M* HOCl, 1 hr; (**D**) 20 μ*M* HOCl, 24 h; (**E**) 50 μ*M* HOCl, 1 h; (**F**) 50 μ*M* HOCl, 24 h. A representative experiment of three is shown.

HOCl pretreatment inhibits PMA-induced von Willebrand factor expression

When endothelial cells were pretreated with increasing concentrations of HOCl prior to stimulation with PMA, a progressive decrease in von Willebrand factor release was observed (Fig. 7). This was significant with 10 μ*M* HOCl, and inhibition was almost complete with 30 μ*M*. At these HOCl concentrations, there was no change in endothelial cell morphology and no apparent toxicity.

DISCUSSION

This is the first reported investigation of the ability of HOCl to modulate neutrophil-endothelial cell adhesion. We have shown that treatment of endothelial monolayers with HOCl does not alter surface expression of E-selectin or P-selectin. ICAM-1 expression was increased with HOCl exposure, but only at higher, more toxic concentrations. A similar pattern was seen for activation of NF-κB,

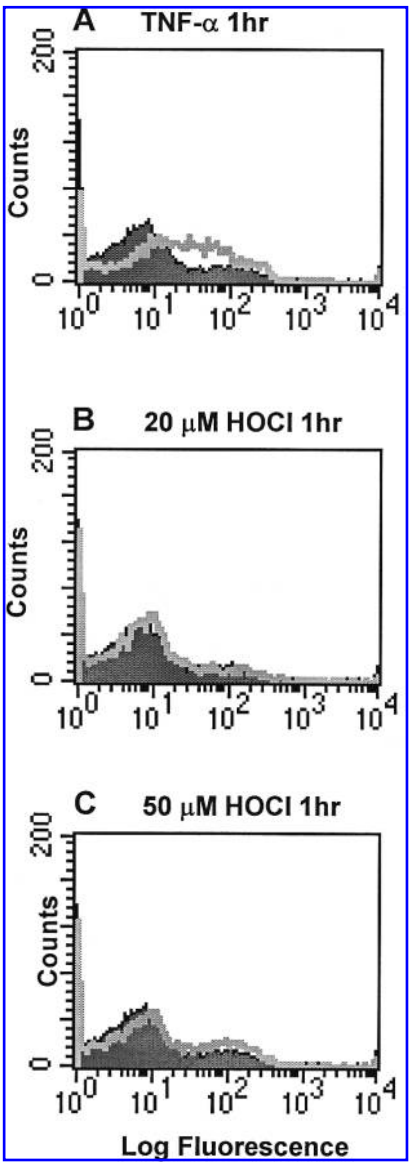

FIG. 4. Effect of HOCl on E-selectin expression on the endothelial cell surface. Confluent HUVEC monolayers were exposed to TNF-α (250 ng/ml) or HOCl for 1 h. E-selectin expression was quantified by flow cytometry using a monoclonal antibody against E-selectin (clone 1.2B6) and a FITC-conjugated secondary antibody. Comparative FACS profiles are shown of control untreated HUVEC (filled histograms) or treated HUVEC (gray line). (**A**) TNF-α, 1 h; (**B**) 20 μ*M* HOCl, 1 h; (**C**) 50 μ*M* HOCl, 1 h. A representative experiment of two is shown.

with no activation at low concentrations but with evidence of some translocation to the nucleus in HUVEC exposed to higher doses of HOCl. Very low concentrations of HOCl could, however, prevent the subsequent release of von Willebrand factor from Wiebel–Palade bodies that also control the expression of P-selectin.

In this way, HOCl contrasts with other oxidants. Treatment with low concentrations of H_2O_2 has been shown to enhance neutrophil adhesion to the endothelium (8, 17, 25, 32) and to increase ICAM-1 expression (5, 18). In one study, 50–1,000 μ*M* H_2O_2 induced a seven- to eightfold increase in neutrophil adhesion within an hour of treatment (18). Concurrent with this, a two- to threefold increase in ICAM-1 surface expression was observed and adhesion was blocked with anti-ICAM-1 antibodies, implying that the increased adhesion was due to the rapid induction of ICAM-1 (18). With HOCl, we observed only a small increase in the adhesion of neutrophils to endothelial cell monolayers with relatively high concentrations of oxidant. There was an associated increase in the expression of ICAM-1, but only after a 24-h incubation period. The expression of ICAM-1 did not, therefore, correlate with increased neutrophil adhesion.

For both E-selectin and ICAM-1, *de novo* protein synthesis is required to increase surface expression (32, 47). Cytokine-induced transcription of these genes relies on the activation of NF-κB (6, 47). Oxidant-mediated activation of this transcription factor has been reported in some cell types (22), and it has been suggested that HOCl could cause activation in endothelial cells (30). Our results suggest that activation of NF-κB is nonspecific and occurs only at relatively toxic concentrations of HOCl. Interestingly, however, the activation of NF-κB and expression of adhesion molecules showed similar dose responses for HOCl. These results contrast with the effect of the cytokine TNF-α, which consistently induced a marked and sustained activation of NF-κB, enhanced the expression of both ICAM-1 and E-selectin, and induced the attachment of neutrophils. When compared with this positive cytokine-mediated response, we feel that although HOCl-mediated activation can be demonstrated, it occurs only within a narrow concentration range that is achievable only in the absence of serum, conditions that are unlikely to be easily dupli-

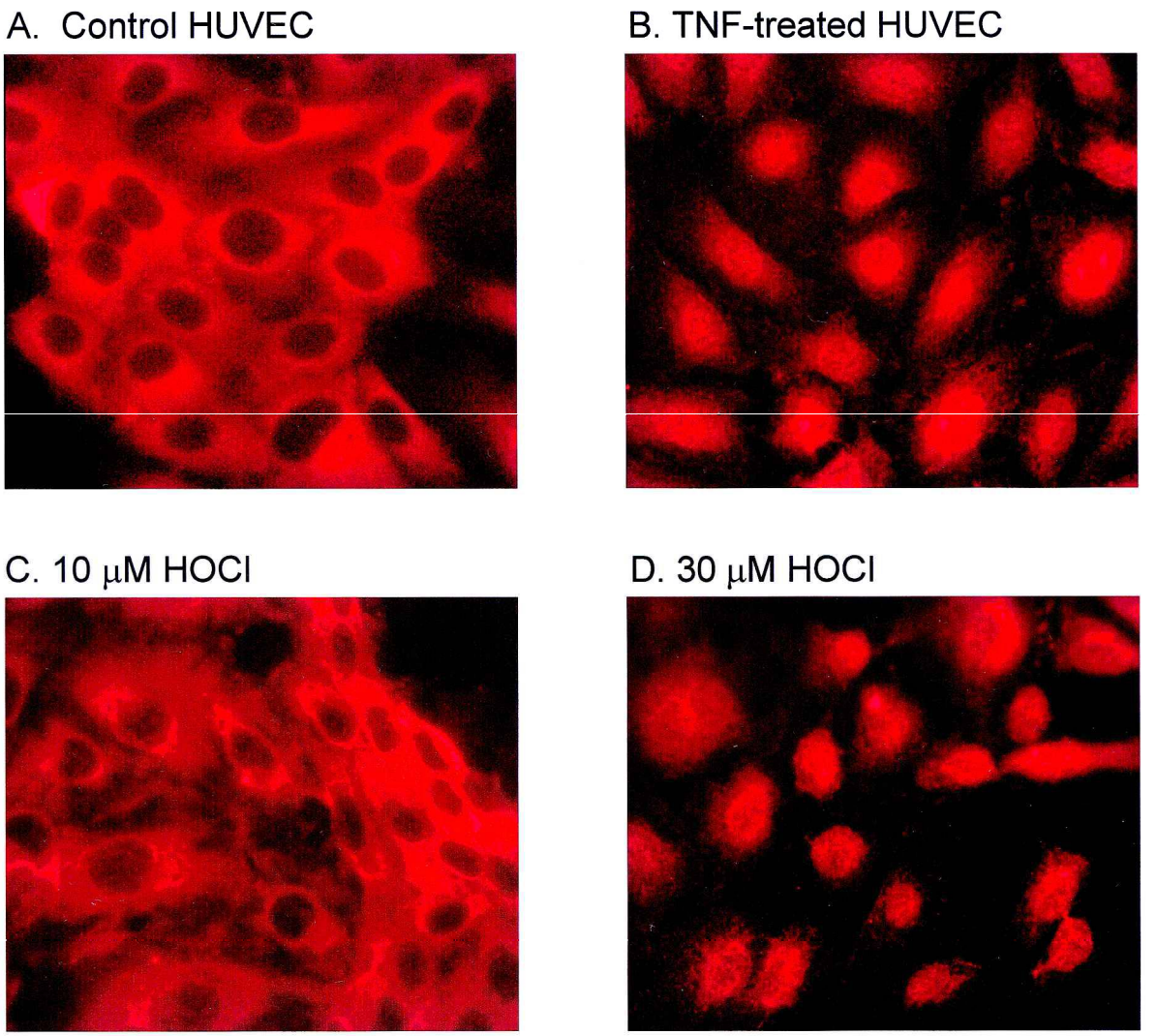

FIG. 5. NF-κB activation by TNF-α and HOCl. The activation of NF-κB in HUVEC was determined by immunolocalization using an antibody to p65, followed by tagging with a fluoro-linked secondary antibody (anti-IgG-Cy3). (**A**) In control HUVEC, the fluorescence is found in the cell cytoplasm, and the nuclei show as black holes. (**B**) After 1 h with TNF-α, all the cells show fluorescence in the nucleus. This was a consistent observation. (**C**) With sublethal concentrations of HOCl, localization of NF-κB did not differ from that in control cells. (**D**) With higher concentrations of HOCl, some regions could be found that showed fluorescence in the nuclear regions. However, in contrast to the results with TNF-α, this effect was seen in some cells only. Magnification = 200×. A color version of this figure can be found online at www.liebertpub.com and also at www.chmeds.ac.nz/research/freerad/publications/NFKB&HUVEC.htm.

cated *in vivo*. In contrast to other studies investigating the effects of HOCl on specific cell signaling responses (39, 40, 42), NF-κB and adhesion molecule expression are unaffected at doses of HOCl that are completely sublethal. For these reasons, we believe that the effects of HOCl on activation of NF-κB, neutrophil adhesion, and expression of adhesion molecules should be interpreted with caution, and the biological relevance considered.

The inhibition of PMA-induced von Willebrand factor release by pretreatment of endothelial cells with HOCl is likely to be more relevant. HUVEC were shown to be extremely sensitive to HOCl in this regard, and sublethal doses of oxidant markedly affected this response. PMA is a potent activator of protein kinase C, and our results could imply that HOCl interferes with this signaling pathway at some point. The ability of chlorine-containing oxidants to inhibit protein kinase C-dependent cell responses has some support in the literature (23). HOCl also reacts rapidly with cell thiols, and thiol oxidation is the pre-

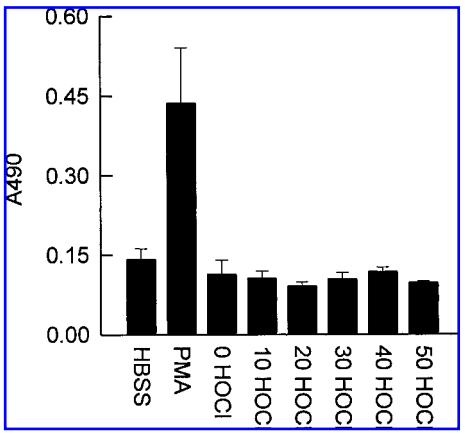

FIG. 6. Effect of HOCl on endothelial cell von Willebrand factor release. HUVEC were exposed to PMA (50 ng/ml) or HOCl (0–50 μ*M*) for 90 min, and supernatants were assayed for von Willebrand factor. Results are expressed as A_{490} and are the means ± SD of five experiments for PMA and three experiments for HOCl. A paired Student's *t*-test demonstrated a significant difference between the HBSS control and PMA treatment ($p < 0.005$). There was no significant difference between the control and the HOCl treatments.

dominant reaction upon exposure to sublethal concentrations of this oxidant (26, 27). Thiol-modifying agents such as diamide and *N*-ethylmaleimide have been shown to inhibit cytokine-induced endothelial cell adhesion molecule expression (7), and HOCl may be able to act in a similar manner. Alternatively, oxidative inactivation of NF-κB has recently been proposed in a study showing that pretreatment of endothelial cells with H_2O_2 reduced subsequent TNF-α-induced ICAM-1 and E-selectin expression (46). Whether any of these mechanisms is responsible for the HOCl-mediated inhibition of von Willebrand factor (and P-selectin) release is unknown, and the ability of HOCl to prevent the expression of adhesion molecules in response to cytokine stimulation may warrant further investigation.

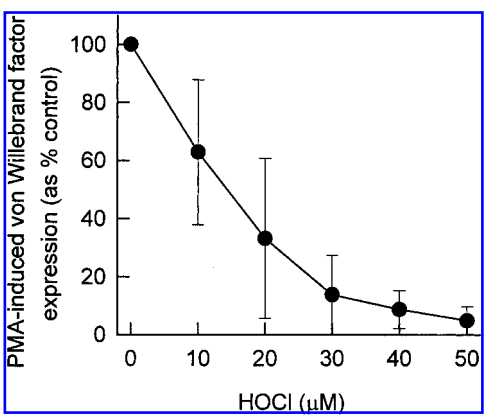

FIG. 7. Effect of HOCl pretreatment on PMA-induced von Willebrand factor release. HUVEC were incubated with HOCl for 30 min in HBSS, and then 50 ng/ml of PMA was added for 90 min. Results are expressed as a percentage of the maximal PMA-induced von Willebrand factor expression in control cells and are the means ± SD of four experiments.

In summary, our results show that HOCl does not function in a manner analogous to H_2O_2 and does not greatly induce the activation of NF-κB nor the expression of adhesion molecules in HUVEC. It is therefore unlikely to enhance the inflammatory response in this way. Instead, we have shown that HOCl pretreatment down-regulates PMA-induced von Willebrand factor release. Further experiments are required to assess whether this results in a decrease in neutrophil-endothelial cell adhesion. Such inhibition *in vivo* could impact on the ability of neutrophils to roll and migrate across the endothelium and thus serve to dampen down the inflammatory response. Whether HOCl can inhibit the expression of ICAM-1 and E-selectin is also important and will be the subject of further investigation. The generation of HOCl in the vascular wall in inflammation and atherosclerosis has been demonstrated (12, 20). The effect of this oxidant on endothelial cells is therefore an important consideration for the understanding of vascular inflammation and the role that endothelial dysfunction may play in the progression of diseases such as atherosclerosis.

ABBREVIATIONS

BSA, bovine serum albumin; CETAC, hexadecyltrimethylammonium chloride; FITC, fluorescein isothiocyanate; HBSS, Hanks' balanced salt solution; H_2O_2, hydrogen peroxide; HOCl, hypochlorous acid; HUVEC, human umbilical vein endothelial cells; ICAM, intercellular adhesion molecule; MPO, myeloperoxidase; NF-κB, nuclear factor-κB; PBS, phosphate-buffered saline; PMA, phorbol

myristate acetate; TMB, 3,3′,5,5′-tetramethylbenzidine; TNF-α, tumor necrosis factor-α.

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