Hypochlorous acid causes caspase activation and apoptosis or growth arrest in human endothelial cells

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We have investigated the effect of hypochlorous acid (HOCl) on cultured human umbilical-vein endothelial cells and shown that, whereas higher concentrations cause rapid necrosis, smaller amounts of this oxidant induce apoptosis or growth arrest. Exposure to 20–40 nmol of HOCl per 1.2×10^5 cells initiated apoptosis that was determined morphologically, by the identification of apoptotic nuclei with Hoechst 33342, and by detection of phosphatidylserine on the outer membrane. Degraded DNA was detected by flow cytometry. HOCl induced caspase activity; specific inhibition of caspases was shown to prevent apoptosis.

No caspase activation could be detected with 50 nmol of HOCl per 1.2×10^5 cells, a dose that caused more extensive necrosis. Lower doses of HOCl, which did not cause cell death, resulted in a transient growth arrest that was apparent with as little as 5 nmol of HOCl per 1.2×10^5 cells. These results show that HOCl can modify cellular responses that are dependent on signal transduction pathways in a manner similar to that of other oxidants.

Key words: DNA degradation, necrosis, phosphatidylserine.

INTRODUCTION

Reactive oxygen species are believed to contribute significantly to cell and tissue injury associated with ischaemia–reperfusion, many inflammatory conditions and exposure to hyperbaric oxygen and radiation [1,2]. More recently it has become clear that, whereas large amounts of reactive oxygen species are damaging and generally cause cell death, a range of cell responses is possible [2]. Sublethal quantities of oxidants can affect various cell components, including stress proteins [3], Ca^{2+} metabolism [1], kinases and phosphatases [4] and can activate the nuclear factor κB (NF- κB) and other transcription factors [5,6]. These lower doses of oxidant can cause cells to enter growth arrest [7,8] or to undergo apoptosis [8,9]. Apoptosis is an active process often described as ‘cell suicide’, which results in the efficient disposal of damaged cells in a contained manner that does not exacerbate inflammation [10,11]. In contrast, necrotic death is uncontrolled and results in the rupture of the cell membrane with leakage of intracellular components; it is pro-inflammatory [11]. Central to the process of apoptosis are the caspases, a unique class of proteinases with thiols at their active site, which cleave at aspartic residues [10,12]. Activation of the caspases, and especially caspase 3, results in the cleavage of various target proteins that are critical for apoptosis [12].

Although the generic term ‘reactive oxygen species’ is widely used, most studies on the effects of sublethal oxidant exposure have been performed with H_2O_2 , organic peroxides and peroxy-nitrite [1–3,7]. Little attention has been given to other biologically relevant oxidative species. A major source of reactive oxidants *in vivo* are the phagocytic cells, particularly neutrophils, which produce vast amounts of superoxide and H_2O_2 on stimulation [13,14]. Through the action of myeloperoxidase, up to 70 % of the H_2O_2 generated by these cells is converted to hypochlorous acid (HOCl) [13]. This oxidant is highly reactive with a broad range of biological molecules and is a highly efficient microbicidal agent [14]. HOCl is also known to be extremely toxic to mammalian cells [15–17] and damaging to tissue components

[18,19], and has been implicated in tissue injury associated with inflammation. Recent studies have demonstrated the presence of HOCl-modified protein in inflammatory bowel disease [20] and in atherosclerotic plaques [21].

HOCl reacts most readily with thiols and thioethers, and generates reactive chloramines on reaction with amine groups [22,23]. We and others have shown that HOCl can enter cells and that the loss of glutathione is an early event after the exposure of red cells or endothelial cells to this oxidant [16,24,25]. This suggests that low doses of HOCl react with cell thiols and could modulate cell processes in a manner similar to that seen with H_2O_2 or peroxy-nitrite. However, most studies on the reaction of HOCl with cells have focused on its toxic nature [15,17,26,27].

We have used human umbilical-vein endothelial cells (HUVEC) as a target system to determine the outcome of exposure to low doses of HOCl. These cells are particularly relevant for this study, because the endothelium is the first point of contact for neutrophils in the inflammatory response and they can be exposed to various amounts of neutrophil-derived oxidants, including HOCl. HUVEC were exposed to reagent HOCl; the effects with respect to cell proliferation and apoptosis were investigated. The activation of caspases was measured and related to the eventual outcome.

EXPERIMENTAL

Materials

Cell culture media and supplies were from Gibco BRL, supplied by Life Technologies (Auckland, New Zealand). HOCl was from Reckitt and Coleman Ltd. (Auckland, New Zealand). Stock solutions were diluted in Hanks balanced salt solution (HBSS; 10 mM PBS, pH 7.4, containing 1 mM CaCl_2 , 0.5 mM MgCl_2 and 1 mg/ml glucose) and were standardized by reaction with thionitrobenzoic acid [28]. Hoechst 33342 was from Molecular Probes (Eugene, OR, U.S.A.). The substrate for caspase-3-like enzymes, Asp-Glu-Val-Asp-7-amino-4-methylcoumarin (DEVD-AMC), was from Bachem (Bubendorf, Switzerland).

Abbreviations used: BrdU, 5-bromo-2'-deoxyuridine; ZVAD-fmk, benzyloxycarbonyl-Val-Ala-Asp(OMe)-CH₂F; DEVD-AMC, Asp-Glu-Val-Asp-7-amino-4-methylcoumarin; HBSS, 10 mM PBS, pH 7.4, containing 1 mM CaCl_2 , 0.5 mM MgCl_2 and 1 mg/ml glucose; HUVEC, human umbilical-vein endothelial cells; PtdSer, phosphatidylserine; NF- κB , nuclear factor κB .

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Figure 1 Release of ^{51}Cr from HUVEC exposed to HOCl

Various amounts of HOCl were added to confluent HUVEC in 24-well plates (approx. 1.2×10^5 cells per well). Symbols: ○, control; □, 10 nmol of HOCl; △, 25 nmol; ▽, 50 nmol; ◇, 100 nmol. Mean $\pm$ S.D. for four to eight experiments are shown. The results obtained with 25, 50 and 100 nmol of HOCl were significantly different from controls as determined by a paired Student's *t* test ($P < 0.05$, $P < 0.01$ and $P < 0.01$ respectively).

The caspase inhibitor benzyloxycarbonyl-Val-Ala-Asp(OMe)-CH₂F (ZVAD) was from Enzyme Systems Products (Livermore, CA, U.S.A.). Phosphatidylserine (PtdSer) exposure was measured with the Apotest™ FITC kit from Nexins Research B. V. (Hoeven, The Netherlands). The cell-proliferation ELISA kit for the determination of 5-bromo-2'-deoxyuridine (BrdU) uptake was from Boehringer Mannheim Corp. (Indianapolis, IN, U.S.A.). ^{51}Cr was from Amersham (Australia) Pty, Ltd. All other reagents and chemicals were from Sigma Chemical Co. (St. Louis, MO, U.S.A.).

Endothelial cell culture

HUVEC were isolated from umbilical cords obtained with informed consent. The cells were extracted by digestion with collagenase and cultured in fibronectin-coated flasks in Medium 199 with 20% (v/v) fetal calf serum and growth supplements as described previously [18]. The cells were identified by their typical cobblestone appearance and morphology, and were used in the second or third passage after primary culture. When grown in 24-well plates, each confluent well contained approx. 1.2×10^5 cells.

Exposure of HUVEC to HOCl

HOCl was diluted in HBSS immediately before addition to the cells. Cultures of HUVEC in 24-well plates were washed four times with HBSS to remove any trace of medium; pre-diluted HOCl was then added in a total volume of 1 ml per well. HOCl was rapidly consumed on addition to the cells: when 35 μM HOCl was added, half of the oxidant was consumed within 1 min. Therefore, when further incubation of the cells was required, the HBSS was removed after 15 min and replaced with complete culture medium. The addition of HOCl was at room temperature; subsequent incubations were at 37 °C.

Estimate of cytotoxicity

The toxicity of HOCl was estimated by measuring the release of ^{51}Cr from HUVEC that had been preincubated for 4–16 h with $^{51}\text{CrSO}_4$. Unincorporated label was removed by washing, and

HOCl was added. Aliquots of the medium were removed at times indicated, spun to remove any detached cells, and the ^{51}Cr in the supernatant was counted in a γ -radiation counter. The released radioactivity was estimated as a percentage of the total from a detergent-lysed sample.

Measurements of apoptosis

Whether HOCl can induce apoptosis was determined by morphological examination, staining of nuclei with Hoechst 33342 dye and detection of PtdSer on the outer membrane.

Morphological changes were assessed by examination of the cells in an inverted phase-contrast microscope between 1 and 6 h after exposure to HOCl. Staining of apoptotic nuclei with Hoechst 33342 was performed with HUVEC cultured on glass microscope slides. Sections of confluent cells were exposed to HOCl and reincubated in medium. At various times up to 6 h, the medium was removed and the cells were fixed with 4% (w/v) paraformaldehyde for 5 min. After being washed, the cells were covered with a solution of 10 $\mu\text{g}/\text{ml}$ Hoechst dye and incubated in the dark for 10 min. The slides were washed, air-dried and mounted in 50% (v/v) glycerol, and the nuclei were examined with a fluorescent microscope.

Cells undergoing apoptosis express PtdSer on the cell surface; this was measured with Annexin V-FITC [29]. Co-staining with propidium iodide was used to measure necrotic cells. The loss of membrane integrity in these cells means that they also bind Annexin V-FITC; staining with propidium iodide allows the differentiation of the Annexin-binding cells into necrotic and apoptotic populations. HUVEC were harvested with trypsin at various times after exposure to HOCl; the detached and adherent cells were pooled. Annexin V was added to the cells in accordance with manufacturer's instructions (2 μl in 200 μl cell suspension, followed by 2 μg of propidium iodide and incubated in the dark for 10 min). The cell fluorescence was determined with a bivariate flow cytometer, and the number of cells in each population was estimated.

DNA fragmentation analysis

DNA degradation was measured by flow cytometry after the cells had been stained with propidium iodide [30]. HUVEC exposed to HOCl were maintained in complete medium for 6 h, harvested with trypsin and pelleted by centrifugation. In some samples the detached and adherent cells were analysed separately. A positive control population of apoptotic cells was generated by a 24 h incubation in serum-free Medium 199. The cell pellets were suspended in 0.5 ml of HBSS and fixed with 4.5 ml of 70% (v/v) ethanol in a tube on ice. The cells were washed once with HBSS, suspended in 0.5 ml of HBSS with 0.5 ml of DNA extraction buffer [0.2 M Na₂HPO₄/0.1 M citric acid (pH 7.8)] for 5 min at room temperature, resuspended in 200 μl of DNA-staining solution (20 $\mu\text{g}/\text{ml}$ propidium iodide/200 $\mu\text{g}/\text{ml}$ RNase A in HBSS) for 30 min and analysed by flow cytometry.

Estimation of caspase activity

The activation of caspase 3 was measured with the specific substrate DEVD-AMC [31]. After exposure to HOCl, the attached cells were harvested by treatment with trypsin and pooled with the detached cells from the supernatant. The cells from three wells (approx. 3.6×10^5) were pooled and stored at -80°C . Immediately before assay the pellets were thawed by the addition of 50 μM DEVD-AMC in 100 μl of buffer [100 mM Hepes/10% (w/v) sucrose/1% (w/v) CHAPS/5 mM dithiothreitol/0.000001% (v/v) Nonidet P40]; the rate of liberation of fluores-

Figure 2 Morphology of HUVEC exposed to HOCl and staining of nuclei with Hoechst 33342

(a) Phase-contrast microscopy of control HUVEC. The cells detach after the addition of HOCl. (b) Blebbing or 'popcorn-like' cells are seen 3 h after addition of 35 nmol of HOCl. (c) The addition of 50 nmol of HOCl resulted in rounded, necrotic cells. (d) Hoechst dye accumulates in control nuclei in HUVEC that appear uniform in shape and intensity. (e) After exposure to 35 nmol of HOCl, the nuclei of 'popcorn-like' cells were fragmented and condensed. In contrast, the nuclei of cells exposed to 50 nmol of HOCl (f) were uniformly stained and not highly fluorescent. Magnification for phase-contrast studies $\times 75$; for Hoechst fluorescence $\times 150$.

cent AMC was monitored with excitation at 370 and emission at 445 [31]. The amount of AMC liberated was calculated from a standard curve (1 μ M AMC corresponds to 1400 fluorescence units).

To inhibit caspase activity in HUVEC, the cells were pre-incubated with 20 or 100 μ M benzyloxycarbonyl-Val-Ala-Asp(OMe)-CH₂F (ZVAD-fmk) (dissolved in DMSO) in medium for 1 h before exposure to HOCl. The medium was replaced with HBSS for the addition of HOCl. After 15 min the cells were returned to conditioned medium containing ZVAD-fmk for further incubation.

Cell proliferation assay

Cell proliferation was measured by the incorporation of BrdU, detected by ELISA with the Boehringer Mannheim kit. HUVEC were seeded into 96-well plates at 8000 per well and left for 12 h. The wells were treated with HOCl in a final volume of 100 μ l per well. The amount of HOCl was calculated such that the dose per cell was equivalent to those used in 24-well plates. (For example, 0.33 nmol of HOCl in 100 μ l with 8000 cells is equivalent to 5 nmol of HOCl per 1.2×10^5 cells in a 24-well plate.) After

exposure of the cells to HOCl for 10 min, the HBSS was removed and replaced with medium. Cell proliferation was measured in the ensuing 48 h by incubation with BrdU for 10 h periods.

RESULTS

Cytotoxicity

Exposure of HUVEC to HOCl resulted in dose- and time-dependent cytotoxicity as measured by the release of ⁵¹Cr from the cells (Figure 1). The higher doses of HOCl used, 50 and 100 nmol per 1.2×10^5 cells, were toxic to the cells; significant release of ⁵¹Cr was detected within 15 min. Complete lysis occurred within 2 h. Lower doses of 15 and 25 nmol were much less toxic and there was minimal or no release of ⁵¹Cr over 4 h. The low level of ⁵¹Cr release with 25 nmol of HOCl (Figure 1) is likely to represent the release of label from a small number of detached cells that were observed by phase-contrast microscopy (results not shown). On further incubation for periods of up to 24 h, the ⁵¹Cr release at all doses was essentially unchanged (results not shown). Morphological examination (Figures 2a–2c) showed that, with doses between 25 and 40 nmol of HOCl per well, the detached cells exhibited characteristics typical of cells

Figure 3 PtdSer exposure on HUVEC as measured by the binding of Annexin V

HUVEC were harvested by treatment with trypsin 6 h after exposure to HOCl, then incubated with Annexin V-FITC and propidium iodide (PI) and analysed by flow cytometry. The dot plots show viable cells (R1), apoptotic cells that bound Annexin V and excluded propidium iodide (R2) and necrotic cells that showed high Annexin V binding and were propidium iodide positive (R3). The number of viable and apoptotic cells decreased at 50 nmol of HOCl, when most of the population was positive with propidium iodide (necrotic). The results shown are from one of six experiments that all gave similar results.

undergoing apoptosis, with the appearance of 'popcorn-like' blebs (Figure 2b). At 50 nmol and above, most of the cells detached from the plate within minutes and were rounded in appearance (Figure 2c); the blebbing features seen with lower doses of HOCl were seldom apparent. At doses below 25 nmol per well there were no apparent long-term morphological changes.

Indices of apoptosis

Staining of the cell nuclei with Hoechst 33342 (Figures 2d–2f) showed intense fluorescence in the nuclei of the blebbing cells, indicative of condensation and fragmentation of the chromatin in these cells (Figure 2e). These cells were commonly seen in populations that had been exposed to doses of HOCl between 25 and 40 nmol per well. In contrast, the nuclei of cells treated with 50 nmol of HOCl per well showed no evidence of chromatin condensation and were evenly stained with Hoechst dye (Figure 2f).

The exposure of PtdSer in the outer layer of the plasma membrane is an indicator of apoptosis [29]; it can be detected by

Figure 4 Distribution of HOCl-treated HUVEC on FACS analysis

Flow cytometry data were collected 6 h after the addition of HOCl. Staining with Annexin V and propidium iodide allowed differentiation into viable (●), apoptotic (■) and necrotic (▲) cell populations. The percentage of apoptotic cells increased between 25–40 nmol of HOCl per 1.2×10^5 cells. With 50 nmol, most cells appeared necrotic. The results shown are representative of six experiments.

the binding of Annexin V to the cells. To assess this, the cells were double-labelled with Annexin V-FITC and propidium iodide (as a marker of membrane permeability) and analysed by flow cytometry. Increasing doses of HOCl caused an increase in the number of cells binding Annexin V and excluding propidium iodide (Figure 3). The apoptotic cells reached approx. 20% of the total cell population at any one time after the addition of 25–40 nmol of HOCl per well (Figures 3 and 4). This extent of PtdSer exposure was equivalent to that seen when the cells had been deprived of serum for 24 h, a more classical inducer of apoptosis (results not shown). PtdSer exposure was first seen 3 h after exposure to HOCl. Necrotic cells were also present; at lower doses of HOCl these might represent secondary necrotic cells, i.e. cells that were apoptotic but with time underwent a loss in membrane integrity. At 50 nmol of HOCl per well, the apoptotic population was less prevalent and necrotic cells seemed to be the major population (Figures 3 and 4).

DNA degradation

DNA fragmentation can be recognized by extraction of the degraded DNA followed by measurement of DNA content by flow cytometry [30]. Apoptotic cells have decreased propidium iodide fluorescence compared with cells in the G₁, S or G₂/M phases of the cell cycle. A marked decrease in fluorescence was seen in serum-deprived apoptotic cells (Figure 5), and in HUVEC exposed to 25–40 nmol of HOCl per 1.2×10^5 cells. The cells containing degraded DNA were in the detached population (Figure 5), whereas the remaining adherent cells, i.e. the viable cells, showed a normal profile.

Caspase activation

The activation of caspase 3 was measurable 3 h after exposure to HOCl (Figure 6). The highest activity was seen with 35 nmol of HOCl per well and was at least 10-fold control levels. There was less activation with 20 nmol of HOCl. With 50 nmol per well there was usually no activation, although caspase activity was

Figure 5 Detection of degraded DNA with propidium iodide

Apoptotic cells have decreased fluorescence and appear in the region below G₁. A significant proportion of these cells was present in a population of serum-deprived cells that appeared mostly apoptotic by microscopic examination. A similar profile was seen in cell populations exposed to 25–40 nmol of HOCl per 1.2×10^5 cells. The degraded DNA was present in the detached cells and not in the remaining adherent (viable) fraction. The results shown are representative of five estimates.

once measured at this dose. Notably, a visual assessment of the HUVEC on this occasion indicated the presence of apoptotic cells.

When HUVEC were pretreated with ZVAD-fmk, a specific chloromethyl ketone inhibitor of the caspases [31], HOCl-mediated caspase activity was completely blocked. This inhibitor also prevented apoptosis at all doses of HOCl. Morphologically there was no indication of membrane blebbing and the exposure of PtdSer was completely prevented (Figure 7). This effect was not due to DMSO, the vehicle for ZVAD-fmk.

Figure 6 Caspase activation in HUVEC exposed to HOCl

Symbols: ○, control; □, 20 nmol of HOCl; △, 35 nmol; ▽, 50 nmol. Caspase activity was detected 3 h after exposure. With 50 nmol of HOCl, activation was seen on only one occasion when cells with apoptotic morphology were also present. Results are means $\pm$ S.D. for five experiments.

Figure 7 Inhibition of PtdSer exposure by ZVAD

The binding of Annexin was assessed 5 h after exposure to 30 nmol of HOCl per well. Histogram plots of the viable and apoptotic populations (see Figure 3) are shown. The results shown are representative of three similar experiments.

Figure 8 Effect of low levels of HOCl on cell proliferation

HUVEC were cultured in 96-well plates at subconfluent levels and exposed to HOCl; proliferation was measured by BrdU uptake in the ensuing 48 h. Symbols: ○, 0–10 h after exposure to HOCl; □, 20–30 h. Results are means $\pm$ S.D. for three experiments. A paired Student's *t* test showed that the cell proliferation between 0 and 10 h was significantly different from the control ($P < 0.05$) at all doses of HOCl.

Inhibition of cell proliferation

The effect of exposure to low levels of HOCl on cell proliferation was determined by uptake of BrdU. At levels of oxidant below 25 nmol per 1.2×10^5 cells, growth arrest was apparent for the first day after the administration of oxidant (Figure 8). Significant growth arrest was observed with the lowest dose of HOCl used (5 nmol). Within 24 h, cell proliferation had returned to control levels. At levels of HOCl that caused cell death by apoptosis or necrosis, cell proliferation was permanently decreased and did not return to control levels within 48 h.

DISCUSSION

We have shown that the outcome of exposure of human endothelial cells to HOCl is dependent on the dose of oxidant used. Low, sublethal doses induce a transient growth arrest, slightly higher amounts initiate apoptosis and as the dose increases the cells become increasingly necrotic. This is a novel finding because it has not previously been shown that exposure to HOCl results in this spectrum of outcomes. In addition to morphological changes typical of apoptosis, these results were confirmed by the detection of PtdSer on the cell membrane, by the activation of caspases and with evidence of DNA degradation.

In this way HOCl seems to act similarly to other oxidants, particularly H_2O_2 and peroxynitrite [7,32,33]. However, considerably smaller amounts of HOCl than other oxidants were required for these effects. With H_2O_2 , for example, approx. 100–500 μM is required to induce growth arrest or apoptosis; millimolar amounts cause necrosis in fibroblasts [7,34], whereas millimolar concentrations of H_2O_2 are required to initiate apoptosis in endothelial cells [35,36]. In comparison, 5–20 μM HOCl caused significant growth arrest and approx. 35 μM gave maximal apoptosis.

That HOCl exposure initiates growth arrest and apoptosis suggests that signal transduction pathways are being activated. When administered as in our experiments, HOCl reacts completely with the cells within minutes of addition. However, growth arrest and apoptosis proceed long after the initial exposure, with caspase activation and PtdSer exposure first being detected after 3 h and growth arrest persisting for the first 24 h.

That caspase activation was necessary for apoptosis to occur was demonstrated by the blocking of this process with ZVAD-fmk. These results imply that a sublethal dose of HOCl can initiate a controlled cell response that is a consequence of primary oxidative events. Other recent evidence also suggests that HOCl can act as a signalling molecule. Vile et al. [37] have shown that low concentrations of HOCl can elevate p53 levels in human skin fibroblasts. HOCl can also induce the expression of apurinic endonuclease (also known as Ref-1) in Chinese hamster ovary cells [38] and can activate NF- κB [39] in a T-lymphocyte cell line. The latter study required higher concentrations of HOCl than we used. However, it was performed in whole cell medium and the effects seen are likely to be due to chloramines.

The reactions of HOCl that determine the varied outcomes remain to be identified. We [24] and others [16] have shown that HOCl reacts readily with glutathione and cell thiols; it is tempting to speculate that the oxidation of a critical thiol group might be involved. However, other reactions are also possible. With regard to caspase activation, it was apparent that HOCl did not activate the caspases directly because this did not occur for at least 2 h after addition. The mechanism of activation could be similar to many other apoptotic stimuli, including H_2O_2 , in which case the release of cytochrome *c* from mitochondria has been invoked [31]. Mitochondrial transport processes are known to be dependent on thiol-containing proteins and it has been suggested that their oxidation could cause apoptosis [8].

The caspases contain thiol groups at their active site, and it is interesting that HOCl can initiate caspase activation, despite their thiol sensitivity. This suggests that there is some selectivity of reaction with cell thiols, because the amount of HOCl that causes maximal activation of the caspases (35–40 μM) also causes a significant loss of cell thiols and glutathione [16,40]. We have found that with these doses of HOCl the cells rapidly regenerate some of the oxidized thiols [40] and it is also possible that caspase thiol oxidation could be reversed within the 2 h before activation occurs. This was less likely with 50 nmol of HOCl per well; the finding that there was no caspase activation with higher doses of HOCl could be due to irreversible oxidative inactivation, as has been seen with H_2O_2 [31,41].

In contrast with the time course of apoptosis and growth arrest, necrosis induced by HOCl occurred within 1 h of its addition, implying that this is induced by the direct and overwhelming reaction of the oxidant with critical cell targets. We have shown that, with more than 50 nmol of HOCl, the ATP levels in HUVEC decrease to approx. 20% of control values [40] and the cells might not have the energy required to drive apoptosis. Higher doses of HOCl could therefore prevent apoptosis through the depletion of ATP or by the oxidative inactivation of caspases but it is unclear whether these reactions contribute to necrotic cell death. We have shown previously that the HOCl-mediated lysis of red cells was correlated strongly with the formation of membrane protein cross-links and was unrelated to thiol oxidation [25]. It is likely that the necrotic death of HUVEC was similarly due to other damaging reactions of HOCl.

Our experiments also have physiological implications for endothelial cells. These cells are intimately involved in the recruitment of leucocytes and are also exposed to stimulated neutrophils in many inflammatory conditions. The ability of HOCl to induce growth arrest or apoptosis in these cells would be advantageous in allowing the repair or removal of damaged cells in a way that did not exacerbate inflammation. The proposal that HOCl can initiate controlled responses also suggests that neutrophils could use their oxidative machinery for cell signalling. Further studies will address whether this can occur.

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