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Identification of proteins susceptible to thiol oxidation in endothelial cells exposed to hypochlorous acid and *N*-chloramines

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ABSTRACT

Hypochlorous acid (HOCl) is a potent oxidant produced by the enzyme myeloperoxidase, which is released by neutrophils under inflammatory conditions. Although important in the immune system, HOCl can also damage host tissue, which contributes to the development of disease. HOCl reacts readily with free amino groups to form *N*-chloramines, which also cause damage *in vivo*, owing to the extracellular release of myeloperoxidase and production of HOCl. HOCl and *N*-chloramines react readily with cellular thiols, which causes dysfunction via enzyme inactivation and modulation of redox signaling processes. In this study, the ability of HOCl and model *N*-chloramines produced on histamine and ammonia at inflammatory sites, to oxidize specific thiol-containing proteins in human coronary artery endothelial cells was investigated. Using a proteomics approach with the thiol-specific probe, 5-iodoacetamidofluorescein, we show that several proteins including peptidylprolyl isomerase A (cyclophilin A), protein disulfide isomerase, glyceraldehyde-3-phosphate dehydrogenase and galectin-1 are particularly sensitive to oxidation by HOCl and *N*-chloramines formed at inflammatory sites. This will contribute to cellular dysfunction and may play a role in inflammatory disease pathogenesis.

1. Introduction

Myeloperoxidase (MPO) produces primarily hypochlorous acid (HOCl), by the reaction of hydrogen peroxide (H_2O_2) with chloride (Cl^-) ions [1]. MPO is released by activated inflammatory cells, which results in the production of HOCl extracellularly [1]. HOCl is a potent bactericidal agent with an important role in the immune system [2]. However, under inflammatory conditions, the high levels of HOCl produced result in damage to host tissue [3], which contributes to the development of a number of diseases, including atherosclerosis, rheumatoid arthritis and some cancers [1,4].

HOCl is a potent oxidant that reacts readily with most biological molecules, including proteins, DNA, and lipids [1,4,5]. Proteins are important targets for HOCl owing to their high reactivity and abundance in biological systems [6]. The free thiol group of Cys and the thioether group of Met are particularly reactive sites [6]. HOCl also reacts readily with amines to form *N*-chloramines [5,7]. The formation of *N*-chloramines via the reaction of HOCl with ammonia (NH_3), histamine and proteins is likely to be important *in vivo*, owing to the extracellular release of MPO and the high reactivity of HOCl [8,9]. *N*-Chloramines retain the oxidizing capacity of HOCl, but display greater selectivity in their reactions than HOCl, particularly with low pK_a thiol proteins [10,11].

Exposure of many different cell types to physiologically and pathologically relevant doses of HOCl results in cell lysis and depletion of reduced glutathione (GSH), cellular thiols, and ATP, as HOCl readily penetrates cell membranes (e.g. [12,13]). *N*-Chloramines also induce damage to cells, though less is known about the cellular targets [9,10,14]. The modification of cellular protein thiols by oxidants results in the inactivation of key intracellular enzymes and the perturbation of various signaling pathways, which is postulated to contribute to disease [15]. Thus, in this study, we performed a global analysis to identify specific thiol-containing cellular proteins targeted by HOCl and model inflammatory *N*-chloramines on exposure to human coronary artery endothelial cells (HCAEC), using a sensitive fluorescent thiol-labeling technique combined with 2D SDS–PAGE. HCAEC were selected owing to evidence implicating MPO-derived oxidants in endothelial damage in atherosclerosis [1].

Abbreviations: DTNB, 5,5′-dithio-bis(2-nitrobenzoic acid); DTT, dithiothreitol; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HBSS, Hank's buffered salt solution; HCAEC, human coronary artery endothelial cells; HistCA, histamine chloramine; HOCl, hypochlorous acid; IAF, 5-iodoacetamidofluorescein; MonoCA, monochloramine (NH_2Cl); MTT, 3-(4,5-dimethyl-2-yl)-2,5-diphenyl-tetrazolium bromide; MPO, myeloperoxidase; RuBPS, ruthenium(II) tris(bathophenanthroline disulfonate); TCA, trichloroacetic acid; TNB, 5-thio-2-nitrobenzoic acid.

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2. Materials and methods

2.1. Materials

Nanopure water was filtered through a four-stage Milli-Q system (Millipore, Lane Cove, NSW, Australia). pH control was achieved using 0.1 M sodium phosphate buffer (pH 7.4) pre-treated with Chelex resin (BioRad, Hercules, CA) to remove contaminating trace metal ions or Hanks buffered salt's solution (HBSS). Unless stated otherwise, chemicals were of analytical reagent grade and obtained from Sigma–Aldrich (St Louis, MO). HOCl (in 0.1 M NaOH, low in bromine, BDH Chemicals, Poole, UK) was standardized at 290 nm using an extinction coefficient 350 M^{-1} cm^{-1} at pH 12 [16]. HistCA and MonoCA were prepared by adding HOCl to a fivefold excess of histamine and ammonium sulfate in HBSS, respectively. *N*-chloramines were quantified by reaction with 5-thio-2-nitrobenzoic acid (TNB) as described previously [7,17]. Under these conditions, complete conversion of HOCl to the respective *N*-chloramine is observed [18,19].

2.2. Cell culture

Human coronary artery endothelial cells (HCAEC) were cultured in HCAEC growth medium (Cell Applications) in an atmosphere of 5% CO_2 at 37 °C. Cells were harvested with trypsin/EDTA (1:250) and centrifugation at 800*g* for 5 min. HCAEC were allowed to adhere overnight to tissue culture plates in HCAEC growth medium at 37 °C. Cells were washed with warm (37 °C) HBSS to remove any cell media prior to addition of HOCl or *N*-chloramines. Any residual oxidant was removed after incubation by washing with HBSS containing 1 mM methionine prior to analysis.

2.3. Quantification of cellular thiols

HCAEC (1×10^5) were plated in 24-well tissue culture plates and incubated for 15 min with 500 μL HOCl or *N*-chloramines (50–200 μM; 250 nmol–1 μmol/10^6 cells). In each case, the oxidant solutions were added to the cells while agitated on an orbital mixer. Cells were lysed after washing by the addition of 500 μL of cold (4 °C) sterile H_2O (Baxter). Cellular thiols were quantified using ThioGlo 1 (Calbiochem, Kilsyth, VIC, Australia) as described previously [17].

2.4. Cellular viability

Cell viability was assessed using a commercial MTT (3-(4,5-dimethyl-2-yl)-2,5-diphenyl-tetrazolium bromide) assay kit (Jomar Bioscience, Kensington, SA, Australia). HCAEC (2×10^4) were plated in 96-well tissue culture plates and incubated for 15 min with 100 μL HOCl or *N*-chloramines (50–200 μM; 250 nmol–1 μmol/10^6 cells), before washing and further incubation of the cells for 4 h with cell media (100 μL) containing (MTT; 10 μL).

2.5. IAF thiol labeling and sample preparation for SDS–PAGE

HCAEC (1×10^5) were plated in 24-well tissue culture plates and incubated for 15 min with 500 μL HOCl and *N*-chloramines (50–200 μM). Briefly, cells were lysed in 125 μL lysis/labeling buffer [35 mM Hepes, pH 7.4, 0.1% v/v Triton X-100, 1× complete protease inhibitors (Roche) and 80 μM IAF (Invitrogen)] for 30 min at 20 °C in the dark, before precipitation of protein by addition of trichloroacetic acid (TCA; 10% w/v) and centrifugation (15 min, 4 °C, 6000*g*). Protein pellets were washed with ice-cold (−20 °C) acetone, and resuspended in gel loading buffer (125 mM Tris, pH 6.8, 20% v/v glycerol, 5% v/v β-mercaptoethanol, 4% w/v SDS, 0.01% w/v bromophenol blue). Samples were reduced by heating at 95 °C for 5 min prior to loading.

2.6. 2D SDS–PAGE

HCAEC (4×10^5) were plated in 6-well tissue culture plates before washing and treatment with 2 mL HOCl and *N*-chloramines (50–200 μM; 250 nmol–1 μmol/10^6 cells) for 15 min and preparation of cell lysates as described [17]. For each treatment, 2 IAF lysate samples were pooled, before precipitation of protein and resuspension at a concentration of 250 μg in 300 μL in rehydration buffer (8 M urea, 4% w/v CHAPS, 0.5% w/v Bio-Lytes, 100 mM DTT, 40 mM Tris, 0.01% w/v bromophenol blue) and loading onto pH 3–10 non-linear IPG strips (BioRad) by overnight passive rehydration [17]. Isoelectric focusing was carried out overnight for 60,000 Vh. The IPG strips were then reduced and alkylated and protein separation performed on 8–16% polyacrylamide gradient gels run overnight at a constant voltage of 90 V, with visualization described previously [17,18]. Analysis of the 2D gel spots was performed with PDQuest™ Advanced 2D gel analysis software version 8.1 (BioRad). The proteins labeled with IAF were analyzed after background subtraction using the Gaussian noise filter with spots detected using a sensitivity of 15.1. The RuBPS images were analyzed after background subtraction with the pepper filter (speckles 200, background 47, median 5 × 3 pixels) with spots detected using a sensitivity setting of 10.7.

2.7. In-gel digestion and protein identification by LC–MS/MS

Lysates which were not labeled with IAF, were separated by 2D SDS–PAGE and spots, corresponding to the proteins susceptible to thiol oxidation, were cut out manually after confirmation of their position in the gels by overlaying previous IAF fluorescence and RuBPS images. In-gel tryptic digestion was performed as described previously [20]. Mass spectrometric analysis was performed at the Bioanalytical Mass Spectrometry Facility (UNSW, Sydney, Australia) as previously [18].

2.8. Statistical analysis

All statistical analyses were performed using Prism 4.0b (GraphPad Software, San Diego, CA) with $p < 0.05$ taken as significant. Details of the specific tests employed are given in the relevant Figure Legends.

3. Results

3.1. HOCl and N-chloramines decrease cellular thiols and viability

Reaction of HOCl, MonoCA, and HistCA with HCAEC (⩽1 μmol/10^6 cells) for 15 min resulted in a dose-dependent loss of thiols, assessed using ThioGlo 1 (Fig. 1A). MonoCA was the most potent oxidant in terms of thiol depletion, with lower concentrations of this oxidant required to induce a significant loss of thiols (Fig. 1A). The extent of consumption of HOCl and each *N*-chloramine by HCAEC under identical conditions was also assessed, using the TNB assay to quantify any residual oxidant remaining in the cell supernatant after incubation. Greater oxidant loss was seen in the presence of HCAEC compared to controls incubated in the absence of cells in each case, with 81 ± 9%, 71 ± 5% and 27 ± 8% of the initial HOCl, MonoCA and HistCA lost, respectively. Experiments were also performed to assess whether the depletion of cellular thiols was accompanied by a loss in the cellular viability of the HCAEC, by measuring the conversion of MTT to formazan by metabolically active cells over 4 h. A loss in cellular viability reflected by a loss in

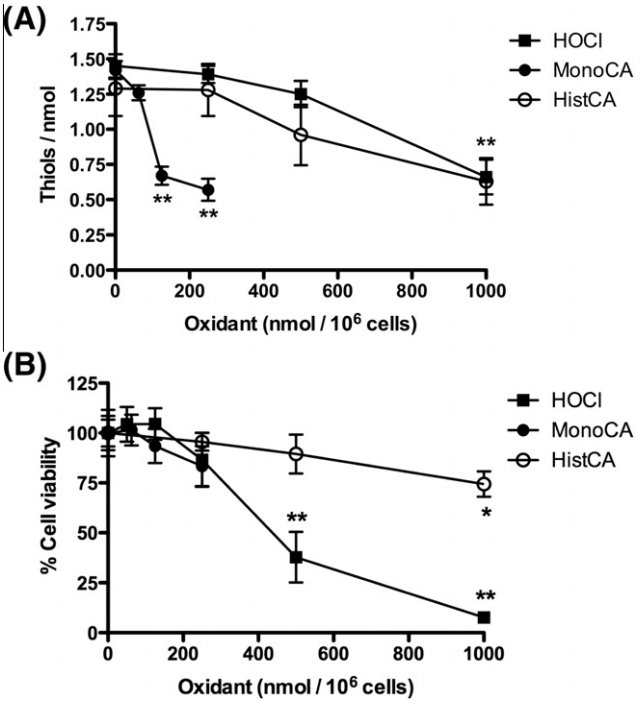

Fig. 1. Loss of thiols and cellular viability on treatment of HCAEC with HOCl and *N*-chloramines. HCAEC were treated with HOCl [closed square], MonoCA [closed circle], and HistCA [open circle] (12.5–200 μM; 50–1000 nmol/10^6 cells) for 15 min before washing and (A) quantification of thiol concentration using ThioGlo 1 or (B) assessment of cell viability using the MTT assay. Data represent the means ± S.E.M. $n \geqslant 3$ with * and ** showing a significant ($p < 0.05$ and 0.01) difference compared to the non-treated control by one-way ANOVA with Dunnett's post hoc test.

metabolic activity was observed on exposure of HCAEC to HOCl and each *N*-chloramine under the conditions described above, compared to control experiments performed with HBSS or the respective parent amine (Fig. 1B).

3.2. Identification of thiol-containing cellular proteins susceptible to oxidation

The nature of the thiol-containing proteins susceptible to oxidation by HOCl and *N*-chloramines was established using the fluorescent thiol-specific probe, IAF, as previously [18]. HCAEC were exposed to HOCl, MonoCA, and HistCA ($\leqslant$1 μmol/10^6 cells) for 15 min before IAF labeling and separation by 2D SDS–PAGE. This treatment resulted in a consistent decrease in staining intensity of $\geqslant$50 fluorescent, IAF-labeled protein spots on comparison of oxidant treated cells to the HBSS or parent amine-treated cells (Fig. 2A and B). No consistent differences in the extent of RuBPS staining were observed with oxidant treatment (Fig. 2C and D), indicating that the changes in IAF fluorescence are unlikely to be due to loss of protein. Fewer protein spots are observed on visualization of the IAF-labeled proteins compared to the RuBPS-stained gels, as IAF is added prior to reduction and alkylation of disulfide bonds, only proteins containing free thiols (not disulfides) are labeled. Exposure of the HCAEC to MonoCA and HistCA also resulted in an increase in the IAF fluorescence associated with a group of protein spots with molecular mass ca. 48 kDa, consistent with the formation, rather than oxidation, of reduced thiols in this case. Treatment of cell lysates with iodoacetamide to alkylate protein thiols prior to IAF labeling resulted in loss of the majority of fluorescent spots (data not shown), consistent with a low extent of non-specific association of the IAF with cellular proteins as previously [18].

Twenty five protein spots were identified to be consistently and significantly susceptible to modification on treatment of the cells with each oxidant as reflected by a $\geqslant$2-fold change in IAF fluorescent staining intensity compared to non-treated cells by multiple t-tests with 90% significance using PDQuest (Fig. 2). These proteins were excised and in-gel digested with trypsin prior to LC-MS sequencing, with the results summarized in Table 1. Sequencing data were obtained for 13 of the protein spots excised from the gel, representing the identification of nine individual proteins: protein disulfide isomerase, protein disulfide isomerase A6, peptidylprolyl isomerase A, thioredoxin domain-containing protein 5, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), galectin-1, β-tubulin, vimentin, and β-actin. In each case, the primary sequences of the proteins identified contain at least one Cys residue. Identification of the protein responsible for a group of IAF-labeled spots with molecular mass ca. 48 kDa was not possible, owing to poor peptide matching and hence non-conclusive sequencing results.

4. Discussion

Although it is becoming increasingly recognized that protein thiols play a key role in the modulation of enzyme activity and various signaling processes in cells [15], in general, there is a lack of global information regarding the nature of thiol-containing proteins targeted in cells exposed to oxidants. In this study, we show that exposure of HCAEC to HOCl and *N*-chloramines formed under inflammatory conditions, results in the oxidation of multiple thiol-containing proteins including GAPDH, protein disulfide isomerase, protein disulfide isomerase A6, thioredoxin domain-containing protein 5, peptidylprolyl isomerase A, galectin-1, vimentin, β-tubulin and β-actin.

Exposure of the cells to MonoCA resulted in the greatest decrease in cellular thiols (with $\leqslant$200 nmol oxidant/10^6 cells), consistent with previous studies showing that this oxidant readily penetrates cells, owing to its lypophilic nature (e.g. [9]). A similar extent of thiol loss was seen with HOCl and HistCA, though the cells appeared to consume >80% of the added HOCl compared to only 25% of the HistCA. This supports previous reports highlighting the greater thiol selectivity of *N*-chloramines [10,11]. The fact that HistCA can discriminate between thiol groups on the basis of their pK_a [11], suggests that HistCA may react in a more selective manner compared to HOCl and MonoCA, though no significant differences in selectivity were apparent from the proteomic analysis.

Although a similar extent of thiol oxidation was observed with HOCl and HistCA, there was a significant difference in the toxicity of these oxidants on assessment of the metabolic activity of the cells determined over a 4 h time period after treatment. This is likely to reflect both the greater consumption of HOCl by the HCAEC, and the greater reactivity of HOCl compared to HistCA. With HOCl, it is well established that reaction with thiols results in the formation of non-reversible oxidation products, including sulfinic and sulfonic acids and sulfenyl-amides [5]. The products formed on reaction of *N*-chloramines with protein thiols have not been studied as widely, though there is evidence for the formation of disulfides, with low molecular mass products and isolated proteins [10] and mixed disulfides (glutathionylation) in cellular systems [11]. Given that disulfides and glutathionylated adducts are reversible thiol modifications in contrast to sulfinic and sulfonic acids, it is possible that the difference in toxicity between HistCA and HOCl reflects the repair of thiol damage during the 4 h incubation period in the cells exposed to HistCA.

GAPDH was identified as a target on exposure of HCAEC to HOCl, MonoCA, and HistCA, which agrees well with previous studies in human umbilical vein endothelial cells (e.g. [12,21,22]).

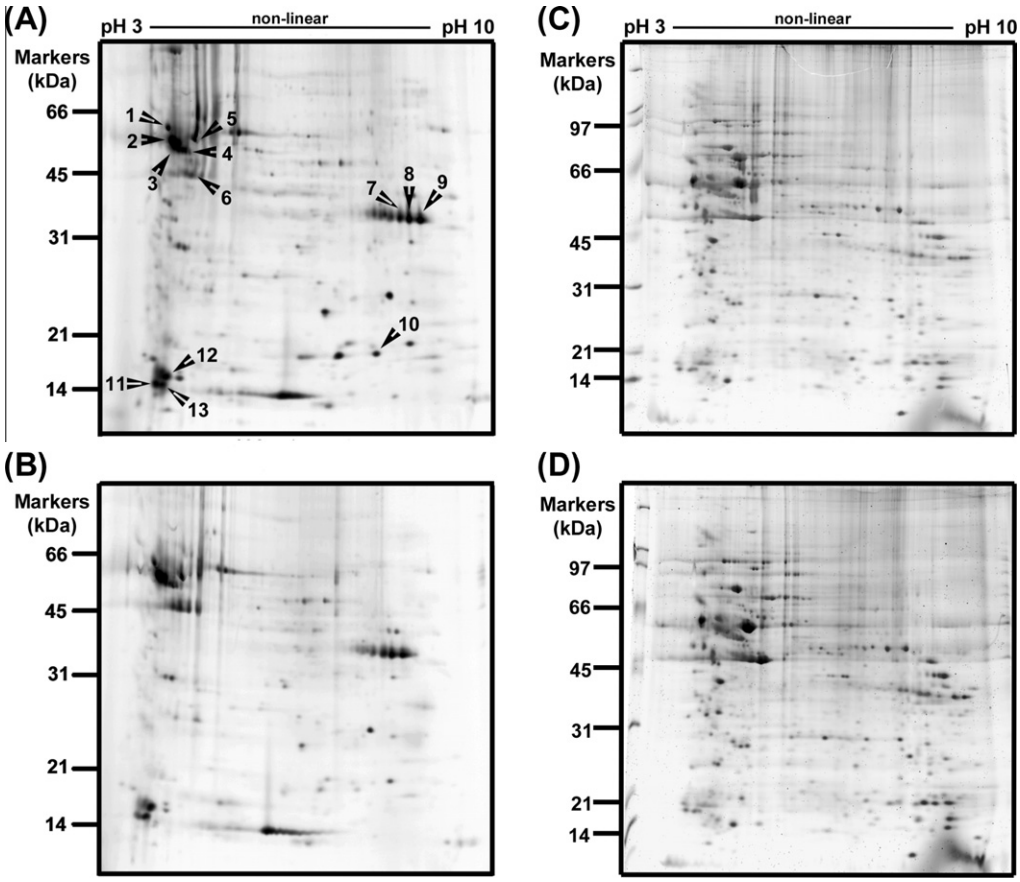

Fig. 2. Representative 2D SDS–PAGE of HCAEC exposed to HistCA showing (A–B) IAF-labeled proteins and (C and D) RuBPS-labeled proteins. Gels represent HCAEC treated with (A–C) histamine (5 μmol/10^6 cells) or (B-D) HistCA (500 nmol/10^6 cells) for 15 min prior to labeling with the fluorescent, thiol-specific tag IAF and separation by 2D SDS–PAGE. IAF-labeled proteins are visualized by scanning at λ_{EX} 488 nm and λ_{EM} 530 nm. Total protein present is visualized by staining with RuBPS and scanning at at λ_{EX} 532 nm and λ_{EM} 605 nm. The protein spots corresponding to those identified by LC–MS/MS are numbered 1–13, as listed in Table 1.

Table 1
Proteins susceptible to thiol oxidation on exposure of HCAEC to HOCl, MonoCA and HistCA.

No.	Identity	NCBI "gI" accession No.	M_r/pI	No. of peptides matched	Sequence coverage (%)	MASCOT score
1	Protein disulfide isomerase	20070125	55294/4.69	9	22	1486
2	Tubulin, β 2C chain	4507729	49907/4.78	13	42	1570
3	Protein disulfide isomerase A6	1710248	46199/4.95	6	20	663
4	Thioredoxin domain-containing protein 5	42794771	44460/5.37	4	12	675
5	Vimentin	62414289	53520/5.06	25	51	2880
6	β-Actin	194376310	38502/5.19	7	22	858
7	GAPDH	7669492	35922/8.58	4	18	727
8	GAPDH	7669492	35922/8.58	3	12	566
9	GAPDH	7669492	35922/8.58	7	30	803
10	Peptidylprolyl isomerase A (cyclophilin A)	10863927	17881/7.82	7	56	651
11	Galectin-1	4504981	14585/5.34	3	28	375
12	Galectin-1	4504981	14585/5.34	4	40	560
13	Galectin-1	4504981	14585/5.34	2	21	287

There was little difference in the extent of GAPDH thiol oxidation observed, as determined by IAF labeling, on treatment of the cells with either HOCl or HistCA, in accord with previous reports that HistCA is one of the most reactive biologically relevant chloramines [11].

Four of the proteins identified, protein disulfide isomerase, protein disulfide isomerase A6, thioredoxin domain-containing protein 5 and peptidylprolyl isomerase A are involved in protein folding. Inactivation of the cellular proteins involved in protein folding by thiol oxidation induced by HOCl and *N*-chloramines may therefore potentially lead to the accumulation of unfolded proteins within cells and subsequent detrimental effects on function. Indeed, the "unfolded protein response" signaling cascade, which is activated in cells on accumulation of unfolded proteins in the endoplasmic reticulum, is a key event in advanced atherosclerotic lesions and other inflammatory disease processes [23].

Evidence was also obtained for the reaction of HOCl and the *N*-chloramines with galectin-1. Galectin-1 is a carbohydrate binding protein with an affinity for β-galactosides, which plays a role in a diverse range of cellular functions, including growth, migration, adhesion and inflammation (reviewed [24,25]). In endothelial cells, galectin-1 is translocated to the outer cell membrane, where it plays a role in the attachment and migration of activated cells over the extracellular matrix [26]. It is postulated that the lectin activity

of galectin-1 is important for the modulation of cell migration, adhesion and motility [24]. This activity is likely to be compromised on treatment of cells with HOCl and *N*-chloramines, as oxidation of galectin-1 results in the loss of lectin activity [27]. Oxidation of galectin-1 may also perturb various inflammatory pathways, owing to the disruption of cell surface glycoprotein binding and cross-linking.

The remaining proteins identified, vimentin, β-tubulin and β-actin, all play a role in maintaining the cytoskeleton of the cell. Oxidation of the thiol residues contained in these proteins by HOCl and *N*-chloramines may disrupt cellular structure. However, it is also possible that the identification of these proteins is a sequencing artifact, owing to the high abundance of these proteins within cells masking the identification of lower abundance proteins with similar molecular mass.

In summary, this study provides evidence for targeting of various proteins on exposure of HCAEC to HOCl, MonoCA and HistCA. The targeting of proteins involved in folding and galectin-1 in particular, highlight potentially novel pathways by which MPO-derived oxidants may promote inflammatory processes and contribute to the pathogenesis of disease. The specificity of HistCA for thiol-containing proteins is of particular significance in the context of atherosclerosis, given the accumulating evidence for a clear role of mast cells, which release histamine, in the pathogenesis of this disease [28].

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References

[1] M.J. Davies, C.L. Hawkins, D.I. Pattison, M.D. Rees, Mammalian heme peroxidases: from molecular mechanisms to health implications, Antioxid. Redox. Signal. 10 (2008) 1199–1234.
[2] M.B. Hampton, A.J. Kettle, C.C. Winterbourn, Inside the neutrophil phagosome: oxidants, myeloperoxidase, and bacterial killing, Blood 92 (1998) 3007–3017.
[3] S.J. Weiss, Tissue destruction by neutrophils, N. Engl. J. Med. 320 (1989) 365–376.
[4] S.J. Klebanoff, Myeloperoxidase: friend and foe, J. Leukoc. Biol. 77 (2005) 598–625.
[5] C.L. Hawkins, D.I. Pattison, M.J. Davies, Hypochlorite-induced oxidation of amino acids, peptides and proteins, Amino acids 25 (2003) 259–274.
[6] D.I. Pattison, M.J. Davies, Reactions of myeloperoxidase-derived oxidants with biological substrates: gaining chemical insight into human inflammatory diseases, Curr. Med. Chem. 13 (2006) 3271–3290.
[7] E.L. Thomas, M.B. Grisham, M.M. Jefferson, Preparation and characterization of chloramines, Methods. Enzymol. 132 (1986) 569–585.
[8] M.B. Grisham, M.M. Jefferson, D.F. Melton, E.L. Thomas, Chlorination of endogenous amines by isolated neutrophils. Ammonia-dependent bactericidal, cytotoxic, and cytolytic activities of the chloramines, J. Biol. Chem. 259 (1984) 10404–10413.
[9] E.L. Thomas, M.B. Grisham, M.M. Jefferson, Cytotoxicity of chloramines, Methods. Enzymol. 132 (1986) 585–593.
[10] A.V. Peskin, C.C. Winterbourn, Taurine chloramine is more selective than hypochlorous acid at targeting critical cysteines and inactivating creatine kinase and glyceraldehyde-3-phosphate dehydrogenase, Free Radical Biol. Med. 40 (2006) 45–53.
[11] A.V. Peskin, C.C. Winterbourn, Histamine chloramine reactivity with thiol compounds, ascorbate and methionine and with intracellular glutathione, Free Radical Biol. Med. 35 (2003) 1252–1260.
[12] J.M. Pullar, C.C. Winterbourn, M.C.M. Vissers, Loss of GSH and thiol enzymes in endothelial cells exposed to sublethal concentrations of hypochlorous acid, Am. J. Physiol. 277 (1999) H1505–H1512.
[13] I.U. Schraufstatter, K. Browne, A. Harris, P.A. Hyslop, J.H. Jackson, O. Quehenberger, C.G. Cochrane, Mechanisms of hypochlorite injury of target cells, J. Clin. Invest. 85 (1990) 554–562.
[14] R.G. Midwinter, F.C. Cheah, J. Moskovitz, M.C. Vissers, C.C. Winterbourn, IκB is a sensitive target for oxidation by cell-permeable chloramines: inhibition of NF-κB activity by glycine chloramine through methionine oxidation, Biochem. J. 396 (2006) 71–78.
[15] Y.M.W. Janssen-Heininger, B.T. Mossman, N.H. Heintz, H.J. Forman, B. Kalyanaraman, T. Finkel, J.S. Stamler, S.G. Rhee, A. van der Vliet, Redox regulation of signal transduction: Principles, pitfalls and promises, Free Radical Biol. Med. 45 (2008) 1–17.
[16] J.C. Morris, The acid ionization constant of HOCl from 5°C to 35°C, J. Phys. Chem. 70 (1966) 3798–3805.
[17] C.L. Hawkins, P.E. Morgan, M.J. Davies, Quantification of protein modification by oxidants, Free Radical Biol. Med. 46 (2009) 965–988.
[18] F.A. Summers, P.E. Morgan, M.J. Davies, C.L. Hawkins, Identification of plasma proteins that are susceptible to thiol oxidation by hypochlorous acid and *N*-chloramines, Chem. Res. Toxicol. 21 (2008) 1832–1840.
[19] C.L. Hawkins, M.J. Davies, Reaction of HOCl with amino acids and peptides: EPR evidence for rapid rearrangement and fragmentation reactions of nitrogen-centered radicals, J. Chem. Soc. Perkin Transact. 2 (1998) 1937–1945.
[20] P.E. Morgan, A.D. Sturgess, A. Hennessy, M.J. Davies, Serum protein oxidation and apolipoprotein CIII levels in people with systemic lupus erythematosus with and without nephritis, Free Radical Res. 41 (2007) 1301–1312.
[21] M.M. Lloyd, D.M. Van Reyk, M.J. Davies, C.L. Hawkins, HOSCN is a more potent inducer of apoptosis and protein thiol depletion in murine macrophage cells than HOCl or HOBr, Biochem. J. 414 (2008) 271–280.
[22] A.V. Peskin, R.G. Midwinter, D.T. Harwood, C.C. Winterbourn, Chlorine transfer between glycine, taurine and histamine: reaction rates and impact on cellular reactivity, Free Radical Biol. Med. 37 (2004) 1622–1630.
[23] I. Tabas, The role of endoplasmic reticulum stress in the progression of atherosclerosis, Circ. Res. 107 (2010) 839–850.
[24] I. Camby, M. Le Mercier, F. Lefranc, R. Kiss, Galectin-1: a small protein with major functions, Glycobiology 16 (2006) 137R–157R.
[25] N. Rubinstein, J.M. Ilarregui, M.A. Toscano, G.A. Rabinovich, The role of galectins in the initiation, amplification and resolution of the inflammatory response, Tissue Antigens 64 (2004) 1–12.
[26] V.L. Thijssen, S. Hulsmans, A.W. Griffioen, The galectin profile of the endothelium: altered expression and localization in activated and tumor endothelial cells, Am. J. Pathol. 172 (2008) 545–553.
[27] R.L. Outenreath, A.L. Jones, Influence of an endogenous lectin substrate on cultured dorsal root ganglion cells, J. Neurocytol. 21 (1992) 788–795.
[28] I. Bot, E.A. Biessen, Mast cells in atherosclerosis, Thromb. Haemost. 106 (2011) 820–826.