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The Role of Myeloperoxidase in Biomolecule Modification, Chronic Inflammation, and Disease

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Abstract

Significance: The release of myeloperoxidase (MPO) by activated leukocytes is critical in innate immune responses. MPO produces hypochlorous acid (HOCl) and other strong oxidants, which kill bacteria and other invading pathogens. However, MPO also drives the development of numerous chronic inflammatory pathologies, including atherosclerosis, neurodegenerative disease, lung disease, arthritis, cancer, and kidney disease, which are globally responsible for significant patient mortality and morbidity.
Recent Advances: The development of imaging approaches to precisely identify the localization of MPO and the molecular targets of HOCl *in vivo* is an important advance, as typically the involvement of MPO in inflammatory disease has been inferred by its presence, together with the detection of biomarkers of HOCl, in biological fluids or diseased tissues. This will provide valuable information in regard to the cell types responsible for releasing MPO *in vivo*, together with new insight into potential therapeutic opportunities.
Critical Issues: Although there is little doubt as to the value of MPO inhibition as a protective strategy to mitigate tissue damage during chronic inflammation in experimental models, the impact of long-term inhibition of MPO as a therapeutic strategy for human disease remains uncertain, in light of the potential effects on innate immunity.
Future Directions: The development of more targeted MPO inhibitors or a treatment regimen designed to reduce MPO-associated host tissue damage without compromising pathogen killing by the innate immune system is therefore an important future direction. Similarly, a partial MPO inhibition strategy may be sufficient to maintain adequate bacterial activity while decreasing the propagation of inflammatory pathologies. *Antioxid. Redox Signal.* 32, 957–981.

Keywords: myeloperoxidase, hypohalous acid, oxidation, inflammation, oxidative stress

Introduction

MYELOPEROXIDASE (MPO) is a key element of the innate immune system and is released primarily by neutrophils to provide defence against invading pathogens. MPO comprises about 5% of the dry mass of the neutrophil and is contained predominantly within the lysosomal azurophilic granules. On neutrophil activation, fusion of lysosomes with phagosomes results in release of MPO, while assembly of an NADPH oxidase complex on the internal membrane surface results in the production of superoxide anions ($O_2^{-\bullet}$), which rapidly dismutate to form hydrogen peroxide (H_2O_2). MPO catalyzes the reaction of H_2O_2 with chloride ions (Cl^-) to form hypochlorous acid (HOCl), which facilitates the destruction of microbes contained within the phagolysosome (170, 292). However, some MPO is also released extracellularly, where the misplaced production of HOCl can promote host tissue damage and the development of disease. This is particularly significant during chronic inflammation, owing to the excessive leukocyte infiltration and activation (50, 265).

Exposure of neutrophils to pathogens, and inflammatory mediators (*e.g.*, cytokines, chemokines, complement proteins, or oxidants such as HOCl), also triggers the release of neutrophil extracellular traps (NETs) (27, 70). NETs are released by a pathway termed ''NETosis,'' and consist of a

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mesh of extracellular DNA, complexed with histones and neutrophil granule proteins, including MPO (261). In addition to its presence on the NET structure, MPO also plays a critical role in triggering neutrophils to undergo NETosis (160). NET-bound MPO retains its activity and can promote bacterial cell killing, or promote tissue injury in the presence of H_2O_2 (181). There is increasing evidence that NETs play a causal role in the development of some inflammatory diseases [reviewed in Bonaventura *et al*. (22)], providing an additional pathway by which MPO could contribute pathologically.

Although HOCl production is believed to be essential for the efficient removal of pathogens, there is little doubt that this and other MPO-derived, reactive species can initiate oxidative tissue damage and cellular dysfunction, and thereby promote disease progression. This is supported by evidence for the presence of MPO, together with specific biomarkers for HOCl-mediated damage, particularly 3-chlorotyrosine (Cl-Tyr), in diseased tissue and inflammatory fluids, which implicates this peroxidase in numerous inflammatory pathologies, as shown in Table 1. Genetic polymorphisms, particularly the MPO-463G > A allele, located in the promoter region of the MPO gene, may also play a role in disease, by influencing MPO transcription levels. This polymorphism has been studied in the context of different diseases and, in general, individuals with the more common G-allele have higher levels of MPO gene expression, and a greater incidence of disease development compared with those with the A-allele (167, 213), although in terms of cancer development, the data are inconsistent [reviewed in Yang *et al*. (295)].

Table 1. Diseases Associated with a Pathological Role of Myeloperoxidase

Disease	*References*
Cardiovascular disease/atherosclerosis	(49, 171, 257, 308)
Myocardial infarction	(163, 217)
Vascular dysfunction	(60, 151, 274)
Lipoprotein modification	(53, 150, 171)
Atrial fibrillation	(139, 216)
Plaque rupture	(240, 247)
Hypertension	(131, 267)
Abdominal aortic aneurysm	(128)
Neurodegenerative disease	(196, 201)
Alzheimer's disease	(80, 258)
Parkinson's disease	(44, 112)
Multiple sclerosis	(78, 79, 167)
Stroke	(45, 245, 300)
Kidney disease	(149)
Chronic kidney disease	(129, 138)
Glomerulonephritis	(177)
Respiratory disease	
Asthma	(32, 111)
Cystic fibrosis	(120, 266, 268)
Chronic obstructive pulmonary disease	(176)
Other diseases	
Rheumatoid arthritis	(236, 278)
Systemic lupus erythematosus	(246)
Autoimmune disease	(239)
Metabolic syndrome/obesity	(6, 277)
Type 2 diabetes	(73)
Nonalcoholic steatohepatitis	(210)
Inflammatory bowel disease/colitis	(40, 75)
Cancer/carcinogenesis	(62, 218, 283)
Noninfectious disease	(104)
Aging	(38)

In this article, we focus on the role of MPO in chronic inflammation and disease, rather than in host defence. We provide an overview of the enzymology, substrate specificity, and oxidant production by MPO, and discuss some examples of how the targeting and modification of specific tissues and biological substrates can modulate cellular function in a detrimental manner to promote the development of inflammatory disease. We also review potential approaches to prevent these damaging reactions, which may have therapeutic value in chronic inflammatory diseases.

Enzymology of MPO and Oxidant Production

Structure of MPO

Mature MPO is a highly cationic (positively charged, pI $\sim$10) dimeric protein with a mass of $\sim$146 kDa. The two dimers are identical and functionally independent. Each molecule consists of two monomer units (73 kDa each) joined by a single disulfide between the Cys153 residues. Each monomer comprises a heavy (58.5 kDa, 467 amino acids) and light chain (14.5 kDa, 106 amino acids) (64), with the former having multiple glycosylation sites. The heavy chains contain a modified iron protoporphyrin IX derivative, which is the active site, with this being located in a deep cleft (302). This restricts access of the iron atom to H_2O_2 and a number of small negatively charged anions (21). A second hydrophobic binding pocket is present at the entrance to the distal heme cavity, with this being the site of oxidation of large substrates *via* the peroxidase cycle (103).

The structure of MPO (and other human peroxidases) has been reviewed (72). The proximal histidine ligand is a key regulator of the redox properties of the heme iron and is hydrogen bonded to an asparagine, which acts as a hydrogen bond acceptor and helps stabilize the resting (ferric, Fe^{3+}) form of the enzyme (72). The redox properties of the heme are markedly affected by a sulfonium ion (covalent) linkage between the heme and Met-243 (301). This withdraws electron density from the heme, due to its positive charge, and distorts the heme from planarity. A distal histidine ligand is also present and is believed to act as an acid/base catalyst with a nearby arginine *via* a hydrogen-bonded network, to polarize the -O—O- bond of H_2O_2 (64), with cleavage of this bond initiating the catalytic cycles of the enzyme (72). The importance of this hydrogen bonding network is indicated by its conservation in other peroxidases (72). Each enzyme also has a conserved asparagine residue adjacent to the distal histidine that appears to align this *via* coordination with a bound Ca^{2+} (72). Crystal structure data indicate that halide substrates bind within this cavity $\sim$3.5 Å from the N^{ε} atom of the distal histidine (20, 64), with this favored at acidic pH values by protonation of the distal histidine and electrostatic interactions (107).

Catalytic cycles of MPO

H_2O_2 reacts with ferric MPO *via* a two-electron reaction to generate Compound I [an oxy-ferryl ($Fe^{IV}=O$) heme species with a porphyrin π radical-cation], and a molecule of H_2O.

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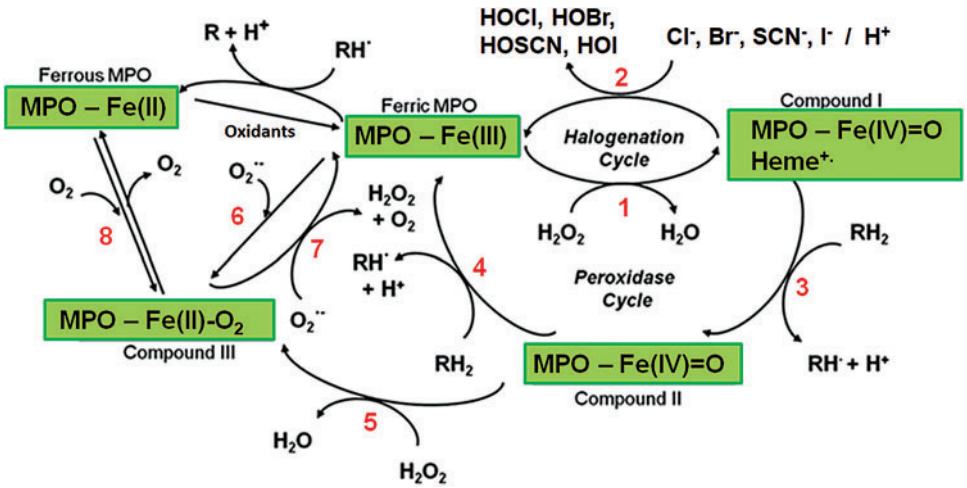

FIG. 1. Generation and reactions of redox intermediates of MPO. RH_2 denotes a classic organic peroxidase substrate; other species also undergo one-electron oxidation by Compound I and Compound II. MPO, myeloperoxidase. Adapted from Nussbaum *et al.* (173). Color images are available online.

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Compound I is then converted back to the ferric form *via* two independent and discrete pathways. The first involves direct, two-electron reduction of Compound I species by halide (Cl^-, bromide ion [Br^-], iodide ion [I^-]) and pseudohalide (thiocyanate ion [SCN^-]) anions. This is commonly termed ''the halogenation cycle'' and results in the formation of hypohalous acids: HOCl, HOBr (hypobromous acid), HOI (hypoiodous acid), and hypothiocyanous acid (HOSCN). The second pathway involves two sequential one-electron reduction reactions, which involve a second enzyme intermediate, Compound II [an oxy-ferryl ($Fe^{IV}=O$) heme species]. Compound II then undergoes a second one-electron reduction to give the ferric species. This second pathway is usually termed ''the peroxidase cycle.'' These processes and the corresponding second-order rate constants are summarized in Figure 1 and Table 2. A further intermediate, Compound III, can be formed *via* reaction of the ferric state with superoxide radical anion ($O_2^{\bullet-}$), or *via* one-electron reduction of the ferric state of the enzyme to the ferrous (Fe^{2+}) form and subsequent O_2 addition. These reactions allow $O_2^{\bullet-}$-dependent catalytic activities of MPO (119).

Substrate specificity: halogenation cycle

As a result of the heme ring distortion and the sulfonium linkage, Compound I of MPO has a high reduction potential for both the Compound I/native enzyme and the Compound I/Compound II couples (301). The Compound II/native enzyme couple has a lower value. These high values allow MPO to oxidize a wide range of substrates *via* the halogenation and peroxidase cycles, although the potentials vary with pH, and impose thresholds on substrate oxidation (235).

Unlike Compounds I and II, Compound III is poorly reactive and is often considered a catalytic ''dead-end,'' although it can induce slow (one-electron) oxidation of ascorbate and paracetamol (acetaminophen) (153) and also

TABLE 2. SELECTED APPARENT SECOND-ORDER RATE CONSTANTS ($M^{-1}\cdot s^{-1}$) FOR THE REACTIONS LISTED IN THE HALOGENATION AND PEROXIDASE CYCLES OF MYELOPEROXIDASE, SHOWN IN FIGURE 1

Reaction No. (Fig. 1)	*Reaction description*	*Rate constant*
1	Native enzyme + H_2O_2 → Compound I	$1.4\times10^{7,a}$
2	Compound I + Cl^- → native enzyme + HOCl	$2.5\times10^{4,a}$
2	Compound I + Br^- → native enzyme + HOBr	$1.1\times10^{6,a}$
2	Compound I + SCN^- → native enzyme + HOSCN	$9.6\times10^{6,a}$
3	Compound I + RH_2 → Compound II + $R^\bullet$	Tyr, $7.7\times10^{5,b}$; Trp, $4.5\times10^{5,b}$; NO_2^-, $2.2\times10^{6,c}$
3	Compound I + $O_2^{-\bullet}$ → Compound II + O_2	$5\times10^{6,d}$
3	Compound I + H_2O_2 → Compound II + $O_2^{-\bullet}$	$7\times10^{4,d}$
4	Compound II + RH_2 → native enzyme + $R^\bullet$	Tyr, $1.6\times10^{4,b}$; Trp, 6.9^b; NO_2^-, 550^c
4	Compound II + $O_2^{-\bullet}$ → native enzyme + O_2 + H_2O	$1\times10^{6,d}$
5	Compound II + H_2O_2 → Compound III + H_2O	50^d
6	Native enzyme + $O_2^{-\bullet}$ → Compound III	$2\times10^{6,d}$
7	Compound III + $O_2^{-\bullet}$ → [native enzyme + H_2O_2] + O_2	$1\times10^{5,d}$
8	Ferrous enzyme + O_2 → Compound III	$1.1\times10^{4,e}$

[a]Measured at pH 7.0 and 15°C [reviewed in Furtmuller *et al.* (72)].
[b]Measured at pH 7.0 and 25°C (109).
[c]Measured at pH 7.0 [reviewed in Arnhold *et al.* (9)].
[d]From Winterbourn *et al.* (291).
[e]Measured at pH 7.0 and 25°C (110).
Br^-, bromide ion; Cl^-, chloride ion; H_2O_2, hydrogen peroxide; HOBr, hypobromous acid; HOCl, hypochlorous acid; HOSCN, hypothiocyanous acid; SCN^-, thiocyanate ions.

$O_2^{\bullet-}$ thereby regenerating ferric MPO and producing H_2O_2 and O_2. MPO can therefore act in a similar manner to a superoxide dismutase (SOD), and the concentration of $O_2^{\bullet-}$ can modulate the activity of MPO (119). Computational modeling suggests that within the neutrophil phagosome (where MPO concentrations have been calculated as ~ 1 m*M*), most of the $O_2^{\bullet-}$ produced by NADPH oxidase is consumed *via* this SOD-like activity, and that efficient recycling of Compound III by $O_2^{\bullet-}$ ensures that phagosomal HOCl production is not constrained (291). However, when MPO concentrations and H_2O_2 fluxes are low, and turnover of the ferric enzyme is rate limiting, Compound III formation can constrain MPO activity (126).

Although H_2O_2 is essential for the catalytic cycles of the enzyme, high concentrations can also inhibit these activities, as it can also act as a competitive substrate for Compound I and Compound II (to give the poorly reactive Compound III), and by inducing irreversible enzyme inactivation (72, 126). Therefore, in the absence of substrates, MPO exhibits catalase-like activity (127). With high H_2O_2 fluxes, where reaction of Compound I with H_2O_2 results in Compound II accumulation, $O_2^{\bullet-}$ appears to maintain chlorinating activity by recycling Compound II to the ferric form (72, 126).

The ability of MPO to oxidize Cl^- with a high rate constant is unique among the peroxidase family, although this anion is not the most favored substrate, as the second-order rate constants for reaction of Compound I with anions reflect the reduction potentials of the various potential substrates, and decrease down the series $SCN^- > I^- > Br^- > Cl^-$. The lower two-electron reduction potentials of Compound I of other peroxidases limit rapid oxidation to SCN^-, I^-, and Br^-, and not Cl^-.

With typical physiological plasma concentrations of these anions (Cl^- ~ 100 to 150 m*M*, Br^- 20 to 100 μM, SCN^- 20 to 150 μM, I^- <0.1 to 1 μM), MPO generates predominantly HOCl and HOSCN (263), with only low levels of HOBr, and minimal HOI formed. It should, however, be noted that this conclusion is dependent on the specific environment, with altered yields of oxidants produced on supplementation/depletion of these anions. Specificity constants and K_m values for each of these anions have been reported (262, 263). The species formed are also pH dependent, with HOBr production by MPO reported to be enhanced at pH values >7, such that at pH 7.8, HOBr may account for up to 40% of the H_2O_2 consumed by MPO (223). The rate of oxidation is also significantly enhanced at acidic pH (9), due to an increased affinity of the anions for the protonated distal histidine (107). This effect is most pronounced for Cl^-, which may indicate that this anion, but not others, may have direct access to the oxyferryl heme center at low pH values (72). There has been debate as to whether free HOCl is formed by MPO, with evidence presented for direct chlorination by an intermediate Compound I-Cl^- complex at low pH (154). However, the chlorination of large proteins and other targets by MPO-H_2O_2-Cl^- suggests that freely diffusible species must be generated, a conclusion supported by studies that quantified HOCl production within neutrophil phagolysosomes, using a rhodamine-based fluorescent probe (3).

Substrate specificity: peroxidase cycle

The MPO peroxidase cycle generates radicals from organic substrates *via* one-electron oxidation by Compounds I and II, and also oxidizes some radicals, including $O_2^{\bullet-}$, nitric oxide radical ($NO^\bullet$), and nitroxides (2, 114, 126, 204), to nonradical products. Although reaction of $NO^\bullet$ with Compounds I and II is facile, consumption of $NO^\bullet$ in plasma at physiologically relevant fluxes (steady state << 1 μM) is likely to occur predominantly *via* reaction with long-lived radicals (*e.g.*, tyrosyl and ascorbyl) generated *via* the peroxidase cycle (56). Nitrite ion (NO_2^-) reacts with Compounds I and II to give nitrogen dioxide radical ($NO_2^\bullet$) (264). A small but significant fraction of NO_2^- has been reported to be oxidized to a species that can induce hydroxylation as well as nitration, a property shared by peroxynitrous acid (ONOOH). In contrast to free ONOOH, the MPO-H_2O_2-NO_2^- system appears to induce hydroxylation only at acidic pH, and CO_2 does not enhance nitration, consistent with an enzyme-bound intermediate (25). ONOOH rapidly converts MPO Compounds I and III, to Compound II. Compound II does not appear to oxidize ONOOH, despite this being thermodynamically favorable (71). The peroxidase reactions of Compound I occur in competition with the halogenation cycle and hence are affected by the concentration of these species relative to the halide/pseudohalide ions.

Physiologically relevant organic peroxidase substrates for MPO include both endogenous compounds (*e.g.*, Tyr, ascorbate, steroidal hormones, and urate) and xenobiotics and drugs. The range of compounds is extensive due to the high reduction potential of Compound I, and to a lesser extent Compound II. Some substrates can react readily with Compound I, but not Compound II (*i.e.*, species with reduction potentials between those for Compound I/Compound II and Compound II/ferric enzyme; often termed ''poor'' peroxidase substrates), with this resulting in an accumulation of Compound II and arrest of the catalytic cycle. Kinetic factors can also influence peroxidase cycle turnover, with rate constants for reaction of substrates with Compound II often significantly lower than with Compound I (155). The halogenation cycle typically occurs more rapidly than the peroxidase cycle, resulting in higher yields of hypohalous acids than radical species, although this is pH dependent (155).

Some radicals generated by the peroxidase cycle can react with the heme moiety and result in enzyme inactivation; this is the mode of action of a number of MPO inhibitors [*e.g.*, thioxanthines (253)]. Radicals may also reduce ferric MPO to ferrous MPO and hence yield Compound III in the presence of O_2; this occurs, for example, during the metabolism of hydroquinone (125) and hydrazides (122). Reaction with the enzyme may also generate protein-derived radicals, *via* hydrogen atom abstraction or addition to aromatic amino acids (226). Radicals may also diffuse from the active site and damage other biomolecules such as lipids (116) and proteins (99).

Reactivity of MPO-Derived Oxidants

Reactions of HOCl and N-chloramines

HOCl is produced under both physiological and pathological conditions, as evidenced by the detection of chlorinated biomarkers, particularly Cl-Tyr, in inflammatory fluids and diseased tissues (Table 1). HOCl is a powerful oxidant and chlorinating agent, and exists in equilibrium with its conjugate base, hypochlorite [$^-$OCl, pK_a 7.59 (166)] at physiological pH. HOCl reacts rapidly with nucleophiles, and

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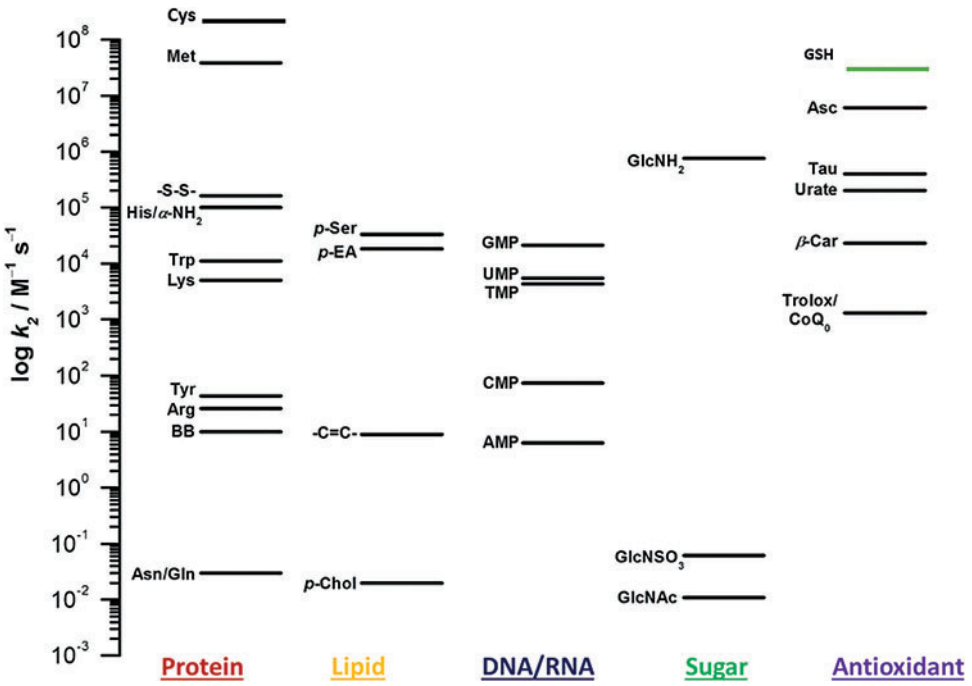

FIG. 2. Plot summarizing the second-order rate constants (on a log scale) for the reactions of HOCl with model compounds of protein, lipid, and carbohydrate components, nucleobases, and antioxidants [reviewed in Pattison and Davies (186)]. The majority of the rate constants were acquired in phosphate-buffered solutions at pH 6.8–7.5 and 20°C–25°C (65, 183, 188, 197, 198, 207, 289); however, that for β-carotene was determined in a detergent solution at pH 4.5 (4). α-NH_2, α-amino group; β-Car, β-carotene; Asc, ascorbate; BB, backbone amides; -C═C-, double bond; CoQ_0, ubiquinol-0; GlcNAc, *N*-acetylated glucosamine; $GlcNH_2$, glucosamine; $GlcNSO_3$, *N*-sulfated glucosamine; HOCl, hypochlorous acid; *p*-Chol, phosphoryl-choline; *p*-EA, phosphoryl-ethanolamine; *p*-Ser, phosphoryl-Ser; -S-S-, disulfide bond. Color images are available online.

especially those with sulfur or nitrogen atoms (*e.g.*, thiols, thioethers, and amines). Selected second-order rate constants, and products generated by HOCl, are shown in Figures 2 and 3 and Table 3.

Cys and Met residues in proteins and glutathione (GSH) are key targets of HOCl (238). Oxidation of Cys yields a sulfenyl chloride (RS-Cl), which reacts rapidly with excess thiol to give the disulfide, or H_2O to yield sulfenic (RSOH), sulfinic (RSO_2H), and sulfonic acids (RSO_3H, cysteic acid); reviewed in Hawkins *et al.* (91). Disulfides (*e.g.*, cystine) can also be oxidized by HOCl, with some of these reactions being very fast due to stabilization of the *S*-chlorinated intermediate

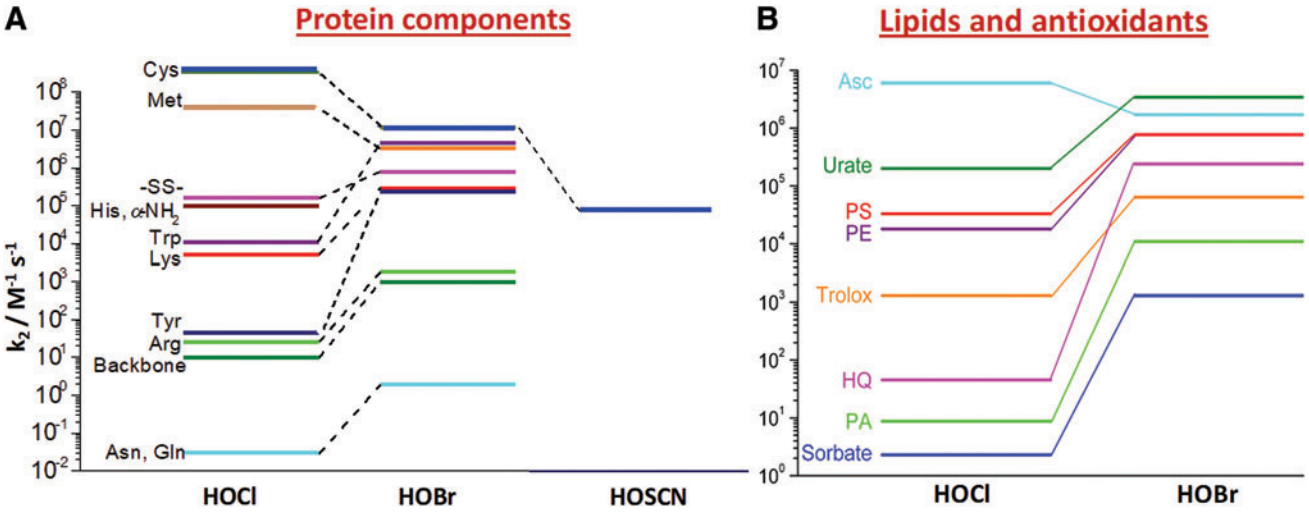

FIG. 3. Plots comparing the known second-order rate constants (on a log scale) for selected reactions of hypohalous acids with model biological substrates. (A) HOCl, HOBr, and HOSCN with model compounds of protein and **(B)** HOCl and HOBr with model lipid components and antioxidants [reviewed in Pattison and Davies (186)]. The majority of the rate constants were determined in phosphate-buffered solutions at pH 7.2–7.5 and 22°C (184, 228). HOBr, hypobromous acid; HOSCN, hypothiocyanous acid; HQ, hydroquinone; PA, pentenoic acid (double bond); PE, phosphoryl-ethanolamine; PS, phosphoryl-Ser. Color images are available online.

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TABLE 3. SUMMARY OF THE SECOND-ORDER RATE CONSTANTS AND PRODUCTS FOR REACTIONS OF HOCL AND HOSCN WITH BIOLOGICALLY RELEVANT THIOLS, SELENOLS, THIOETHERS AND SELENIDES

	Second-order rate constants $k_2/M^{-1} \cdot s^{-1}$		*Products*	
Substrate	*HOCl*	*HOSCN*	*HOCl*	*HOSCN*
Cysteine (Cys)	$3.6 \times 10^{8,a}$	$7.8 \times 10^{4,b}$	Sulfenyl chloride (RS-Cl); sulfenic acid (RS-OH); sulfinic/sulfonic acids; disulfides (RS-SR′)	Sulfenyl thiocyanate (RS-SCN); sulfenic acid (RS-OH); sulfinic/ sulfonic acids; disulfides (RS-SR′)
Glutathione (GSH)	$1.3 \times 10^{8,a}$	$2.5 \times 10^{4,b}$	GSSG; glutathione sulfonic acid; glutathione sulfonamide; mixed disulfides (GS-SR)	GSSG; mixed disulfides (GS-SR)
Methionine (Met)	$3.4 \times 10^{7,a}$	$<10^{3,b}$	Methionine sulfoxide (MetSO); methionine sulfone ($MetSO_2$); dehydromethionine	No reaction
Selenocysteine (Sec)[c]	ND	$1.24 \times 10^{6,d}$	Selenyl chloride (RSe-Cl); selenenic acid (RSe-O -/RSe-OH); seleninic/selenonic acids; diselenides (RSe-SeR′); mixed RSe-SR′	Selenyl thiocyanate (RSe-SCN); selenenic acid (RSe-O -/RSe-OH); seleninic/selenonic acids; diselenides (RSe-SeR′); mixed RSe-SR′
Selenomethionine (SeMet)	ND	$2.8 \times 10^{3,d}$	Methionine selenoxide (MetSeO)	Methionine selenoxide (MetSeO)

[a]At 22°C, pH 7.4 (183, 238).
[b]At 25°C, pH 7.4 (65).
[c]Products are not fully characterized, postulated on the basis of the chemistry of related thiol compounds.
[d]At 22°C, pH 7.4 (230).
GSH, glutathione; GSSG, oxidized glutathione; ND, not determined.

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by either remote lone pairs of electrons or by the neighboring sulfur atom when the orbitals are favorably oriented (117). These reactions yield thiosulfinates [RS(O)SR′] and eventually RSO_2H and RSO_3H with cleavage of the disulfide bond (Carroll *et al.*, unpublished; Karimi *et al.*, unpublished). HOCl can also induce the formation of sulfenamide (RSNR′), sulfinamide [RS(O)NR′], and sulfonamides [$RS(O)_2NR'$] (86), *via* nucleophilic attack of Lys or Arg side-chains on RS-Cl or sulfenic or RSO_2H intermediates. Glutathione sulfonamide, formed from GSH, is a sensitive marker for MPO-mediated reactions (86) due to the high rate constant for oxidation of GSH (238).

The high susceptibility of Cys residues to oxidation results in inactivation of enzymes (*e.g.*, creatine kinase and glyceraldehyde-3-phosphate dehydrogenase, GAPDH) that have Cys residues, and particularly those with low pK_a Cys residues in their active sites, with inactivation occurring in parallel with thiol depletion (194, 199). Conversely, HOCl can activate the proforms of matrix metalloproteinases (*e.g.*, MMP-7) *via* oxidation of the Cys residue in the ''cysteine switch'' domain of pro-MMP-7 (68).

Oxidation of Met side-chains by HOCl yields the sulfoxide (and the sulfone with large excesses) (90, 189). These species are readily formed, but are poor markers for MPO damage as they are also produced by other oxidants. Met oxidation can result in enzyme inactivation in some cases (*e.g.*, lysozyme and α_1-antitrypsin), but not others (*e.g.*, soybean trypsin inhibitor) (90).

Amines (and to a lesser extent amides and guanidines) are readily converted to *N*-chlorinated species (chloramines, RR′NCl). Further reaction with HOCl can result in the formation of dichloramines ($RNCl_2$), particularly with high excesses of oxidant (249). Chloramines are formed on reaction of free amino acids at the α-amino group, and on the amine or guanidine groups of Lys, His, and Arg [reviewed in Hawkins *et al.* (91)]. In proteins, where the α-amino group is part of an amide (peptide) bond, reaction is less rapid (183). Reaction can also occur with the indole nitrogen of Trp residues. With backbone amides and the amide side-chains of Gln and Asn, chloramides are formed with high HOCl excesses (183).

Amine groups on other biological molecules react similarly. The sulfonated β-amino acid, taurine, which is present in neutrophils and muscle cells at high concentrations, reacts rapidly with HOCl to give long-lived taurine chloramines [reviewed in Marcinkiewicz and Kontny (152)]. Chloramines are also formed rapidly at the amines present on nucleobases, nucleosides, nucleotides, RNA, and DNA (89), with reaction occurring at both the primary (exocylic) amines in cytosine, adenosine, and guanosine, and the secondary heterocyclic amines in thymidine, uridine, and guanosine (89). The amine head groups of amine-containing phospholipids (*e.g.*, phosphatidylethanolamine and phosphatidylserine) also react to give chloramines, but these are not formed to a significant extent at the quaternary amine of phosphatidylcholine (269).

HOCl reacts rapidly with aminosugars (*e.g.*, glucosamine) and more slowly with the corresponding amide or sulfonamide species (205). These reactions can occur with both low-molecular-mass species, and also sugars in large glycosaminoglycans (*e.g.*, hyaluronan, heparin, chondroitin-, heparan-, dermatan-, and keratin-sulfates) (205), and these species when present on glycoproteins and proteoglycans [*e.g.*, perlecan (118)].

Chloramines and chloramides retain the oxidizing capacity of the parent oxidant, and can induce further reactions although at slower rates (248, 249). Some of these reactions regenerate the parent amine (*e.g.*, *via* halogen transfer or

radical reactions), while others result in loss or modification of the amine and formation of carbonyl or nitrile functions (88, 90, 140, 192, 249). A major pathway for primary amines is loss of HCl to give an imine and subsequent hydrolysis of this species to give ammonium ions and a carbonyl [reviewed in Hawkins *et al.* (91)]. Hydrolysis of the chloramines formed on the imidazole ring of His is a potential pathway for the formation of 2-oxo-His (91) and related carbonyl compounds from nucleobases. Chloramines and chloramides can also decompose thermally, and in the presence of one-electron reductants (*e.g.*, Fe^{2+}, Cu^{+}, and $O_2^{-\bullet}$) to give nitrogen-centered (aminyl) radicals ($RNH^{\bullet}$) and Cl^{-}. Aminyl radicals can induce hydrogen atom abstraction from other bonds resulting in further oxidative damage (*e.g.*, Hawkins and Davies (87, 88)].

As chloramines and chloramides react more slowly and selectively than HOCl, these can induce damage at sites remote from their site of formation, and hence may be major mediators of damage (192, 193, 248). These species can oxidize Cys and Met residues on peptides, including GSH (192, 193). The reduced reactivity of these species results in some differences in the pattern of damage, with little evidence for the formation of glutathione sulfonamide (86). Low pK_a Cys residues on enzymes and protein are particularly susceptible to damage (192, 193), and this may play a role in the induction of cellular apoptosis (193, 248, 271) and the inactivation of specific intracellular enzymes (193, 248).

HOCl can undergo addition reactions with aromatic compounds and double bonds, including the side-chains of Tyr and Trp, nucleobases, and unsaturated fatty acid side-chains [reviewed in Hawkins *et al.* (91) and Pattison and Davies (185)]. Reaction with the Tyr phenolic side-chain yields Cl-Tyr, and 3,5-dichloro-tyrosine (diCl-Tyr) at high molar excesses. These long-lived products are widely used as highly characteristic, and specific, biomarkers of HOCl-mediated reactions [reviewed in Winterbourn (290)]. These may be formed *via* intermediate chloramines, as well as *via* direct reactions (90). Tyr residues are also oxidized to the dimer species *o-o′* dityrosine (di-Tyr) in low yield, probably *via* radical reactions (90). The indole side-chain of Trp can be converted to the 2-oxindole, possibly by addition of HOCl across the C2–C3 bond and subsequent hydrolysis (69, 91). Neighboring groups can modulate these reactions with cyclized products detected in proteins, when the group neighboring the Trp residue is a Gly or Ala (69).

With nucleobases, stable products featuring carbon/halogen bonds can be formed in addition to (unstable) chloramines. These include 5-chlorocytosine, 5-chloro(2′-deoxy)cytidine, 5-chlorouracil, 8-chloroadenine, 8-chloro(2′-deoxy)adenosine, and 8-chloro(2′-deoxy)guanosine [reviewed in Hawkins *et al.* (92)]. Some of these (*e.g.*, 5-chlorouracil, 5-chlorocytidine, and 8-chloroguanosine) have been detected in biological samples, and have been used as biomarkers of HOCl (11, 100, 172, 243). Hydroxylated and ring-opened nucleobase products can also be formed (158, 285).

Addition of HOCl across double bonds (*e.g.*, in unsaturated fatty acids and cholesterol) gives chlorohydrins [RCH=CHR′ + HOX → RCH(X)-CH(OH)R′] (10). These can undergo further reaction to yield epoxides. The formation of these species on phospholipids can modulate the structure of cell membranes and result in cell lysis (269, 270). Similar species are formed on lipoproteins (34). The rate constants for the generation of these species are, however, slow relative to other reactions of HOCl (186, 229). In contrast, plasmalogen lipids, which contain a vinyl ether linkage, react rapidly with HOCl (229) and this can result in cleavage of the ether linkage to give an α-halogenated aldehyde and a lysophospholipid (251). Elevated levels of chlorinated aldehydes have been detected in a number of biological samples (*e.g.*, human atherosclerotic lesions, lipoproteins, and cardiac tissue) and used as markers of HOCl-mediated damage (251). These α-halogenated aldehydes have significant biological effects, and may act as signaling molecules (251) and inhibitors of enzymatic reactions (156).

Rate constants have been determined for many of the reactions of HOCl. Much of the data have been obtained at pH 7–7.4, although some data have been reported at higher and lower pH values. These data indicate that HOCl, rather than its anion (^{-}OCl), is the more reactive species, and many reactions therefore occur more rapidly at lower pH values. Some reactions, however, show a complex pH dependence (*e.g.*, the imidazole ring of His) due to the presence of multiple ionizable groups on both the oxidant and target (183). The available kinetic data allow a ''pecking order'' to be determined for HOCl with different targets, and comparison with other oxidants (*e.g.*, HOBr or HOSCN) (183, 186). As proteins and GSH are the most abundant targets within cells (in terms of dry mass, and also concentration of reactive sites), these are likely to be the major targets of initial oxidation. It should, however, be noted that the extent of reaction at a particular site and its biological importance do not necessarily coincide, as cells can sustain significant damage to nonessential sites, whereas highly selective damage at a limited set of sites may be lethal.

There are much lower levels of many antioxidants and protective systems extracellularly (82), and this can result in different targets being important. Thus, the plasma concentration of GSH (low μM) is very different to that within most cells (typically 2–10 mM), and the number of Cys (and to a less extent Met) residues on extracellular proteins is low. However, even under these conditions, proteins still appear to be the major target (190), although the sites of damage may differ compared with that within cells, with disulfide bonds being more significant targets extracellularly (and also potentially in the endoplasmic reticulum) where they can be abundant, compared with the cell cytosol (117).

Reactions of HOBr and HOSCN

HOBr is a potent oxidant and brominating agent, and has similar reactivity to HOCl except with unsaturated residues [reviewed in Hawkins and Rayner (93) and Rayner *et al.* (202)]. Under normal physiological conditions, the formation of HOBr by MPO accounts for only a small minor proportion of the H_2O_2 consumed (165, 263), and therefore, a detailed discussion of HOBr chemistry is not provided here; Figure 3 summarizes some of the key data. In contrast, SCN^{-} is a favored substrate for MPO, resulting in the formation of this oxidant under normal physiological conditions (165, 263). HOSCN can also be formed by direct reaction of SCN^{-} with HOCl and HOBr (12, 168). The pK_a of HOSCN has been reported as 5.3 or 4.85 (169), with the latter more likely to be correct, so at pH 7.4 the major form is the (less reactive) conjugate base, $OSCN^{-}$. Although the nature of the oxidizing

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species has been debated, with various groups proposing the formation of thiocyanogen $(SCN)_2$, trithiocyanate $(SCN)_3^-$, cyanosulfurous acid (HO_2SCN), cyanosulfuric acid (HO_3SCN), and cyanide (CN^-), there is considerable evidence for the formation of HOSCN [reviewed in Barrett and Hawkins (16)]. Radical formation [$SCN^\bullet$, $OSCN^{-\bullet}$, and/or $(SCN)_2^{-\bullet}$] *via* the peroxidase cycle has also been proposed (143), although this is likely to be disfavored thermodynamically [reviewed in Barrett and Hawkins (16)].

Kinetic and product studies indicate that HOSCN is much less reactive and hence more selective than HOCl and HOBr, with thiols being the major targets for HOSCN (16, 187, 230). Kinetic data indicate that these reactions are $\sim 10^4$-fold slower than with HOCl (Table 3) (230, 238). The major initial products formed by HOSCN with thiols are believed to be RS-SCN species, with these reacting rapidly either with H_2O to give RSOH, or another thiol to give a disulfide (16, 17, 144). This targeting can result in selective damage to thiol-dependent enzymes [and particularly those with low pK_a thiols (8, 17, 232)], and GSH within cells (17, 144). For example, the selective nature of this oxidant results in more efficient (by 10- to 1000-fold) inactivation of membrane ATPases than with identical concentrations of HOCl (8). Exposure of endothelial cells to HOSCN can induce tissue factor activity (276) and promote adhesion molecule expression (275), possibly *via* site-specific oxidation of redox-sensitive thiols and NF-κB activation (178). If this is correct, multiple genes may be upregulated following cell exposure to HOSCN (275).

HOSCN also reacts rapidly with the selenol group (RSeH) of selenocysteine (Sec), with these reactions being 10- to 100-fold faster than for the sulfur analog, Cys (Table 3) (231). This can result in inactivation of key Sec-dependent enzymes such as thioredoxin reductase (TrxR) and glutathione peroxidase (231). Limited evidence has also been presented for slow reaction with aromatic residues (Tyr, His, and Trp), but these reactions are unlikely to be of major significance within cells due to the abundance of GSH and protein thiols (23).

Decay of HOSCN yields a complex mixture of species, including significant levels of cyanate, OCN^- (8). This species is also formed from the decomposition of urea (hence is of significance in subjects with uremia), and *via* reaction of CN^- with MPO (54). Cyanate undergoes adduction reactions with Cys, and the side-chain amine group of Lys, to give a carbamylated product [homocitrulline, $RN\text{-}C(O)NH_2$], which has been suggested as a surrogate biomarker for HOSCN *in vivo* (280). Homocitrulline formation has been detected in uremia-induced damage, and on dysfunctional lipoproteins [reviewed in Sirpal (227)]. There is limited evidence for the reaction of HOSCN or OCN^- with other targets, including amino sugars, phospholipid head groups, or nucleobases (16, 256), although $(SCN)_2$ may add across double bonds to give products analogous to chlorohydrins (81).

Reactions of other MPO-derived oxidants

$NO_2^\bullet$ is formed by the oxidation of nitrite, NO_2^-, by MPO in the presence of H_2O_2 (58, 264). $NO_2^\bullet$ is a key intermediate in MPO-mediated nitration of Tyr residues, probably *via* MPO-mediated tyrosyl radical formation and radical/radical termination with $NO_2^\bullet$ (264). $NO_2^\bullet$ has been implicated in peroxidase-mediated peroxidation of low-density lipoproteins (LDL) in the presence of NO_2^-, both *in vitro* (29) and in inflammation models (309). Nitryl chloride (NO_2Cl) has been postulated to be formed by activated leukocytes, possibly *via* indirect reaction of HOCl with NO_2^- (57). This reaction is slow [$k \sim 10^4\ M^{-1}\cdot s^{-1}$ (180)] compared with other HOCl reactions and may be of limited relevance in cells (287), although NO_2Cl can nitrate and chlorinate phenols, including Tyr, (58), and DNA bases (43), as well as generating Tyr dimers (58) *in vitro*. A role for NO_2Cl in lipid peroxidation has been discounted (29).

Singlet oxygen ($^1\Delta_g$, $^1O_2$), the first excited singlet state of molecular O_2, has been suggested to be formed by activated leukocytes, although the specificity of the probes used has been questioned [reviewed in Klebanoff (130)]. However, there is evidence that $^1O_2$ may arise from peroxidase reaction products, including *via* reaction of HOCl with H_2O_2 (115). $^1O_2$ formation by neutrophils undergoing phagocytosis, and activated macrophages, has also been reported using compounds that (specifically) trap $^1O_2$ (7). Reaction of HOCl with H_2O_2 is kinetically slow, compared with other reactions at physiological concentrations [reviewed in Tarr and Valenzeno (244)], and thus, it has been concluded that $^1O_2$ is unlikely to be formed in high yields in the neutrophil phagosome (291). HOCl can also react with lipid hydroperoxides to yield $^1O_2$ *via* peroxyl radical intermediates (162), although it has been suggested that this may not be important (179).

MPO can generate phenoxyl radicals from phenols in the presence of H_2O_2, in *in vitro* model systems, cells, and animal models (98). MPO can generate di-Tyr, *via* radical/radical termination of tyrosyl radicals, and free tyrosyl radicals have been reported to oxidize protein Tyr residues, thereby yielding di-Tyr crosslinks with proteins (99). Protein Tyr residues do not appear to be directly oxidized by MPO, presumably because of steric interactions (254). Tyrosyl radicals can react with ascorbate and unsaturated phospholipids, resulting in the oxidation of these species (98). MPO-derived tyrosyl radicals are potential initiators of LDL lipid peroxidation *in vitro* (219), but the relevance of this process *in vivo* has been questioned (309). Other phenols and related species can also be converted to radicals [*e.g.*, Kettle *et al.* (121, 123)]. Acetaminophen (paracetamol)-derived radicals can initiate LDL lipid peroxidation in the presence of MPO or neutrophils (116). Phenol itself is a good substrate for MPO, although the lipid oxidation observed in cells may be mediated by glutathionyl radicals ($GS^\bullet$) formed *via* hydrogen-abstraction from GSH by initial phenoxyl radicals (24). Urate is also a good substrate for MPO (221), with this resulting in the formation of urate radicals that subsequently yield urate hydroperoxides (182). $HO^\bullet$ may be generated indirectly *via* reaction of $O_2^{-\bullet}$ with HOCl (31), however, the physiological relevance of this process is questionable, due to the modest levels of $O_2^{-\bullet}$ *in vivo* and the reactivity of HOCl with other targets (291).

Cellular systems

The reactivity of HOCl with cells *in vitro*, including numerous types of mammalian cells (202), bacteria (85, 292), and yeast (33), has been studied extensively, and there is no doubt as to the damaging nature of this oxidant. HOBr and

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HOSCN also react with cells, although there are key differences in the cytotoxicity and selectivity when compared with HOCl (93, 187). With HOSCN, these differences have led to the proposal that *in vivo* SCN^- supplementation may have therapeutic value, by altering both the nature and extent of MPO-induced damage (41, 187).

Exposure of cells to HOCl results in cell death, in a cell type-, time-, and dose-dependent manner, *via* a range of different pathways [reviewed in Rayner *et al*. (202)]. HOCl can induce direct cell lysis by reacting with the cell membrane, as demonstrated with red blood cells, with HOCl-induced hemolysis attributed to the modification of membranes and cytoskeletal proteins rather than lipid oxidation (269). Leukocytes, including monocytes and macrophages, are also sensitive to HOCl-induced damage, with this characterized by mitochondrial dysfunction, release of proinflammatory signaling molecules, and necrotic cell death (142, 203, 297). With endothelial cells, HOCl treatment also leads to mitochondrial dysfunction and perturbation of signaling cascades, although in this case, there is evidence for both necrosis and apoptotic cell death (141, 273). HOCl-induced cytotoxicity with physiologically relevant oxidant doses has also been reported in lung and bronchial epithelial cells (77), chondrocytes (284), fibroblasts (102), and vascular smooth muscle cells (VSMC) (113).

The variation in the sensitivity of different cells, or between the same cell type from different donors, to HOCl, has been attributed, at least in part, to differences in the concentration of GSH, which is rapidly depleted by this oxidant (113, 142, 199, 272, 297). Cell exposure to HOCl also results in the rapid oxidation of thiol-containing proteins, which causes loss of enzymatic activity (194, 242). One example is GAPDH, an abundant, thiol-dependent enzyme, which is known to be targeted by HOCl and related oxidants in multiple cells [*e.g*., Lloyd *et al*. (141, 142), Pullar *et al*. (199), and Yang *et al*. (297)], with this leading to decreased glycolytic activity and reduced ATP production (144). Targeting of thiol proteins can perturb Ca^{2+} homeostasis by various pathways, including by modulation of the activity of sarco/endoplasmic reticulum (SR) Ca^{2+} ATPase (SERCA) (46, 61), L-type Ca^{2+} channels (83, 296), and the ryanodine receptor (RyR) (296).

The extent of the Ca^{2+} perturbations arising from HOCl exposure can influence the extent of cell survival, which may be related to mitochondrial dysfunction and changes in mitochondrial membrane potential ($\Delta\Psi_m$) and permeability (288). Altered Ca^{2+} fluxes within the cells also influence signaling pathways involving tyrosine phosphorylation (220). Phosphorylation cascades can be dysregulated by the direct reaction of HOCl with phosphotyrosine phosphatases, as these contain a highly reactive thiol in their active site (108). This can enhance the extent of phosphorylation of mitogen-activated protein kinases (MAPK), including extracellular signal-regulated kinase (ERK 1/2) and p38, which play a critical role in controlling cellular differentiation, growth, and survival pathways (135, 161, 286).

The nuclear factor erythroid 2-related factor 2 (Nrf2)/antioxidant response element (ARE) pathway is an important protective and adaptive response system that can be activated in cells by HOCl (195, 282, 293). This activation may replenish depleted intracellular GSH pools, as the expression of glutamate cysteine ligase catalytic subunit (GCLC), glutathione synthetase (GS), and glutathione reductase (GR) is regulated by Nrf2. Activation of Nrf2 also induces heme oxygenase 1 (HO-1) as well as enzymes involved in GSH metabolism, which have been reported to be upregulated in cells exposed to HOCl, with a resultant decreased cytotoxicity (195, 282).

HOCl therefore appears to induce cell dysfunction and death *via* multiple pathways, involving rapid loss of intracellular GSH, an accumulation in cytosolic Ca^{2+}, triggering of mitochondrial dysfunction by decreasing $\Delta\Psi_m$, enzyme inactivation, and a loss of ATP stores. This culminates in activation of cellular stress responses and cell death. The specific mechanism of cell death following HOCl exposure in different cell types can also influenced by the oxidant concentration, with high HOCl concentrations being typically associated with necrosis, and low concentrations with apoptosis, particularly in endothelial cells (141, 199, 240, 271, 273).

Apoptotic cell death induced by HOCl is characterized by increased binding of Annexin V, DNA fragmentation, and release of mitochondrial cytochrome *c* and other proapoptotic proteins. This has been attributed to the mitochondrial translocation of proapoptotic Bax (284). HOCl-induced apoptosis can also occur by caspase-independent pathways, as evidenced by a lack of efficacy of the pan-caspase inhibitor Z-VAD-fmk in preventing cell death. This may be related to the capacity of HOCl to oxidize the active site Cys of caspases, thereby inhibiting their protease activity (141, 142, 284).

Targets of MPO Oxidants *In Vivo* and Disease

Although there is compelling *in vitro* evidence that HOCl plays an important role in the induction of tissue damage and cell death (202), less is known regarding the mechanisms that may occur during MPO-mediated tissue damage *in vivo*. Typically, the involvement of MPO in pathologies has been inferred by the detection of elevated levels of the enzyme, or biomarkers of HOCl in the circulation, inflammatory fluids, urine, or diseased tissues (Table 1). These data, although important, do not readily allow the identification of the molecular targets or specific cellular pathways susceptible to MPO and its oxidants. In the sections below, we focus primarily on the role of MPO in cardiovascular, neurological, and respiratory diseases, where there is significant interest in therapeutic modulation of MPO activity. Data on other inflammatory pathologies are available in the references cited in Table 1.

Vascular endothelium

The vascular endothelium is a primary target for MPO, HOCl, and biomolecules modified by HOCl such as LDL, high-density lipoprotein (HDL), or chlorinated lipids (150, 156, 171, 247). In atherosclerosis, which is characterized by endothelial dysfunction, there is significant evidence for elevated levels of enzymatically active MPO, and multiple markers of MPO-induced damage in diseased tissue [reviewed in Nicholls and Hazen (171) and Teng *et al*. (247)]. The observation that the extent of staining by an antibody raised against HOCl-modified proteins correlates with disease severity [as assessed by intimal thickening in human lesions (94, 95)], together with the observation that MPO is

present at particularly high levels in the rupture-prone, shoulder regions of lesions (49), strongly supports a role of HOCl in the development of atherosclerosis.

MPO-derived oxidants can readily induce endothelial dysfunction, as shown by the exposure of arterial rings *ex vivo* to HOCl with this resulting in impaired endothelium-dependent relaxation (237, 303). This results from a reduction in the bioavailability of $NO^{\bullet}$, as a result of alterations in the expression, localization, activity, or substrate availability for endothelial nitric oxide synthase (eNOS) (237, 303). Similar observations have been reported in experiments with HOCl-modified LDL (1, 174). MPO can also induce endothelial dysfunction *via* HOCl-independent pathways, including by directly reducing $NO^{\bullet}$ bioavailability (56, 206), or mediating damage to extracellular matrix proteins following its transmigration through the endothelium (13). In addition, MPO can induce the collapse of the endothelial glycocalyx, which promotes the recruitment and activation of neutrophils (151). Taken together, these data corroborate and support clinical data showing that serum MPO levels can independently predict endothelial dysfunction in humans [*e.g.*, Vita *et al*. (274)].

In addition to effects on $NO^{\bullet}$ bioavailability, MPO can influence endothelial function by activating intracellular signaling cascades, which alter vascular function and promote inflammation. For example, MPO is believed to contribute to the development of pulmonary arterial hypertension by its ability to activate the Rho-kinase signaling pathway, which impairs vasorelaxation to increase right ventricular pressure (131). Similarly, activation of calpain by MPO increases inflammation by upregulating the expression of vascular cell adhesion molecules (VCAM), thereby promoting leukocyte adhesion to the endothelium (60). Calpain also plays a role in $NO^{\bullet}$ regulation, which may be an additional pathway by which MPO can induce endothelial dysfunction (60).

The presence of MPO in the subendothelium has been implicated in plaque destabilization and thrombogenesis (200, 247) as HOCl promotes endothelial cell apoptosis (141, 273) and the release of tissue factor at sublethal oxidant doses (240). HOCl and MPO-H_2O_2-Cl^- directly damage extracellular matrix components such as fibronectin, laminins, perlecan, and basement membrane materials, both *in vitro* and *in vivo*, with this having adverse effects on associated endothelial and smooth muscle cell function, viability, and gene expression (175, 208). This direct oxidation may act in concert, or synergistically, with MPO-mediated activation of MMPs, as these species can destabilize plaques by their enzymatic activity (106). This may be further exacerbated by HOCl-mediated inactivation of the tissue inhibitors of metalloproteinases (TIMPs) (279). These *in vitro* studies support clinical observations on the colocalization of HOCl-modified proteins with macrophage MPO expression in lesions from patients who experienced sudden cardiac death (241), and help rationalize the prognostic value of MPO as a predictor of greater risk of myocardial infarction in patients with acute coronary syndrome (14, 26).

Lipoproteins

Modification of LDL and HDL by MPO and its oxidants can promote the proatherogenic properties of LDL, while compromising the cardioprotective effects of HDL, and thereby contribute to the development of atherosclerosis [reviewed in (150), Nicholls and Hazen (171), and Teng *et al*. (247)]. Evidence to support MPO-induced LDL modification comes from detection of increased levels of Cl-Tyr on LDL isolated from human lesions (97), with more recent data showing LDL-MPO complexes in the plasma of patients with atherosclerosis, particularly those with high levels of MPO in their circulation (233). In addition, HOCl-modified LDL is present in the plasma of patients undergoing hemodialysis (52).

HOCl-modified LDL can initiate a cascade of proinflammatory and atherogenic events (150). HOCl-modified LDL is recognized by macrophage scavenger receptors, including the class B scavenger receptors CD36 and SR-B1 (157), resulting in the accumulation of cholesterol and cholesteryl esters and foam cell formation (96). Exposure of macrophages to HOCl-LDL activates stress-related signaling responses, including Nrf2, to increase the expression of antioxidant genes (30) or promoting cell death by apoptosis (59). HOCl-modified LDL can also promote damage to endothelial cells by stimulating increased macrophage adhesion (134) and reducing the formation of $NO^{\bullet}$ by eNOS delocalization (174) and uncoupling (1). Recent studies also provide evidence for a potential role for MPO-modified LDL in the resolution of inflammation in endothelial cells, *via* the induction of resolvin D1 (55).

Dysfunctional HDL extensively oxidized by MPO and HOCl is present in human atheroma and the circulation of patients with cardiovascular disease (18, 105, 191, 310). There is evidence indicating that MPO binds to apolipoprotein A1 (apoA1) in plasma, with this promoting HDL dysfunction (310). Modification of apoA1 both impairs the cholesterol acceptor function of HDL (18, 310) and promotes proinflammatory activity of endothelial cells (105, 260). These effects are also apparent on infusion of apoA1 pretreated with MPO-H_2O_2-Cl^- into atherosclerosis-prone mice (101). In this case, in contrast to observations with native apoA1, apoA1 oxidized by MPO failed to decrease macrophage number in the lesions or promote a noninflammatory macrophage phenotype (101). Finally, HOCl-induced modification of HDL inhibits the migration and proliferation of VSMC (311). This suggests that MPO-dependent HDL modifications can potentially promote inflammation, lesion development, and plaque instability in atherosclerosis.

Brain and central nervous system

MPO-induced endothelial dysfunction is also important in other pathologies, particularly neurological diseases, as it can contribute to the disruption and altered permeability of the blood/brain barrier (259). There is evidence for increased expression of MPO in the brains of patients with Alzheimer's disease (AD) (80, 212), Parkinson's disease (PD) (44), and multiple sclerosis (MS) (167). Recent studies demonstrate that the MPO expression is elevated in brain regions affected by neurodegeneration in both AD and PD (76). This supports previous data showing colocalization of MPO with β-amyloid peptide in senile plaques (212), and elevated levels of enzymatically active MPO and Cl-Tyr in the brain tissue of patients with AD (80). Similarly, Cl-Tyr and HOCl-modified proteins are elevated in the brains of mice treated with

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the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) to induce loss of dopaminergic neurons, as a model of PD (44).

The links between MPO and neurodegeneration in AD and PD are not well understood, but this may be related to the activation of microglia or astrocytes, which can produce MPO [*e.g.*, Maki *et al.* (148)]. In AD, neurodegeneration and memory defects have been directly linked to the aberrant expression of MPO by astrocytes, and correlate with phospholipid oxidation, in humans and *in vivo* AD models (148). Whether phospholipid oxidation is causal in the disease, or merely indicative of an elevated oxidative stress as a result of increased MPO expression, is not clear. However, *in vitro* studies indicate that neuronal cells are highly susceptible to HOCl, with exposure resulting in apoptotic or necrotic cell death, depending on the concentration and exposure conditions (298, 299). These cells also release proinflammatory cytokines, including TNFα and IL-1β on HOCl exposure, thereby exacerbating neuroinflammation (137). In addition, HOCl may contribute indirectly to the development of PD by chlorinating the amine and catechol groups of dopamine to form chlorodopamines, which can selectively kill dopaminergic neurons by inhibiting mitochondrial respiration (112).

MPO has also been implicated in MS, a degenerative central nervous system disease characterized by the demyelination and transection of neuron axons, with this causing lesions (scleroses) throughout the brain and spinal cord (167). MPO has been detected in MS lesion macrophages and microglia (78), with HOCl production postulated to accelerate damage to the myelin sheath, neuronal damage, and ultimately neurodegeneration (67). Similar observations are reported in experimental autoimmune encephalomyelitis (EAE), a well-characterized *in vivo* model of MS [reviewed in Pravalika *et al.* (196) and Ray and Katyal (201)].

Lungs/airway

MPO and HOCl overproduction is strongly associated with several respiratory pathologies, including cystic fibrosis (CF) and chronic obstructive pulmonary disorder (Table 1). Peroxidase-mediated reactions are also important in asthma, although eosinophil peroxidase (EPO) and increased production of HOBr may be the dominating process in mediating lung injury (5). In CF, MPO activity in circulating neutrophils correlates with both airway obstruction and sputum production, consistent with MPO-derived oxidants contributing to airway injury (74). Moreover, it has been demonstrated that sputum and bronchoalveolar fluid from patients with CF contain active MPO and various markers of HOCl-induced damage, including both chlorinated and brominated proteins (250).

GSH is depleted in the airways of children with CF, and associated with increased levels of GSH sulfonamide, a specific oxidation product of HOCl, and GSSG (oxidized glutathione) (124). These *in vivo* data are consistent with results from an *ex vivo* model of rabbit lung perfusion, where infusion of low concentrations of HOCl (1 μM) resulted in a marked depletion of GSH and other low-molecular-mass thiols (84). This oxidation was associated with increased vascular permeability, pulmonary artery pressure, and lung fluid retention (84). This corroborates *in vivo* studies demonstrating increased airway epithelial permeability on exposure to MPO-derived oxidants, and helps to rationalize the leakage of plasma proteins observed in the airway lumen of CF patients (209). As thiols are established targets of MPO-derived oxidants, there is interest in the development of thiol-based therapeutics for use in CF (77, 268).

MPO-derived oxidants are also indirectly implicated in exacerbating inflammation and lung damage in CF, as HOCl targets the neutrophil-derived, protective protein calprotectin, in the airways of CF patients, which facilitates bacterial growth by compromising the chelation of essential metal ions (147). Similarly, HOCl can promote airway damage and remodeling by increasing protease activity, by inactivating protease inhibitors such as α_1-antiproteinase, while activating latent collagenase and the metalloproteinase gelatinase (19). Finally, the systemic oxidative stress and endothelial dysfunction associated with increased MPO activity in patients with CF suggest that these individuals may also be at greater risk of developing cardiovascular pathologies [reviewed in Reverri *et al.* (211)].

Modulation of MPO-Induced Damage

The strong evidence for a role of MPO in inflammatory pathologies has led to significant interest in the development of inhibitors and approaches to modulate MPO activity *in vivo* as a therapeutic strategy. This has resulted in the development of several highly potent, irreversible inhibitors [reviewed in Lazarevic-Pasti *et al.* (136) and Soubhye *et al.* (234)]. Other approaches to modulate MPO-induced damage involve limiting substrate availability, promoting H_2O_2 removal, or supplementation with alterative MPO substrates [*e.g.*, SCN^- which has shown efficacy in *in vivo* CF models (41, 42)]. We provide below a brief overview of these strategies; for further details, and information on specific diseases, the reader is referred to the referenced reviews.

Limiting MPO substrate availability

As Cl^- is highly abundant *in vivo* and therefore difficult to modulate, H_2O_2 availability is a key factor in determining the extent of MPO-derived oxidant formation. H_2O_2 is efficiently removed by enzymes, including peroxiredoxins, catalase, and glutathione peroxidases, in competition with MPO. H_2O_2 levels can also be limited by inhibition of the NADPH oxidase complexes that generate $O_2^{-\bullet}$, and hence, H_2O_2 *via* dismutation (304). In the vasculature, it has been reported that $NO^\bullet$ can suppress NADPH oxidase activity in endothelial cells by promoting the *S*-nitrosylation of the key p47phox subunit of the enzyme, thereby limiting H_2O_2 levels (222).

MPO can also be diverted from HOCl production by supplementation with alternative substrates, such as NO_2^- or SCN^- (263, 264). Oxidation of these anions yields alternative, potentially less damaging oxidants, and they can also react directly with HOCl (see Substrate specificity: halogenation cycle section). Supplementation with NO_2^- has been proposed as a therapeutic strategy in neurodegenerative diseases, based on its ability to inhibit MPO-induced oxidative damage in neuronal cells *in vitro* (145). However, in other cell types, inhibition of MPO by NO_2^- promoted neutrophil-induced DNA strand breakage (132). Addition of SCN^- to cell cultures exposed to MPO-H_2O_2-Cl^- or HOCl *in vitro* reduces cellular damage and death (41, 294), although it is known that high (bolus) concentrations of

HOSCN can be damaging and toxic [reviewed in Pattison *et al*. (187) and Rayner *et al*. (202)]. Supplementation with SCN^- has been reported to be protective in inflammatory disease models *in vivo* [reviewed in Chandler and Day (41) and Day (51)], and high SCN^- levels have been reported to have a marked anti-inflammatory effect and decrease bacterial loads in a murine CF model; these positive effects were associated with a restoration of GSH levels in the lungs (42). This may arise from upregulation of TrxR within the lung tissue, which can detoxify HOSCN, and hence preserve GSH (42). Similarly, supplementing atherosclerosis-prone mice, which overexpress human MPO, with SCN^- resulted in a marked decrease in plaque area (164). These protective effects have been detected at SCN^- levels that can be readily achieved and tolerated in humans (164), but more work is needed to assess the mechanism(s) involved, and particularly whether this arises from an alteration in HOCl levels in these animals.

Nonselective MPO inhibition

MPO inhibition is an alternative approach, and a large number of compounds that prevent MPO-induced damage in a nonselective manner have been reported. Some of these lack specificity for MPO, including general heme poisons such as azide and CN^-, and hence are unlikely to be of therapeutic relevance. Other inhibitors include various drugs, chelators, antioxidants, scavengers, and natural products, including various flavonoids/polyphenols. There are many compounds that can act as peroxidase substrates and divert the catalytic activity of MPO from its halogenation cycles. One approach is to use substrates that react readily with Compound I, but not Compound II, thereby resulting in a blocking of the enzymatic cycle, and less halide oxidation. This approach may have limited efficacy *in vivo*, as high concentrations would be required to compete with halide oxidation. Physiologically relevant concentrations of acetaminophen (paracetamol; $<130\,\mu M$) inhibit oxidant production by MPO-H_2O_2-Cl^- (133), although acetaminophen has also been reported to enhance HOCl production by recycling Compound II and Compound III (153). Similarly, a major dietary flavonoid, quercetin, can attenuate MPO-induced, endothelial dysfunction both *in vitro* and *in vivo* (146, 224), possibly by inhibiting MPO activity by acting as a cosubstrate (225). However, quercetin can also act by alternative pathways, such as by activation of Nrf2 and upregulation of HO-1 (224), attributed to quercetin oxidation products (47). Other *in vitro* studies have shown that the green tea polyphenol, epigallocatechin gallate (EGCG), can preserve endothelial function (252), with this reported to occur *via* binding of EGCG to the heme group of MPO and an accumulation of Compound II (252).

Cyclic nitroxides (*e.g*., TEMPO and derivatives) can also act as peroxidase substrates and react with Compound I to inhibit HOCl formation, as these species react slowly with Compound II (114, 204). This inhibition (as above) might be mitigated by reductants, such as $O_2^{\bullet-}$, that can recycle Compound II by reducing this to the ferric form (see above), but nitroxides can also act as SOD mimetics, suggesting efficacy as MPO inhibitors even in the presence of $O_2^{\bullet-}$ and/or absence of SOD. This is supported by studies showing nitroxide-dependent inhibition of HOCl production in an *in vitro* cardiomyocyte model of myocardial inflammation (39) and *ex vivo* in human plasma (114).

The peptide *N*-acetyl lysyltyrosylcysteine amide (KYC) is postulated to be a selective, reversible MPO inhibitor, which binds to the active site and reacts with Compounds I and II to form a tyrosyl radical that is efficiently scavenged by the adjacent Cys forming the KYC disulfide (305). This inhibitor has low toxicity, and is able to prevent HOCl formation and associated damage both *in vitro* (305) and in various *in vivo* disease models (218, 300, 306, 307). KYC reduced brain damage in murine models of stroke (300), and decreased the disease score in murine EAE models, which was associated with a decreased extent of protein oxidation and a restoration of the blood/brain barrier (306). Importantly, the protective effect of KYC was not seen in an analogous EAE model in MPO knockout mice, which supports the action of KYC being specific, and attributable to its MPO inhibitory capacity (306). KYC was also shown to improve vasodilatation in sickle cell disease mice (307) and inhibit tumor formation in a murine lung cancer model (218).

Other examples of nonselective MPO inhibitors include the copper-containing plasma protein ceruloplasmin, which binds strongly to MPO and inhibits its activity (233), and heparin, a polyanionic glycosaminoglycan widely used as anticoagulant, that binds (cationic) MPO electrostatically (48). The MPO binding capacity of heparin results in its release from blood vessels (15), a property that is reported to reflect plaque burden in patients with stable coronary artery disease (215).

Finally, a number of compounds have been identified that may modulate MPO-induced damage by acting as competitive targets for HOCl. This strategy is only likely to be successful when high concentrations of these ''scavengers'' can be achieved. One potential species is taurine, which is present in high concentrations within neutrophils (152), with supplementation with taurine shown to decrease macrophage infiltration, elastin fragmentation, and MMP activation associated with the overexpression of MPO in an *in vivo* model of abdominal aortic aneurysms (128). Similarly, some selenium-containing compounds can react rapidly with MPO-derived oxidants (36), with the resulting selenoxides able to be catalytically recycled by cellular reductants and antioxidant enzymes (37). Although there are positive associations with selenium supplementation and beneficial disease outcomes, further studies are required to specifically assess whether this is related to a reduction in MPO-induced damage (35).

Selective, irreversible MPO inhibitors

''Suicide'' substrates are likely to be the most specific and effective MPO inhibitors. The development and utilization of mechanism-based MPO inhibitors as therapeutic agents have been reviewed recently (136, 234). Briefly, hydrazines and hydrazides ($RNHNH_2$ and $RCONHNH_2$, respectively) inhibit MPO *via* irreversible heme destruction (126). Thus, 4-aminobenzoic acid hydrazide (ABAH) (122) induces heme loss *via* generation of ferrous MPO and subsequent reactions of this species (28). This compound has been used extensively *in vitro*, and to a lesser extent *in vivo* due to its toxicity, to examine the role of MPO in inducing cell dysfunction. However, ABAH supplementation can decrease inflammation and vascular oxidative stress in atherosclerosis-prone mice, improve endothelial function, and significantly reduce lesion development and neointima formation (255). ABAH

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has also shown to reduce infarct size and neuronal deficit in a murine model of stroke (66), and reduce disease severity in murine EAE, as a model of MS (67).

Thioxanthines are a class of compounds that have attracted significant clinical attention. These can covalently (and irreversibly) modify the heme groups of MPO and effectively inhibit HOCl production (253). These compounds have high potency and react rapidly with Compounds I and II of MPO, in a two-step mechanism (281). Studies in a murine model of peritoneal inflammation have shown that 2-thioxanthine is an effective inhibitor and decreases HOCl production *in vivo* (253). More recently, AZM198, a thioxanthine derivative developed by AstraZeneca, inhibits MPO activity in a tandem stenosis model of atherosclerotic plaque instability in apolipoprotein E knockout ($ApoE^{-/-}$) mice (200). This results in an increase in the fibrous cap thickness in unstable plaques and highlights the potential utility of MPO inhibition as a clinical strategy to improve outcomes in high-risk coronary artery disease patients (200). AZM198 may also have therapeutic value in the prevention of MPO-induced adverse pulmonary vascular function, by mitigating the MPO-dependent activation of Rho-kinase that is associated with increased pulmonary arterial hypertension (131).

Conclusions and Future Perspectives

There is now considerable evidence that strongly supports a role for MPO in the development of many inflammatory pathologies, which can result from effects mediated directly by MPO or from the excessive generation of MPO-derived oxidants, and particularly HOCl. However, there are still significant gaps in our knowledge as to the mechanisms by which MPO appears extracellularly in inflamed tissues. Multiple pathways have been identified, including release from phagolysosomes, *via* the formation of neutrophil and other leukocyte extracellular traps (''NETosis'' and related processes), or dysfunctional intracellular trafficking. Understanding these pathways is important as they may be amenable to therapeutic manipulation. Furthermore, there is still controversy as to precisely which cells (and phenotypes) release MPO. In particular, the role of tissue ''macrophage-like'' cells is disputed and is worthy of further study (159, 214, 241). This is of particular relevance in the light of data from novel imaging modalities (such as bis-5HT-DTPA-Gd molecular magnetic resonance imaging [MPO-Gd MRI]) used to detect MPO *in vivo*, which have indicated that distinct macrophage subtypes may be important (214). Understanding the nature and role of different phenotypes, and subsequent manipulation of these, may provide routes to modulating tissue damage.

Further study also appears warranted with regard to the pattern and concentrations of oxidants formed by MPO under both physiological and pathological conditions, and how this is affected by the levels of different anions (halides or pseudohalide oxidized by the halogenation cycle) and oxidizable substrates (species that react with Compounds I and II in the peroxidase cycle). The role of MPO intermediates in consuming the critical biological signaling molecule $NO^{\bullet}$, and generating nitrating species ($NO_2^{\bullet}$ from NO_2^-), is also of considerable interest and relevance, particularly in the light of the many reports of the presence of nitrated biomolecules at sites of inflammation (63).

The growing body of evidence for a role for MPO in multiple human pathologies (Table 1) has also sparked considerable interest in the development and use of inhibitors of MPO. However, whether therapeutic interventions that inhibit oxidant production, or alter the nature of oxidants generated, can improve disease outcomes and survival in humans is yet to be firmly established. Promising results have been obtained in a number of *in vivo* disease models, including thioxanthines (AZM198) in cardiovascular disease, which influence the thickness of the fibrous cap and reduce intraplaque hemorrhage, which may be relevant for high-risk patient cohorts (200). Similarly, the tripeptide KYC has demonstrated efficacy reducing disease severity in animal models of stroke (300), EAE (306), sickle cell disease (307), and lung cancer (218).

However, the impact of long-term inhibitor use on immune function is of concern, and needs to be assessed. The thioxanthines have been shown to slow, but not prevent, the killing of *Staphylococcus aureus* by neutrophils (253); studies with other MPO inhibitors (such as the KYC peptide), and alternative bacterial strains or targets (*e.g.*, yeasts), have not appeared in the literature. These data suggest that either residual MPO activity is sufficient for bactericidal activity or that other mechanisms can compensate for the inhibition of MPO. Thioxanthines inhibit thyroid peroxidase and lactoperoxidase to a much lesser extent than MPO as judged by *in vitro* assays [*e.g.*, 10% inhibition of thyroid peroxidase as concentrations that completely inhibit MPO (253)], and only marginally affected the formation of thyroid hormones *in vivo* (253), suggesting that ''off-target'' activity may not be a major limiting factor.

There may also be value in supplementation with anions, such as SCN^-, which can act as alternative substrates for MPO, as the production of less potent, and slower reacting, oxidants such as HOSCN may reduce tissue damage while maintaining a capacity to control infection (41, 51). However, further work is clearly needed to assess the efficacy and consequences of such a strategy as this only reduces the extent of damage, with HOSCN (at least *in vitro*) being toxic to some cell types at high levels (93, 202).

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Abbreviations Used

$\Delta\Psi_m$ = mitochondrial membrane potential
$^1O_2$ = molecular oxygen in its $^1\Delta_g$ excited singlet state
ABAH = 4-aminobenzoic acid hydrazide
AD = Alzheimer's disease
apoA1 = apolipoprotein A1
Br^- = bromide ion
CF = cystic fibrosis
Cl^- = chloride ion
Cl-Tyr = 3-chloro-tyrosine
CN^- = cyanide
di-Tyr = *o-o′* dityrosine
EAE = experimental autoimmune encephalomyelitis
EGCG = epigallocatechin gallate
eNOS = endothelial nitric oxide synthase
$Fe^{IV}=O$ = oxy-ferryl species
GAPDH = glyceraldehyde-3-phosphate dehydrogenase
GSH = glutathione
H_2O_2 = hydrogen peroxide
HDL = high-density lipoprotein
HO-1 = heme oxygenase 1
HOBr = hypobromous acid
HOCl = hypochlorous acid
HOI = hypoiodous acid
HOSCN = hypothiocyanous acid
KYC = *N*-acetyl lysyltyrosylcysteine amide
LDL = low-density lipoprotein
MMP = matrix metalloproteinase
MPO = myeloperoxidase
MS = multiple sclerosis
NETs = neutrophil extracellular traps
$NO^\bullet$ = nitric oxide radical
NO_2^- = nitrite ion
NO_2Cl = nitryl chloride
Nrf2 = nuclear factor erythroid 2-related factor 2
$O_2^{\bullet-}$ = superoxide radical anion
OCN^- = cyanate
ONOOH = peroxynitrous acid
PD = Parkinson's disease
RS-Cl = sulfenyl chloride
RSOH = sulfenic acid
RSO_2H = sulfinic acid
RSO_3H = sulfonic acid
SCN^- = thiocyanate ions
$(SCN)_2$ = thiocyanogen
Sec = selenocysteine
SOD = superoxide dismutase
SR = sarco/endoplasmic reticulum
TrxR = thioredoxin reductase
VSMC = vascular smooth muscle cells

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