The many roles of myeloperoxidase: From inflammation and immunity to biomarkers, drug metabolism and drug discovery

Arno G. Siraki

Faculty of Pharmacy & Pharmaceutical Sciences, University of Alberta, Edmonton, AB, Canada

ARTICLE INFO

Keywords:
Myeloperoxidase
Neutrophil extracellular traps
Biomarkers
Drug metabolism
Drug development

ABSTRACT

This review provides a practical guide to myeloperoxidase (MPO) and presents to the reader the diversity of its presence in biology. The review provides a historical background, from peroxidase activity to the discovery of MPO, to its role in disease and drug development. MPO is discussed in terms of its necessity, as specific individuals lack MPO expression. An underlying theme presented throughout brings up the question of the benefit and burden of MPO activity. Enzyme structure is discussed, including accurate masses and glycosylation sites. The catalytic cycle of MPO and its corresponding pathways are presented, with a discussion of the importance of the redox couples of the different states of MPO. Cell lines expressing MPO are discussed and practically summarized for the reader, and locations of MPO (primary and secondary) are provided. Useful methods of MPO detection are discussed, and how these can be used for studying disease processes are implied through the presentation of MPO as a biomarker. The presence of MPO in neutrophil extracellular traps is presented, and the activators of the former are provided. Lastly, the transition from drug metabolism to a target for drug development is where the review concludes.

1. Myeloperoxidase – a historical timeline to the present

The initial identification of myeloperoxidase (MPO) occurred by empirical observations of a chemical reaction. The following observations have been summarized from the original article [4,5]. What led up to the discovery of MPO include the observation of a colour change when guaiac tincture (a mixture of guaiacum and alcohols) changed colour in the presence of pus (Klebs, E. 1868; Struve, H., 1872). Others later found that such colour changes occurred in leucocytes and bone marrow (Brandenburg, K., 1900; Meyer, E., 1903). Furthermore, the peroxidase nature of MPO was reflected by the requirement of $\text{H}_2\text{O}_2$ for the oxidation of Nadi reagent (a mixture of N,N-dimethyl-p-phenylenediamine and 1-naphthol, identified by Linossier, G., 1898). Lastly, catalase, which degraded $\text{H}_2\text{O}_2$, also prevented the formation of indophenol blue (Agner, K. 1941). Its green color and peroxidase activity earned this enzyme the name of verdoperoxidase, and its discovery was assigned to Kjell Agner in 1941, from which the above summary and citations were acquired [4]. Nauseef has also provided an excellent fundamental studies of Klebanoff [11,12].

The name myeloperoxidase (MPO) was suggested by Theorell (1943) and was used later in a Methods in Enzymology article dedicated to MPO [8]. The reason for this was to associate the source organ/tissue name with the protein rather than a physical feature. It was initially abbreviated as MyPO (to not confuse it with milk peroxidase, which turns out to be lactoperoxidase). However, in this review, the conventional MPO abbreviation will be adhered to. Soon after its discovery, MPO was studied to “ascertain whether it has a special function in combating infectious diseases” by Agner, where it was observed that the enzyme disappeared from leukocytes during severe infection; mice survived treatment of tetanus toxin only if the toxin was first oxidized with MPO [9]. The idea of such peroxidase activity being bactericidal was also shown earlier by utilizing phenolic oxidation by MPO for bacterial killing [10]. The functional role of MPO in neutrophil-mediated destruction of bacteria has been ascribed to the fundamental studies of Klebanoff [11,12].

1.1. Enzymes related to MPO

MPO belongs to the peroxidase family of enzymes. As this review is focused solely on MPO, only a brief summary of other peroxidases will...
be discussed.

Thyroid peroxidase has a nucleotide sequence similarity of 58% compared to the functional protein sequence of MPO. The sequence similarity near the haem active site between thyroid peroxidase and MPO is 74%. However, the entire amino acid sequence bears 44% homology with MPO, indicating that these proteins are of the same gene family [13]. Thyroid peroxidase is localized to the thyroid gland and is only involved in thyroid hormone synthesis.

Eosinophil peroxidase is more closely related to MPO in structure and function, even though it is a monomer. This enzyme is located in eosinophils and is considered mainly responsible for generating hypobromous acid to assist in microbial killing. Eosinophil peroxidase shares approximately 70% amino acid sequence homology with MPO [14].

Lactoperoxidase, unlike MPO, has a single chain of 80 kDa containing a single haem. It shares approximately 51% amino acid sequence homology with MPO [15]. Lactoperoxidase is found in milk, tears, saliva, and mucus of the respiratory tract [16].

The most recently discovered enzyme in this family is the somewhat esoteric vascular peroxidase 1 (VPO1) [7]; it is relatively challenging to acquire or isolate. Studies have shown that it is upregulated and is activated in chronic cardiac fibrosis more so than MPO. Fibrosis in a myocardial infarction model was attenuated when VPO1 siRNA was used [17]. A timeline of important discoveries involving MPO is shown in Fig. 1.

2. MPO: it’s in (most of) our genes

MPO was cloned from human acute myelogenous leukemia cells, revealing that a single gene was responsible for its encoding [45]. MPO was determined to be located on chromosome 17 [46,47]. Studies by Morishita et al. reported that the human MPO gene was composed of 12 exons and 11 introns and located at band 17q23.1. In addition, a single mRNA transcription initiation site was identified, which shared homology with sequences found on the S’-promoter region for the proto-oncogene, c-myc. During differentiation of mouse or human promyelocytic leukemia cells to granulocytes or monocytes, it has been observed that mRNA of MPO and c-myc are both decreased in response to granulocyte-colony stimulating factor [48,49]. Interestingly, other related peroxidase enzymes, including eosinophil peroxidase, lactoperoxidase, and thyroid peroxidase, appear to cluster around the same region, where eosinophil peroxidase is localized 34 kb from the MPO and lactoperoxidase genes [50].

MPO deficiency was first reported about 50 years ago and was highlighted by a patient with a candidiasis infection [51]. This patient appeared to be only deficient in neutrophil MPO, as eosinophil peroxidase (a similar enzyme found in eosinophils) was present. Interestingly, the MPO deficient neutrophils functioned normally otherwise (e.g., phagocytosis was normal) and were able to slowly but eventually kill S. aureus. This observation suggests that the respiratory burst is more significant in bactericidal activity, but fungal infections require additional support by MPO activity. Early studies have shown that both neutrophils and monocytes lacking MPO have a longer respiratory burst rate, extent and duration [52].

The incidence of MPO deficiency in the general population is 1 in 2000 to 4000 in the United States and Europe [53–55]. The complete deficiency of MPO is characterized by the absence of MPO-related peptides in neutrophils; the partial absence is due to less MPO activity, for a lower overall amount of the protein is visible to the naked eye in neutrophil pellets (Fig. 2) [1,56]. Interestingly, MPO deficiency in Japan appears to be far rarer: approximately 1 in 60 000 for complete deficiency and 1 in 20 000 for partial deficiency [57]. Certain dogs have been noted to have MPO deficiency [58]. The deficiency in MPO is also
3. Do we need MPO?

Despite the apparent importance of MPO in microbial killing, it seems that about 50% of MPO-deficient individuals remain asymptomatic and do not have remarkable health concerns (other than those described above). The MPO deficiency may become more critical if the individual is afflicted with other disease states or mutations. On the other hand, secondary MPO deficiency can result from various disease states or xenobiotic exposure [65]. Indeed, there may be benefits to lower MPO activity. An Alzheimer’s disease mouse model (5XFAD) demonstrated improved cognitive, memory, and proinflammatory activity after producing bone marrow deficient MPO [66]. MPO can oxidize high-density lipoprotein, which can lead to its dysfunction and atherosclerotic plaques [67]. However, a study of mice infected with Salmonella proposed that MPO actually mitigated collateral (host) damage in this model [68].

4. Enzyme structure

The MPO protein is a tetramer, with a total estimated molecular weight of 150 000 kDa. The tetramer is composed of two halves (hemi-MPO), composed of two heavy (~60 kDa) and two light (~15 kDa) components [69]. Previously, a range of molecular weights was reported at 120 000–160 000 kDa [48]. More accurate masses are described later (see below). Human MPO is reported to have 467 amino acids in the large chain and 105 amino acids in its short chain.

It is unique in the peroxidase enzyme family, where MPO has three covalently linked amino acid linkages from the protein chain to the protoporphyrin IX (haem) active site (Fig. 3) [2,70]. The fifth coordination position of the iron is occupied by the imidazole group of histidine, similar to other peroxidases [71]. The three amino acid bonds to the haem have been associated with its green colour, as MPO was originally called verdoperoxidase (Agner, 1940). A curious observation of a protein referred to as “spleen green” protein was identical to MPO [69]. Site-directed mutation studies of MPO revealed that the Met243 → Gln mutation changed the haem absorption peak to 410 nm, approximately 20 nm (blue-shifted) from the native peak at 429 nm, which resembles other peroxidases. The sulphonium linkage by Met243 to the haem produces a distortion of planarity and ligand binding characteristics, which is unique to MPO [72].

5. MPO is a glycoprotein

With the advances in glycomics, it is essential to discuss MPO glycosylation and how this affects structure and function. MPO has 38 glycopeptides, and all five expected sites for glycosylation were found to be glycosylated. These sites contain an asparagine residue (Asn323, Asn355, Asn391, Asn483, and Asn729) which is why they are N-linked glycosylation sites [73,74]. Another study was carried out to characterize MPO glycosylation sites [75]. There appear to be specific critical amino acids glycosylated, all of which are Asn residues: Asn323, Asn355, Asn391, Asn483, and Asn729. The glycosylation included phosphorylated N-glycans (Asn323) and paucimannose (Asn483). These in-depth studies have provided a more accurate picture of the mass of MPO, although the glycosylation masses are relatively close to earlier findings and are summarized in Table 1 [73].

Furthermore, the unusual (truncated) glycosylation pattern on MPO along with the heterogeneity of MPO glycosylation may affect antibody responses in complex systems, such as cancer or autoimmune diseases. The difference in antibody recognition between wild-type and MPO-deficient individuals could provide insights into the role of MPO in these diseases.

Table 1

| Glycosylation site (Asn) | Peptide mass (digestion with PNGase-F) | Ref. [73] |
|--------------------------|-------------------------------------|----------|
| 323                      | 1433                                |          |
| 355                      | 1558, 1575, 1058                    |          |
| 391                      | 2126                                |          |
| 483                      | 1053                                |          |
| 729                      | 1965                                |          |

Table 2

| MPO component                  | Average MW |
|-------------------------------|------------|
| Holoenzyme                    | 144170.9   |
| Monomer/hemi-MPO              | 72085.5    |
| Haem (prothetic group)        | 616.5      |
| Heavy chain                   | 53401.5    |
| Light chain                   | 12375.9    |
6. MPO catalytic cycle

MPO is considered to have two main activities: oxidation of halogens (halogenation cycle) to form hypohalous acids (such as hypochlorous acid) and the peroxidase cycle. As shown in Fig. 3, the resting enzyme is ferric MPO (1). Typically, for the cycle to begin, a source of H₂O₂ is required to “activate” the enzyme to form Compound I. This form of MPO has an oxidized iron (4+ valence) and a delocalized haem radical (ferryl haem). Compound I can either be reduced to the resting state by oxidizing (pseudo)halogens or go through the peroxidase pathway to Compound II:

Resting MPO [Fe(III)] + H₂O₂ → Compound I MPO [Fe(IV=O)●+] + H₂O

(1)

Compound I MPO [Fe(IV=O)●+] + halogen → Resting MPO [Fe(III)] + hypohalous acid

(2)

The halogenation cycle is unique to MPO in terms of its ability to oxidize chloride. Although the latter is a relatively poor substrate for MPO, its abundance in biological fluids (e.g., physiological NaCl levels are ~ 100 mM) allows for its oxidation to occur. It has been proposed, however, that less abundant (pseudo)halogens, like thiocyanate (micromolar amounts in the blood), are more easily oxidized and thus do not require the millimolar quantities of chloride [76]. This notion is supported by the electron donation potential as indicated by the second-order rate constant for Compound I reduction, where thiocyanate (SCN⁻) is the best reductant (most easily oxidized) for Compound I, followed closely by iodide, bromide, and two orders of magnitude slower for chloride (at neutral pH) [77]. Indeed, the bromination of a phenolic substrate (phenol red commonly used in cell culture) because of the Br⁻ contaminant of laboratory-grade NaCl, has been observed [78].

Although the halogenation cycle is considered the antimicrobial pathway because of the generation of hypohalous acids, there are far more electron substrates for MPO that are metabolized via the peroxidase pathway. Many free radical molecules are produced through this pathway. It is important to emphasize that although these pathways are described as distinct, they can feed into each other. For example, the presence of ascorbic acid and other cellular MPO substrates can ensure that MPO is in its resting state such that it can catalyze chlorination reactions. This is also true of certain xenobiotics and drugs [79]. There is a significant overlap in xenobiotic oxidation of MPO with the cytochrome P450 mixed-function oxidases (P450), which are the predominant drug metabolizing enzymes (and have a similar haem active site). Once Compound I MPO is formed, it is capable of substrate (X) oxidation to produce an initial X free radical (X●):

Resting MPO [Fe(III)] + H₂O₂ → Compound I MPO [Fe(IV=O)●+] + H₂O

(3)

Compound I MPO [Fe(IV=O)●+] + X → Compound II MPO [Fe(IV=O)] + X●

(4)

Compound II of MPO is less oxidizing than Compound I. If organic substrates (such as drugs or amino acids) have a favourable redox potential, they can reduce Compound II to Resting MPO. It is possible that the X● may undergo additional catalytic oxidation to produce a two-electron oxidized metabolite (X(O)●):

Compound II MPO [Fe(IV=O)] + X → Resting MPO [Fe(III)] + X●

(5)

However, a dismutation reaction can also occur that does not require enzymatic catalysis. This will happen at different rates but is usually a thermodynamically favourable reaction:

X● + X● → X + X(O)

(6)

Lastly, Compound III MPO is an intermediate that has been considered catalytically inactive, but it may have more value concerning hydroxylations of xenobiotics. To produce Compound III, superoxide is required:

Resting MPO [Fe(III)] + O₂●− → Compound III MPO [Fe(III)–O₂●−] → Compound III MPO [Fe(II)–O₂]

(7)

Compound III has been considered inactivated, based on studies in activated neutrophils [80]. Moreover, it has been referred to as a “dead-end” in terms of the more prominent functions of MPO [81], though Compound III has been implicated in oxidizing ascorbic acid [82], acetaminophen [83], salicylic acid [84], dimethyltryptamine [85], lysieric acid diethylamide (LSD, [86], phenol and phenolic drugs [87, 88], melatonin [89,90], phenylalanine [91], and possibly phenytoin [92]. An interesting reaction catalyzed by isoniazid and related hydrazines led to the generation of Compound III, where subsequent hydroxylation reactions may occur [93]. This observation was confirmed and likely contributed to identifying 4-aminobenzoic acid as the first potent MPO inhibitor that first converted MPO to Compound III before the loss of activity [94]. A short list of some substrates that MPO oxidizes is shown in Table 3.

The catalytic cycle for MPO is shown in Fig. 4. The reduction potentials are also indicated for the relevant redox couple. The higher (more positive) the reduction potential is, the more potent an oxidant is formed. The Compound I/Resting reduction potential (1.16 V) means that MPO can oxidize many compounds. Note that the Compound II/I reduction potential is even higher, which can oxidize numerous xenobiotics and endobiotics. Although these pathways are traditionally regarded as distinct, it is essential to note that the formation of hypochlorous acid, for example, is capable of chlorinating xenobiotics and endobiotics. The latter two may end up appearing to be “oxidized,” although the initial intermediate would be a chlorinated molecule.

7. Location, location, location

The best known location for MPO is within the primary or azurophilic granules of neutrophils, as this is the cell in which its peroxidase activity is expressed.
activity was discovered. Neutrophils, however, are end-differentiated and do not synthesize MPO. Monocytes also express MPO, though to a significantly lesser degree than neutrophils. Macrophage expression of MPO has been controversial. Early studies showed that MPO in monocytes granules are lost when differentiated into macrophages in vitro - activity was discovered. Neutrophils, however, are end-differentiated and do not synthesize MPO. Monocytes also express MPO, though to a significantly lesser degree than neutrophils. Macrophage expression of MPO has been controversial. Early studies showed that MPO in monocytes granules are lost when differentiated into macrophages in vitro.

Some studies suggest that MPO expression occurs in peripheral blood lymphocytes of mice and humans [105,106]. These reports indicated that lymphocytes had a higher mRNA level than neutrophils or monocytes but had significantly lower protein expression. Interestingly, in chronic HIV cases, there appears to be a statistically significant higher expression of MPO in CD4 and CD8 T lymphocytes. The functional significance of MPO in lymphocytes is yet unknown and more studies are required to understand how HIV infection, which causes immunosuppression, relates to MPO.

Other cells that express MPO include cells in the CNS, such as astrocytes, microglia, and neurons. Studies from human autopsies or animal models have revealed that MPO from these cells is associated with neuroinflammation, and epilepsy (reviewed in [107]). To get a clearer picture of the cellular origins of MPO, one must go to the bone marrow. MPO mRNA synthesis and translation occurs in the late myeloblast or promyelocytes, the precursor to granulocytes [101, 102]. A significant subset of bone marrow CD34+ stem cells has been reported to express MPO, although circulating CD34+ cells lacked MPO entirely, suggesting the former would have been committed to granulocyte development [103]. Therefore, MPO synthesis occurs in the bone marrow and not in circulation or within tissues (normally). The steps in processing MPO after translation have been elegantly outlined by Nauseef are summarized here briefly [104]. Readers are directed to that publication for in-depth biosynthesis and processing.

- Step 1 – preMPO is the initial translation product in the endoplasmic reticulum.
- Step 2 – glycosylation of preMPO to form apopMPO
- Step 3 – acquisition of haem to form proMPO (haem acquisition → catalytically active)
- Step 4 – exit from the endoplasmic reticulum
- Step 5 – proteolytic processing to form hemi-MPO.
- Step 6 – Within the primary/azurophilic granule, the dimerization of the hemi-MPO takes place through a disulfide bond (Cys319) to produce mature MPO.

The most abundant site of MPO synthesis begins in the bone marrow promyelocytes and in specific populations of CD34+ stem cells [103]. In addition, some studies suggest that MPO expression occurs in peripheral blood lymphocytes of mice and humans [105,106]. These reports indicated that lymphocytes had a higher mRNA level than neutrophils or monocytes but had significantly lower protein expression. Interestingly, in chronic HIV cases, there appears to be a statistically significant higher expression of MPO in CD4 and CD8 T lymphocytes. The functional significance of MPO in lymphocytes is yet unknown and more studies are required to understand how HIV infection, which causes immunosuppression, relates to MPO.

Other cells that express MPO include cells in the CNS, such as astrocytes, microglia, and neurons. Studies from human autopsies or animal models have revealed that MPO from these cells is associated with Alzheimer’s disease, multiple sclerosis, stroke or cerebral ischemia, Parkinson’s disease, depression or bipolar disorder, bacterial meningitis, neuroinflammation, and epilepsy (reviewed in [107]).

8. Cell lines containing MPO

Commercially available cell lines that highly express MPO are expectedly cell lines isolated from leukemia patients. However, not all leukemia cell lines express MPO or express MPO at different levels. Specific examples of cell lines that highly express MPO include HL-60, NB4, Kasumi-1, and MOLM13. Examples of low MPO-expressing cell lines include K562, U937, KG1, and OCI-AML3 [108]. A cell line that has been used as a good model for human macrophage precursors is THP-1.

---

**Table 4. Related leukemia cell lines and their relative expression of MPO.**

| Cell line   | Disease                          | MPO relative expression |
|-------------|----------------------------------|-------------------------|
| HL-60       | Acute Myeloblastic Leukemia, (myelocytic) | ↑↑↑                     |
| NB-4        | Acute Myeloblastic Leukemia, (myelocytic) | ↑↑↑                     |
| Kasumi-1    | Acute Myeloblastic Leukemia, (myelocytic) | ↑↑↑                     |
| MOLM-13     | Acute Myeloid Leukemia, (monocytic) | ↑↑↑                     |
| K-562       | Chronic Myelogenous Leukemia     | ↑↑↑                     |
| KG-1        | Acute Myeloid Leukemia, (myelocytic) | ↑↑↑                     |
| OCI-AML3    | Acute Myeloid Leukemia, (myelocytic) | ↑↑↑                     |
| U937        | Acute Myeloid Leukemia, (monocytic) | ↑↑↑                     |
| THP-1       | Acute Myeloid Leukemia, (monocytic) | ↑↑↑                     |

Information in this table is derived from [108-110]. The disease was verified using ATCC (USA) and DSMZ (Germany).
cells. The reason is that they can be differentiated into macrophages and demonstrate inflammasome function. Although THP-1 cells are leukemia cells, they are acute monocytic leukemia. No MPO activity has been reported in this cell line, and our lab has not detected any MPO expression (unpublished data). One study investigated the role of MPO inhibition for chemotherapy using cytarabine (AraC) in vitro and in vivo using the MOLM13 cell line. MPO inhibition in cells highly expressing MPO resulted in sensitization to AraC, which appeared to result from mitochondrial redox changes resulting in oxidative stress [109]. For reference, the reader is directed to Table 4 that lists related acute myeloid leukemia cell lines. As can be observed, myelocytic cell lines typically express MPO, whereas monocytic cell lines do not.

8.1. Secondary sites of MPO localization

It should be noted that neutrophils, through chemotaxis (e.g., IL-8, TNFα), can infiltrate organs and catalyze inflammatory reactions, including the release and activation of MPO. This is clear in specific instances of liver injury. What may complicate the matter in this case is the activation of liver-resident macrophages (Kupffer cells) which may express MPO during M1 polarization during inflammation (discussed above). Otherwise, Kupffer cells or macrophages do generally not express MPO. Another important site of MPO is in the atherosclerotic lesions in the blood vessel endothelium. It has been shown that MPO plays an important role in the formation of the plaques (discussed below). Other locations for MPO include the heart and the CNS.

Neurodegenerative conditions appear to have a significant association with the expression of MPO. Although cells will show a basal level of MPO, as demonstrated from human brain homogenate, disease states have the propensity to induce expression of MPO similar to phenomena in liver Kupffer cells described above. In Alzheimer’s disease patient brain autopsies, the presence of MPO in the frontal cortex is increased (detected by Western blot). Importantly, biochemical markers of MPO activity were also found, indicating not only the presence but activity also were increased in Alzheimer’s disease patient brain autopsies. From a disease pathology point of view, MPO was associated with amyloid β deposits [111]. MPO has been found human saliva, though peroxidase activity can also be derived from lactoperoxidase also found in tears, saliva, and milk [112]. The presence of MPO in healthy and disease states is summarized in Fig. 5.

9. MPO usefulness as a biomarker in disease states

9.1. Detection strategy

In order to be helpful as a biomarker, MPO must be effectively detected in the specimen of interest. MPO can be detected through several methods, which are either direct or indirect (but specific). Direct methods involve using commercially available antibodies for immunoassays, including ELISA, Western blot, or microscopy. Cytochemical staining, however, is usually accomplished by using MPO substrates (e.g., 3,3′-diaminobenzidine, o-dianisidine), which undergo metabolism upon adding H₂O₂ to form coloured products (usually red-brown). The consumption of H₂O₂ through the use of a H₂O₂ electrode is also possible, although competing reactions with catalase must be considered.

Indirect detection focuses on products of MPO, chiefly hypochlorous acid (HOCl), considered to be the main physiological product of MPO. HOCl can oxidize amino acids such as tyrosine to form 3-chloro-tyrosine [113,114]. 3,5-Dichlorotyrosine is also a suitable and stable detection compound lost green fluorescence upon reacting with HOCl in HepG2 compound (TPY) could detect micromolar HOCl ratiometrically. This fluorescent detection is usually a desirable strategy in cell studies due to providing a simple means for cell and tissue imaging. There are some challenges, however, since fluorescent yield, solubility, and permeability need to be considered in the useful implementation of such probes. A recent study demonstrated that a large lipophilic, charged compound (TPY) could detect micromolar HOCl ratiometrically. This compound lost green fluorescence upon reacting with HOCl in HepG2 cells [120]. One study used a dopamine scaffold to generate a fluorescent product that demonstrated specificity for hypochlorite and responded to exogenous HOCl (NaOCl) addition to cells [121,173]. An acute model of mouse liver ischemia showed fluorescent detection of a probe, though this required the exposure of the organs (i.e., reflection of the abdominal wall) for visualization. An interesting colorimetric probe based on the reaction with HOCl and glutathione could provide a
Fig. 6. Fluorescent and magnetic resonance detection methodology. MPO peroxidase activity catalyzes the one-electron oxidation of the substrate, which converts it to a free radical metabolite. Although the free radical metabolite was proposed to bind to protein [126], there is more evidence to suggest that a quinoneimine or o-quinone serotonin metabolite is the proximate electrophile that will bind to protein [127,128]. (Modified from [126]).

snapshot of the balance between the oxidant (HOCl) and antioxidant (glutathione) status of the cell or tissue [122]. Other approaches may be better suited for in vivo approaches, whereas in vitro approaches are more diverse [123]. Further information on such probes was recently described in [124].

Methods have employed MPO substrates conjugated with fluorescent or paramagnetic probes for fluorescent microscopy and magnetic resonance imaging, respectively [125]. As shown in Fig. 6, a substrate molecule (serotonin shown here) is conjugated with the desired probe initially via chemical synthesis [126], which then can be used in the test specimen for active MPO detection. A key consideration here is that MPO activity is needed, which indicates the need for an immunological process for the successful detection of active MPO localization.

9.2. Leukemia

MPO serves as a valuable marker for acute myelogenous leukemia, as alluded to above in describing cell lines (see “Cell lines containing MPO”). Acute myelogenous leukemia is characterized by a rapid increase in the myeloid cells associated with an arrest in their maturation. Its diagnosis was initially based solely on the pathological and morphological examination of the bone marrow and the blood. However, with growing knowledge about the heterogeneity of acute myelogenous leukemia, subcategorization into nine subtypes has been established by the French-American-British group, depending on the particular myeloid lineage, morphologic appearance and the reactivity with three histochemical stains, including MPO, Sudan black, and the nonspecific esterase α-naphthylacetate and naphthylbutyrate. Six (M1 to M4, M4EO and M6) out of the nine acute myelogenous leukemia subtypes are MPO-positive [129,130]. MPO expression in this disease is associated with a favourable outcome, in addition to its aforementioned role as a biomarker.

9.3. Cardiovascular diseases

MPO has been considered an important biomarker in cardiovascular health for about two decades. Cardiovascular diseases in which increased MPO levels have been detected include acute coronary syndrome, heart failure, pulmonary arterial hypertension, hypertension, heart transplant and graft rejection, diabetes mellitus, atrial fibrillation, and atherosclerosis (reviewed in [131]). MPO serum level is a predictor for future cardiovascular events and adverse outcomes in patients with acute coronary [31,132]. It has been known for some time that macrophage and neutrophil activity in the plaques of coronary blood vessels leads to oxidation and destabilization of those lesions [133]. Unstable plaques in thrombi have been found to contain MPO-positive monocytes and neutrophils [134].

Concerning MPO, it is well established to be the catalyst for oxidation of LDL and HDL, where oxidation of the latter is the primary concern. Tyrosine residues in this molecule serve as facile targets of oxidation by MPO and its products [135]. In a study evaluating Chinese coronary artery disease patients, there was a difference in comparison to US patients; there was no increase in MPO mediated oxidation of HDL observed, though several limitations were discussed, such as ambient air quality [136,137]. Prediabetic patients affected by adverse cardiovascular outcomes also demonstrated that MPO levels were correlated with cardiovascular risk factors. As such metabolic diseases are multifactorial, it should be noted that these subjects also had increased blood pressure, body-mass index, waist-to-hip ratio and dyslipidemia, which sets the stage for atherosclerosis [138].

The pathogenic role for MPO in cardiovascular diseases is reasonably established. The use of MPO as a prognostic indicator of cardiovascular disease status will likely continue to gain momentum. It remains to be seen if MPO will be used solely as a biomarker of cardiovascular disease status, or become a therapeutic target.

9.4. Neurodegenerative diseases

MPO has been associated with the neuropathology observed in Alzheimer’s disease, multiple sclerosis, stroke, Parkinson’s disease, depression, and traumatic brain injury and epilepsy. As described above, the peripheral blood MPO concentration appears to correlate with disease progress or intensity. In Alzheimer’s disease patients, MPO peripheral blood concentration was significantly higher in these patients compared to control [139]. Furthermore, this biochemical measurement is considered reflective of neutrophil activity in this disease [140]. (See “Secondary sites of MPO localization” section above for more details on MPO and Alzheimer’s disease). Similarly, inhibition of MPO in experimental autoimmune encephalitis – which shares similarity to multiple sclerosis as a neuroinflammatory autoimmune disease – reduced disease severity [141]. Both the levels of MPO and its activity reflected by 3-chlorotyrosine were reduced by an experimental MPO inhibitor, which correlated with disease severity, including demyelination, oligodendrocyte regeneration, and neurogenesis [142]. In Parkinson’s disease, elevated MPO has been detected in functionally relevant brain regions compared to healthy controls, including the putamen, caudate nucleus, and substantia nigra [143]. In a transgenic mouse model expressing human MPO, significant damage to α-synuclein was observed characteristic of MPO-catalyzed oxidants. In human Parkinson’s disease patients, post-mortem analysis also revealed elevated levels of MPO present in the substantia nigra [144]. A mouse model of Parkinson’s disease (using MPTP) showed that treatment with a plant extract (Tomentosin) reduced proinflammatory cytokines and MPO levels.
SLE is an autoimmune disease that can be expressed with systemic symptoms or limited to one specific organ, e.g., skin, kidney, blood vessels. The serum profile is characterized by two main types of auto-antibodies: one against a cell’s nuclear components, including single-stranded or double-stranded DNA, histones, and histone-DNA complexes, referred to as anti-nuclear antibodies (ANAs). The other class of antibodies is directed towards cytoplasmic lysosomal components of neutrophils and monocytes, including the enzymes such as proteinase-3 and MPO. These are collectively termed anti-neutrophil cytoplasmic antibodies (ANCAs). Within this subset, specific antibodies towards a single protein, i.e., anti-MPO = p-ANCA and anti-proteinase-3 = c-ANCA, have been identified in patients with specific variants of SLE. The presence of these antibodies is used to diagnose certain rare forms of vasculitis, which include three different forms of polyangiitis, renal nephritis, and drug-induced vasculitis [151]. Anti-MPO antibodies in particular, will be discussed.

One study showed that high titers of anti-MPO antibodies are observed with SLE or a drug-induced SLE-like disease, drug-induced vasculitis or nephritis, and idiopathic vasculitides [152]. In these instances, i.e., where anti-MPO antibodies are observed, ANA is usually absent. It has been reported that anti-MPO antibodies have been reported in an inconsistent manner in SLE patients, which has been ascribed to detection methodology. The same study, however, reported that anti-MPO is deposited in the kidney glomerular basement membrane, the first study to do so [153].

Drugs can produce a rare, unpredictable adverse drug reaction (idiosyncratic drug reaction), which can produce vasculitis characterized by the presence of anti-MPO. Although rare, approximately one hundred drugs have been associated with drug-induced SLE. Some notable drugs which have been definitively associated with this condition includes hydralazine, isoniazid, procainamide, methylidopa (withdrawn), quinidine, minocycline, and chlorpromazine [154,155]. In a study of seven patients taking hydralazine, six were positive for ANA. Five of the seven showed anti-MPO antibodies. Interestingly, anti-MPO was normalized after six months after drug withdrawal. Leukopenia was also observed in these patients [156]. Hydralazine appears to be the drug most recently and often reported that report both anti-MPO and ANAs [157-159]. The role of anti-MPO antibodies in either idiopathic SLE or drug-induced lupus is considered to involve mechanisms that activate neutrophils leading to the NET formation and tissue damage. Interestingly, a study reported statistically higher MPO plasma levels in SLE patients compared to healthy patients, although this did not correlate with disease severity [160].

### 10. Neutrophil extracellular traps (NETs) at the crossroads of defence and disease

The foundational publication regarding NETs was reported by the Zychlinsky group [161]. The NETs are composed of a web of chromatin (DNA) and proteins that form a matrix that can trap invading pathogens. NET formation has been synonymously referred to as NETosis, which implies a form of cell death occurs. Although debatable, suicidal NETosis has been found to occur with certain stimuli (e.g., phorbol 12-myristate 13-acetate), which is characterized by NET release accompanied by cell lysis. On the other hand, vital NETosis involves a more regulated but rapid release of NETs induced by appropriate stimuli (bacterial lipopolysaccharide, for example), through toll-like receptor 2/4 activation [162]. A third NETosis pathway involved mitochondrial DNA release and is dependent on reactive oxygen species and granulocyte-macrophage colony stimulating factor [163]. NET formation involves three significant steps: 1) activation to produce superoxide (and subsequently H$_2$O$_2$) via NADPH oxide; 2) nuclear and granule membrane destabilization and release of respective contents into the cytosol; 3) mixing of nuclear and granule contents and complete loss of their membranes; 4) cell contraction and release of NETs [164]. What results in this process is termed NETosis (initially coined in [165]). Thus, the NET can provide defence against pathogens, such as bacteria and fungi [166]. Significant to this review is the involvement of MPO in the chromatin (DNA) strands of NETs. MPO is considered to exert its oxidative capacity to cause damage to invading microbes, or host tissue, within the scope of the NET. Importantly, individuals lacking MPO appear not to be capable of forming neutrophil extracellular traps (NETs) [38]. In addition, the superoxide (and subsequently H$_2$O$_2$) generated by the respiratory burst (NADPH oxidase) is also required to produce NETs together with the fundamental role of MPO [167]. The re-addition of H$_2$O$_2$ to chronic granulomatous disease (patients lacking NADPH oxidase activity) neutrophils reactivated their capacity to form neutrophil extracellular traps [168]. This may partially explain the reason behind the inability to combat C. Albicans infection.

Linking the previous topic of ANCA (anti-MPO antibodies), there is a relationship between ANCA and NETosis. Studies on this topic first were indicated over a decade ago when examining vasculitis, where the NET formation was proposed to trigger vasculitis and anti-MPO formation [170]. A recent study reported a role for the TIM-3 surface receptor on T-cells as a critical contributor to NET maintenance in MPO-ANCA-associated vasculitis [171]. This is an essential connection between the adaptive and innate immune systems via NETs. The NET formation, where MPO can have an important role, has appeared in numerous reports of diseases and morbidities. NET formation and complement (C5, C5) were observed in COVID-19 patients and were associated with disease severity. MPO-DNA levels were significantly higher in severe vs. mild COVID-19 cases [43]. A study investigating thrombosis mechanisms in COVID-19 patients reported increased circulating NET formation in these patients, including increased MPO-DNA complexes (MPO associated with NETs) [44]. As a catalyst for thrombotic events in COVID-19, NETs were reported to be an exciting interplay between platelets and neutrophils in this disease [42]. As previously discussed, circulating MPO levels are an independent risk factor associated with cardiovascular disease. NETs, as a more complex structure (that also includes MPO), are involved in cardiac inflammation, myocardial infarction, and atrial fibrillation, though it is unclear if they are the cause or the consequence of these morbidities [172,173].

Furthermore, higher NET levels were detected in the plasma of Brazilian Alzheimer’s disease patients compared to healthy controls, which may be associated to complement system activity [174]. Importantly, damage-associated molecular patterns (such as mitochondrial
DNA, fibronectin extra domain A, and galectin-3) released during tissue damage may activate NETs through interaction with neutrophil surface proteins [172]. In addition, NETs can be triggered by cytokines [175], platelets [176], nitric oxide and peroxynitrite [177], and numerous microcrystals (of urate, calcium, cholesterol, and silica) [178], and numerous microbes (bacteria, viruses, yeast, parasites) [179]. The activation step for NETs as well as inducers of NETs is shown in Fig. 7.

Overall, the involvement in NETs in many disease processes is continually evolving. As NETs are a heterogeneous mixture of components, including different enzymes, singling out the specific contribution of damage may activate NETs through interaction with neutrophil surface proteins (e.g., vesi

**11. Drug metabolism and MPO inhibition**

Drug metabolism reactions catalyzed by MPO have been known for some time. The fact that MPO is a haem enzyme, similar to the P450 enzyme family, also suggests its drug metabolism capacity. Neutrophil metabolism of drugs, specifically via the concerted action of NADPH oxidase and MPO, has been proposed to have a role in their side effects and adverse drug reactions [35,36]. MPO is unique in that it has at least four mechanisms to catalyze reactions mimicking P450-like drug metabolism: 1) its peroxidase activity [84]; 2) oxidizing equivalents via HOCI/OCl generation, and 3) superoxide radical catalyzed hydroxylation [84].

**Simplified Hydroxylation reactions of MPO:**

\[
\text{MPO} + \text{RH} \rightarrow \text{ROH} + \text{H}^+ \quad (9)
\]

\[
\text{R}^+ + \text{H}_2\text{O} \rightarrow \text{ROH} + \text{H}^+ \quad (10)
\]

An example of this reaction is the phenol parahydroxylation mechanism to form hydroquinone and eventually benzoquinone. This pathway proposes a localized carboxylation reaction with H2O to produce a hydroxylated product [180], although a superoxide mechanism was initially suggested [181].

\[
\text{R} - \text{NH}_2 + \text{HOCI} \rightarrow \text{RNHCl} \rightarrow \text{RNH}^- + \text{Cl}^- \rightarrow \text{RN} = \text{O} \rightarrow \text{RNHOH} \quad (11)
\]

The example of amine-containing drugs is used, however, this can apply to other compounds such as the amine of nucleosides. Indeed, a primary radical is observed (an aminyl radical in this instance) [182, 183], though the question of the products may vary. There will be an aldehyde product in the degradation pathway of these chloramines [184,185]. The latter may undergo reduction to an alcohol to produce a putative hydroxylated product. However, if the aldehyde (in this case, nitroso intermediate) is highly reactive, free products will likely not be observed in a biological matrix, as observed with sulfamethoxazole and procainamide [186,187].

\[
\text{RH} + \text{O}_2^+ + 2\text{H}^- \text{MPO} \rightarrow \text{ROH} + \text{H}_2\text{O} \quad (12)
\]

The superoxide-dependent reaction involves an initial MPO Compound III formed between superoxide and resting MPO (in the ferric state). This is followed by a subsequent reduction by superoxide molecule of the MPO Compound III to produce a hydroxylated enzyme similar to P450, where superoxide is bound to ferrous MPO to interact with the substrate [84]. It is important to note that unlike P450, where a radical pair (both the enzyme and substrate) are caged, so the free radial (drug) is not diffusible to the medium, MPO substrates usually are not caged. For example, aromatic carbon oxidation reactions of MPO, where molecular oxygen favourably adds to the radical, forming a peroxy radical (as seen with lipid peroxidation):

\[
\text{R} - \text{C} + \text{O}_2 \text{MPO} \rightarrow \text{R} - \text{C} + \text{H}^- \text{MPO} \rightarrow \text{R} - \text{C} - \text{O} - \text{O}^- \quad (13)
\]

Numerous compounds can be oxidized by MPO through one of these processes, including olefin oxidation (epoxidation) [188], drug oxidation such as carbon oxidation [189] and alyamine oxidation [190–193]. Recently, the oxidation of edaravone, a pyrazolone antioxidant drug, was detected as a carbon radical [79]. A relevant functional group with respect to this review that undergoes oxidation by MPO is that of the hydrazines and hydrazides. In particular, this scaffold has provided research tools that have paved the road to new drugs, which turn out to be MPO inhibitors.

**11.1. The evolution of MPO-catalyzed drug metabolism towards MPO inhibitors**

The seminal study by Kettle and Winterbourn on benzoic acid hydrazides led to 4-aminobenzoic acid hydrazide as a tool to study the effect of MPO inhibition [94,194]. Almost 700 publications have cited the use of 4-aminobenzoic acid hydrazide to date. A mechanistic study of inhibition revealed that 4-aminobenzoic acid hydrazide catalyzed ester bond cleavage between the haem and amino acid protein chain [195]. Although quite valuable for enzymatic, cellular, and short-term in vivo animal studies, 4-aminobenzoic acid hydrazide is not ideal as a drug
candidate as hydrazides have undesirable side effects, which must be outweighed by their benefit to be clinically used (such as the anti-mycobacterial drug, isoniazid).

A detailed review outlining patents that have been filed since 2003 reported 15 MPO inhibitor patents [3]. Studies have come quite a long way since hydrazides, and there is considerable chemical heterogeneity for MPO inhibitors. Computational and experimental screening efforts directed towards identifying reversible and irreversible MPO inhibitors have led to diverse scaffolds [196–199]. These chemical scaffolds include triazolopyridine macrocycles, peptides, furic acids, indole alkylamines, thioxanthines, thioxodihydroxazinolones, triazolopyrimidines, and thiopyrimidinones [3]. Despite this chemical diversity, the most favourable scaffolds thus far are based on 2-thioxanthine and 2-thiopyrimidinone. A 2-thiopyrimidinone (PF06282999, Fig. 8) produced by Pfizer did not progress after Phase I clinical trials, in spite of desirable potency and pharmacokinetics parameters [3]. Work by Kettle and scientists at AstraZeneca demonstrated the potent MPO inhibition of 2-thioxanthines, which act as mechanism-based inhibitors. The initial radical product forms a haem-adduct before it is able to leave the MPO active site [39]. In this sense, the radical of the inhibitor is effectively caged, resulting in haem inactivation. The 2-thioxanthines demonstrated sub-micromolar IC50 values. Studies on aromatic hydroxamates revealed novel reversible inhibition [200]; however, it appears the utility of MPO inhibitors in disease settings favours irreversible inhibition. Even though 2-thioxanthines and a 2-thiopyrimidone advanced to clinical trials, none have yet passed phase II. There is currently a clinical trial for an AstraZeneca MPO inhibitor (AZD4831, Fig. 8) being run at the Mayo Clinic, which is currently in phase II (Clinical trial: NCT03611153 [201]). The study aims to determine if a single dose of this compound can affect hemodynamics in heart failure patients. It appears as though MPO inhibition is currently aimed at ameliorating cardiovascular disease, although work should continue to attenuate oxidative stress which is a key component in many of the diseases discussed in this review.

12. Concluding comments

MPO has remarkable diversity in the spectrum of its functions in health and disease. After reading this review, and considering clinical MPO inhibitors are being evaluated, one may be tempted to suppose that we may be better off without this enzyme. However, this is over simplistic, since the healthy *milieu intérieur* must be achieved with the normal presence and activity of MPO. Indeed, reflecting on the fact that most individuals express MPO indicates a fundamental (protective) function, and perhaps regulation of over active MPO is more desirable. When disease and pathobiology comes into play, MPO inhibition becomes a viable target, though clinical trials will be telling exactly how well this is achieved.

---

**Funding source**

Select parts of this review, where relevant articles were cited, were funded by the Natural Sciences and Engineering Research Council (NSERC) grant number RGPIN-2014-04878 and RGPIN-2020-06305.

---

**Appendix A. Supplementary data**

Supplementary data to this article can be found online at https://doi.org/10.1016/j.redox.2021.102109.

---

**References**

[1] W.M. Nauseef, Diagnostic assays for myeloperoxidase deficiency, in: M.T. Quinn, F.R. DeLeo, G.M. Bokoch (Eds.), Neutrophil Methods and Protocols, Humana Press, Totowa, NJ, 2007, pp. 225–230.
[2] G. Battistuzzi, et al., Influence of the covalent heme–protein bonds on the redox thermodynamics of human myeloperoxidase, Biochemistry 50 (37) (2011) 7987–7994.
[3] J. Soubyre, P. Van Antwerpen, F. Dufranne, A patent review of myeloperoxidase inhibitors for treating chronic inflammatory syndromes (focus on cardiovascular diseases, 2013–2019), Expert Opin. Ther. Pat. 30 (8) (2020) 595–608.
[4] K. Agner, Verdoperoxidase, in: F.F.N.a.C. Werkman (Ed.), In Advances in Enzymology and Related Areas of Molecular Biology, 2006, pp. 137–148.
[5] K. Agner, Verdoperoxidase, In Advances in Enzymology and Related Areas of Molecular Biology, 1943, pp. 137–148.
[6] W.M. Nauseef, Myeloperoxidase in human neutrophil host defence, Cell Microbiol. 16 (8) (2014) 1146–1155.
[7] G. Cheng, et al., Identification and characterization of VPO1, a new animal heme-containing peroxidase, Free Radic. Biol. Med. 45 (12) (2008) 1662–1694.
[8] A.C. Maehly, MYELOPEROXIDASE. Methods in Enzymology 2 (1955) 794–801.
[9] K. Agner, Detoxicating effect of verdoperoxidase on toxins, Nature 159 (4034) (1947) 271.
[10] S. Kohjima, Studies ON peroxidase. II. The effect of peroxidase on the bactericidal action of phenols, J. Biochem. 14 (1) (1931) 95–109.
[11] S.J. Klebanoff, Myeloperoxidase-halide-hydrogen peroxide antibacterial system, J. Bacteriol. 95 (6) (1968) 2131–2138.
[12] S.J. Klebanoff, Myeloperoxidase: contribution to the microbial activity of intact leukocytes, Science 169 (3950) (1970) 1095–1097.
[13] S. Kimura, M. Ikeda-Saito, Human myeloperoxidase and thyroid peroxidase, two enzymes with separate and distinct physiological functions, are evolutionarily related members of the same gene family, Proteins: Structure, Function, and Bioinformatics 3 (2) (1998) 113–120.
[14] K. Sakamaki, et al., Molecular cloning and characterization of a chromosomal gene for human eosinophil peroxidase, J. Biol. Chem. 243 (1-2) (1997) 3190–3197.
[15] Z. Liu, et al., Vascular peroxidase 1 is a novel regulator of cardiac fibrosis after myocardial infarction, Redox Biol. 22 (2019) 101151.
[16] R. Fischer, Der mikrochemische Nachweis der Peroxydase und Pseudoperoxydase in tierischen Geweben, Archiv für mikroskopische Anatomie 83 (1) (1913) A175.
[17] K. Agner, Verdoperoxidase: a ferment isolated from leukocytes, Acta Physiol. Scand. 2 (1941) 1–62.
[18] J. Schultz, K. Kaminski, Myeloperoxidase of the leukocyte of normal human blood. I. Content and localization, Arch. Biochem. Biophys. 96 (3) (1962) 465–467.
[19] F.R. DeLeo, G.M. Bokoch (Eds.), Neutrophil Methods and Protocols, Humana Press, Totowa, NJ, 2007, pp. 225–230.
[20] W.M. Nauseef, Diagnostic assays for myeloperoxidase deficiency, in: M.T. Quinn, F.R. DeLeo, G.M. Bokoch (Eds.), Neutrophil Methods and Protocols, Humana Press, Totowa, NJ, 2007, pp. 225–230.
[21] R.J. Falk, J.C. Jennette, Anti-neutrophil cytoplasmic autoantibodies with specificity for myeloperoxidase in patients with systemic vasculitis and idiopathic necrotizing and crescentic glomerulonephritis, N. Engl. J. Med. 318 (25) (1988) 1682–16828.
[22] X. Bosch, A. Gullabert, J. Font, Antineutrophil cytoplasmic antibodies, Lancet 368 (9533) (2006) 404–418.
[23] C.G.M. Kallenberg, A.H. Leontine Mulder, J.W. Cohen Tervaert, Antineutrophil cytoplasmic antibodies: a still-growing class of autoantibodies in inflammatory disorders, Am. J. Med. 95 (6) (1992) 675–682.
[24] A. Daugherty, et al., Myeloperoxidase, a catalyst for lipoprotein oxidation, is markedly elevated in low density lipoprotein isolated from human atherosclerotic intima, J. Clin. Invest. 94 (1) (1994) 427–444.
[25] S.L. Hazen, J.W. Heinecker, 3-Chlorotyrosine, a specific marker of myeloperoxidase-catalyzed oxidation, is markedly elevated in low density lipoprotein isolated from human atherosclerotic intima, J. Clin. Invest. 99 (9) (1997) 2075–2081.
A.G. Siraki

Redox Biology 46 (2021) 102109

[28] M. Yagmurca, et al., Erythrocyte prevents doxorubicin-induced cytotoxicity in rats, Pharmacol. Res. 48 (4) (2003) 377–382.

[29] B. Ky, et al., Early increase in brain tissue markers predict subsequent cardiotoxicity in patients with breast cancer treated with doxorubicin, taxanes, and trastuzumab, J. Am. Coll. Cardiol. 63 (8) (2014) 809–816.

[30] M.L. Brennan, et al., Prognostic value of myeloperoxidase in patients with chest pain, N. Engl. J. Med. 349 (17) (2008) 1595–1604.

[31] S. Baldus, et al., Myeloperoxidase serum levels predict risk in patients with acute coronary syndromes, Circulation 108 (12) (2003) 1440–1445.

[32] S. Pennathur, et al., Mass spectrometric quantification of 3-nitrotyrosine, oxytyrosine, and ε-oxoarginine in human breast tissue, Free Radic. Res. 50 (12) (2016) 1340.

[33] A.K. Tiden, et al., Two-thiolanes are mechanism-based inactivators of myeloperoxidase that block oxidative stress during inflammation, J. Biol. Chem. 286 (43) (2011) 37578–37589.

[34] I.M. Kooter, et al., The sulfonium ion linkage in myeloperoxidase: direct resonances in high-spin ferric hemoproteins: substrate binding in horseradish peroxidase, Biochim. Biophys. Acta Protein Proteomics 1804 (10) (2010) 2046–2053.

[35] T. Ravnsborg, G. Houen, P. Højrup, The glycosylation of myeloperoxidase, Biochim. Biophys. Acta Protein Proteomics 1804 (10) (2010) 2046–2053.

[36] K.R. Reiding, et al., Neutrophil myeloperoxidase harbors distinct site-specific modifications in its glycosylation, J. Biol. Chem. 279 (51) (2004) 52439–52446.

[37] G.J. Dalen, et al., Thiocyanate and chloride as competing substrates for myeloperoxidase, Biochim. Biophys. Acta 1327 (2) (1997) 487–492.

[38] P.G. Furthmüller, U. Bumer, C. Obinger, Reaction of myeloperoxidase compound I with chloride, bromide, iodide, and thiocyanate, Biochemistry 37 (51) (1998) 17923–17930.

[39] A. Morgan, et al., Caution for the routine use of phenol red – it is more than just a pH indicator, Chem. Biol. Interact. 310 (2019) 108738.

[40] R. Volkman, et al., Myeloperoxidase deficiency inhibits cognitive decline in the 5xFAD mouse model of Alzheimer’s disease, Front. Neurosci. 13 (990) (2019).

[41] J. Kudoh, et al., Assignment of the myeloperoxidase gene MPO to human chromosome 17: relationship to the 15;17 translocation of acute promyelocytic leukemia, Oncogene 1 (3) (1987) 319–324.

[42] B.J. van der Walt, J.M. van Zyl, A. Kriegler, Aromatic hydroxylation during the chlorination in atherosclerotic lesions and generates dysfunctional high-density lipoprotein, Chem. Res. Toxicol. (2021).

[43] M. Yagmurca, et al., Erdosteine prevents doxorubicin-induced cardiotoxicity in patients with breast cancer treated with doxorubicin, taxanes, and trastuzumab, J. Am. Coll. Cardiol. 63 (8) (2014) 809–816.

[44] W.F. Reynolds, et al., MPO and APOE polymorphisms interact to increase risk for AD in Finnish males, Neurology 55 (9) (2000) 1284–1290.

[45] J.P. Uetrecht, Idiosyncratic drug reactions: possible role of reactive metabolites of myeloperoxidase, Adv. Drug Deliv. Rev. 8 (4) (1991) 265–273.

[46] F. Gentilini, et al., A nonsense mutation in the myeloperoxidase gene is associated with up-regulation of surface expression of CD11b in mouse neutrophils, FEBS Lett. 50 (12) (2001) 1340–1349.

[47] Y. Zuo, et al., Neutrophil extracellular traps and thrombosis in COVID-19, Front. Immunol. 11 (2020) 6793.

[48] J. Kudoh, et al., Assignment of the human myeloperoxidase gene (MPO) to chromosome 17: relationship to the 15;17 translocation of acute promyelocytic leukemia, Oncogene 1 (3) (1987) 319–324.

[49] J. Inazawa, et al., Assignment of the myeloperoxidase gene MPO to human chromosome 17, Cytogenet. Cell Genet. 63 (3) (1994) 150–155.

[50] A.G. Siraki, et al., Erythrocyte prevents doxorubicin-induced cytotoxicity in rats, Pharmacol. Res. 48 (4) (2003) 377–382.

[51] B. Ky, et al., Early increase in brain tissue markers predict subsequent cardiotoxicity in patients with breast cancer treated with doxorubicin, taxanes, and trastuzumab, J. Am. Coll. Cardiol. 63 (8) (2014) 809–816.

[52] M.L. Brennan, et al., Prognostic value of myeloperoxidase in patients with chest pain, N. Engl. J. Med. 349 (17) (2008) 1595–1604.

[53] S. Baldus, et al., Myeloperoxidase serum levels predict risk in patients with acute coronary syndromes, Circulation 108 (12) (2003) 1440–1445.

[54] S. Pennathur, et al., Mass spectrometric quantification of 3-nitrotyrosine, oxytyrosine, and ε-oxoarginine in human breast tissue, Free Radic. Res. 50 (12) (2016) 1340.

[55] A.K. Tiden, et al., Two-thiolanes are mechanism-based inactivators of myeloperoxidase that block oxidative stress during inflammation, J. Biol. Chem. 286 (43) (2011) 37578–37589.

[56] I.M. Kooter, et al., The sulfonium ion linkage in myeloperoxidase: direct resonances in high-spin ferric hemoproteins: substrate binding in horseradish peroxidase, Biochim. Biophys. Acta Protein Proteomics 1804 (10) (2010) 2046–2053.

[57] T. Ravnsborg, G. Houen, P. Højrup, The glycosylation of myeloperoxidase, Biochim. Biophys. Acta Protein Proteomics 1804 (10) (2010) 2046–2053.

[58] K.R. Reiding, et al., Neutrophil myeloperoxidase harbors distinct site-specific modifications in its glycosylation, J. Biol. Chem. 279 (51) (2004) 52439–52446.

[59] G.J. Dalen, et al., Thiocyanate and chloride as competing substrates for myeloperoxidase, Biochim. Biophys. Acta 1327 (2) (1997) 487–492.

[60] P.G. Furthmüller, U. Bumer, C. Obinger, Reaction of myeloperoxidase compound I with chloride, bromide, iodide, and thiocyanate, Biochemistry 37 (51) (1998) 17923–17930.

[61] A. Morgan, et al., Caution for the routine use of phenol red – it is more than just a pH indicator, Chem. Biol. Interact. 310 (2019) 108738.

[62] R. Volkman, et al., Myeloperoxidase deficiency inhibits cognitive decline in the 5xFAD mouse model of Alzheimer’s disease, Front. Neurosci. 13 (990) (2019).

[63] J. Kudoh, et al., Assignment of the myeloperoxidase gene MPO to human chromosome 17: relationship to the 15;17 translocation of acute promyelocytic leukemia, Oncogene 1 (3) (1987) 319–322.

[64] K. Morishita, et al., Chromosomal gene structure of human myeloperoxidase and lactoperoxidase on human chromosome 17, Cytogenet. Cell Genet. 63 (3) (1994) 150–155.

[65] A.G. Siraki, et al., Erythrocyte prevents doxorubicin-induced cytotoxicity in rats, Pharmacol. Res. 48 (4) (2003) 377–382.

[66] B. Ky, et al., Early increase in brain tissue markers predict subsequent cardiotoxicity in patients with breast cancer treated with doxorubicin, taxanes, and trastuzumab, J. Am. Coll. Cardiol. 63 (8) (2014) 809–816.

[67] M.L. Brennan, et al., Prognostic value of myeloperoxidase in patients with chest pain, N. Engl. J. Med. 349 (17) (2008) 1595–1604.

[68] S. Baldus, et al., Myeloperoxidase serum levels predict risk in patients with acute coronary syndromes, Circulation 108 (12) (2003) 1440–1445.

[69] S. Pennathur, et al., Mass spectrometric quantification of 3-nitrotyrosine, oxytyrosine, and ε-oxoarginine in human breast tissue, Free Radic. Res. 50 (12) (2016) 1340.
