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OXIDATIVE METABOLISM OF AMSACRINE BY THE NEUTROPHIL ENZYME MYELOPEROXIDASE

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Abstract—Oxidative metabolism of the anti-cancer drug amsacrine 4′-(9-acridinylamino)methanesulphan-*m*-anisidide has been suggested to account for its cytotoxicity. However, enzymes capable of oxidizing it in non-hepatic tissue have yet to be identified. A potential candidate, that may be relevant to the metabolism of amsacrine in blood and its action in myeloid leukaemias and myelosuppression, is the haem enzyme myeloperoxidase. We have found that the purified human enzyme oxidizes amsacrine to its quinone diimine, either directly or through the production of hypochlorous acid. In comparison, the 4-methyl-5-methylcarboxamide derivative of amsacrine, CI-921 9-[[2-methoxy-4[(methylsulphonyl)-amino]phenyl]amino)-*N*,5-dimethyl-4-acridine carboxamide, reacted poorly with myeloperoxidase, although it was oxidized by hypochlorous acid. Detailed studies of the mechanism by which myeloperoxidase oxidizes amsacrine revealed that the semiquinone imine free radical is a likely intermediate in this reaction. Oxidation of amsacrine analogues indicated that factors other than their reduction potential determine how readily they are metabolized by myeloperoxidase. Both amsacrine and CI-921 inhibited production of hypochlorous acid by myeloperoxidase. CI-921 acted by trapping the enzyme as the inactive redox intermediate compound **II**. Amsacrine inhibited by a different mechanism that may involve conversion of myeloperoxidase to compound **III**, which is also unable to oxidize Cl^-. The susceptibility of amsacrine to oxidation by myeloperoxidase indicates that this reaction may contribute to the cytotoxicity of amsacrine toward neutrophils, monocytes and their precursors.

Amsacrine [4′-(9-acridinylamino)methanesulphon-*m*-anisidide‖; NSC 249992] (Fig. 1) is an anticancer drug that is clinically effective against myeloid and acute lymphatic leukaemias [1]. Its 4-methyl-5-methylcarboxamide derivative, CI-921 (NSC 343499) (Fig. 1), is currently undergoing phase II trials and has been selected for further development chiefly because of its greater activity against experimental solid tumours and better dispositional characteristics [2]. Like most antineoplastic drugs, amsacrine has many potentially fatal side effects, the most important of which is the suppression of bone marrow function. The cytotoxic effects of amsacrine are proposed to arise from its ability to intercalate with DNA and stabilize the formation of a cleavable complex with topoisomerase II, which results in double strand breaks of the DNA [3]. However, even though there is a correlation between cleavable complex formation and amsacrine's cytotoxic effects, there is considerable doubt concerning the events responsible for cell death [4].

Several authors have proposed that metabolic products of amsacrine contribute to its cytotoxicity [5–8]. It is extensively metabolized in blood [9] and the liver [10] to produce conjugates with thiols. In the liver, cytochrome P450 promotes oxidation of this drug to its quinone diimine, *N*1′-methanesulphonyl-*N*4′-(9-acridinyl)-3′-methoxy-2′,5′-cyclohexadiene-1′,4′-diimine (mAQDI, Fig. 1) [5, 10]. This product undergoes facile reduction and conjugate addition of glutathione to the diimine ring [11]. In addition, mAQDI and its hydrolysis product mAQI are more cytotoxic toward L1210 cells than the parent compound [5]. It has also been suggested that cytotoxicity of amsacrine involves its redox cycling with mAQDI, in a mechanism that is analogous to that of Adriamycin® [6, 7]. This suggestion is based on the finding that a ternary complex between DNA, amsacrine and Cu^{2+} results in rapid cleavage of the DNA through the generation of oxygen free radicals [8]. Recently, however, it was found that there is no detectable metabolism of amsacrine to mAQDI by cultured AA8 tumour cells [12]. It was therefore proposed that the parent compound is responsible for antitumour activity *in vivo*, at least in tumours with limited oxidative capacity. Thus, before oxidation products can be invoked in its cytotoxicity toward non-hepatic tissues, it must be established that other pathways besides cytochrome P450 oxidation are available for the metabolism of amsacrine.

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‖ Abbreviations: amsacrine, 4′-(9-acridinylamino)-methanesulphon-*m*-anisidide; CI-921, 9-[[2-methoxy-4[(methylsulphonyl)-amino]phenyl]amino)-*N*,5-dimethyl-4-acridine carboxamide; mAQDI, *N*1′-methanesulphonyl-*N*4′-(9-acridinyl)-3′-methoxy-2′,5′-cyclohexadiene-1′,4′-diimine; MPO, myeloperoxidase; QDI, quinone diimine; AMSA, fully reduced amsacrine analogue; mAQDI·, the semiquinone imine of mADQI; AH_2, a reducing substrate for MPO; s.c.e., saturate calomel electrode; n.h.e., normal hydrogen electrode.

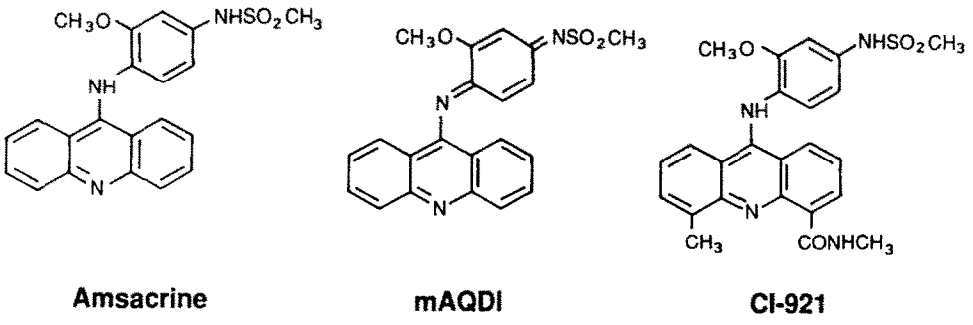


Fig. 1. The structures of amsacrine, mAQDI and CI-921.

Recently, a microsomal enzyme from HL-60 promyelocytic leukaemia cells was shown to double amsacrine-dependent topoisomerase-associated DNA lesions [13]. Neither the active metabolite, which was transient, nor the enzyme responsible for oxidation of the drug, was identified. A potential candidate for the metabolism of amsacrine is the haem enzyme myeloperoxidase (MPO). This is the most abundant protein in neutrophils, constituting 5% of their dry weight, and it is also found in monocytes [14]. In HL-60 cells, approximately half the MPO is located in the cytoplasm, while the remainder is packaged in primary granules [15]. MPO uses H_2O_2 to catalyse the direct oxidation of many xenobiotics including phenol, hydroquinone, paracetamol and amodiaquine [16–18]. These latter two drugs are oxidized to quinone imines which have been implicated in their cytotoxicity [19]. MPO also uses H_2O_2 to convert Cl^- to hypochlorous acid (HOCl). This is the major strong oxidant produced by neutrophils [14], reacting rapidly with biological molecules and xenobiotics [20]. We therefore undertook this investigation to determine whether MPO can metabolize amsacrine and CI-921. In addition, since HOCl plays a central role in host defence, we evaluated the effects of amsacrine and CI-921 on its production by MPO.

MATERIALS AND METHODS

Materials. Amsacrine and CI-921 were supplied by Warner-Lambert Laboratories (Ann Arbor, MI, U.S.A.) as the isethionate salt. Other amsacrine analogues and their quinone diimines were synthesized in the Cancer Research Laboratory, Auckland University School of Medicine, Auckland, New Zealand. mAQDI was prepared, as required, by adding an equimolar concentration of HOCl to amsacrine with continuous mixing. Myeloperoxidase was purified to a purity index (A_{430}/A_{280}) of greater than 0.75 as described previously [21], and its concentration was determined using ε_{430} 91,000 $M^{-1}\,cm^{-1}$ [22]. HOCl was purchased from Reckitt and Colman (N.Z.) Ltd, Avondale, New Zealand. Its concentration was determined by reacting it with monochlorodimedon (ε_{290} 19,000 $M^{-1}\,cm^{-1}$) and measuring ΔA_{290} due to the production of dichlorodimedon [21]. All other chemicals were purchased from the Sigma Chemical Co. (St Louis, MO, U.S.A.)

Methods. Oxidation of amsacrine was measured by either recording the changes in its absorption spectrum between 300 and 600 nm, or by continuously monitoring ΔA_{434}(ε_{434} 12,000 $M^{-1}\,cm^{-1}$) [7]. Maximum rates of oxidation were determined from the linear part of the progress curves. Lag periods were calculated by extrapolating the linear part of the progress curve back to where it intersected the tangent for the initial rate. Products were identified by their absorption spectra and by HPLC analysis essentially as described elsewhere [12], directly and after further reaction with glutathione. For analysis of the CI-921 products the acetonitrile content of the mobile phase was increased to 23%. Under these conditions retention times were: CI-921, 32.4 min; quinone diimine, 29.3 min; and 5′,6′-glutathione conjugates, 5.9/6.65 min. Oxidation of the amsacrine analogues was determined by measuring the loss in absorbance at the wavelength that had the maximum absorbance in the visible region (λ_{max}). The difference in the molar absorption coefficients for the amsacrine analogues and their corresponding quinone diimines were determined by measuring $\Delta A\lambda_{max}$ after the analogues had been completely oxidized by MPO. In all cases the spectral changes for the oxidation of the analogues had similar characteristics as for amsacrine. All reactions were carried out in 50 mM sodium phosphate buffer. One-electron reduction potentials of the amsacrine analogues were determined by pulse radiolysis as described previously by Anderson *et al* [23].

Conversion of H_2O_2 to HOCl was continuously monitored by measuring the loss of H_2O_2 with a YSI model 25 oxidase meter fitted with a YSI 2510 oxidase probe (Yellow Springs Instrument Co., Yellow Springs, OH, U.S.A.) as described previously [24]. Methionine was included in the reaction system to scavenge HOCl and prevent it from inactivating the enzyme. Reactions were carried out in 50 mM sodium phosphate buffer, pH 7.4, containing 140 mM NaCl and 300 μM methionine, and started by adding MPO. Initial rates were determined by drawing a tangent to the initial part of the curve for H_2O_2 loss. Replicates for H_2O_2 loss in the absence of drugs had a standard deviation of less than ± 5% (N = 4). I_{50} values were calculated by fitting a rectangular

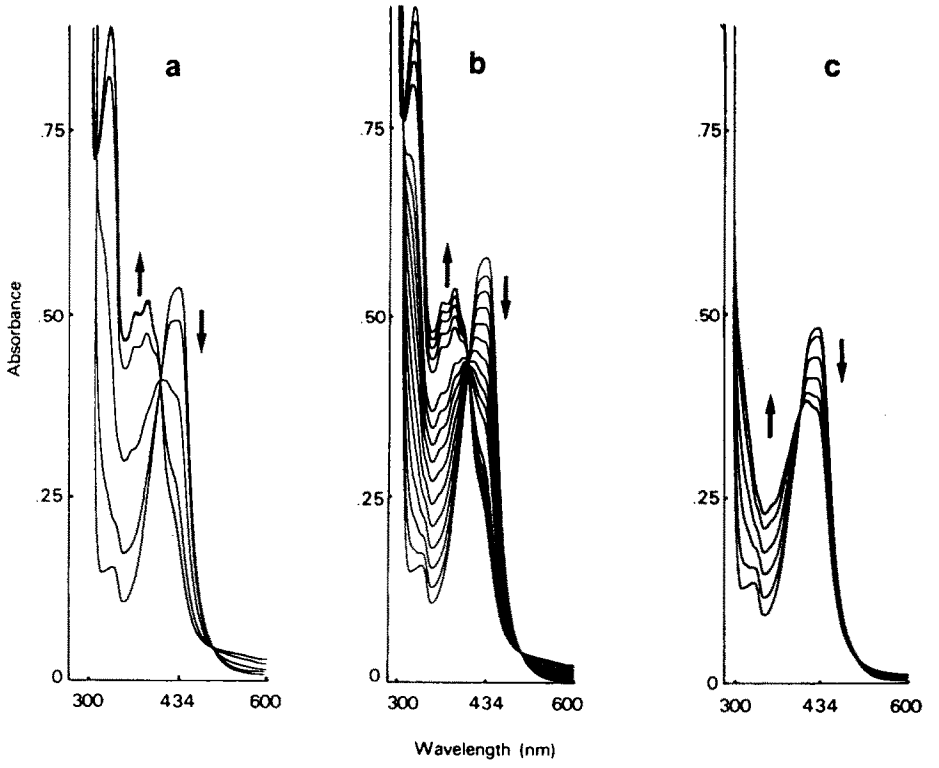


Fig. 2. Oxidation of amsacrine by myeloperoxidase. (a) Oxidation of 45 μM amsacrine by 20 nm MPO and 45 μM H_2O_2 in 50 mM sodium phosphate buffer (pH 7.4) containing 100 mM Cl^- at 25°. (b) As for (a) but without Cl^- and the concentrations of amsacrine and H_2O_2 were 50 μM. (c) As for (a) but with the addition of 1 mM methionine. Isotonicity was maintained with 33 mM Na_2SO_4. Reactions were started by adding H_2O_2 and scans were recorded every 2 min at a speed of 5 nm/sec. Arrows indicate the direction of the changes in absorbance.

hyperbola to the dose–response curves using non-linear regression. The correlation coefficient was always greater than 0.96. Conversion of H_2O_2 to HOCl was also determined by replacing methionine with taurine, and measuring the production of taurine chloramine with 2-nitro-5-thiobenzoate (ε_{412} 13,600 M^{-1} cm^{-1}) [25]. Reactions were stopped after 1 min with 100 μg/mL of catalase, and the loss of H_2O_2 was compared to the formation of taurine chloramine. The activity of MPO was also determined by measuring ΔA_{440} due to the oxidation of guaiacol [26].

RESULTS

Myeloperoxidase-dependent oxidation of amsacrine and CI-921

In the presence of the MPO/H_2O_2/Cl^- system, amsacrine was rapidly oxidized to a stable product (Fig. 2a). No oxidation occurred in the absence of either MPO or H_2O_2. The distinct isosbestic points indicate that there was no appreciable accumulation of intermediates in this reaction. In the absence of Cl^-, amsacrine was oxidized to a product with the same spectral characteristics as that formed in the presence of Cl^- (Fig. 2b). The rate of oxidation, however, was much slower. This demonstrates that MPO is able to directly oxidize amsacrine. Direct oxidation by MPO also occurred in the presence of physiological concentrations of Cl^-, since methionine, which scavenges HOCl, was unable to completely inhibit the loss of amsacrine (Fig. 2c). When stoichiometric amounts of reagent HOCl were added to amsacrine the spectral changes were identical to those promoted by MPO. These spectral characteristics indicated that in each case the product was mAQDI [23]. This was confirmed by HPLC analysis (results not shown).

The MPO/H_2O_2/Cl^- system also oxidized CI-921, giving spectral changes similar to those for amsacrine. However, the rate of oxidation was about 60% slower than with amsacrine. Identical spectral changes occurred with reagent HOCl. Another contrast with amsacrine was that CI-921 reacted poorly with MPO/H_2O_2. To achieve similar rates of oxidation the concentration of MPO had to be increased 20-fold.

Direct oxidation of amsacrine by myeloperoxidase

When oxidation of amsacrine by MPO/H_2O_2 was followed at 434 nm there was a distinct lag phase in its conversion to mAQDI (Fig. 3). The maximum rate attained was dependent on the concentrations of MPO (not shown) and amsacrine (Fig. 4). With

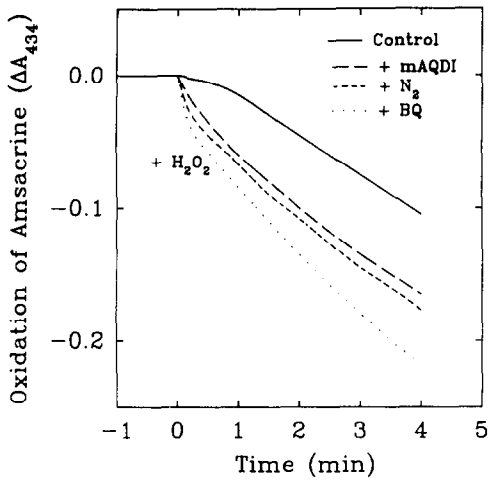


Fig. 3. Effects of quinone diimine, benzoquinone (BQ) and oxygen on the oxidation of amsacrine. Oxidation of 50 μM amsacrine by 20 nM MPO and 50 μM H_2O_2 in 50 mM sodium phosphate buffer (pH 7.4) at 25° (———) was also determined in the presence of 50 μM mAQDI (— —) or 50 μM (BQ) (. . . . .), or under an atmosphere of nitrogen (----). Reactions were started by the addition of H_2O_2.

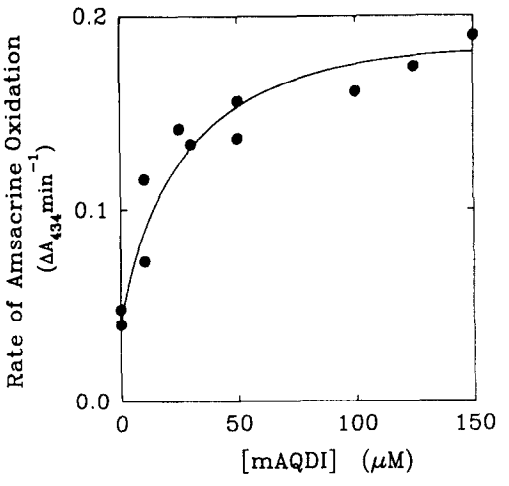


Fig. 5. Effect of quinone diimine on the maximum rate of oxidation of amsacrine. Conditions were as described in Fig. 3 except the concentration of MPO was 30 nM.

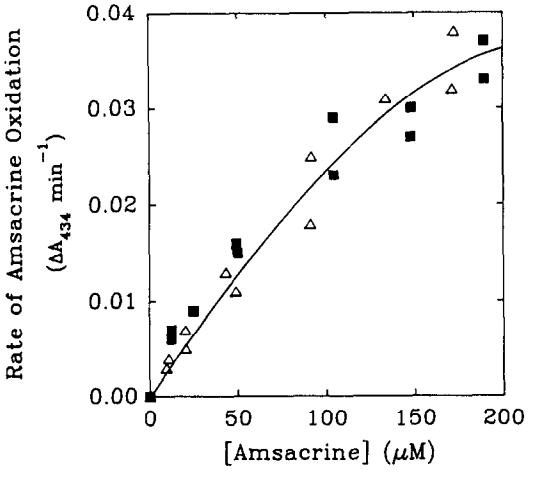


Fig. 4. Effects of the concentration of amsacrine and Cl^- on the maximum rate of oxidation of amsacrine by myeloperoxidase. Amsacrine was oxidized by 20 nM MPO and 100 μM H_2O_2 in the presence (△) or absence of 100 mM Cl^- (■) in 50 mM phosphate buffer containing 2 mM methionine. Other conditions were as described in Fig. 2.

methionine present to scavenge HOCl and prevent it from reacting with amsacrine, physiological concentrations of chloride had no effect on the rate of oxidation (Fig. 4).

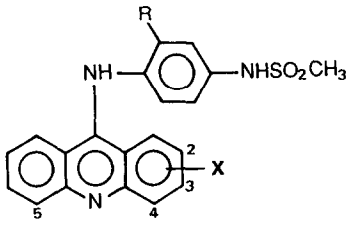
The oxidation of hydroquinone by MPO also shows a lag phase that is abolished by adding benzoquinone or by eliminating oxygen from the reaction system [27]. To determine if MPO oxidizes amsacrine and hydroquinone by the same mechanism, we investigated the effects of benzoquinone, mAQDI and an atmosphere of nitrogen on the oxidation of amsacrine (Fig. 3). All these conditions abolished the lag phase and greatly enhanced the rate of oxidation. The effect of mAQDI was concentration dependent having a K_m of 20 μM (Fig. 5). Another similarity to the oxidation of hydroquinone was that the rate of amsacrine oxidation increased slightly when the pH was increased between 5 and 8 (data not shown). Thus, we conclude that MPO oxidizes amsacrine by the same mechanism as previously outlined for hydroquinone [27]. Superoxide dismutase had no effect on the rate of oxidation (data not shown).

Direct oxidation of amsacrine analogues by myeloperoxidase

The ability of MPO to oxidize several other analogues of amsacrine was also examined. Changing the substituent on the anilino ring had little affect on the rate of oxidation of the analogues compared to amsacrine (Table 1). Only 3′-N$(CH_3)_2$ (Table 1, **4**) had a significant effect in that it decreased oxidation by 30%. However, all substituents on the acridine ring decreased the rate of oxidation. There was no obvious relationship between their electron-donating or -withdrawing capacity, as measured by Hammett constants (σ) [28], and their effect on the rate of oxidation.

With horseradish peroxidase, reactivity of aromatic compounds is generally related to their relative ease of oxidation, with steric effects having little influence [29, 30]. We therefore determined the one-electron reduction potentials of the quinone diimines (QDI) and the semiquinone imines (QDI$^{\cdot}$). Consistent with previous findings for the two-electron reduction potentials of the amsacrine analogues ($E_{1/2}$ QDI/AMSA) [31], changes in the substituents on the anilino ring had a large effect on the one-electron

Table 1. Relative rats of oxidation of amsacrine analogues, and their one- and two-electron reduction potentials



No.	R	X	σ	Relative rate of oxidation	$E_{1/2}$ (mV)	$E(1)$ (mV)	$E(2)$ (mV)
1	3'-OCH_3	H	−0.27	1.0	280*	−85†	915†
2	2'-OCH_3	H	—	1.1	ND	ND	ND
3	3'- H	H	0	0.91	330*	−130	1060
4	3'$N(CH_3)_2$	H	−0.60	0.7	195*	+25†	635†
5	3'-OCH_3	4-CH_3	−0.07	0.35	263*	−100	896
6	3'-OCH_3	4-OCH_3	0.12	0.20	278*	−91	917
7	3'-OCH_3	4-$CONHCH_3$	0.35	0.0	243*	−88	844
8	3'-OCH_3	4-CH_3	−0.07	<0.1	240*	+45	705
		5-$CONHCH_3$	0.35				
9	3'-OCH_3	4-$CONH_2$	0.28	0.12	ND	ND	ND
10	3'-OCH_3	4-CN	0.68	0.21	ND	ND	ND

Amsacrine, CI-921 and other analogues (20 μM) were oxidized with 40 nM MPO and 100 μM H_2O_2 in 50 mM sodium phosphate buffer pH 7.4 at 25°. Their maximum rates of oxidation were compared to that for amsacrine (3.5 μM/min). One-electron reduction potentials of the couple QDI/QDI$^{\cdot}$ ($E(1)$ vs n.h.e. at pH 7.4 in aqueous solution) were determined by pulse radiolysis, and used in conjunction with the two-electron reduction potentials of the couple QDI/AMSA ($E_{1/2}$ vs s.c.e. at pH 4.5 in 40% acetronitrile) [31] to calculate the one-electron reduction potentials for the couple QDI$^{\cdot}$/AMSA ($E(2)$ vs n.h.e. at pH 7.4 in aqueous solution). σ indicates the Hammett constants for substituents **R** (σ_p) on the anilino ring and **X** (σ_m) on the acridine ring [28].

* Values from Ref. 31.

† Values from Ref. 23. All other values were determined in this investigation.

ND, not determined.

reduction potentials. The greater the electron-donating capacity of these substituents, the lower the one-electron reduction potential of the semiquinone imines ($E(2)$ QDI$^{\cdot}$/AMSA). In contrast, the reduction potential for the couple QDI/QDI$^{\cdot}$ ($E(1)$) increased with the electron-donating capacity of the substituents. Except for CI-921 (**8**), substituents on the acridine ring had little effect. Surprisingly, the ease of oxidation by MPO was not related to the one-electron reduction potentials of the quinone imines or the semiquinone imines. In fact, while amsacrine reacted readily with MPO, CI-921, which is more prone to oxidation, was not metabolized under the conditions of our assay. On the basis of these results, we conclude that electronic effects do not determine the relative ability of amsacrine and its analogues to be oxidized by MPO.

Inhibition of HOCl *production by amsacrine and CI-921*

A number of anti-inflammatory drugs that have oxidizable groups are potent inhibitors of MPO-dependent production of HOCl [32]. These drugs act by promoting the accumulation of compound **II**, which is a redox intermediate of MPO that is unable to oxidize Cl^-. Inhibition is reversible, because compound **II** can be reduced back to the active enzyme. Since amsacrine is directly oxidized by MPO, it should also affect the production of HOCl by this enzyme. Indeed, we found that amsacrine inhibited the chlorination activity of MPO (Fig. 6), causing 50% inhibition of the initial rate of H_2O_2 loss (I_{50}) at a concentration of 1.2 μM (Fig. 7). However, it inhibited to a maximum of only 75%. At all concentrations of amsacrine, H_2O_2 was stoichiometrically converted to HOCl, as measured by the production of taurine chloramine (results not shown) [25]. Ascorbate was unable to reverse the inhibition, which indicates that accumulation of compound **II** was not responsible for the decrease in HOCl production [32]. Inhibition was not irreversible, because after chlorination in the presence of amsacrine, the ability of MPO to oxidize guaiacol was unaffected (results not shown).

CI-921 also inhibited the conversion of H_2O_2 to HOCl (Fig. 6), having an I_{50} of 2.2 μM (Fig. 7). However, in contrast to amsacrine, CI-921 inhibited MPO by greater than 90% and inhibition was reversed by ascorbate. We therefore conclude that although CI-921 was poorly oxidized by MPO, it must promote the accumulation of compound **II**.

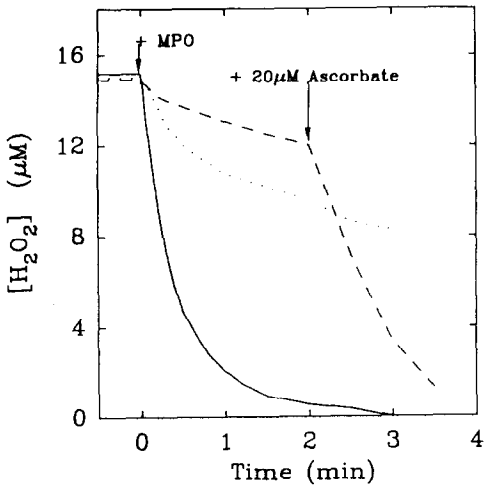


Fig. 6. The effects of amsacrine and CI-921 on the loss of H_2O_2 catalysed by myeloperoxidase in the presence of Cl^-. The chlorinating activity of MPO was determined by measuring the loss of H_2O_2 in the absence (———) and in the presence of 10 μM amsacrine (.....) or CI-921 (----) at 25°. Reactions were started by adding 15 nM MPO to 15 μM H_2O_2 in 10 mM sodium phosphate buffer (pH 7.4) containing 140 mM NaCl and 300 μM methionine. Ascorbate was added to reaction systems containing either amsacrine or CI-921 as indicated by the arrow.

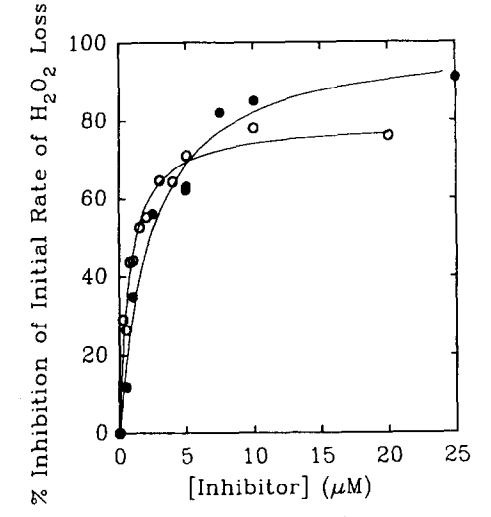


Fig. 7. The effects of amsacrine and CI-921 on the initial rate of H_2O_2 loss catalysed by myeloperoxidase in the presence of Cl^-. Inhibition was calculated from the decrease in the initial rate of H_2O_2 loss. Conditions were as described in Fig. 6. (○) amsacrine and (●) CI-921.

DISCUSSION

We have shown that MPO readily oxidizes amsacrine to mAQDI either directly, or through its production of HOCl. Even in the presence of physiological concentrations of Cl^-, amsacrine reacts with MPO. Although direct oxidation is slower than oxidation by MPO-derived HOCl, it is still considerable and is most likely to predominate *in vivo*, as HOCl would be preferentially scavenged by a myriad of biological molecules. From these results, it is likely that amsacrine will be oxidized by neutrophils and monocytes, and their precursor cells in the bone marrow that contain MPO, to produce mAQDI. We therefore propose that oxidative metabolism by MPO should be considered as a possible mechanism of amsacrine's cytotoxicity. In comparison to amsacrine, CI-921 reacts poorly with MPO, although it is readily oxidized by MPO-derived HOCl.

Our results indicate that the semiquinone imine, mAQDI$^{\cdot}$, is a likely intermediate in the formation of mAQDI by MPO. It is generally accepted that reducing substrates (AH_2) are metabolized by MPO in two successive one-electron oxidations to give free radical intermediates (Fig. 8, reactions 1–3) [33]. In contrast, Cl^- is converted to HOCl by compound **I** in a single two-electron oxidation (reaction 4) [14]. Oxidation of amsacrine is characterized by a distinct lag phase that is abolished by benzoquinone, mAQDI or by removing oxygen. This mirrors oxidation of hydroquinone by MPO [27]. With hydroquinone there is a lag phase because benzosemiquinone produced in reactions 2 and 3 converts MP^{3+} to compound **III** (reaction 5), which is outside the classical peroxidase cycle (reactions 1–3). However, as benzoquinone accumulates it converts compound **III** back to MP^{3+} and thereby increases the rate of oxidation. Given the similarity between the oxidation of hydroquinone and amsacrine, it is most likely that mAQDI$^{\cdot}$ is generated by MPO. With amsacrine, superoxide could not have been responsible for inhibiting MPO during the lag phase because superoxide dismutase did not alter the rate of oxidation.

We probed the mechanism of direct oxidation further by determining the relative rates of oxidation of a series of amsacrine analogues, and found a wide variability in their metabolism by MPO. While amsacrine and analogues that had different substituents on the anilino ring were readily oxidized, substituents on the acridine ring decreased the rate of metabolism. In fact CI-921 (Table 1, **8**) and the monosubstituted 4-methylcarboxamide derivative (Table 1, **7**) were not oxidized to any appreciable extent under the reaction conditions. In contrast to previous work on the metabolism of aromatic substrates by peroxidases [29, 30, 32], oxidation of the amsacrine analogues was not dependent on their one-electron reduction potentials. This may indicate that steric effects of the amsacrine analogues influence the extent of their oxidation by MPO. Although CI-921 was poorly oxidized by MPO, it inhibited production of HOCl by promoting the accumulation of compound **II**. This signifies that CI-921 reduces compound **I** to compound **II**, but is unable to reduce compound, **II** back to the ferric enzyme. Thus, binding of the amsacrine analogues to compound **II** may be an essential prerequisite for reduction of this intermediate.

We found that amsacrine and CI-921 are both

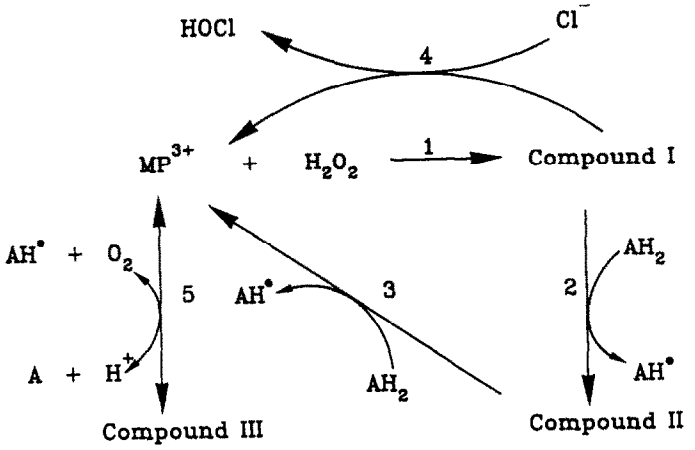


Fig. 8. The reactions of myeloperoxidase. AH_2 is a reducing substrate such as amsacrine or hydroquinone. Double arrowheads indicate equilibrium reactions.

good inhibitors of MPO-dependent production of HOCl; Yet they act by different mechanisms. Amsacrine inhibition is not reversed by ascorbate and reaches only 75% of maximum. Since the enzyme is not irreversibly inactivated, it must be converted to an intermediate, other than compound **II**, that is unable to oxidize Cl^-. One possibility is that the semiquinone imine converts MPO to compound **III** (reaction 5). Even though MPO plays a central role in microbial killing, it is not absolutely essential as judged by the good health of MPO-deficient individuals [34]. Thus, amsacrine and CI-921 are unlikely to compromise host defence; particularly since inhibition by CI-921 is reversible, and amsacrine allows approx. 25% of maximal activity so that a significant amount of HOCl should be produced in the presence of these drugs.

If MPO-dependent metabolism of amsacrine has an impact *in vivo*, it is most likely restricted to the blood and bone marrow due to the reactive nature of mAQDI. The free radical mAQDI$^{\cdot}$ will be more reactive than mAQDI, and should also be considered to contribute to any adverse reactions associated with MPO-dependent metabolism of amsacrine. Analogous radicals have been implicated in the toxicity of several quinoid anticancer drugs [35], paracetamol [19] and amodiaquine [16, 36]. In the blood, amsacrine is extensively metabolized to form adducts with protein thiols [9]. This has been proposed to at least in part occur through the formation of mAQDI, which interacts with plasma proteins to form thioether conjugates [10]. Given the susceptibility of amsacrine to oxidation by MPO, it is indeed possible that this haem enzyme contributes to the metabolism of amsacrine in blood. Interestingly, while a lower dose of CI-921 than amsacrine is required for cytotoxicity to several tumour cell lines, human and mouse bone marrow cells have similar sensitivity to both compounds [37]. A possible explanation for the greater than expected relative toxicity of amsacrine to these bone marrow cells may be the involvement of MPO metabolism in addition to topoismerase II-mediated effects.

Conversely, several independent studies suggest that attention should be focused on a role for MPO in the anticancer action of amsacrine. Firstly, oxidative metabolism by a microsomal protein potentiates the drug's capacity to promote DNA lesions in isolated nuclei of HL-60 cells [13]. In these cells MPO exists predominantly as a monomeric form of about 79 kDa [15], which is associated with the endoplasmic reticulum and packaged in azurophilic granules [38]. Secondly, a protein of 76 kDa has been implicated in the cytotoxicity of amsacrine toward another human myeloid leukemic cell line, KMB-3 [39]. Finally, MPO has recently been reported to be bound to DNA in the nuclei of HL-60 cells and neutrophils [40]. Hence, an oxidative mechanism of damage to DNA involving MPO and amsacrine may contribute to the action of this drug against acute myeloid leukaemia.

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