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# Comparison of HOCl traps with myeloperoxidase inhibitors in prevention of low density lipoprotein oxidation

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**Abstract**

In this study, the production of the highly toxic oxidant hypochlorous acid (HOCl) by the phagocytic enzyme myeloperoxidase (MPO) was quantitated and the concomitant alterations of low density lipoprotein (LDL) were analyzed in view of the potential role of LDL in atherosclerosis. Using the monochlorodimedone assay, it was found that HOCl is produced in micromolar concentrations. The kinetics of the decrease of tryptophan fluorescence appeared to be a sensitive method to monitor LDL alterations under near in vivo conditions. Therefore, this method was used to subsequently compare the effectiveness of MPO inhibitors that block production of HOCl with compounds that act as HOCl traps. The efficiency of MPO inhibitors to prevent LDL damage increased in the series benzohydroxamic acid < salicylhydroxamic acid < 3-amino-1,2,4-triazole < sodium azide < potassium cyanide < *p*-hydroxy-benzoic acid hydrazide, while for the HOCl traps the protective efficiency increased in the series glycine < taurine < methionine. We conclude that HOCl traps may have high potential therapeutic impact in vivo due to their low toxicity, although high concentrations of them would have to reach sites of inflammation. In contrast, only low concentrations of a specific MPO inhibitor would be required to irreversibly inhibit the enzyme. © 2000 Elsevier Science B.V. All rights reserved.

*Keywords:* Low density lipoprotein; Methionine; Myeloperoxidase; *p*-Hydroxy-benzoic acid hydrazide; Taurine; Tryptophan fluorescence

## 1. Introduction

It is now well accepted that oxidative modification of low density lipoprotein (LDL) contributes to the pathology of atherosclerosis [1,2]. Recently, attention has focused on the role of the heme enzyme myeloperoxidase (MPO) in this process, which uses $H_2O_2$ and $Cl^-$ to catalyze the production of the reactive and cytotoxic oxidant hypochlorous acid (HOCl). Evidence for the involvement of MPO in atherosclerosis comes from the observation that it is present in an active form in human atherosclerotic tissue [3] and that chlorinated tyrosine derivatives, which are thought to be specific products of MPO, have been detected during all stages of the development of atherosclerosis [4].

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LDL contains both a protein and a lipid component, either of which may be a target for oxidative damage during atherosclerosis, and consequently a number of studies have investigated the effects of HOCl on these moieties. For recent reviews, see Kettle and Winterbourn or Schaur et al. [5,6]. HOCl is known to undergo a variety of oxidation and chlorination reactions with biological molecules. Indeed all oxidizable groups are subject to oxidation by HOCl, and if these groups are required for the integrity of the target, damage ensues. The issue is not what it can attack, but what it does attack and where [7].

Hazell et al. [8] have reported that HOCl treatment of LDL results in oxidation of lysine residues, leading to aggregation, and that this may occur preferentially to lipid oxidation. There is evidence that certain lysine residues of apoprotein B-100 (apo B-100) are required for recognition by LDL receptors [1], and that loss of a critical number of these lysines will lead to recognition by the acetyl-LDL or scavenger receptor [9].

Because of the many known effects of HOCl, modulation of MPO function has considerable potential for therapeutic intervention in numerous pathologies. In this study, we have focused on MPO as a rational target for the prevention of human atherosclerosis and other inflammatory diseases. The effectiveness of inhibitors that block production of HOCl or compounds that act as HOCl traps can easily be monitored by the decrease of tryptophan fluorescence. This is a sensitive and virtually continuous method to study the kinetics of LDL oxidation under near in vivo conditions.

While cysteine is the amino acid most susceptible to modification by HOCl, tryptophan residues are also easily oxidized [10] and are part of sequential oxidation events in LDL. It was shown in this study that in the time scale of tryptophan destruction significant HOCl production can be observed, which is associated with major alterations in the overall structure of the LDL particle. On the other hand, lipid peroxidation in terms of thiobarbituric acid reactive substances formation was found to be comparably slow to tryptophan destruction. Therefore tryptophan destruction caused by the $MPO/H_2O_2/Cl^-$ system was monitored fluorimetrically as a marker of LDL modification to compare the HOCl-trapping amino acids taurine, methionine and glycine with the following classes of MPO inhibitors: the heme–enzyme inhibitors 3-amino-1,2,4-triazole (ATZ), potassium cyanide and sodium azide, the peroxidase inhibitors salicylhydroxamic acid (SHA) and benzohydroxamic acid (BHA) [11] as well as the MPO inhibitor *p*-hydroxy-benzoic acid hydrazide (pHBAH) [12].

The amino sulphonic acid taurine was chosen because it is exceptionally abundant in the cytosol of inflammatory cells and especially in neutrophils. In leukocytes, a taurine concentration of 35 mmol/l has been reported [13] which may protect cells from self-destruction during processes that generate oxidants. The major function of taurine in leukocytes is probably to trap HOCl. Taurine reacts with HOCl to produce the long-lived taurine chloroamine, which has been suggested to work as a specific signaling molecule of activated neutrophils [14].

## 2. Materials and methods

All concentrations refer to final concentrations if not otherwise stated. Human MPO (EC 1.11.1.7) from Calbiochem (Bad Soden, Germany) was used throughout the study, except in the monochlorodimedone (MCD) experiments, which were performed with MPO obtained from Professor Inge Olsson (Lund Hospital, Department of Medicine, Lund, Sweden) [15].

MCD (1,1-dimethyl-4-chloro-3,5-cyclohexanedione), ATZ, pHBAH, BHA, SHA, glycine and methionine were purchased from Sigma-Aldrich Chemicals (Deisenhofen, Germany). Taurine and sodium azide were from Fluka Chemicals (Buchs, Switzerland) and potassium cyanide and $H_2O_2$ were from Merck (Darmstadt, Germany). Solutions of these compounds were diluted daily from a stock solution.

### 2.1. Plasma and LDL preparation

Plasma preparation was performed as described elsewhere [16]. Briefly, blood drawn from normolipidemic donors after overnight fasting was collected and the plasma was separated immediately by low spin centrifugation. After pooling the plasma, a sucrose solution was added and aliquots were stored at −80°C in the dark until lipoprotein preparation (up

to 2 months). Plasma stored for 5 weeks under this conditions showed no changes in the oxidizability indices, as determined by the conjugated diene method [17].

LDL was obtained by ultra-centrifugation using a single step discontinuous gradient in a Beckman NVT 65 rotor at 60 000 rpm for 2 h at 10°C [17]. The LDL solution was filtered through 0.2 μm syringe filters into sterile vials and stored at 4°C under argon in the dark up to 2 weeks.

LDL was desalted to remove EDTA by gel filtration with an Econo-Pac 10 DG column supplied by Bio-Rad using phosphate-buffered saline (PBS, 150 mmol/l NaCl, 10 mmol/l $NaH_2PO_4*H_2O$, pH 6.0) as eluent. The LDL concentration was determined by the CHOD-PAD method using the cholesterol kit supplied by Boehringer-Mannheim. A factor of 3.16 was used to convert mg cholesterol/ml into mg LDL total mass/ml.

### 2.2. Determination of the HOCl concentration

The chlorination of MCD, used to measure the production of HOCl, was performed in PBS pH 6.0 as described previously [15,18].

### 2.3. Dynamic light scattering (DLS)

DLS [19,20] uses diffusion to determine the size distribution of particles. Each particle acts as a scattering center and if several particles are within the sample volume, the scattered waves will interfere from destructive to constructive at a certain fixed point. But the relative positions of the particles change due to diffusion, leading to changing interference and a stochastically fluctuating intensity signal at the fixed point. The speed of diffusion and thereby the rate of detected intensity change, which can be transformed into an autocorrelation function, depends on the size of the particles in the sample. A distribution of diffusion coefficients can be calculated from the autocorrelation function by means of an inverse Lapace transformation. Assuming spherical symmetry, these coefficients can be expressed as hydrodynamic radii according to the Stokes–Einstein relation.

DLS experiments were performed on a laboratory built goniometer with an $Ar^+$ Laser (BeamLok 2060-5S, Spectra Physics, Darmstadt, Germany) at a wavelength of 514 nm and 400 mW output which was reduced by a beam splitter to about 200 mW. The detection optics consisted of single mode fibers (OZ from GMP, Zürich, Switzerland), an ALV/SO-SIPD/DUAL photomultiplier with pseudo cross correlation setup (ALV, Langen, Germany), and an ALV-5000 multiple tau digital correlator with ALV-5000/FAST digital correlator for fast expansion (both ALV, Langen, Germany). Two polarizers, one in front and one behind of the sample cell, guarantee that only vertically polarized light impinges the sample and only vertically polarized light reaches the detector. The program ALV-5000/E (ALV, Langen, Germany) was used for data acquisition.

All experiments were performed at a scattering angle of 90°. LDL was diluted to 0.25 mg/ml with PBS pH 6.0 and the reaction was started by HOCl addition. Samples were incubated at 37°C for 30 min to assure that the aggregation process was completed. Autocorrelation functions were measured 10 times for 30 s at 37°C and the size distributions were calculated using the Optimized Regularization Technique (ORT) software package [21] developed at the Institute of Physical Chemistry, University of Graz, Austria.

### 2.4. Determination of TBArS by high performance liquid chromatography (HPLC)

LDL was diluted to 0.25 mg/ml with PBS and 2.0 μg/ml of MPO was added (except control). The reaction was started by addition of 0.5 mM $H_2O_2$ at 25°C and was stopped with 0.15 M phosphoric acid after certain time intervals and immediately frozen at −20°C. MDA was determined as the thiobarbituric acid derivative using a slight modification of the method described by Wong et al. [22]. Because of the high dilution of MDA, 500 μl samples (instead of 50 μl plus 450 μl $H_2O$) were used.

### 2.5. Tryptophan fluorescence

LDL exhibits a fluorescence in the UV region due to the 37 tryptophan residues contained in apo B-100. LDL vitamin E fluorescence (Ex/Em = 290/323 nm) does not interfere with the tryptophan fluorescence even at high concentrations [23]. The LDL

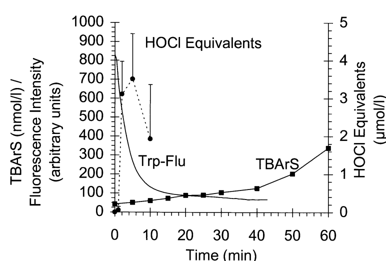


Fig. 1. Time course of equivalents formation of HOCl and comparison of tryptophan decomposition with TBArS formation during modification of LDL by MPO. HOCl equivalents were determined at 0.5 µg/ml MPO, 50 µmol/l $H_2O_2$, PBS pH 6.0, 25°C using the MCD assay. The number of independent experiments was seven (1 min: $n=3$). Tryptophan decomposition was determined fluorimetrically and TBArS formation by HPLC. Identical conditions were used for both experiments: 2.0 µg/ml MPO, 0.5 mmol/l $H_2O_2$, 0.25 mg/ml LDL, PBS pH 6.0, 25°C, $n=2$.

oxidation product di-tyrosine is also not likely to interfere, because it emits fluorescent light at a much higher wavelength (Em = 410 nm).

The decrease of LDL-bound tryptophan fluorescence in presence of MPO was measured on a Perkin Elmer LS 50 B Luminescence Spectrometer using the time drive method at an emission wavelength of 331 nm, with excitation set to 282 nm. For the direct comparison of tryptophan destruction with TBArS formation emission and excitation, slits were set to 5 nm, LDL was diluted to 0.25 mg/ml total mass with PBS (pH 6.0, 150 mmol/l NaCl, 10 mmol/l $NaH_2PO_4*H_2O$) and 2.0 µg/ml of MPO was added. The reaction was started by addition of 0.5 mmol/l $H_2O_2$ and the kinetics of modification were measured every 0.5 min at 25°C.

For the comparison of HOCl traps with MPO inhibitors, experiments were performed at a temperature of 37°C and concentrations of all components were reduced in order to get closer to in vivo conditions: 12.5 µg/ml LDL total mass in PBS pH 6.0 was mixed with 0.1 µg/ml MPO and HOCl traps and MPO inhibitors, respectively. The initial fluorescence intensity value was recorded (= 100%, Fig. 1) and subsequently the reaction was started by addition of 0.1 mmol/l $H_2O_2$ (zero time). After mixing for 0.1 min, the next data point was collected and then the kinetics of LDL modification were measured every 0.5 min with emission and excitation slits set to 15 nm. A Continuous curve was obtained from discrete time points by the standard curve smoothing function of the Microsoft Excel 7.0 software package.

The fluorometry of tryptophan destruction used in this study proved to be a suitable method for the kinetic determination of apo B-100 modification of LDL. A comparison of this method with UV absorption photometry [24] shows a good correlation. The high sensitivity of fluorometry allowed us to reduce the concentrations by one order of magnitude. Under these conditions, fluorescence changes due to light scattering are negligible, but the general kinetic pattern remained as in the previous study.

## 3. Results and discussion

### *3.1. HOCl as a modifying agent*

The relevance of MPO and HOCl in the pathogenesis of atherosclerosis is currently not completely understood, but it appears likely that HOCl causes modification of both the protein and lipid moiety of LDL, via a number of different oxidation and chlorination reactions, leading to preferential uptake of LDL by macrophages.

Estimates of HOCl formation by neutrophils, as measured by its reaction with nitrogenous compounds to form stable chloramines [25,26] or by the chlorination of 1,3,5-trimethoxybenzene [27], have varied from 28 to 72% of the oxygen consumed depending on the stimulus [26,28]. It has been emphasized that these are minimum values which do not take into account the competing effect of proteins and other cellular HOCl scavengers in the assay system [27]. In one study, $5\times10^6$ human neutrophils ingesting opsonized zymosan produced 88.3 nmol of HOCl during a 2 h incubation at 22°C [28]. In our study, a concentration dependent increase in HOCl production by the $MPO/H_2O_2/Cl^-$ system was found using the MCD assay for the determination of HOCl formation: at a concentration of 50 µmol/l $H_2O_2$ and an enzyme concentration of 0.5 µg/ml MPO, a maximum of about 3.5 µmol/l HOCl equivalents appeared after 5 min (Fig. 1). After 10 min, the level of HOCl decreased to about 2 µmol/l.

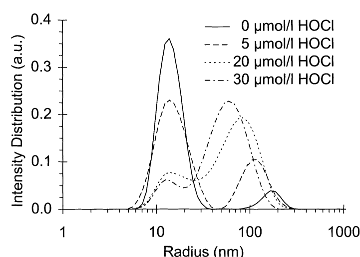


Fig. 2. DLS of native LDL and LDL treated with increasing concentrations of HOCl. Size distribution of LDL weighted by the scattering intensity Di($R$) vs. hydrodynamic radius $R$ determined by DLS experiments. The calculation assumes spherical symmetry. Native LDL (0.25 mg/ml) and LDL incubated for 30 min with 5, 20 and 30 μmol/l HOCl at 37°C are shown.

### 3.2. Aggregation of LDL

Treatment of native LDL with reagent HOCl in the concentration range of 5–30 μmol/l led to major alterations in the overall structure of LDL as detected by DLS (Fig. 2). A mean radius of 13.9 nm was found for native LDL with a second small population of an impurity at 170 nm. Formation of aggregates with a radius of about 120 nm was already found upon addition of the lowest HOCl concentration used (5 μmol/l), while the corresponding peak of the native LDL population decreased in height, but remained detectable at the same particle size. Further increase of the HOCl concentration (up to 30 μmol/l) resulted in a continued decrease of the native LDL peak while the corresponding aggregate peak increased dramatically in height. The position of the aggregate peak showed a shift to smaller radii from about 120 nm down to about 60 nm. The peak widths do not necessarily give a direct measure for the actual width of the distribution. Especially the distribution of native LDL might be sharper than found, but cannot be resolved sufficiently by means of DLS. Nevertheless, this method provides peak positions quite accurately and gives only a slight trend to excessive values. The peak height corresponds to the scattering intensity which increases with $R^6$ while the particle mass increases with $R^3$. According to this behavior, the small population of native LDL found at 170 nm is an impurity or aggregate of very low concentration. Furthermore, the growing height at decreasing aggregate peak radii indicates increasing concentrations of aggregates which become progressively compact. This behavior is in agreement with a reaction-limited aggregation mechanism.

### 3.3. Protein modification versus lipid modification

Since the MPO/$H_2O_2$/$Cl^-$ system causes modification of a variety of different proteins, including apo B-100 [8,29–31], and since significant alterations in the overall structure of the LDL particle were found (Fig. 2), two approaches were used for their characterization: decomposition of tryptophan residues (as a sensitive and virtually continuous method to study the overall apo B-100 oxidation) was determined fluorimetrically and the rate of TBArS formation (as end products of lipid peroxidation) was investigated by HPLC (Fig. 1).

While tryptophan, the most susceptible amino acids with respect to oxidation by HOCl, was largely destroyed in less than 10 min, the kinetics of TBArS formation showed a lag phase with minor LDL lipid modification, followed by a slow increase of TBArS formation. The onset of significant TBArS formation commenced not earlier than after 40 min, i.e. there was a gap of about 30 min between both phenomena (Fig. 1). Studies investigating lipid peroxidation of LDL either by monocytes and neutrophils [32] or by hypochlorite and the MPO/$H_2O_2$/$Cl^-$ system [8], respectively, also indicated lipid peroxidation as a minor process. Intensive lipid peroxidation of LDL and liposomes induced by exogenous HOCl generated by the MPO/$H_2O_2$/$Cl^-$ system was only observed by Panasenko et al. [33] but in these experiments rather high $H_2O_2$ concentrations (4 mmol/l) were used.

### 3.4. Evaluation of the kinetic curves of tryptophan degradation

Monitoring of tryptophan fluorescence appeared to be a sensitive method to study the kinetics of LDL oxidation under near in vivo conditions. Therefore, this method was used to determine the effectiveness of inhibitors that block production of HOCl or compounds that act as HOCl traps. Common to all experiments with the complete MPO/$H_2O_2$/$Cl^-$ system was a general kinetic pattern as displayed in Fig. 3. Only the complete MPO/$H_2O_2$/$Cl^-$ system with both substrates, $Cl^-$ and $H_2O_2$, was able to induce

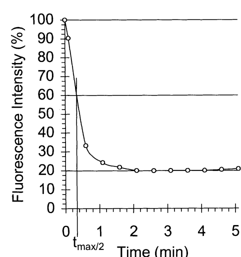


Fig. 3. Time course of tryptophan fluorescence changes and calculation of $t_{max/2}$. 12.5 μg/ml LDL in PBS pH 6.0 at 37°C was mixed with 0.1 μg/ml of MPO and the reaction was started by addition of 0.1 mmol/l $H_2O_2$. $t_{max/2}$, a unit for the velocity of tryptophan oxidation in MPO-induced LDL modification, is the time needed to observe a reduction in fluorescence of 50% of the difference between initial and residual fluorescence intensity.

significant LDL–tryptophan modification, indicating that HOCl was the modifying agent. The typical course of tryptophan fluorescence changes was highly reproducible and generally consisted of a rapid decrease, leveling off into a bottom value. Control experiments without LDL showed that the residual fluorescence is contributed by impurities contained in the buffer and the other components of the reaction mixture. Thus virtually all tryptophan residues are oxidized under these conditions. Tryptophan destruction appeared to be a fast process, largely completed in less than 3 min in the absence of inhibitors.

The parameter 'half-time' ($t_{max/2}$ [34]) was used to characterize the fluorescence changes in quantitative terms for practical purposes. It is defined as the time needed to observe a reduction in fluorescence of 50% of the difference between initial and residual fluorescence intensity. $t_{max/2}$ may be considered as a parameter for the velocity of tryptophan destruction in MPO-induced LDL modification and its kinetics are not supposed to follow a first order law. Evaluation of $t_{max/2}$ is shown in Fig. 3. The final fluorescence decrease was nearly identical for all inhibitors (except azide) no matter which concentration was used. Differences occurred only with respect to the value of $t_{max/2}$.

### 3.5. Intervention strategies

The only source of MPO are phagocytes (neutrophils and monocytes/macrophages) and the importance of macrophages in the pathogenesis of atherosclerosis has been pointed out repeatedly (for a review, see Roessner [35]). Quite in contrast to monocytes/macrophages, the participation of neutrophils in the pathogenesis of atherosclerosis is only poorly studied, but it is known that PMNs can damage endothelium in vivo and in vitro [36,37] and possibly oxidize LDL already in the blood circulation [38,39]. For the prevention of MPO-induced modification of LDL in the development of atherosclerosis, a non-toxic and potent inhibitor for this enzyme needs to be found and therefore several strategies for potential therapeutic interventions on neutrophil function have been proposed [40].

Our study focused on the interference of agents with two major points of the inflammatory process: inhibition of neutrophil function by preventing oxidant production by MPO and trapping of products of activated neutrophils, such as MPO-derived HOCl. Therefore concentrations of all components were reduced dramatically to get closer to in vivo conditions and MPO-induced destruction of LDL-bound tryptophan was monitored sensitively via its fluorescence.

### 3.6. Inhibition of LDL-bound tryptophan destruction by prevention of oxidant production by MPO

#### 3.6.1. ATZ, cyanide and azide

The porphyrin inhibitor ATZ was found to be a weak inhibitor of MPO-induced LDL modification compared to azide and cyanide. It showed only a low retardation of the kinetics in terms of an slightly increased $t_{max/2}$ in the concentration range from 2.5 to 50 μmol/l (Fig. 4A). It was suggested that ATZ, which also functions as a catalase and glutathione peroxidase inhibitor [41], causes suicide inactivation of lactoperoxidase by covalent binding to the protein moiety rather than to the heme groups [42].

Azide and cyanide were found to be quite effective in the modulation of MPO-induced tryptophan destruction (Fig. 4A): cyanide (0–50 μmol/l) strongly delayed LDL modification in the higher concentration range from 20 to 50 μmol/l. Azide showed the same general kinetic course as described for the other inhibitors, but additionally a concentration dependent reduction of the extent of total tryptophan destruction was found, possibly due to inactivation of the enzyme. At an azide concentration of 50 μmol/l, the value of tryptophan destruction only reached

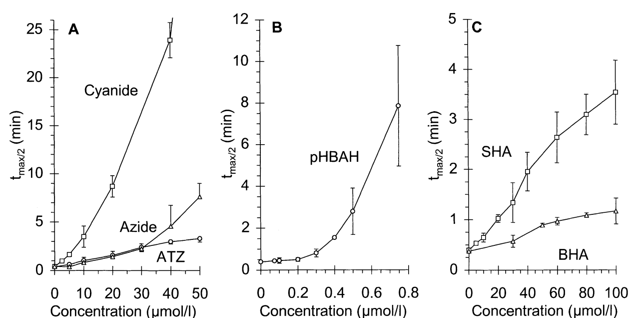


Fig. 4. The effect of MPO inhibitors on the modification of LDL by the $MPO/H_2O_2/Cl^-$ system. 12.5 μg/ml LDL in PBS pH 6.0 at 37°C was mixed with 0.1 μg/ml of MPO and varying concentrations of MPO inhibitors. A: ATZ, potassium cyanide and sodium azide. B: pHBAH. C: SHA and BHA. Reactions were started by addition of 0.1 mmol/l $H_2O_2$ and $t_{max/2}$ for the inhibition of LDL modification was determined (50 μmol/l KCN: $t_{max/2} = 62.85$ min). Data are represented as means and S.D. of three experiments (0.4 μmol/l pHBAH: $n = 2$).

68% when compared with the total fluorescence decrease in absence of azide (data not shown). At the same azide concentration, a significant retarding effect on MPO-induced LDL modification was found: $t_{max/2}$ was increased by a factor of about 18, when compared to the control in absence of azide (7.6 min versus 0.42 min). In cases of such a dual effect (reduction of velocity and of the extent of the reaction), $t_{max/2}$ will not represent the full potency of the inhibitor, rather the efficacy would be underestimated. In conclusion, all three heme–enzyme inhibitors have little therapeutic relevance, due to their non-specific mechanisms of action on all heme proteins.

#### 3.6.2. Aryl hydroxamic acids and pHBAH

Among the two aryl hydroxamic acids SHA and BHA, which are regarded as specific inhibitors of MPO [11,43], SHA was significantly more effective than BHA (Fig. 4C). At a concentration of 100 μmol/l BHA, $t_{max/2}$ was decreased by a factor of 3.2 compared to SHA (3.54 min versus 1.17 min). In another study, binding of SHA was found to be about three orders of magnitude stronger than that of BHA [11]. These differences in SHA and BHA binding to MPO were also reflected in our experiments, but to a lesser extent.

In our study, the suicide inhibitor pHBAH was found to be considerably more potent than the aryl hydroxamic acids SHA and BHA. It showed the strongest retarding effect on MPO-induced tryptophan destruction, even at concentrations between 0.3 and 0.75 μmol/l (Fig. 4B). Kettle et al. [12], who studied the mechanism of the inhibitory effect of benzoic acid hydrazides, suggested that pHBAH may be oxidized by MPO to a radical that reduces the enzyme to its ferrous intermediate [44]. The potent MPO inhibitor *p*-aminobenzoic acid hydrazide [44] was excluded from our studies since preliminary experiments indicated that it directly interacts with LDL (data not shown).

### 3.7. Inhibition of LDL modification by trapping the products of activated neutrophils

Concentrations of methionine, taurine and glycine in the range from 10 μmol/l up to 40 μmol/l were found to inhibit tryptophan destruction in terms of an increased $t_{max/2}$. At a concentration of 40 μmol/l, which is three orders of magnitude below the physiological value of taurine in leukocytes [13], $t_{max/2}$ was increased by a factor of about 44 compared to the control in absence of taurine (18.1 min versus 0.41 min). Methionine showed an even stronger retarding effect on MPO-induced LDL modification among the HOCl traps used (factor 103), while glycine was the least effective (factor 13, Fig. 5).

All results of the intervention experiments are summarized in Table 1. The concentrations of individual MPO inhibitors and HOCl traps which caused an arbitrarily chosen 10-fold increase of $t_{max/2}$ are shown ($10 \times t_{max/2}$). These values were estimated by graphical interpolation from the figures.

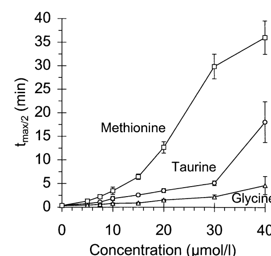


Fig. 5. The effect of the amino acids methionine, taurine and glycine on the modification of LDL by the $MPO/H_2O_2/Cl^-$ system. 12.5 μg/ml LDL in PBS pH 6.0 at 37°C was mixed with 0.1 μg/ml of MPO and varying concentrations of methionine, taurine and glycine, respectively. Reactions were started by addition of 0.1 mmol/l $H_2O_2$ and $t_{max/2}$ for the inhibition of LDL modification was determined. Data are represented as means and S.D. of three experiments.

Table 1
Concentrations of individual inhibitors causing a 10-fold increase of $t_{max/2}$

| | Inhibitor | | | | | | Trap | | |
|---|---|---|---|---|---|---|---|---|---|
| | BHA | SHA | ATZ | Azide | Cyanide | pHBAH | Glycine | Taurine | Methionine |
| $10\times t_{max/2}$ (μmol/l) | $>100$ | $>100$ | $>50$ | 38.3 | 11.2 | 0.6 | 35.2 | 23.5 | 10.1 |

Values were estimated by graphical interpolation from the figures.

Taurine is present as a free amino acid in many animal tissues and is not incorporated into proteins nor into other macromolecules. Taurine is exceptionally abundant in the cytosol of inflammatory cells and especially in neutrophils. In leukocytes, a concentration of 35 mmol/l has been reported [13]. Taurine protects neutrophils from self-destruction during processes that generate oxidants. The major function of taurine in leukocytes is to trap chlorinated oxidants (HOCl), while in other cells it possibly acts as an enantiostatic agent [45].

Lampert and Weiss [46] have concluded that HOCl generated by the $MPO/H_2O_2/Cl^-$ system of intact human neutrophils is able to chlorinate taurine to its relatively stable monochloroamine derivative (TauNHCl). The ability of taurine to protect biomembranes attacked by HOCl was investigated by Koyama et al. [47] using canine erythrocytes which have been pre-treated with HOCl. Of the amino acids tested, taurine was the most effective in inhibiting attack by HOCl followed by glycine and alanine. During the incubation of HOCl-treated erythrocytes with different concentrations of taurine, an appreciable amount of TauNHCl was detected. The favored reaction with HOCl complies with early reports of Grisham et al. [48], describing rapid reaction of taurine with HOCl to form relatively non-toxic taurine chloroamines, which have still chlorinating ability, but at a much slower rate. We conclude from this study that endogenous taurine is a powerful trap of HOCl, which is able to control the biological activity of chloride dependent MPO.

The inhibitory effect of methionine in terms of an increased $t_{max/2}$ is more than twice as high as the effect of taurine. In accordance with our data, Winterbourn [49] found that methionine is a more efficient competitor for the chlorination of MCD by the $MPO/H_2O_2/Cl^-$ system than taurine. This can be explained by the fact that methionine is able to bind up to 3 mol of HOCl. Nevertheless, the results on taurine might be more important because of its known role in neutrophils and of the high concentration to be expected in an inflammatory focus.

### 3.8. *Relevance for the in vivo situation*

The results of this study contribute to the potential therapeutic impact of HOCl traps and especially taurine in vivo, by protecting certain targets from attack by potent chlorinated oxidants [50] due to their generally assumed low toxicity. The trapping of HOCl may be important under pathological conditions in order to plan rational therapeutic strategies in particular for retarding inflammatory processes by modulation of the oxidative burst of neutrophils and monocytes/macrophages. Inhibitors of MPO may have an even greater potential to block oxidative reactions of HOCl than traps such as taurine. Large concentrations of HOCl traps would have to reach sites of inflammation for them to compete with the endogenous targets for HOCl, whereas only very low concentrations of a MPO inhibitor would be required to irreversibly inhibit the enzyme. Thus development of MPO inhibitors with high specificity and low toxicity is a challenging goal for the future. A major issue would be the investigation of the effect of the compounds used on LDL oxidation by phagocytes.

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