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Sulphoacetaldehyde as a product of taurine chloramine peroxidation at site of inflammation

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Summary. It has been reported, that sulphoacetalhehyde is formed in the fagocytozing PMNs and its production is taurine monochloramine mediated. Since H_2O_2 and secreted MPO are present in the medium the non- and enzymatic peroxidation of taurine of its mono- and dichloramines were examined within the pH range 5.3–7.4. The formation of sulphoacetaldehyde was observed in nonenzymatic hydrolysis of taurine N,N-dichloramine (pH 5.3) as well as for monochloramine at pH 7.4. It was found also that its formation was accelerated in the presence of H_2O_2, in the MPO/H_2O_2 and in the full system containing Cl^-. Additionally it was shown that also horseradish peroxidase (HRP) could catalyze sulphoacetaldehyde production. The sulphoacetaldehyde formation in the examined systems was confirmed with the use of ^{1}HNMR spectra of separated 2,4-dinitrophenylhydrazone derivative. Our results suggest that both non- and ezymatic processes could contribute to the sulphoacetaldehyde formation at site of inflammation.

Keywords: Amino acids – Sulphoacetaldehyde – Taurine dichloramine – Myeloperoxidase – Horseradish peroxidase

Introduction

Neutrophils (PMNs) during phagocytosis release reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2) and superoxide radical ($HO_2^{\cdot}$). In the presence of hydrogen peroxide, chloride ions in the medium are oxidized to hypochlorite (HOCl/OCl^-) with neutrophil enzyme – myeloperoxidase (MPO). The reactive intermediate – hypochlorite forms less reactive chloramines when reacted in the medium with amino groups of proteins and amino acids. It was shown that N-chloramines of α-amino acids are unstable and decompose to aldehydes with consecutive decarboxylation (Thomas et al., 1986; Zgliczyński et al., 1971). Conversely, taurine (2-aminoethanesulphonic acid), which is present in most mammalian tissues in

mM concentration forms in physiological conditions much more stable monochloramine. The taurine monochloramine could, in turn, dismutate to dichloramine and taurine when pH of the fagolysosome is temporarily decreased. Recently, it has been shown that taurine monochloramine produced by the activated PMNs slowly decomposes to sulphoacetaldehyde and that the process is enhanced in the presence of liver homogenate (Cunningham et al., 1998). Thus the oxidative deamination of the physiological low molecular amines may lead to the formation of aldehydes which are involved in processes of lipoprotein modification (Paliński et al., 1996; Hazen et al., 1997). In the locus of inflammation the ROS and chloramine production is followed by the secretion of MPO into the medium. Therefore, as it was suggested (Olszowski et al., 1999a), it seems possible that taurine derivatives could react with an excess of H_2O_2 as well as interact with the secreted MPO in the presence of chlorides. In the present report we examined the sulphoacetaldehyde formation in the model reactions in which taurine, its N-mono- and N,N-dichloroderivatives were oxidized nonenzymatically (H_2O_2) or enzymatically by myeloperoxidase (MPO)/H_2O_2 and horseradish peroxidase (HRP)/H_2O_2 systems.

Material and methods

Acetonitrile, methanol, methionine, taurine, HCl, and ferric chloride were obtained from Fluka Chemie AG (Switzerland); sodium dibasic and monobasic phosphate, sodium hypochlorite and hydrogen peroxide from Aldrich (Germany); horseradish peroxidase VI (E.C. 1.11.1.7), myeloperoxidase (from human leucocytes E.C.1.11.1.7), catalase (from bovine liver E.C. 1.11.1.6), N-methyl-benzothiazolone hydrazone (MBHT), cysteic acid, NaOH from Sigma (USA). NaOCl was prepared prior to use from 0.5 M stock solution and standardized iodometrically. H_2O_2 solutions were prepared from 30% solution prior to use and standardized manganometrically. 5 mM sulphoacetaldehyde standard solutions were obtained in the reaction of cysteic acid with 5 mM HOCl in the phosphate buffer at pH 5.3 within 24 h at room temperature. Stoichiometric conversion of cysteic acid was confirmed with MBTH method (Paz et al., 1965). All solutions were prepared using water obtained from EASY pure RF Compact Ultrapure Water System, Barnsted, USA (18 mΩ).

Chlorination of taurine

10 mM taurine N-monochloramine stock solution was prepared prior to use by addition of 5 mL of 20 mM HOCl in 0.2 M phosphate buffer pH 7.4, in 20 μl portions under stirring to 5 mL of 20 mM taurine in 0.2 M phosphate buffer pH 7.4. 10 mM taurine N,N-dichloramine was prepared by adding 1.5 mL of 130 mM HOCl in 0.02 M phosphate buffer in one portion to 8.5 mL of 20 mM taurine in 0.2 M phosphate buffer pH 5.3. Mono- and dichloramine concentrations were determined spectrophotometrically at 252 nm and 300 nm (ε = 415 $M^{-1}cm^{-1}$, 300 $M^{-1}cm^{-1}$, respectively).

Production of sulphoacetaldehyde

Sulphoacetaldehyde was produced: (A) nonenzymatically (H_2O_2) or (B) using MPO/H_2O_2 or HRP/H_2O_2 systems. (A) – 5 mM taurine mono- or dichloramine in appropriate

phosphate buffers (pH 7.4 or 5.3) were incubated with H_2O_2 (5–10 mM) for 168 h at room temperature. To stop the reaction 300 μl samples of the incubation mixtures were added to the 200 μl water solution containing catalase (decomposition of an access H_2O_2) and 5 mM methionine (mono- or dichloramine decomposition). Aliquots (50–200 μl) of the final solution were used for aldehyde assay with use of N-methyl Benzothiazolone Hydrazone (MBHT) method (Paz et al., 1965) and HPLC assay. (B) 5 mM taurine, taurine monochloramine and MPO (A_{424}/A_{280nm} = 0.7) or 1.3 μM horseradish peroxidase (HRP) in 0.1 M appropriate phosphate buffers (pH 7.4 or 5.3) with or without 0.2 M sodium chloride were incubated with 5 mM H_2O_2 for 168 h at room temperature. Hydrogen peroxide solution was added in 50 μl portions to keep its maximal concentration below 1 mM. After each portion samples were incubated 20 min to complete taurine monochloramine formation. Providing the best conditions for taurine monochloramine formation, initial concentrations of reactants follow the formula $[H_2O_2]/[Cl^-][H^+] = 800$ in the examined enzymatic systems. To stop the reaction 300 μl samples of the incubation mixtures were added to 200 μl solution containing catalase and 5 mM methionine. Aliquots of the final solution were used for aldehyde assay with use of MBHT. Additionally the modified HPLC assay (Anderson et al., 1997) of resulting cyanine dye was used to confirm the specific sulphoacetaldehyde formation in the reaction mixtures.

Sulphoaceticaldehyde 2,4-phenylhydrazone synthesis

50 mL of the reaction mixtures (A) or (B) after 48 h of incubation were mixed with 5 mL 0.2% 2,4-dinitrophenylhydrazine (DNPH) in 2 M HCl and then incubated for 2 h. Solutions were applied to the column filled with SiO_2 and adsorbed material and dried at 37°C under vacuum. A fraction enriched in the resulting 2,4-phenylhydrazone was eluted with methanol, concentrated under vacuum and the final product was separated using thin layer chromatography (SiO_2).

Spectrophotometric measurements were performed with a Hitachi U-2000 Spectrometer (Japan). ^{1}HNMR spectrum was acquired on a Bruker AMX 500 spectrometer, HPLC assays using of HPLC Kontron system on Nucleosil C-18 (4.6 × 250 mm) column in isocratic conditions (1:1 v/v 5 mM phosphate buffer pH 7.0 and acetonitrile, flow 0.8 mL/min, detection at 598 nm).

Results

The formation of sulphoacetaldehyde in all presented sytems was confirmed with HPLC method. The HPLC profiles of its cyanine derivative showed the same peak (5.8 min) as observed for the control solution of the sulphoacetaldehyde formed from cysteic acid (data not shown). Additionally the sulphoacetaldehyde formation was confirmed by means of ^{1}HNMR spectra of its 2.4-dinitrophenylhydrazone derivative isolated from the reaction mixtures. The ^{1}HNMR spectrum in Fig. 1 reveals the presence of the doublet (CH_2) at 3.517 and 3.538 ppm and triplet (B) for (–CH = N–) at 8.033, 8.045 and 8.058 ppm – the system of signals characteristic of the aldehyde derived part of 2,4-dinitrophenylhydrazone.

It has been recently proved that sulphoacetaldehyde is a product of taurine monochloramine decomposition at pH 7.4 and that the starting mM taurine monochloramine concentration results in the final μM concentration of the aldehyde within 8 h of the reaction course (Cunningham at all., 1998). Since not only taurine monochloramine but also dichloramines and H_2O_2 are the products of the neutrophile (PMN) respiratory burst it was reasonable

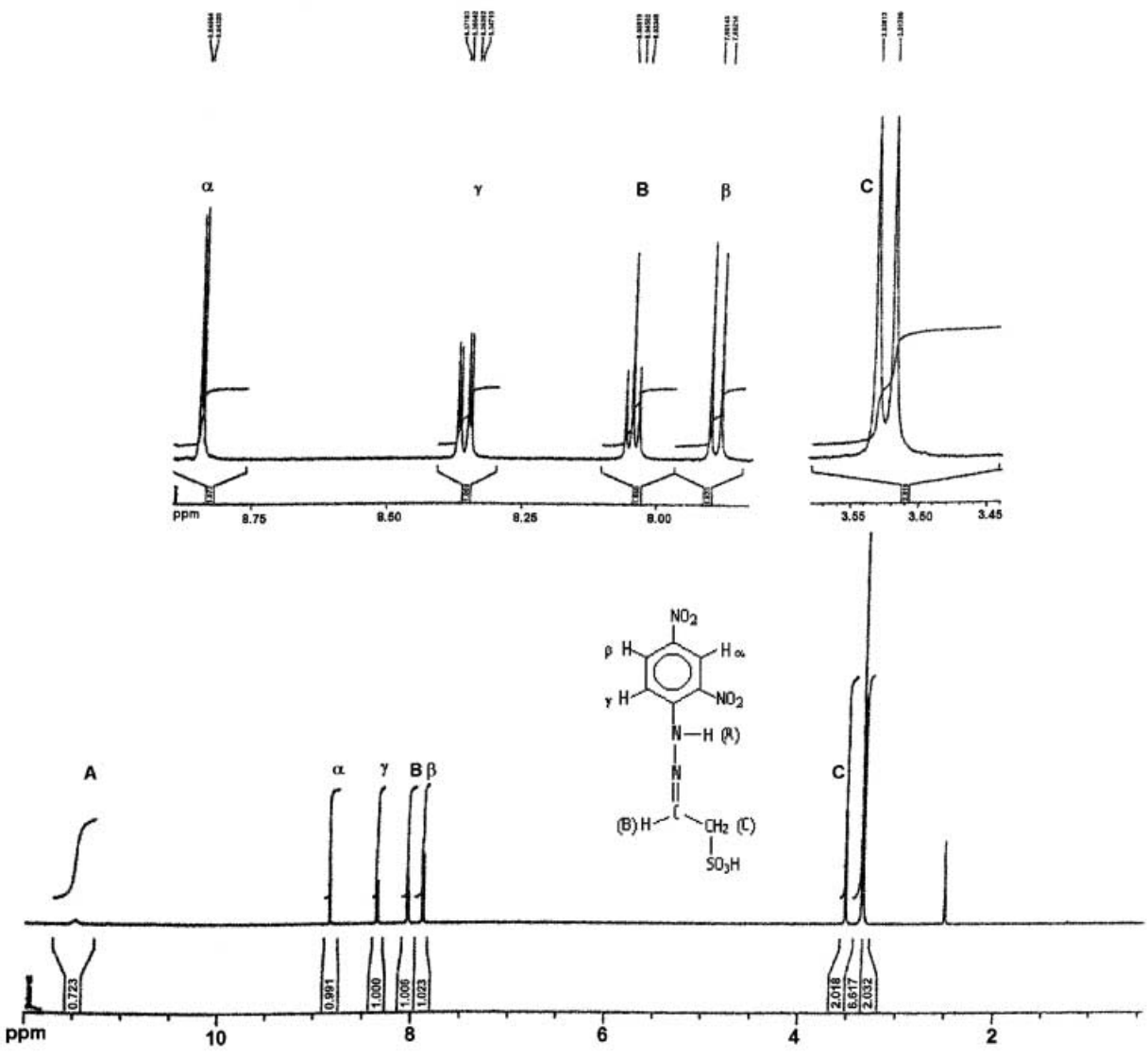

Fig. 1. [1]HNMR spectrum of the 2,4-dinitrophenylhydrazine derivative of sulphacetaldehyde. Sample contained 1 mg of 2,4-dinitrophenylhydrazone derivative of sulphoacetaldehyde in 1 mL of DMSO. Other details in Methods section

to check whether sulphoacetaldehyde could be formed in the H_2O_2 presence from the both taurine mono- and dichloramines. Data in Fig. 2 suggest that the sulphoacetaldehyde production from taurine dichloramine is more efficient than that observed for taurine monochloramine in the systems with or without H_2O_2. It is also shown that the production of sulphoacetaldehyde from taurine dichloramine is accelerated in the presence of H_2O_2 and depends on its concentration. This effect is profound for the first 48 h of the reaction course. The sulphoacetaldehyde depletion, observed for longer incubation times, may be interpreted in terms of further H_2O_2 – mediated aldehyde group oxidation. Sulphoacetaldehyde is also produced in the myeloperoxidase

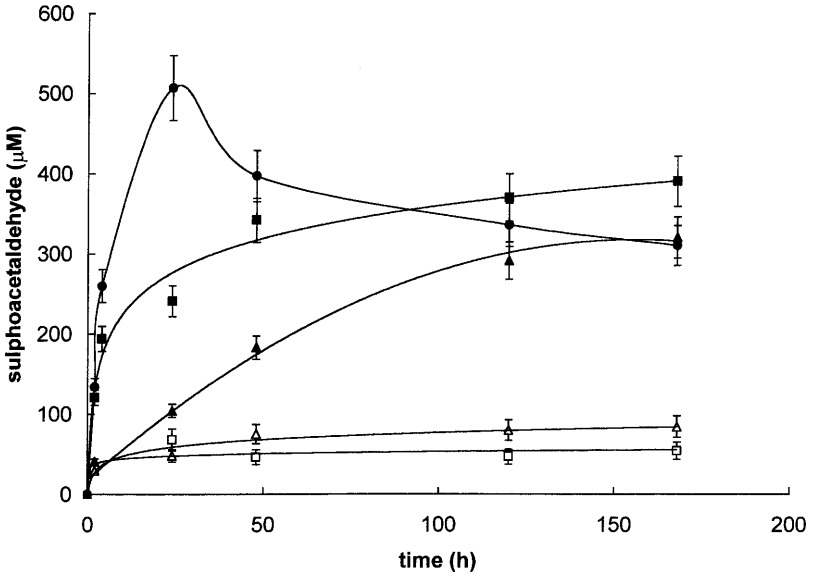

Fig. 2. Sulphoacetaldehyde formation in the nonenzymatic systems. Samples contained 5 mM taurine monochloramine (△), 5 mM H_2O_2 and monochloramine (□) in the 0.2 M phosphate buffer pH 7.4; 5 mM taurine dichloramine (▲), 5 mM taurine dichloramine with 5 mM (■) or 10 mM H_2O_2 (●) in the 0.2 M phosphate buffer pH 5.3

(MPO)/H_2O_2/Cl^- system at pH 5.3 (Fig. 3). The formation of the stoichiometric amounts of taurine monochloramine within the first hour of the reaction was monitored with the increase of the absorbance at 250 nm, and its complete dismutation to dichloramine derivative within 24 h was observed (data not shown). Since the formation of sulphoacetaldehyde (Fig. 3) is proceeding for longer than 24 h it may be concluded that its synthesis in the system is partially dichloramine mediated and has the nonenzymatic character. It was also proved that in the medium without chlorides sulphoacetaldehyde could be produced from taurine in the MPO/H_2O_2 and HRP/H_2O_2 systems. The highest yield of the sulphoactaldehyde formation in the enzymatic systems was observed at pH 7.4 for MPO/H_2O_2 and at pH 6.6 for HRP/H_2O_2, respectively (Fig. 4, 5). The latter result correlates with the maximal activity of HRP in the examined pH range. It was also found at (data not shown) that in these enzymatic systems in the medium without Cl^- the rate of aldehyde production is comparable for taurine and taurine monochloramine. These facts taken together suggest that in the MPO/H_2O/Cl^- system the sulphoacetic aldehyde is produced in several separate reactions – nonenzymatic; taurine mono- and dichloramine hydrolysis, H_2O_2 mediated taurine, taurine mono- or dichloramine oxidation and enzymatic in the MPO/H_2O_2 and HRP/H_2O_2 systems.

Discussion

The present results provide evidence that sulphoacetaldehyde is produced not only from taurine monochloramine but also from dichloramine and that the

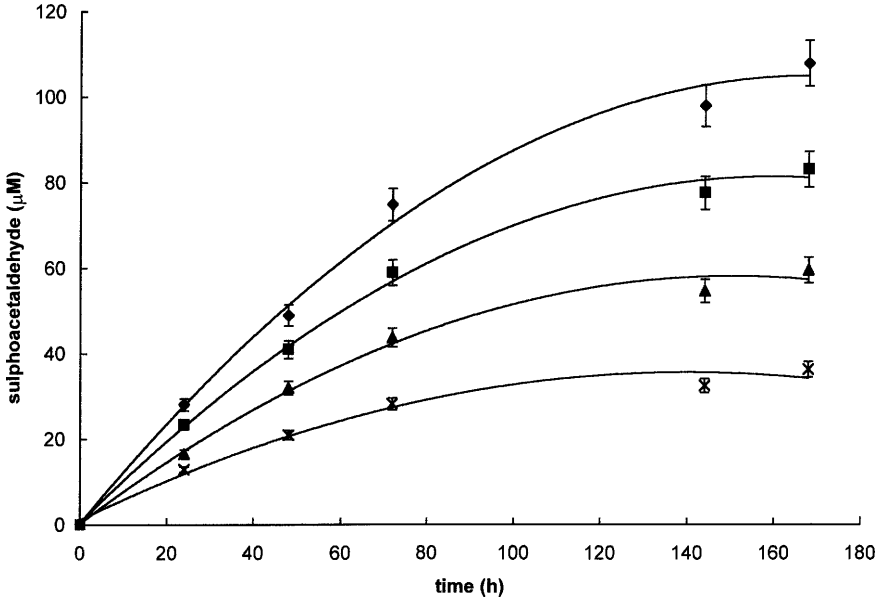

Fig. 3. Sulphoacetaldehyde formation in the MPO/H_2O_2/Cl^- system. Samples contained in 0.2 M phosphate buffer pH 5.3 with 0.2 M NaCl 200 nM MPO, 5 mM taurine and 1 (×), 2 (▲), 3 (■), 4 (◆) mM H_2O_2

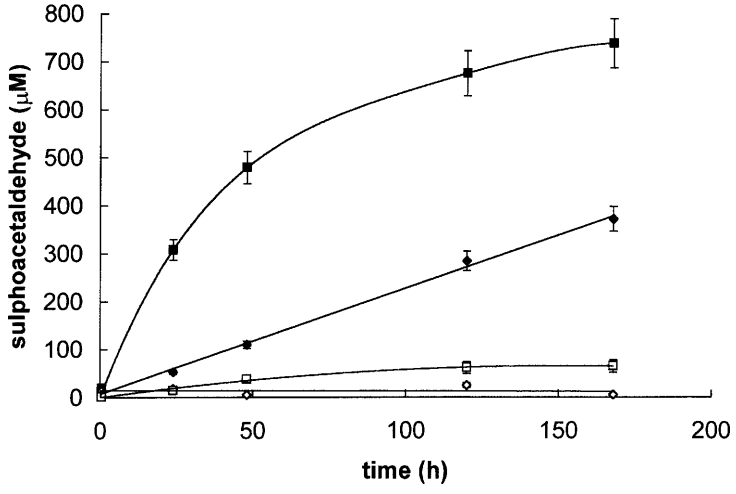

Fig. 4. Sulphoacetaldehyde formation in the MPO/H_2O_2 system. Samples contained 5 mM taurine and 4 mM H_2O_2 in 0.2 M phosphate buffer pH 7.4 with (■) and without (□) 200 nM MPO and in the 0.2 M phosphate pH 5.3 with (◆) or without MPO (◇)

latter process is accelerated in H_2O_2 presence. This effect suggests other than proposed mechanism for hydrolysis of monochloramine (Cunningham et al., 1998; Hazen et al., 1998) involving elimination of HCl with concomitant imine formation. It was demonstrated that taurine monochloramine spontaneously forms N-centered radicals (Hawkins et al., 1998) and is involved in the initiation of the deamination process. Moreover, it was shown that taurine

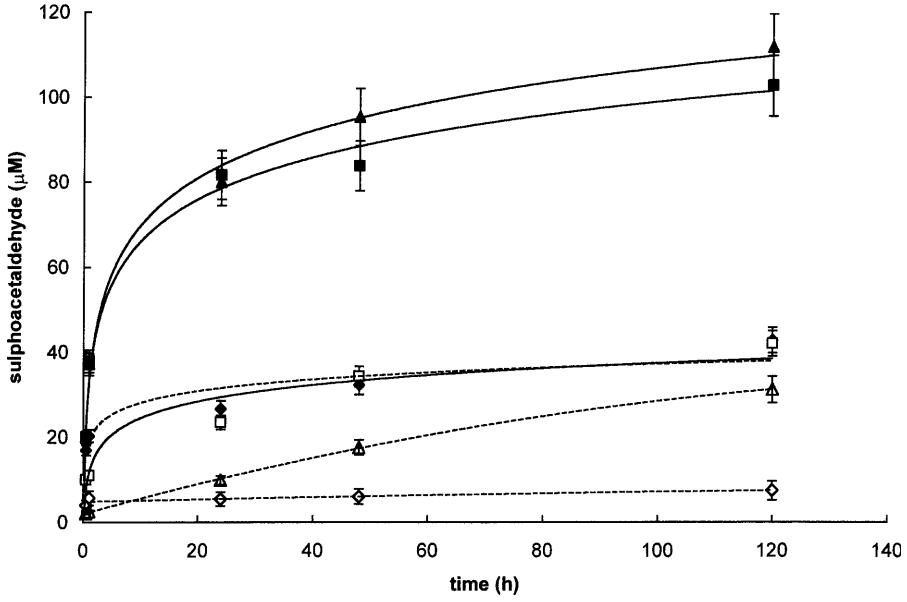

Fig. 5. Sulphoacetaldehyde formation in the HRP/H_2O_2 system. Samples contained 1.3μM HRP, 5 mM taurine, 5 mM H_2O_2 in 0.2 M phosphate buffers of pH 5.3 (◆), 6.6 (▲), 7.4 (■), and respectively samples in which HRP was omitted 5.3 (◇), 6.6 (△), 7.4 (□)

monochloramine with H_2O_2 but not separately form the efficient (iso)luminolic chemiluminescent system (Olszowski et al., 1999b). The latter results suggest that H_2O_2 oxidizes taurine chloramine to form the unstable intermediate which in turn efficiently oxidizes the (iso)luminol ring. The plausible radical mechanism involving hydrogen peroxide taurine mono- and dichloramine initiation is still under investigation.

In the acidic solution (pH = 5.3) the MPO/H_2O_2/Cl^- system catalyzes the monochloramine formation which via dichloramine is oxidized to sulphoacetaldehyde. Therefore the MPO in these conditions seems to serve as a source of reactant (dichloramine) for further nonenzymatic deamination. Similarly, in the neutral solution MPO/H_2O_2/Cl^- system catalyzes the taurine monochloramine formation that undergoes slow hydrolysis to the aldehyde.

Thus, our results also suggest that the presence of chlorides is not necessary for sulphoacetic production in the MPO/H_2O_2 system and that chloramines are not the only possible intermediates. In general, it seems possible that the tissue originated peroxidases present in the inflammation loci could catalyze the slow taurine or taurine monochloramine deamination. It seems probable that taurine and taurine monochloramine oxidation is initiated by oxoferryl MPO and HRP complexes although it is still unclear whether taurine or its chloroderivatives could serve as the true hydrogen donor. At present, only one example of the aliphatic amine is known which could serve as a true reactant in HRP peroxidation process (Sayre et al., 1996). The alternative mechanism (Hoogland et al., 1998) involving oxygen or superoxide mediated taurine oxidation could be excluded since H_2O_2 is crucial for the completion of the reaction and respectively the presence of SOD does

not influence the sulphoacetaldehyde formation (data not shown). In all examined system the conversion of taurine and its chloroderivatives leads to μM level of aldehyde concentration within 24 h. Our results suggest that all examined processes, enzymatic or nonenzymatic, could contribute to the sulphoacetaldehyde production in the inflammation loci. The processes can be of physiological significance in tissues where neutrophils accumulate (e.g. after γ ray irradiation or in chronic inflammation) due to the relatively high taurine content in these cells. On the other hand, it seems possible that sulphoacetaldehyde is formed in the acidic medium of PMN vacuoles as well in the neutral medium after the release of vacuolar content. Thus, in vivo, the sulphoacetaldehyde production could be accelerated during acidosis. In our opinion, the presented model reactions mimic local conditions in chronic inflammation with respect to the duration of the process and local oxidant concentrations.

Acknowledgements

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