Effectiveness of Taurine in Protecting Biomembrane against Oxidant

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The effect of taurine in protecting biomembrane attacked by hypochlorous acid (HOCl) was examined using canine erythrocytes which had been pre-treated with HOCl. In the treatment, most of the HOCl was consumed as a result of its reaction with a number of electrophilic substances, such as free amino groups ($-NH_2$) in the membrane, whereas hemoglobin inside the cells was not oxidized. The lysis of HOCl-treated erythrocytes was dependent on the concentration of HOCl and on the incubation time at 37 °C. Taurine inhibited the lysis at 37 °C in a dose dependent manner. During the incubation of HOCl-treated erythrocytes with taurine, an appreciable amount of monochlorotaurine (TauNHCl) was detected in the supernate. This suggests that taurine might remove the oxidized chlorine from HOCl-treated erythrocytes, resulting in the production of TauNHCl. The effect of taurine on the removal of Cl^+ moiety was further examined using Sepharose gel with free amino groups. Taurine removed Cl^+ moiety from HOCl-treated Sepharose gel, and the yield of TauNHCl depended on the concentration of taurine and the incubation time. These results indicate that taurine might inhibit the hemolysis by scavenging the oxidized chlorine moiety from the HOCl-treated erythrocytes. Inhibition of the HOCl-induced hemolysis was also observed with other amino acids. The concentrations of taurine, α-alanine, β-alanine and glycine required for 50% inhibition of the lysis were 18, 30, 34, and 40 mM, respectively; thus taurine was the most effective inhibitor of all the amino acids. These results clearly indicate that taurine could be effective in inhibiting the biomembrane damage caused by HOCl.

Keywords taurine; monochlorotaurine; amino acid; biomembrane; erythrocyte; biomembrane damage; lysis; oxidant; hypochlorous acid; protection

Taurine is a sulfur-containing β-amino acid present in high concentrations in animal tissues such as retina and neutrophils.[1] In these organs, various oxidants are generated both photolytically and enzymatically.[2–5]

Hypochlorous acid (HOCl), which is a powerful oxidant, is produced by the oxidation of chloride ions (Cl^-) catalyzed by myeloperoxidase in neutrophils.[6–8] HOCl can directly oxidize a variety of biologically significant substances, such as carbohydrates, nucleic acids, peptide linkages and amino acids. Taurine is thought to play a significant role in protecting neutrophils against oxidative attack resulting from excessive HOCl.[9,10] This protection is attributed to the effect of taurine as a trap for HOCl or for a competing amine that did not yield toxic N–Cl derivatives. Taurine effectively inhibited the lysis of human erythrocytes caused by HOCl generated in neutrophils.[11,12] This effect is also attributed to scavenging HOCl in the medium. Wright *et al.* reported the role of taurine as a scavenger of HOCl in biological systems.[13] HOCl is a potent oxidizing agent whose biocidal properties are well known, especially in the disinfection of municipal water systems. Kitano and Yoshimura reported that HOCl used to disinfect water in swimming pools caused corneal damage.[14] In addition, it has been reported that taurine is effective in healing corneal damage caused by HOCl in rabbits.[15]

In the present study, we examined the effect of taurine on biomembrane damage caused by HOCl using canine erythrocytes that had been pre-treated with HOCl. A new protection mechanism of taurine against HOCl attack will be discussed below.

Materials and Methods

Materials The taurine used in this study was a standardized product (51 A.M, No. 796) recognized by the Ministry of Welfare, which had been synthesized in our laboratory. L-Glycine, L-α-alanine, L-β-alanine, sodium hypochlorite solution (NaClO) and *N,N*-diethyl-*p*-phenylenediamine sulfate (DPD) were purchased from WAKO Pure Chemical Industries. Sepharose gel with free amino groups (EAH-Sepharose gel 4B) was purchased from Pharmacia LKB. These and the other chemicals used were reagent grade commercially available.

Cells Fresh blood from a healthy beagle dog was drawn into heparinized tubes. Red blood cells were separated by centrifugation at $550 \times g$ for 5 min and washed three times with 40 mM phosphate buffered isotonic saline solution (pH 7.4, PBS). The packed cells were then diluted with PBS to make a red blood cell suspension of 4×10^8 cells/ml. This erythrocyte suspension was kept at 4 °C and was used as the stock solution for the experiments.

Quantitative Determination of Hemolytic Behavior The amount of oxidized chlorines (Cl^+) was determined using the DPD ferrous titrimetric method.[16] Free available chlorine reacts instantly with DPD indicator to produce a red color. By subsequent addition of a small amount of iodide ion, monochloramine (–NHCl) reacts instantly with DPD indicator. Further addition of iodide ion to excess causes dichloramine ($-NCl_2$) to react with DPD indicator. In the titrimetric procedure, decolorization by standard ferrous ammonium sulfate (FAS) titrant is instantaneous, thereby enabling each step to be performed more rapidly.

One ml of HOCl solution at various concentrations was added to 1 ml of erythrocyte suspension (4×10^8 cells/ml) in a test tube at 4 °C. After treatment for 5 s, these erythrocytes were then separated by centrifugation at $550 \times g$ for 1 min and washed once with PBS in order to remove the free HOCl and the oxidized soluble substances such as a methemoglobin. The washed erythrocytes were then diluted with PBS to 4×10^7 cells/ml. HOCl-treated erythrocytes were stable for at least 1 h at 4 °C. HOCl-treated erythrocytes (10^7 cells/ml) were incubated with varying concentrations of taurine or other amino acids over a 4 h period at 37 °C. HOCl-induced hemolytic behavior was assayed by measuring the absorbance of released hemoglobin at 416 nm.

The percentage (I) of inhibition of the lysis by taurine and other amino acids after 4 h of incubation was calculated according to the formula:

$$I\,(\%) = \frac{T-E}{T-C} \times 100$$

where T and E are the percent of the lysis of HOCl-treated erythrocytes with and without amino acids, respectively, and C is the percent of the lysis of untreated erythrocytes.

Determination of Cl^+ Removed from HOCl-Treated Erythrocytes and from HOCl-Treated Sepharose Gel by Taurine To prepare the HOCl-treated Sepharose gel, 1 ml of the gel with from 7 μmol to 11 μmol of free amino groups was used. The Sepharose gel was washed three times and diluted ten times with PBS. One ml of 2.8 mM HOCl was added to 1 ml of the Sepharose gel suspension in a test tube at 4 °C. Since the amount of amino groups in the Sepharose gel was greatly in excess of

HOCl, a part of each amino group was oxidized by HOCl, thereby producing monochloroamino groups (–NHCl). HOCl-treated Sepharose gel was then separated by centrifugation at 550 × *g* for 5 min and washed three times with PBS. HOCl-treated Sepharose gel was diluted with PBS to the same concentration as before the HOCl-treatment. HOCl-Treated Sepharose gel suspension was kept at 4 °C.

HOCl-treated erythrocyte suspension (4×10^8 cells/ml) or HOCl-treated Sepharose gel suspension of 2 ml was incubated with 2 ml of PBS and 4 ml of varying concentrations of taurine over a 4 h period or a 2 h period at 37 °C.

The amount of TauNHCl that was yielded in the supernate by taurine scavenging Cl^+ was determined using the DPD ferrous titrimetric method[16] or by measuring the absorbance of TauNHCl at 252 nm.[13] TauNHCl was so stable that by measuring it we were able to determine the amount of Cl^+ moiety removed.

Results and Discussion

As shown in Fig. 1, the lysis of HOCl-treated erythrocytes was dependent on the concentration of HOCl and the incubation time. Although the highest concentration of HOCl (423 μM) caused the rapid lysis of erythrocytes, hemoglobin inside the cells did not change to an oxidized hemoglobin such as methemoglobin, judging from the absorption spectra. For concentrations below 213 μM, HOCl attacked the membrane of the erythrocytes, causing a gradual lysis. The lysis of HOCl-treated erythrocytes followed the swelling of the cells due to the acceleration of membrane permeability for water and ions.

HOCl is an exceedingly powerful oxidant known to react rapidly with amines ($R–NH_2$) and sulfhydryl-containing molecules (R–SH).[17] These reactions yield chloramines (R–NHCl) and disulfide compounds (R–SS–R) as follows:

$$R–NH_2 + HOCl \rightarrow R–NHCl + H_2O$$

$$2R–SH + HOCl \rightarrow R–SS–R + H_2O + H^+ + Cl^-$$

For instance, HOCl reacting with $R–NH_2$ molecules, such as taurine, glycine or alanine, led to the formation of the respective chloramines, as detected by ultraviolet spectrophotometry (λ_{max}, 252 nm).[13] In the same way, HOCl may attack and decompose $–NH_2$ and –SH groups on the membrane of the target cell, leading to lysis by denaturation of the membrane. Therefore, the lysis of erythrocytes may be due primarily to the gradual decomposition of amines ($–NH_2$) and sulfhydryl-containing molecules (R–SH) of membrane components. The lipid peroxidation of the membrane may also be responsible for the lysis.

The effect of taurine on HOCl-induced lysis is shown in Fig. 2. The gradual lysis of erythrocytes previously treated with HOCl was remarkably inhibited by the addition of taurine. This inhibition was dependent on taurine concentration, and complete inhibition of the lysis was attained at the highest concentration of 160 mM.

Previous reports have indicated that taurine effectively inhibits the lysis of human erythrocytes caused by HOCl generating in neutrophils, as well as the lysis caused by HOCl in a cell-free system.[11,12] This effect of taurine has been attributed mainly to scavenging HOCl in the medium. In our study, however, HOCl and other oxidized substances such as a methemoglobin in the medium were previously removed by washing HOCl-treated erythrocytes. Accordingly, our data suggest that taurine effectively inhibits the lysis by suppressing the denaturing process of the membrane attacked by HOCl.

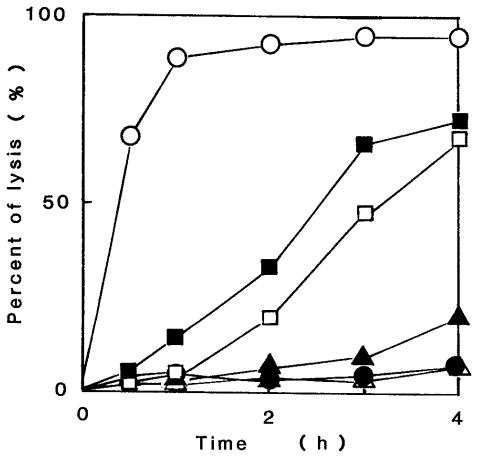

Fig. 1. HOCl-Induced Lysis of Erythrocytes

△, 0 μM HOCl (final concentration); ●, 70 μM HOCl; ▲, 105 μM HOCl; □, 140 μM HOCl; ■, 213 μM HOCl; ○, 423 μM HOCl. The erythrocytes (10^7 cells/ml) were treated with varying concentrations of HOCl at 37 °C.

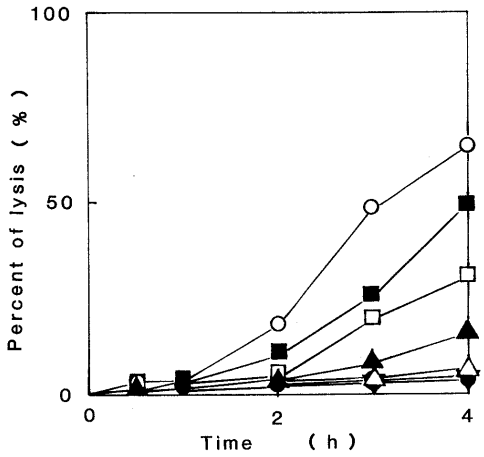

Fig. 2. Effect of Taurine on Inhibition of a HOCl-Induced Lysis

○, 0 mM (final concentration); ■, 5 mM taurine; □, 20 mM taurine; ▲, 40 mM taurine; △, 120 mM taurine; ▼, 160 mM taurine; ●, control. HOCl-treated erythrocytes (10^7 cells/ml) were incubated at 37 °C in PBS with varying concentrations of taurine. Intact erythrocytes were incubated at 37 °C in PBS as the control. The final concentration of HOCl used in HOCl-treatment was 140 μM.

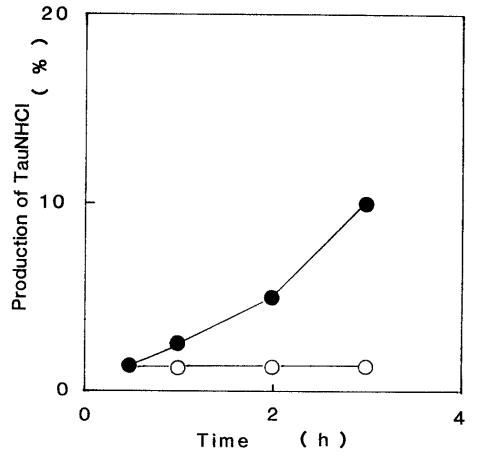

Fig. 3. Effect of Taurine on Removal of the Oxidized Chlorine (Cl^+) from HOCl-Treated Erythrocytes

○, 0 mM taurine; ●, 160 mM taurine. HOCl-Treated erythrocytes (10^8 cells/ml) were incubated in PBS with taurine. The final concentration of HOCl used in HOCl-treatment was 0.175 mM. Percentage of removed Cl^+ moiety was calculated by determination of the amount of TauNHCl using the DPD ferrous titrimetric method.

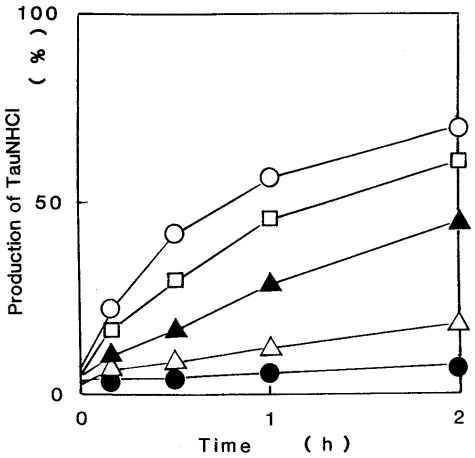

Fig. 4. Effect of Taurine on Removal of the Oxidized Chlorine (Cl^+) from HOCl-Treated Sepharose Gel

●, 0 mM taurine (final concentration); △, 10 mM taurine; ▲, 20 mM taurine; □, 40 mM taurine; ○, 80 mM taurine. HOCl-treated Sepharose gel was incubated with varying concentrations of taurine at 37 °C. The final concentration of HOCl used in HOCl-treatment was 0.7 mM. Percentage of removed Cl^+ moiety was calculated from the absorbance of TauNHCl at 250 nm.

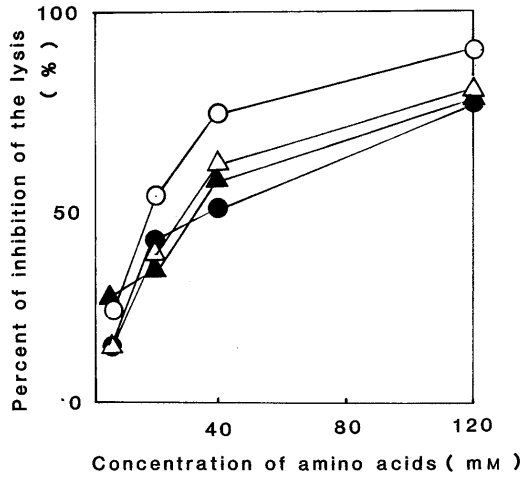

Fig. 5. Effect of Various Amino Acids on Inhibition of the HOCl-Induced Lysis

○, taurine; ●, glycine; △, α-alanine; ▲, β-alanine. HOCl-treated erythrocytes (10^7 cells/ml) were incubated in PBS with various amino acids at 37 °C. Concentration of HOCl used in HOCl treatment was 0.7 mM.

A gradual increase of the production of TauNHCl was observed when HOCl-treated erythrocytes were incubated with taurine as shown in Fig. 3. The results suggest that taurine removes the oxidized chlorine (Cl^+) from HOCl-treated erythrocytes, resulting in a yield of TauNHCl.

It has been reported that ascorbate, methionine, hypotaurine, $FeSO_3$, KCN and Na_2SO_3 all reduced TauNHCl with equal stoichiometry, and that cysteine, glutathione and KI also reduced TauNHCl at a ratio of 2 mol of reductant to 1 mol of TauNHCl.[18] Thomas *et al.* reported that TauNHCl in the extra medium was transported into erythrocytes by the anion-transport system.[11] Steady-state concentrations of TauNHCl would be difficult to detect exactly in complex biological systems. However, our data indicate that taurine removed the Cl^+ moiety from HOCl-treated erythrocytes, resulting in the production of TauNHCl and the inhibition of the lysis of HOCl-treated erythrocytes.

Figure 4 shows the effect of taurine on removal of the Cl^+ moiety from HOCl-treated Sepharose gel. Transfer of the electrophilic Cl^+ moiety from monochloramine groups (–NHCl) inHOCl-treated Sepharose gel to the nucleophilic acceptor, taurine, resulted in the production of TauNHCl. The yield of TauNHCl depended on the concentration of taurine and the incubation time. These results suggest that taurine has the role of removing Cl^+ moiety from monochloroamine groups in the membrane proteins and lipids of erythrocytes attacked by HOCl.

Figure 5 shows the effect of various amino acids on the inhibition of a HOCl-induced lysis. Other amino acids such as glycine, α-alanine and β-alanine also inhibited the lysis of HOCl-treated erythrocytes. The concentrations of taurine, α-alanine, β-alanine and glycine required for 50% inhibition of the lysis of erythrocytes were 18, 30, 34, and 40 mM, respectively. Although inhibition of the lysis of HOCl-treated erythrocytes by amino acids might be mainly due to the removal of Cl^+ moiety, taurine is the most potent reagent of all amino acids tested here.

Cytotoxicity assays of the MPO–H_2O_2–Cl^--system were performed in the presence of taurine, glycine, serine and valine.[12] Each of these compounds efficiently inhibited the lysis of human red blood cells by HOCl generated in neutrophil polymorphonuclears (PMN). Among these compounds, taurine inhibited the lysis most effectively, and it is therefore though to be the most powerful inhibitor in the reaction with HOCl generated in PMN.

Pasantes-Morales *et al.* reported the effect of taurine on the protection of isolated rod outer segments from frog retina against structural damage induced by illumination and oxidants.[19–21] Taurine protected also cultured cells against retinol-induced by ferrous sulfate-induced damage and/or swelling.[22,23] In the cells of a variety of marine species, taurine is believed to contribute significantly to the maintenance and regulation of cell volume by counteracting the osmotic effects of high salt concentrations in extracellular fluid.[24,25] These reports suggest that taurine directly exerts a protective effect on the biomembrane attacked by HOCl.

Taurine is thought to have a dual role in biomembrane protection against HOCl. First, it scavenges HOCl and removes the oxidized chlorine (Cl^+) by reacting with chloroamines (R–NHCl or R–NCl_2) in the membrane components attacked by HOCl *via* its amino group to inhibit the denaturation of the membrane. Second, it might also exert a direct protective effect by preventing the ionic and water shifts that result in cellular damage.

These results suggest that taurine could be effective in protecting the biomembrane against oxidants such as HOCl, and may be clinically useful in the treatment of corneal damage caused by oxidants such as HOCl.

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