Korean J. of Pharmacology
Vol. 28, No. 2, pp. 201-212, 1992

Inhibitory Effect of Taurine on HOCl-and NH_2Cl-induced Degradation of Hyaluronic Acid

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ABSTRACT

Effect of exogenous taurine on HOCl, NH_2Cl and other oxidants-induced degradation of hyaluronic acid was investigated. The scavenging action of taurine on HOCl, NH_2Cl and other oxidants was examined. The antioxidant action of taurine was also compared with that of thiol compounds.

Viscosity of hyaluronic acid was markedly decreased by HOCl and NH_2Cl on a dose dependent fashion. The degradative effect of HOCl on hyaluronic acid was greater than that of NH_2Cl. Taurine effectively inhibited HOCl-and NH_2Cl-induced degradation of hyaluronic acid in a dose dependent fashion. The degradative effect of HOCl was markedly inhibited by DMSO. Fe^{2+} plus H_2O_2-induced degradation of hyaluronic acid was inhibited by catalase and DMSO but not affected by taurine. The degradative action of xanthine and xanthine oxidase was effectively inhibited by SOD and catalase but not affected by taurine. HOCl was significantly decomposed by taurine, DMSO, GSH and MPG. Both absorbance of HOCl at 250 nm and absorbance of NH_2Cl at 242 nm were significantly increased by the addition of taurine. Interaction of NH_2Cl with GSH or MPG showed an initial peak absorbance, but these absorbances were gradually decreased with time. $OH\cdot$ production in the presence of Fe^{2+} and H_2O_2 was inhibited by catalase and DMSO but not affected by taurine. Taurine did not affect $^1O_2$ production by U.V. irradiation which is responsible for DABCO and DABA. GSH and MPG markedly inhibited the degradative action of HOCl.

These results suggest that the protective action of taurine on oxidants-induced damages of tissue components, including degradation of hyaluronic acid may be attributable to both its scavenging action on HOCl and NH_2Cl and the complex formation of taurine with HOCl or NH_2Cl without scavenging action on oxygen free radicals. Sulfhydryl group of taurine appears to show partially a protective action on HOCl-and NH_2Cl-induced degradation.

Key Words: Taurine, HOCl, NH_2Cl, Hyaluronic acid

INTRODUCTION

HOCl is highly reactive, being able to oxidize many biological molecules, especially thiol groups (Albrich *et al.*, 1981; Cuperus *et al.*, 1985). Biologically generated HOCl appears to play a role in a central mechanism of host defense against infection (Fantone and Ward, 1982). In the phagolysosomes of activated neutrophils, they act to kill ingested microorganisms. However, extracellulary generated HOCl is cytotoxic and is thought to be a major factor in the destruction of tissues in chronic inflammatory conditions such as rheumatoid arthritis and emphysema (Matheson *et al.*, 1981; Halliwell and Gutteridge, 1989b). Antimicrobial, cytotoxic and cytolytic activities of HOCl producing myeloperoxidase system may also be mediated by NH_2Cl and possibly by other lipo-

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philic N-Cl derivatives (Thomas, 1979a; Grisham *et al.*, 1984a; Bernofsky, 1991). Lipophilic derivatives such as NH_2Cl penetrate the hydrophobic barrier of biological membranes and appear to oxidize membrane and intracellular components (Thomas *et al.*, 1983; Grisham *et al.*, 1984b).

Taurine (2-aminoethanesulphonic acid) occurs at high concentrations in many tissues exposed to elevated levels of pro-oxidants (Jacobsen and Smith, 1968; Wright *et al.*, 1986) and has been shown to exhibit antioxidant properties (Alvarez and Storey, 1983; Pasantes-Morales *et al.*, 1985; Wright *et al.*, 1986). Taurine deficiency appears to predispose the heart to enhanced formation of malondialdehyde caused by doxorubicin administration (Harada *et al.*, 1990). It is also suggested that taurine maintaines retinal membrane integrity by preventing both lipid peroxidation in photoreceptors and membrane destabilization (Pasantes-Morales *et al.*, 1986). On the other hand, some experiments show that the cytoprotective effect of taurine is associated with membrane stabilization rather than inhibition of lipid peroxidation (Wright *et al.*, 1986).

Intracellular taurine concentrations of human neutrophils and lymphocytes are 22 mM and 35 mM (Jacobsen and Smith, 1968; Grisham *et al.*, 1984a), respectively. Stimulated neutrophils release taurine to the extracellular medium. Taurine is known to protect neutrophils, erythrocytes, plasma component and tissues against oxidative attack by acting as a trap for HOCl and by competing with endogenous NH_4^+ which also reacts with HOCl to yield NH_2Cl (Grisham *et al.*, 1984a; Thomas *et al.*, 1985). It is reported that exogenous taurine effectively decrease the killing rate of myeloperoxidase system on Escherichia coli (Thomas, 1979b). Taurine chloramines formed by interaction of taurine with HOCl or NH_2Cl are considered to act as endogenous cytoprotective substances (Thomas *et al.*, 1985). However, they could act as a mediator of neutrophil toxicity under a certain biological conditions (Thomas, 1979b). TauNHCl is the slow reacting oxidant. In inflammatory exudates, endogenous removing system for taurine chloramines is low and then TauNHCl and other anionic chloramines may accumulate (Thomas *et al.*, 1985). Taurine chloramines could mediate cytotoxicity to tissue components in the presence of NH_4^+ and amines.

In the present study, effect of exogenous taurine on HOCl, NH_2Cl and other oxidants-induced degradation of hyaluronic acid which is present at synovial fluid and act as a joint lubricant (Halliwell *et al.*, 1988) was investigated. Scavenging action of taurine on HOCl, NH_2Cl and other oxidants was examined. Antioxidant action of taurine was also compared with that of thiol compounds.

MATERIALS AND METHODS

Hyaluronic acid (Grade III from human umbilical cord), taurine, superoxide dismutase (from bovine blood, SOD), catalase (from bovine liver), dimethyl sulfoxide (DMSO), 1, 4-diazabicyclo (2.2.2) octane (DABCO), 3-methylamino-benzoic acid (DABA), glutathione (reduced from, GSH), N-(2-mercaptopropionyl)-glycine (MPG), xanthine oxidase (from buttermilk), 2-α deoxyribose and 1, 3-diphenyl isobenzofuran (DPBF) were purchased from Sigma Chemical Co.. NaOCl was obtained from Shinyo Pure Chemicals Co., Ltd.; xanthine from E. Merck; 2-thiobarbituric acid from Fluka AG; $FeSO_4$ from Avondale Laboratories; H_2O_2 from Junsei Chemical Co., Ltd.. Other chemicals were of analytical reagent grades.

Viscometry

Viscosity of hyaluronic acid was measured using a modified Cannon capillary viscometer. The reaction mixtures contained 1 mg/ml hyaluronic acid, 150 mM KCl, 50 mM KH_2PO_4 buffer, pH 7.5 and other compounds. The viscosity change was measured at 25°C and expressed as a flow time (sec).

Preparation and assay of HOCl

HOCl was prepared immediately before use by adjusting NaOCl to pH 6.2 with diluted H_2SO_4 (Green *et al.*, 1985). The concentration of HOCl was determined using a molar extinction coefficient of 142 at 291 nm (Thomas *et al.*, 1986b).

Preparation and assay of NH_2Cl

4.8 ml of distilled water was added to 5 ml of

40 mM NH_4Cl in 10 mM phosphate buffer, pH 8.0. One volume of NaOCl was added to 4 volume of the above amine solution at 4℃. The concentration of NH_2Cl was determined using a molar extinction coefficient of 42.9 at 242 nm (Thomas *et al.*, 1986a).

Assay of xanthine oxidase activity

Ten microliters of crude xanthine oxidase was placed in a cuvette which contains 3 ml of mixture containing 40 mM Tris-maleate, pH 7.0, 100 mM KCl, 5 mM $MgCl_2$ and 0.4 mM xanthine. Spectrophotometric recording at 290 nm (the peak absorbance of uric acid) was carried out at 25℃ for 1 min with a BECKMAN DU 70 spectrophotometer, and the slope of the initial linear portion of the curve was measured. By use of the molar extinction coefficient of urate (1.24×10^4/M/cm), the amount of urate generated was calculated. One unit of xanthine oxidase activity was defined as 1 μM urate produced per minute (Greenwald and Moy, 1979).

Assay of the thiobarbituric acid reactivity of 2-α deoxyribose

Amount of hydroxyl radical generated was estimated from the thiobarbituric acid (TBA) reactivity of 2-α deoxyribose (Gutteridge, 1981; Halliwell and Gutteridge, 1981). The reaction mixtures contained 1 mM 2-α deoxyribose, 5 μM iron (II), 0.1 mM H_2O_2, 150 mM KCl, 50 mM NaH_2PO_4 buffer, pH 7.4 and other compounds in a final volume of 1.0 ml. After 30 min of incubation, the reaction was stopped by adding 1.0 ml of 1% TBA on 50 mM NaOH and 1.0 ml of 2.8% trichloroacetic acid. The reaction mixtures were heated in a boiling water bath for 10 min. After cooling to the room temperature, the reaction mixtures were centrifuged at 3000 rpm for 10 min. The fluorescence was read at the wavelengths of excitation, 532 nm and emission, 553 nm.

Assay of DPBF oxidation

Conversion of DPBF (1, 3-diphenyl isobenzofuran) to DBB (dibenzoyl benzene), which is responsible for $^1O_2$, was measured at 415 nm (Marnett *et al.*, 1979). The reaction mixtures contained 1 μM HOCl, 1 mM thiol compounds, 67 μM DPBF, 150 mM KCl, 50 mM KH_2PO_4 buffer, pH 7.5 and other compounds.

RESULTS

Inhibitory effect of taurine on HOCl-or NH_2Cl-induced degradation of hyaluronic acid

HOCl and NH_2Cl are powerful oxidizing agents and they oxidize membrane, intracellular components and tissue components. In inflammed joint, hyaluronic acid is depolymerized by the oxidants and the synovial fluid losses its lubricating properties (McCord, 1974). As can be seen in Fig. 1 and 3, HOCl and NH_2Cl decreased the viscosity of hyaluronic acid in a dose dependent fashion. The decrease of viscosity of hyaluronic acid is attributed to depolymerization (Lee *et al.*, 1985). Viscosity of intact hyaluronic acid was 21.9±0.5 (S. D.) sec, n=5. The degradative effect of HOCl on hyaluronic acid was greater than that of NH_2Cl.

Taurine effectively inhibited HOCl-induced

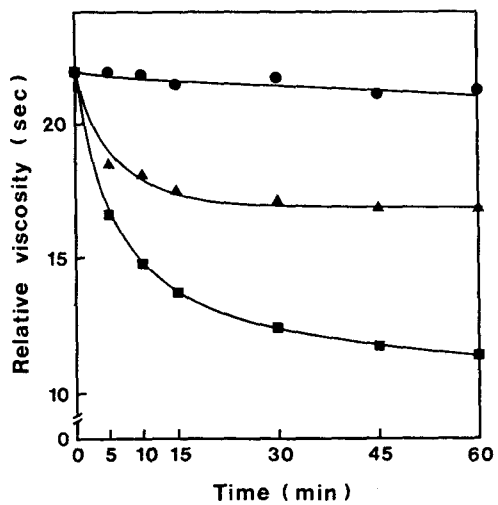

Fig. 1. Degradation of hyaluronic acid by HOCl. The reaction mixtures contained 1 mg/ml hyaluronic acid and 1 or 5 μM HOCl. Experimental conditions were the same as described in Materials and Methods. Values were expressed as flow time (sec) and viscosity of intact hyaluronic acid was 21.9±0.5 sec(S.D.), n=5. Values are means of 5 experiments. ●, none and ▲, 1 μM; ■, 5 μM HOCl.

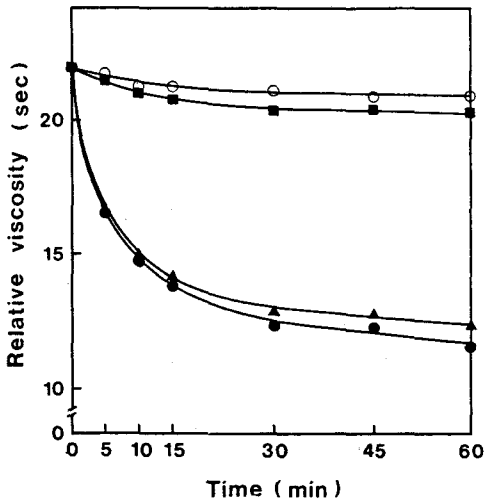

Fig. 2. Inhibitory effect of taurine on HOCl-induced degradation of hyaluronic acid. The reaction mixtures contained 1 mg/ml hyaluronic acid, HOCl and varying concentration of taurine. Values are means of 5 experiments. ●, none; ▲, 0.1 mM; ■, 1 mM; ○, 10 mM taurine in the presence of 5 μM HOCl, respectively.

degradation of hyaluronic acid in a dose dependent fashion (Fig. 2) and 1 mM taurine almost completely inhibited 5 μM HOCl-induced degradation. The stated amounts of taurine alone did not affect viscosity of hyaluronic acid (data not shown). Inhibitory effect of taurine on NH_2Cl-induced degradation of hyaluronic acid was similar with that on the degradative effect of HOCl and 10 μM NH_2Cl-induced degradation was almost completely inhibited by 1 mM taurine (Fig. 4).

HOCl-induced degradation of hyaluronic acid was markedly inhibited by DMSO, a scavenger of HOCl.

Effect of taurine on Fe^{2+} plus H_2O_2-and xanthine oxidase system-induced degradation of hyaluronic acid

The reaction of Fe^{2+} with H_2O_2, a Fenton reaction may be an important source of OH • in biological system (Halliwell, 1978). Highly reactive OH • is known to damage directly most types of cellular macromolecules (Fridovich, 1978). Fig. 5 showed that 5 μM Fe^{2+} plus 0.1 mM H_2O_2 signifi-

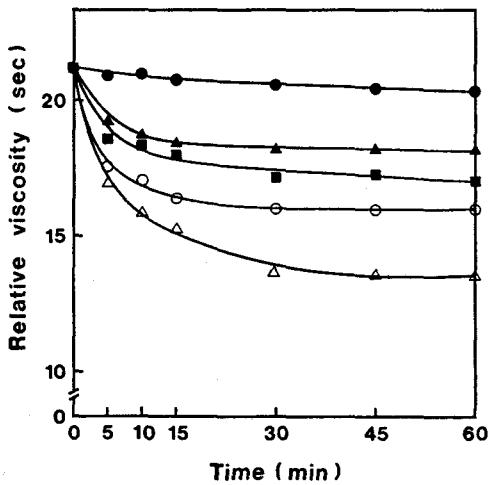

Fig. 3. Degradation of hyaluronic acid by NH_2Cl. The reaction mixtures contained 1 mg/ml hyaluronic acid and varying concentration of NH_2Cl. Values are means of 5 experiments. ●, none and ▲, 1 μM; ■, 2.5 μM; ○, 5 μM; △, 10 μM NH_2Cl.

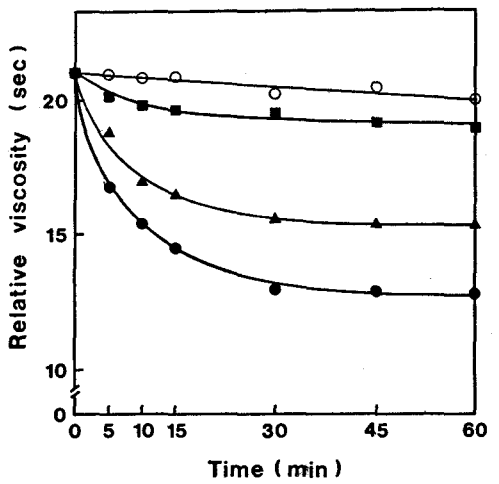

Fig. 4. Inhibitory effect of taurine on NH_2Cl-induced degradation of hyaluronic acid. The reaction mixtures contained 1 mg/ml hyaluronic acid, NH_2Cl and varying concentration of taurine. Values are means of 5 experiments. ●, none; ▲, 0.1 mM; ■, 1 mM; ○, 10 mM taurine in the presence of 10 μM NH_2Cl, respectively.

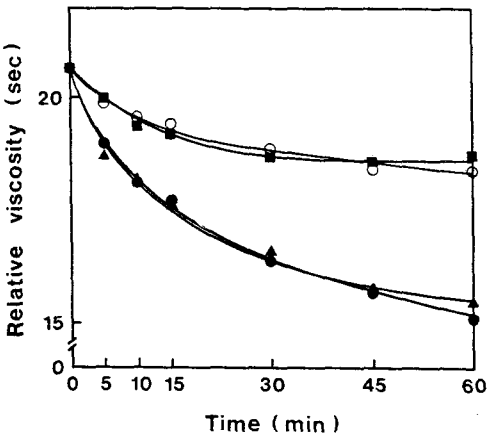

Fig. 5. Effects of taurine, catalase and DMSO on Fe^{2+} plus H_2O_2-induced degradation of hyaluronic acid. The reaction mixtures contained 1 mg/ml hyaluronic acid, $FeSO_4$, H_2O_2 and other compounds. Values are means of 5 experiments. ●, none; ▲, 10 mM taurine; ■, 10 μg/ml catalase; ○, 1 mM DMSO in the presence of 5 μM $FeSO_4$ and 0.1 mM H_2O_2, respectively.

cantly decreased viscosity of hyaluronic acid. The degradative action of 5 μM Fe^{2+} plus 0.1 mM H_2O_2 was inhibited by 10 μg/ml catalase, a scavenger of H_2O_2 and 1 mM DMSO, a scavenger of OH · and HOCl. However, 10 mM taurine did not affect the degradative action of Fe^{2+} plus H_2O_2.

Effect of taurine on degradation of hyaluronic acid by xanthine and xanthine oxidase system which easily and rapidly produces oxygen free radicals was investigated. The degradative action of xanthine and xanthine oxidase was effectively inhibited by 10 μg/ml SOD, a scavenger of O_2^- and 10 μg/ml catalase but not affected by 10 mM taurine (Fig. 6).

Decomposing action of taurine on HOCl and NH_2Cl

HOCl showes a peak absorbance at the wavelength, 291 nm (Weiss *et al.*, 1982). In the present study, effects of taurine, DMSO and thiol compounds on HOCl decomposition were investigated. As shown in Fig. 7, absorbance of 5 μM HOCl was siginificantly decreased by the addition of

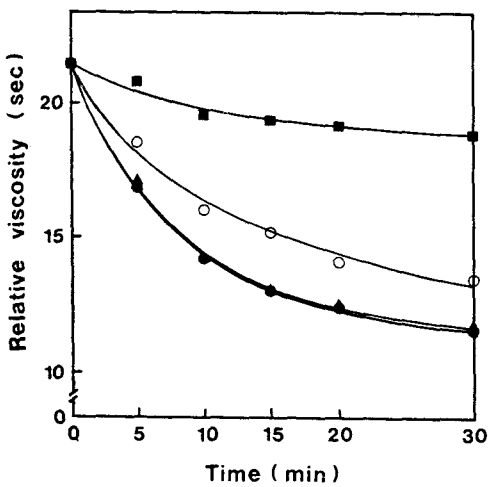

Fig. 6. Effects of taurine, SOD and catalase on xanthine/xanthine oxidase-induced degradation of hyaluronic acid. The reaction mixtures contained 1 mg/ml hyaluronic acid, xanthine, xanthine oxidase and other compounds. Values are means of 4~5 experiments. ●, none; ▲, 10 mM taurine; ■, 10 μg/ml SOD; ○, 10 μg/ml catalase in the presence of 0.25 mM xanthine and 13.2 mU/ml xanthine oxidase, respectively.

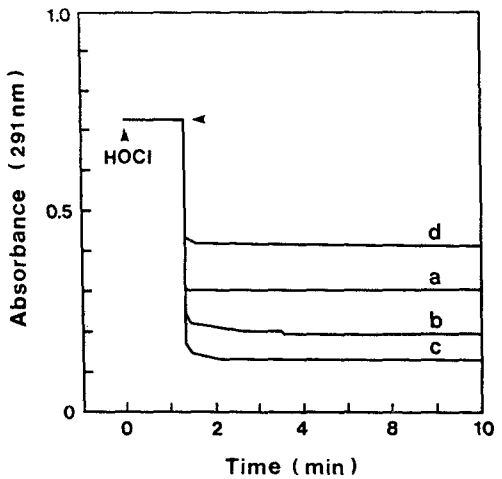

Fig. 7. The decomposition of HOCl by taurine. The reaction mixtures contained 5 μM HOCl, 150 mM KCl, 50 mM potassium phosphate buffer, pH 7.5 and other compounds. Compounds were added at the arrow points. Decomposition of HOCl was read spectrophotometrically at the wavelength 291 nm. a, 5 mM taurine; b, 1 mM GSH; c, 1 mM MPG; d, 1 mM DMSO in the presence of 5 μM HOCl, respectively.

5 mM taurine, 1 mM DMSO, 1 mM GSH and 1 mM MPG.

Taurine chloramine can be quantitated directly by measuring its absorbance at 250 nm (Weiss *et al.*, 1982). Fig. 8 showed that absorbance of HOCl was markedly increased by adding taurine at 250 nm.

NH_2Cl showes a peak absorbance at 242 nm (Thomas *et al.*, 1986a). In this wavelength, absorbance of NH_2Cl was significantly increased by the addition of taurine. Interaction of NH_2Cl with thiol compounds GSH and MPG showed an initial peak absorbance, but these absorbances were gradually decreased with time (Fig. 9).

Effect of taurine on the decomposition of reactive oxygen species

The scavenging action of taurine on OH · and singlet oxygen ($^1O_2$) was examined. OH · formed in reaction can be sensitively detected with the thiobarbituric acid (TBA) reactivity of 2-α deoxyribose (Halliwell and Gutteridge, 1989a). Increased TBA reactivity of deoxyribose by 5 μM Fe^{2+} plus 1 mM H_2O_2 was inhibited by 30 μg/ml catalase and 10 mM DMSO but not affected by 10 mM taurine (Table 1).

Conversion of DPBF (1,3-diphenzylisobenofuran) to DBB (dibenzoyl benzene) has been widely used as a monitor for $^1O_2$ at 415 nm (Marnett *et al.*, 1979; Singh, 1981). U.V. irradiation is known as an

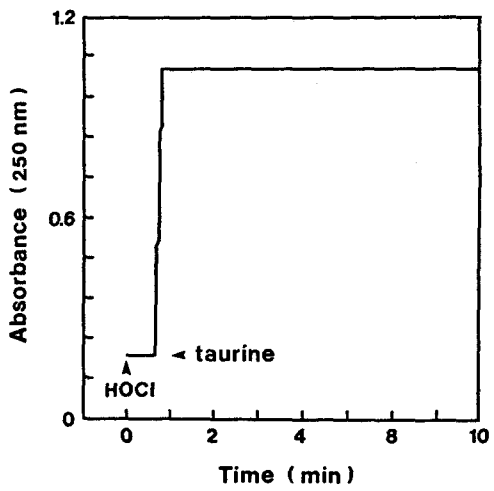

Fig. 8. The formation of complex of taurine and HOCl. The reaction mixture contained 5 μM HOCl, 5 mM taurine, 150 mM KCl and 50 mM potassium phosphate buffer, pH 7.5. Compounds were added at the arrow points. Formation of complex of taurine and HOCl was read spectrophotometrically at the wavelength 250 nm.

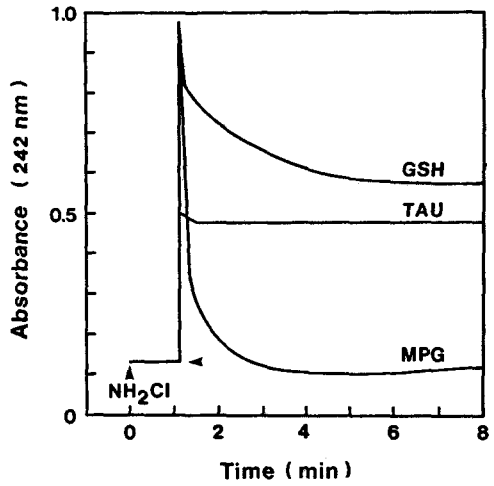

Fig. 9. The formation of complex of taurine and NH_2Cl. The reaction mixtures contained 10 μM NH_2Cl, 5 mM taurine(or 1 mM thiol compounds), 150 mM KCl and 50 mM potassium phosphate buffer, pH 7.5. Compounds were added at the arrow points. Formation of complex of taurine and NH_2Cl was read spectrophotometrically at the wavelength 242 nm.

Table 1. Effects of taurine, catalase and DMSO on deoxyribose degradation by Fe^{2+} plus H_2O_2-dependent OH · formation

Compounds	Amount of TBA-reactive product formed (fluorescent units)
5 μM Fe^{2+} + 1 mM H_2O_2	42.1 ± 0.9
+ Taurine 10 mM	41.4 ± 1.4
+ Catalase 30 μg/ml	8.7 ± 0.7
+ DMSO 10 mM	31.7 ± 0.7

Deoxyribose degradation by Fe^{2+} plus H_2O_2 was measured as described in Materials and Methods and expressed as the fluorescent unit at the wavelengths of excitation, 532 nm and emission, 553 nm. Values are means ± SEM of 5 experiments.

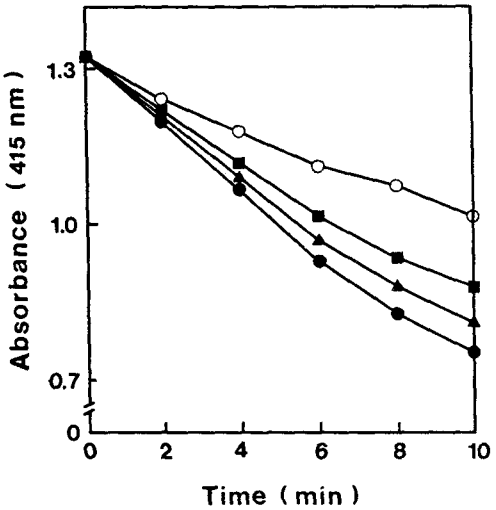

Fig. 10. Effects of taurine, DABCO and DABA on the oxidation of DPBF by U.V. irradiation. The reaction mixtures contained 67 μM DPBF, 150 mM KCl, 50 mM potassium phosphate buffer, pH 7.5 and other compounds. Oxidation of DPBF by U.V. irradiation was read spectrophotometrically at 415 nm. Values are mean absorbances of 5 experiments. ●, none; ▲, 10 mM taurine; ■, 10 mM DABCO; ○, 10 mM DABA.

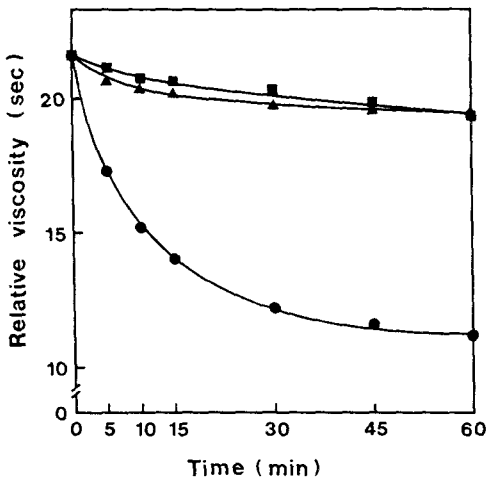

Fig. 11. Inhibitory effects of thiol compounds on the degradative action of HOCl. The reaction mixtures contained 1 mg/ml hyaluronic acid, HOCl and thiol compounds. Values are means of 4 experiments. ●, none; ▲, 1 mM GSH; ■, 1 mM MPG in the presence of 5 μM HOCl, respectively.

effective source of $^1O_2$. Effect of taurine on oxidation of DPBF by U.V. irradiation was observed. The result represented in Fig. 10 showed that oxidation of DPBF by U.V. irradiation was inhibited by 10 mM DABCO and 10 mM DABA, quenchers of $^1O_2$ but not affected by 10 mM taurine.

Effects of thiol compounds on degradation of hyaluronic acid by HOCl

Since thiol compounds appear to have a protective action against the oxidative tissue injury, effect of thiol compounds on HOCl-induced degradation of hyaluronic acid was examined. The results of Fig. 11 indicated that 1 mM of GSH and MPG markedly inhibited the degradative action of 5 μM HOCl. The same amounts of GSH and MPG alone had not effect on viscosity of hyaluronic acid.

DISCUSSION

Oxidants appear to be implicated in the tissue damage in various pathological conditions (Leibovitz and Siegel, 1980). Destruction of the joint components associated with inflammation may be one of the situations where oxidants are involved (Weissman *et al.*, 1980). In the inflammatory situation, oxidants would be released from the phagocytic cell infiltrating into the inflamed sites (Fantone and Ward, 1982). Stimulated neutrophils release oxidants including H_2O_2 and secrete cytoplasmic-granule components including myeloperoxidase into the intracellular phagolysosome compartment and the extracellular medium (Badwey and Karnovsky, 1980). Myeloperoxidase (MPO) catalyzes oxidation of Cl^- by H_2O_2 to yield HOCl (Harrison and Schultz, 1976),

$$H_2O_2 + Cl^- + H^+ \xrightarrow{MPO} HOCl + H_2O$$

which reacts rapidly with nitrogen compounds to

yield derivatives containing the nitrogen-chlorine (N-Cl) bond (Stelmaszynska and Zgliczynski, 1978).

$$HOCl + NH_4^+ \longrightarrow H_2O + H^+ + NH_2Cl$$

N-Cl derivatives retain the two oxidizing equivalents of H_2O_2 or HOCl and are powerful oxidizing agents (Grisham *et al.*, 1984a).

HOCl is highly reactive and react with most biological molecules, degrading structural proteins and inactivating enzymes (Weiss, 1989). HOCl readily inactivates the major plasma protease inhibitor α_1-antitrypsin (Wasil *et al.*, 1987) and can activate neutrophil collagenase (Capodici and Berg, 1989). Thus, HOCl may promote tissue damage directly by facilitating proteolysis at inflammatory sites. In inflammatory conditions such as rheumatoid arthritis, many neutrophils are accumulated at synovial fluid (Halliwell *et al.*, 1988) and release oxidants. However, since synovial fluid has little antioxidant system, oxidants are not detoxified and react with joint components to cause damage (McCord, 1974). In particular, hyaluronic acid is depolymerized and synovial fluid losses its lubricating properties, causing friction in the joint (Kofoed and Barcelo, 1978). NH_2Cl is also highly reactive oxidizing agent and this cell penetrable oxidant is known to oxidize membrane and intracellular components (Grisham *et al.*, 1984b).

HOCl and NH_2Cl markedly decreased viscosity of hyaluronic acid in a dose dependent fashion. In endogenous amines containing biological condition, the oxidative toxicity of the myeloperoxidase-H_2O_2-Cl^- system is mediated by NH_2Cl and possibly by other lipophilic N-Cl derivatives (Thomas, 1979a; Thomas *et al.*, 1983). It is reported that bactericidal activity of the supernatant from activated neutrophils is enhanced by the addition of NH_4^+ (Grisham *et al.*, 1984a). Thus, cytolytic activity of cell penetrable NH_2Cl may be greater than that of cell unpenetrable HOCl. However, in the reaction medium did not contain amine the degradative effect of HOCl was greater than that of NH_2Cl.

Taurine is present at high concentrations in many tissues (Jacobsen and Smith, 1968; Wright *et al.*, 1986). Taurine appears to protect the oxidative injury to cell membrane, intracellular components and cells by illumination, retinol, iron plus ascorbate, CCl_4, hypoxia and drugs through inhibition of lipid peroxidation and particularly, stabilization of membrane (Pasantes-Morales and Cruz, 1985; Sawamura *et al.*, 1986; Wright *et al.*, 1986; Harada *et al.*, 1990). On the other hand, protection of lymphoblastoid cells from iron plus ascorbate-induced damage by taurine is considered to be associated with an action on stabilization of membrane rather than inhibition of lipid peroxidation (Pasantes-Morales *et al.*, 1985). Thus, the antioxidant mechanism of taurine is still unclear. Human neutrophils contain 22 mM of taurine (Grisham *et al.*, 1984a). When neutrophils are incubated with phorbol myristate acetate for 1 h at 37℃, they release about 30% of the intracellular taurine.

Polylysine, taurine and α-amino acids prevent oxidation of bacterial components by HOCl or by lipid soluble N-Cl derivatives of bacterial components (Thomas, 1979b). Previous reports (Grisham *et al.*, 1984a) suggest that taurine protects membrane, intracellular components and tissue components against oxidative attack by acting as a trap for HOCl and by competing with endogenous NH_4^+ for reaction with HOCl.

$$HOCl + RNH_2\,(\text{Taurine}) \longrightarrow H_2O + RNHCl$$

$$NH_2Cl + RNH_2 \longrightarrow NH_3 + RNHCl$$

However, at high concentrations, TauNHCl kills bacteria over a period of hours, either as the result of slow diffusion through bacterial membranes or the reaction with bacterial products or components to yield lipophilic N-Cl derivatives (Grisham *et al.*, 1984a; Grisham *et al.*, 1984b). Taurine significantly inhibited HOCl and NH_2Cl-induced degradation of hyaluronic acid in a dose dependent fashion. The inhibitory effect of taurine may be ascribed to the decomposing action on HOCl and the complex formation of taurine and HOCl or NH_2Cl. Absorbances of HOCl and NH_2Cl alone were markedly increased by the addition of taurine at the wavelength which has a peak absorbance for taurine chloramine (Fig. 8 and 9). Thus, this finding supports formation of taurine chloramine by interaction of taurine with HOCl or NH_2Cl.

In inflammatory conditions such as rheumatoid

arthritis, many neutorphils are accumulated at synovial fluid. Besides this phenomenon the iron content of synovial fluid rises sharply (Sorensen, 1978; Ogilvie-Harris and Fornaiser, 1980). It is well established that iron catalyzes the Haber-Weiss reaction between O_2^- and H_2O_2, effectively yielding reactive oxygen species(Gutteridge *et al.*, 1981). OH • and $^1O_2$ are highly reactive oxygen species and play a major role in the oxidative tissue injury (Kellogg and Fridovich, 1977; McCord and Day, 1978). Furthermore, metal ion-oxygen complex have also been proposed as proximate reactive species for the oxidative tissue damage including lipid peroxidation (Pederson and Aust, 1975; Minotti and Aust, 1987). In the present study, taurine did not inhibit the degradative actions of oxygen free radicals and possible iron-oxygen complex. Iron (II) plus H_2O_2-induced TBA reactivity of deoxyribose and U.V. irradiation-induced oxidation of DPBF were not affected by taurine. Accordingly, these findings suggest that taurine has not the scavenging action on reactive oxygen species. The result also supports that taurine-iron complex has very poor stability (Wright *et al.*, 1986).

Free sulfhydryl groups are essential for the maintenance of cellular functions. Ether exogenous thiol compounds or free sulfhydryl components in tissue may act as a protective molecule against chemicals- or X-ray irradiation-induced cytotoxicity through the competitive inactivation of free radicals (Jeon *et al.*, 1986; Lee *et al.*, 1991). Thiol compounds also appear to scavenge HOCl. In addition, taurine has sulfhydryl group in its chemical structure. Effects of thiol compounds on HOCl-induced degradation of hyaluronic acid were the same as action of taurine. The finding suggests that sulfhydryl group of taurine may play a protective role in HOCl-and NH_2Cl-induced degradation.

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= 국문초록 =

HOCl과 NH_2Cl에 의한 Hyaluronic Acid의 변성에 있어서 Taurine의 억제 효과

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외인성 taurine이 HOCl, NH_2Cl과 그밖의 산화성 물질에 의한 hyaluronic acid의 변성에 미치는 영향을 관찰하였다. HOCl, NH_2Cl과 그밖의 산화성 물질에 대한 taurine의 제거 작용을 조사하였다. Taurine의 항 산화 작용을 또한 치올 화합물의 작용과 비교 관찰하였다.

Hyaluronic acid의 점성도는 HOCl과 NH_2Cl에 의하여 뚜렷하게 용량에 따라 감소하였다. Hyaluronic acid에 대한 HOCl의 변성 효과는 NH_2Cl에 의한 것보다 현저하였다. Taurine은 HOCl과 NH_2Cl에 의한 hyaluronic acid의 변성을 효과적으로 용량에 따라 억제하였다. HOCl의 변성 효과는 DMSO에 의하여 뚜렷하게 억제되었다. Fe^{2+}과 H_2O_2에 의한 hyaluronic acid의 변성은 catalase와 DMSO에 의하여 억제되었으나 taurine의 영향은 받지 않았다. Xanthine과 xanthine oxidase의 변성 작용은 SOD와 catalase에 의하여 효과적으로 억제되었으나 taurine의 영향은 받지 않았다. HOCl은 taurine, DMSO, GSH와 MPG에 의하여 유의하게 분해되었다. 파장 250 nm에서의 HOCl의 흡광도와 파장 242 nm에서의 NH_2Cl의 흡광도는 taurine의 첨가로 유의하게 증가하였다. NH_2Cl과 GSH 또는 MPG의 상호 작용으로 초기에 최대의 흡광도가 관찰되었으나, 이러한 흡광도는 반응 시간에 따라 점차적으로 감소하였다. Fe^{2+}와 H_2O_2의 존재하에서 $OH\cdot$의 생성은 catalase와 DMSO에 의하여 억제되었으나 taurine의 영향은 받지 않았다. DABCO와 DABA에 반응하는 자외선 조사에 따른 $^1O_2$의 생성은 taurine의 영향을 받지 않았다. GSH와 MPG는 HOCl의 변성 작용을 뚜렷하게 억제하였다.

이상의 결과로 부터 hyaluronic acid의 변성을 포함한 조직 구성성분의 산화성 손상에 있어서 taurine의 보호 작용은 산소 유리 라디칼에 대한 제거 작용과 관계없으며 HOCl과 NH_2Cl에 대한 제거 작용 그리고 taurine과 HOCl 또는 NH_2Cl의 복합체 형성에 기인할 것으로 시사된다. Taurine의 치올 기가 HOCl과 NH_2Cl에 의한 변성에 부분적으로 보호 작용을 나타 낼 것으로 추정된다.