Quantitative Evaluation of the Effectiveness of Taurine in Protecting the Ocular Surface against Oxidant

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Quantitative evaluation of the effectiveness of taurine against ocular surface damage caused by hypochlorous acid (HOCl) was investigated using albino rabbits. The activity of lactate dehydrogenase (LDH) released from ocular tissues into meniscus tears at eye irritation was used as an index of ocular surface damage. Instead of collecting meniscus tears directly with a glass micropipette, a new sampling method, where 150 μl of saline was instilled into the cul-de-sac of rabbit eyes and collected all of the diluted tears within 10 s, was developed.

The LDH activity after serial instillations of HOCl increased dose-dependently with increasing HOCl concentration. After serial instillation of taurine, HOCl was instilled in the same way. Pre-application of taurine effectively suppressed ($p<0.01$, $n=11$) the HOCl-induced LDH release as compared to saline, suggesting that the residual taurine in ocular surface tissues was still effective in protecting the tissues against HOCl by scavenging HOCl. LDH activity at 30 min after post-application of taurine was significantly lower ($p<0.01$, $n=10$) than that in the case of saline. This result indicates that taurine is effective in protecting the ocular surface after it has been attacked by HOCl. LDH activity in meniscus tears became a good index of quantitatively estimating ocular surface damage due to HOCl by devising the new sampling method. By using this method, we were able to prove objectively and quantitatively that taurine is effective in protecting the ocular surface against HOCl. It was suggested that taurine is clinically useful in the treatment of ocular surface damage caused by oxidants, such as HOCl.

Keywords taurine; hypochlorous acid; ocular surface damage; albino rabbit; oxidant; protection

Hypochlorous acid (HOCl) is thought to play a significant role as a potent bactericidal agent derived from the myeloperoxidase–H_2O_2–chloride-system in neutrophils.[1,2] On the other hand, Weiss *et al.* have indicated that HOCl plays a critical role in cellular cytotoxicity and connective tissue damage.[3] HOCl is a potent oxidizing agent whose biocidal properties are well known, especially in the disinfection of municipal water systems. It can directly oxidize a variety of biologically significant substances, such as carbohydrates, nucleic acids, peptide linkages, and amino acids. Kitano *et al.* reported that HOCl used to disinfect water in swimming pools and chlorine gas such as Cl_2 or NCl_3 which is yielded in indoor pools caused corneal damage.[4–6] However, there is no eyedrop for protecting ocular surface tissues against HOCl.

Taurine plays a significant role in protecting neutrophils against oxidative attack by excessive HOCl.[7,8] This protection is attributed to the effect of taurine as a trap for HOCl or a competing amine that does not yield toxic N-Cl derivatives. Wright *et al.* reported the role of taurine as a scavenger of HOCl in biological systems.[9] In our previous study using canine erythrocyte membranes, we reported that taurine could be effective in protecting biomembranes against HOCl attack, and that it may be useful clinically in the treatment of corneal damage caused by oxidants such as HOCl.[10] Using a histochemical or morphological method, Yoshimura *et al.* indicated that taurine is effective in protecting ocular surface tissues against HOCl.[11] However, the concentration of HOCl used in the previous report was 5.6 mM, which was higher than that actually used in swimming pools (0.14—2.8 mM).[4] A quantitative and more sensitive estimation method of HOCl-induced ocular surface damage is thought to be required in order to evaluate the effect of taurine in protecting the ocular surface against lower concentrations of HOCl.

There are several methods for evaluating eye irritation, which have been performed by macroscopic examination of the ocular surface (Draze test[12]) and by histochemical or morphological examination of the enucleated eye.[5,6,13] However, these methods are inadequate for accurate quantitative estimation of ocular surface damage. Imayasu *et al.* reported that lactate dehydrogenase (LDH) activity in the tear fluid of rabbit eyes is useful (a good index) for estimating corneal damage caused by wearing contact lenses, mechanical scratching, and dropping contact lense cleaner.[14] Hiraki *et al.*[15,17] measured reduced glutathione (GSH), ascorbic acid, and serum albumin exuded into tear fluid from ocular tissues *in vivo*, in order to estimate ocular surface damage caused by topical ophthalmic agents. We developed a new meniscus tear sampling method and an estimated method of HOCl-induced ocular surface damage by measuring LDH activity or the amount of GSH in tear fluid. Using our method, we evaluated quantitatively the effectiveness of taurine on the ocular surface damage caused by HOCl.

Materials and Methods

Materials Taurine used in this study was a product (51 A.M, No. 796) which had been synthesized in our laboratory. Sodium hypochlorite solution (NaClO), and *N,N*-diethyl-*p*-phenylenediamine sulfate (DPD) were purchased from Wako Pure Chemical Industries. The chemicals used in this study were reagent grade commercial products.

Meniscus Tear Sampling Method and Measurement of LDH Activity in Meniscus Tears The meniscus tear samples of normal albino rabbits (males, 2.5—4.0 kg) were collected by the following two sampling methods: the micropipette method and the saline pool method. In the micropipette method, 2-μl of tear sample was collected carefully with a 2-μl glass micropipette within 30 s in order to avoid inclusion of LDH released by stimulus of or damage to ocular surface tissues at a tear sampling. The 2-μl tear sample was diluted to 50 μl, and was kept at 5 °C.

In the saline pool method, 150 μl of saline was poured into the cul-de-sac of a rabbit eye and all of tear the sample was collected from the meniscus tears. The LDH activity in 80 μl of the tear samples was measured with a Hitachi automatic analyzer (7150 type).

Measurement Values of LDH and GSH Release into Meniscus Tears by HOCl Treatment Various HOCl solutions were prepared by diluting NaClO solution with 40 mM phosphate-buffered isotonic saline solution (pH 7.4, PBS) and were determined by using the DPD ferrous titrimetric method.[18]

After serial instillation of 150 μl of HOCl solution at various concentrations for 30 s, the ocular surface was washed once with 150 μl of saline in order to remove the free HOCl and some oxidized water-soluble substances, then all the meniscus tear samples were collected at a certain interval over 120 min. Thirty μl and 80 μl tear samples were used for measuring the GSH amount and LDH activity, respectively. The GSH amount in meniscus tears was determined using the method reported by Hiraki *et al.*[19]

Quantitative Evaluation of Effectiveness of Taurine on Ocular Surface Damage due to HOCl Effect of Pre-application of Taurine: At 30 min after serial instillation of 150 μl of isotonic taurine solution (pH 7.0) at various concentrations, or saline, 150 μl of HOCl solution (4.2 mM or 0.7 mM) was instilled into the cul-de-sac of rabbit eyes. At a certain time after HOCl treatment, meniscus tear samples were collected using the saline pool method, and the LDH activity of each was measured.

Effect of Post-application of Taurine: After serial instillation of 150 μl of 0.7 mM HOCl solution for 30 s, the ocular surface was washed with saline, and at once 150 μl of 240 mM taurine, or saline, was instilled in the cul-de-sac of rabbit eyes. After 30 min, meniscus tear sample was collected and the LDH activity was measured.

Results and Discussion

Accurate sampling of a meniscus tear is required in order to quantitatively estimate ocular surface damage. Imayasu *et al.* reported that LDH activity in meniscus tears collected with 2-μl glass micropipettes is a good index to estimate corneal damage.[14] In this micropipette method, 2 μl of tear sample mustbe collected carefully within 30 s. Because the meniscus tear volume is small, 7.5 ± 2.5 μl,[20] it was difficult to collect meniscus tears within 30 s. In testing protecting agents, meniscus tears were diluted, so the LDH activity in meniscus tears may have been lowered.

Our saline pool method, in which all of the meniscus tears were collected within 10 s after pooling the tears in the cul-de-sac of the rabbit eye with 150 μl of saline, has advantages over the conventional micropipette method: first, it is easy to collect meniscus tears; second, the LDH value is thought to be the least sensitive to dilution by tear secretion induced by eye irritation and the residual sample in the cul-de-sac.

LDH activities in normal rabbits' meniscus tears collected by the two sampling methods are shown in Table I. The mean value of LDH activity with the micropipette method was 1381 ± 781 U/l ($n=21$), which is close to the LDH value (1352 ± 1098 U/l, $n=22$) in normal rabbit meniscus tears reported by Imayasu *et al.*[14] The mean LDH value obtained by the saline pool method was 58.5 ± 32.3 U/l ($n=42$), which corresponds to the original LDH concentration of 1237 U/l calculated from the normal resident volume (7.5 μl) in meniscus tears. The value is close to that obtained by the conventional micropipette method, showing that the mean LDH value obtained by the saline pool method reflects the real LDH value in a meniscus tear.

TABLE I. Each LDH Activity in Normal Rabbit Meniscus Tears Collected by Two Meniscus Tear Sampling Methods

	LDH activity	
	Micropipette method[a]	Saline pool method[b]
Mean value (U/l)	1381	58.9 (1237)[c]
C.V. (%)	56.6	55.2
Number of eyes (n)	21	42

a) The method of collecting the meniscus tears directly with 2-μl of glass micropipette within 30 s. *b*) The method of collecting meniscus tears with 150 μl of saline pooled for 10 s in the cul de sac of rabbit eyes. *c*) 58.9 (U/l) × 157.5/7.5 = 1237; 7.5 μl: Initial volume based on the normal resident volume in the cul de sac of rabbit eyes.

LDH and GSH released from ocular surface tissues into meniscus tears after HOCl treatment are shown in Fig. 1. LDH activity reached a maximum value after 5 min, suggesting that the damage to ocular surface tissues by HOCl occurred rapidly. On the other hand, GSH disappeared quickly after the HOCl treatment and then increased gradually for 15 min, suggesting that the GSH in meniscus tears was consumed by interacting with HOCl. These observations clearly indicate that GSH was inadequate for use as an index of ocular surface tissue damage caused by HOCl.

Figure 2 shows the time course of release of LDH from

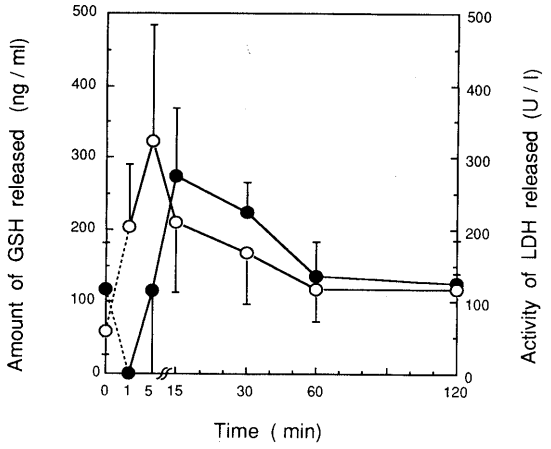

Fig. 1. Release Behaviors of LDH and GSH from the Ocular Surface after HOCl Treatment

○, LDH; ●, GSH. The concentration of HOCl used in HOCl Treatment was 1.12 mM. Each value represents the mean ± S.D. ($n=6$–10).

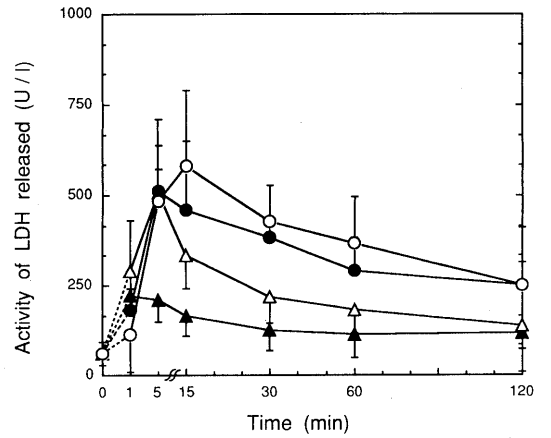

Fig. 2. Time Course of Release of LDH from Ocular Surface Tissues as a Function of HOCl Concentration

▲, 1.15 mM HOCl; △, 2.3 mM HOCl; ●, 4.6 mM HOCl; ○, 9.2 mM HOCl. Each value represents the mean ± S.D. ($n=6$–7).

ocular surface tissues as a function of HOCl concentration. HOCl-induced LDH release exhibited a peak at 5—15 min, and then LDH activity decreased gradually. The LDH activity after serial instillations of HOCl increased dose-dependently with increasing HOCl concentration, which indicates that the LDH activity is available as a good index of ocular surface damage due to HOCl. With higher concentrations of HOCl (more than 4.6 mM), LDH activity was about three times the initial level, even at 120 min after HOCl treatment, suggesting that ocular surface tissues were destroyed even 120 min after the treatment. These observations indicate that HOCl could react with components of ocular surface tissues quickly and potently, resulting in damage to ocular surface tissue.

Imayasu *et al.* reported that LDH activity was parallel to the Draze test scores in the ocular surface damage caused by wearing contact lenses, mechanical scratching, and dropping of contact lens cleaner.[14] In a histochemical or morphological examination of enucleated rabbit eyes, Yoshimura *et al.*[5] indicated that HOCl attacked ocular surface tissues quickly and potently, resulting in the dose-dependent damage of these tissues. These reports support the presumption that an increase of LDH activity in meniscus tears directly reflects damage to ocular surface tissues, and the use of LDH activity as an index of ocular surface damage provides reasonable quantitative evaluation of the effectiveness of taurine on ocular surface damage due to HOCl.

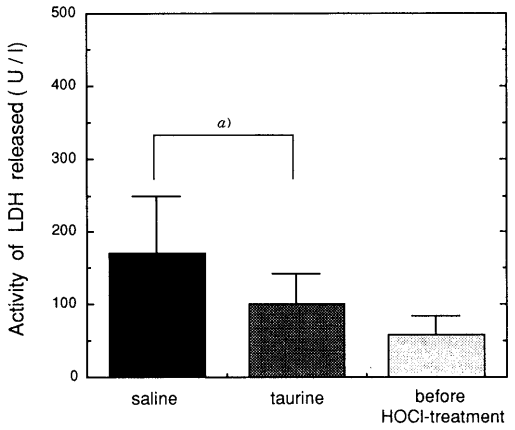

Fig. 3. Effect of Pre-application of Taurine on Oular Surface Damage Caused by HOCl

a) $p<0.01$, paired T-test. Saline was used as a control and the concentration of taurine was 240 mM. HOCl used in HOCl treatment was 0.7 mM. Each sample was applied 30 min before HOCl treatment. Each value was LDH activity 30 min after HOCl treatment. Each value represents the mean ± S.D. ($n=11$).

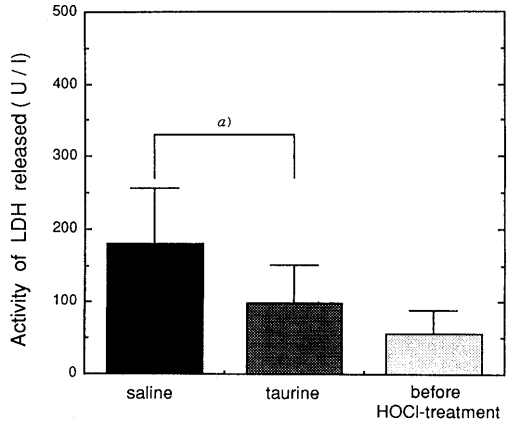

Fig. 4. Effect of Post-application of Taurine on the Ocular Surface Damage Caused by HOCl

a) $p<0.01$, paired T-test. Saline was used as a control and the concentration of taurine was 240 mM. HOCl used in HOCl treatment was 0.7 mM. Each sample was applied 30 s after HOCl treatment. Each value was LDH activity 30 min after HOCl treatment. Each value represents the mean ± S.D. ($n=10$).

The effect of the pre-application of taurine on the ocular surface damage caused by HOCl is shown in Fig. 3. At 30 min after serial instillation of 240 mM taurine, 0.7 mM HOCl was treated. LDH activity at 30 min after HOCl treatment was significantly ($p<0.01$, $n=11$) lower than that of saline. Pre-application of taurine effectively suppressed the HOCl-induced LDH release, suggesting that the residual taurine in ocular surface tissues was still effective in protecting the tissues from HOCl induced damage. Several reports have suggested that taurine efficiently inhibited the lysis of human erythrocytes (HRBC) caused by HOCl generated in neutrophils, as well as the lysis caused by HOCl in a cell-free system,[21,22] suggesting that taurine could effectively protect HRBC from attack by HOCl by scavenging HOCl in the medium.

Figure 4 shows the effect of the post-application of taurine on the ocular surface damage induced by HOCl. After serial instillation of 0.7 mM HOCl for 30 s, ocular surface tissues were washed once with saline in order to remove the free HOCl and the oxidized meniscus tear components, then 240 mM taurine was instilled in the same way. LDH activity at 30 min after the post-application of 240 mM taurine was significantly ($p<0.01$, $n=10$) lower than that in the case of saline. This result indicates that taurine is effective in arresting the progression of damage of ocular surface tissues that have already been attacked by HOCl. We have indicated that taurine effectively inhibited the lysis of canine erythrocytes that had been pre-treated with HOCl by scavenging the oxidized chlorine moiety from the HOCl-treated erythrocytes and that such pre-treatment had a direct protective effect on the erythrocyte membranes.[10] In a histochemical or morphological examination of enucleated rabbit eyes, Yoshimura *et al.* indicated that taurine is effective in healing corneal damage caused by the high concentration of HOCl (5.6 mM).[11] Using our quantitative evaluation, which was performed using LDH activity in meniscus tears as the index of ocular surface damage, we could indicate that the application of taurine is effective against ocular surface damage caused by a low concentration of HOCl (0.7 mM) which is actually used in swimming pools. These results suggest that the application of taurine in swimming pools may significantly ameliorate ocular discomfort.

Conclusions

We developed a new meniscus tear sampling method, and by measuring LDH activity in meniscus tears we quantitatively estimated the ocular surface damage caused by HOCl. Our established method is thought to be available in the estimation of ocular surface damage caused by various eye irritation substances. We showed that taurine could effectively protect ocular surface tissues from damage induced by HOCl and arrest the progression of tissue damage that had already been caused by HOCl. These observations suggest that taurine is clinically useful

in the treatment of ocular surface damage caused by oxidants such as HOCl.

References and Notes

1) B. M. Babior, *N. Engl. J. Med.*, **298**, 659 (1978).
2) J. A. Badway and K. L. Kalnovsky, *Annu. Rev. Biochem.*, **49**, 695 (1980).
3) S. J. Weiss, *N. Engl. J. Med.*, **320**, 365 (1989).
4) S. Kitano and S. Yoshimura, *Nihon No Ganka*, **56**, 539 (1985).
5) H. Yoshimura, T. Yoshizawa, T. Sakimoto and S. Kitano, *Nihon Ganka Kiyou*, **37**, 975 (1986).
6) H. Yoshimura, T. Kiyohara, T. Yoshizawa, T. Sakimoto and S. Kitano, *Nihon Ganka Kiyou*, **37**, 1141 (1986).
7) S. J. Weiss, R. Klein, A. Slivka and M. Wei, *J. Clin. Invest.*, **70**, 598 (1982).
8) E. L. Thomas, M. B. Grishim, D. F. Melton and M. M. Jefferson, *J. Clin. Invest.*, **72**, 441 (1983).
9) E. L. Wright, T. T. Lin, Y. Y. Lin, J. A. Sturman and G. E. Gaull "Taurine: Biological Actions and Clinical Perspective," Alan R. Liss, Inc., 1985, pp. 137—147.
10) K. Nakamori, I. Koyama, T. Nakamura, T. Yoshida, M. Umeda and K. Inoue, *Chem. Pharm. Bull.*, **38**, 3116 (1990).
11) H. Yoshimura, J. Shoji and S. Kitano, *Nihon Ganka Kiyou*, **54**, 1797 (1989).
12) J. H. Draize, "Application of Safety of Chemicals in Foods, Drugs, and Cosmetics," Association of FDA Officials of U.S.A., 1959, p. 49.
13) Jan A. M. A. Dormans and Marinus J. Van Logten, *Toxicol. Appl. Pharmacol.*, **62**, 251 (1982).
14) M. Imayasu, T. Hirata, S. Mitunaga, S. Kotani and H. Hamano, *Atarashii Ganka*, **7**, 297 (1990).
15) S. Hiraki, T. Ishida, Y. Yamada and Y. Nakamura, *Atarashii Ganka*, **3**, 383 (1986).
16) T. Ishida, S. Hiraki and Y. Nakamura, *Nichigankaishi*, **91**, 63 (1987).
17) S. Hiraki, T. Ishida and M. Karino, *Nichigankaishi*, **92**, 135 (1988).
18) A. P. H. A., A. W. W. A. and W. P. C. F., "Standard Method for the Examination of Water and Wastewater," 14th ed., ed. by M. A. Franson, American Public Health Association, Washington, 1975, pp. 329—332.
19) S. Hiraki, Y. Yamada and Y. Nakamura, *Atarashi Ganka*, **2**, 294 (1985).
20) S. S. Chrai, T. F. Patton, A. Mehta and J. R. Robinson, *J. Pharm. Sci.*, **62**, 1112 (1973).
21) E. L. Thomas, M. B. Grisham, D. F. Melton and M. M. Jefferson, *J. Biol. Chem.*, **260**, 3321 (1985).
22) F. Dallergri, A. Ballestrero, G. Frumento and F. Datrone, *Immunology*, **55**, 639 (1985).