Ophthalmic Research

Ophthalmic Res 1996;28(suppl 2):16–25

Yoshiji Ohta[a]
Hiroshi Okada[b]
Yoshinao Majima[b]
Isao Ishiguro[a]

Departments of
[a] Biochemistry and
[b] Ophthalmology, School of Medicine, Fujita Health University, Toyoake, Aichi, Japan

Anticataract Action of Vitamin E: Its Estimation Using an in vitro Steroid Cataract Model

Key Words
Methylprednisolone
Steroid cataract
Vitamin E
Oxidative damage
Membrane dysfunction
Cultured rat lens

Abstract
The aim of this study was to estimate the anticataract action of vitamin E using an in vitro methylprednisolone (MP)-induced cataract model. The same severity of early cortical cataract was induced in lenses isolated from male Wistar rats aged 6 weeks by incubation with MP (1.5 mg/ml) in TC-199 medium. The cataractous lenses showed slight increases in lipid peroxide (LPO) content and Na^+/K^+ ratio and slight decreases in reduced glutathione (GSH) content and glyceraldehyde-3-phosphate dehydrogenase (GAP-DH), a sensitive index of oxidative stress, and Na^+,K^+-ATPase activities. When the cataractous lenses were further incubated in TC-199 medium with and without vitamin E (250 μg/ml) for 48 h, the progression of cataract was prevented in the vitamin E-treated lenses, but not in the vitamin E-untreated lenses. The vitamin E-untreated lenses showed a decrease in vitamin E content and an increase in water content in addition to further increases in LPO content and Na^+/K^+ ratio and further decreases in GSH content and GAP-DH and Na^+,K^+-ATPase activities. In contrast, the changes of these components and enzymes except for GSH were attenuated in the vitamin E-treated lenses. From these results, it can be estimated that vitamin E prevents in vitro cataractogenesis in rat lenses treated with MP by protecting the lenses against oxidative damage and loss of membrane function.

KARGER
E-Mail karger@karger.ch
Fax + 41 61 306 12 34
http://www.karger.ch

0030–3747/96/0288–0016$10.00/0

Yoshiji Ohta, PhD
Department of Biochemistry
School of Medicine
Fujita Health University
Toyoake, Aichi 470-11 (Japan)

Introduction

It has been demonstrated that vitamin E prevents cataractogenesis in a variety of in vivo and in vitro cataract models [1–3]. Creighton et al. [4] have reported that vitamin E partially prevents opacification of rat lenses cultured with methylprednisolone (MP). Recently, we have shown that vitamin E content decreases with an increase in lipid peroxide (LPO) content and decreases in reduced glutathione (GSH) content and glyceraldehyde-3-phosphate dehydrogenase (GAP-DH) activity at the late stage of cataractogenesis in cultured rat lenses with MP treatment [5]. In addition, we have reported that vitamin E can prevent cataract progression in rat lenses cultured with MP when the vitamin treatment is conducted at the early stage of the cataractogenesis, but not at the late stage [6]. However, it is not still enough to estimate the preventive action of vitamin E on this in vitro cataractogenesis in MP-treated rat lenses.

We, therefore, attempted to estimate the anti-cataract action of vitamin E using this in vitro MP-induced cataract model. Namely, we examined the preventive effect of vitamin E on cataract progression and changes of vitamin E, LPO, GSH, and water contents, Na^+/K^+ ratio, and GAP-DH, which is known to be sensitive to oxidative stress [7], and Na^+/K^+-ATPase activities in rat lenses with early cortical cataract induced by MP treatment following further incubation in a culture medium containing MP. Vitamin E is well known to function not only as a chain-breaking antioxidant but also as a membrane stabilizer [8, 9]. Mayman et al. [10] have reported using bovine lenses cultured with dexamethasone that the dysfunction of the cation pump via Na^+,K^+-ATPase might be related to cataractogenesis. Accordingly, Na^+/K^+ ratio, water content, and Na^+/K^+-ATPase activity in lenses were checked to estimate the membrane function in addition to the determinations of vitamin E, LPO, and GSH contents and GAP-DH activity in the tissues.

Materials and Methods

Chemicals

MP (6α-methylprednisolone 21-hemi-succinate) was purchased from Sigma Chemical Co. (St. Louis, Mo., USA); powder TC-199 and fetal calf serum (FCS) from Gibco Laboratories Life Technologies Inc. (Grand Island, N.Y., USA); a mixture of antibiotics (penicillin, streptomycin, and fungisone) from Bio-Whittaker (Walkersville, Mass., USA); vitamin E dissolved in HCO-60 and HCO-60 from Esai Co. (Tokyo, Japan); ATP, 3-phosphoglycerate, and 3-phosphoglycerate kinase from Boehringer Mannheim (Tokyo, Japan). Other chemicals used were commercially available and of reagent grade.

Animals

Male Wistar rats aged 6 weeks were used throughout. The animals were purchased from SLC Co. (Hamamatsu, Japan). All animals received human care in compliance with the institution's guidelines.

Isolation and Culture of Lenses

Eyes were enucleated from rats immediately after sacrifice by cervical dislocation. Lenses were dissected from the eyes using the posterior approach. Each isolated lens was placed in one well of a Falcon plastic culture plate (24 wells) (Becton Dickinson Labware, Franklin Lakes, N.J., USA) which contained 2 ml of a culture medium consisting of TC-199/bicarbonate buffer (pH 7.4), 10% FCS, 0.1 ml of a mixed solution of antibiotics, and 5.6 m*M* glucose (basic medium). They were incubated at 37 °C under 90% moisture and 95% air-5% CO_2 gas atmosphere for 24 h. A transient increase in lens wet weight was observed during this incubation as described in our previous report [11], but the incubated lenses showed the same wet weight as fresh lenses dissected from the eyes. Then, each of the resulting transparent lenses was incubated in 2 ml of the basic culture medium containing MP (1.5 mg/ml) for 24 h to induce early cataracts around the cortical equator or in 2 ml of the basic medium for the same period as the control. Next, each of the MP-treated lenses with the same severity of cortical opacity was incubated in 2 ml of the basic medium containing MP (1.5 mg/ml) with and without vitamin E (250 μg/ml) for 24 and 48 h, while each of the MP-untreated clear

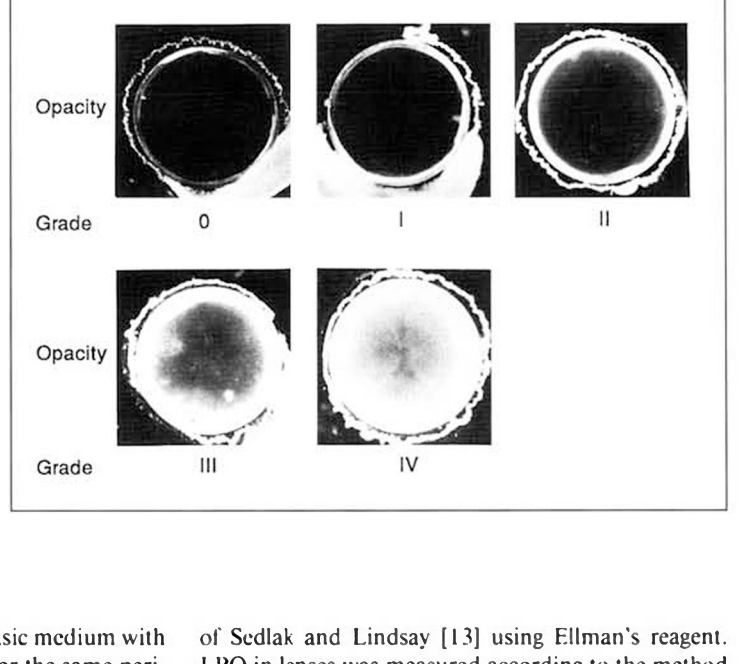

Fig. 1. Gradation of opacity in rat lenses cultured with MP. Grade 0 = No opacity; grade I = faint cortical opacity around the equator; grade II = cortical opacity slightly spreading from the equator toward the center part of lens; grade III = cortical opacity spreading nearby the center part of lens; grade IV = cortical opacity covering the whole part of lens.

lenses was incubated in 2 ml of the basic medium with and without vitamin E (250 μg/ml) for the same period. During these incubations, PM-untreated clear lenses showed no significant change in wet weight. The volume of a vitamin E solution in HCO-60, a detergent, added to the medium was 10 μl. When lenses with and without MP treatment were not incubated with vitamin E, 10 μl of a HCO-60 solution used for dissolution of vitamin E was added to the medium. Immediately after observation of opacity, lenses were lightly blotted on a filter, weighed, and then kept in a small plastic tube with a tight cap which was filled with nitrogen gas at −80°C until use.

Estimation of the Severity of Lens Opacity

Lens opacity was checked by the dark field photographic method described in our previous reports [5, 6, 11], using a dissecting microscope, Olympus SHZ-ILL, equipped with an automatic microscope camera, Olympus PM-10AD (Olympus Optical Co., Tokyo, Japan). The severity of lens opacity was estimated based on the opacity gradation as shown in figure 1.

Measurements of Vitamin E, GSH, LPO, and Water in Lenses

Vitamin E in lenses was measured by the method of Abe et al. [12] using high-performance liquid chromatography with fluorescence detection. The amount of vitamin E measured is expressed as that of α-tocopherol (α-Toc). GSH in lenses was measured by the method of Sedlak and Lindsay [13] using Ellman's reagent. LPO in lenses was measured according to the method of Ohkawa et al. [14] using 2-thiobarbituric acid reactione except that 1.0 m*M* ethylenediaminetetraacetic acid was added to the reaction mixture. The amount of LPO measured is expressed as that of malondialdehyde (MDA). Water in lenses was determined as follows: Lenses were dried at 100°C in an electric oven for 30 h after checking their wet weight and then their dry weight was checked again after cooling. Water content in lenses was estimated by subtracting their dry weight from their wet weight. This content is expressed as the percentage of water contained in lenses.

Measurement of Na^+/K^+ Ratio in Lenses

Na^+ and K^+ in lenses were determined using a Hitachi model 710 automatic electrolytes analyzer (Hitachi Co., Tokyo, Japan). Na^+/K^+ ratio in lenses was calculated based on the contents of Na^+ and K^+ in the tissues.

Assays of GAP-DH and Na^+,K^+-ATPase in Lenses

GAP-DH in lenses was assayed according to the method of Bergmeyer et al. [15]. The oxidation of NADH to NAD^+ following the conversion of 1,3-diphosphoglycerate to glyceraldehyde via the enzyme was measured in the reaction mixture consisting of 1.1 m*M* ATP, 6.2 m*M* 3-phosphoglycerate, 0.2 m*M* NADH, 0.9 m*M* ethylenediaminetetraacetic acid, 2.0 m*M* $MgCl_2$, 13 units of 3-phosphoglycerate kinase, and

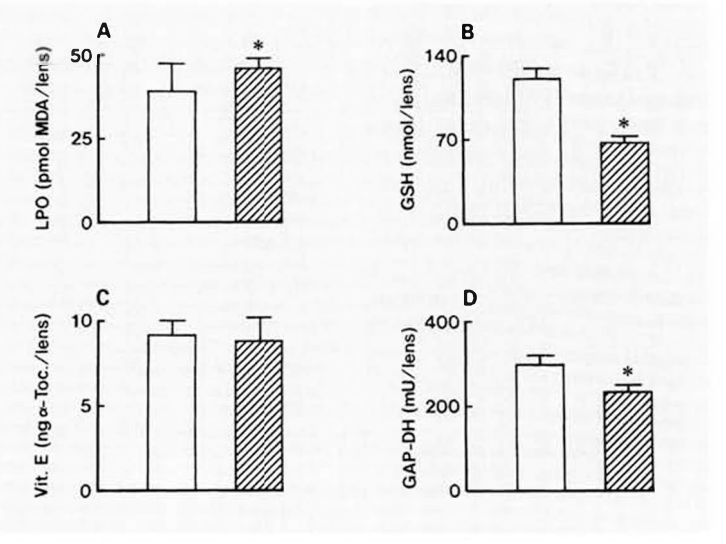

Fig. 2. LPO (**A**), GSH (**B**), and vitamin E (**C**) contents and GAP-DH activity (**D**) in rat lenses with MP-induced grade II opacity and without opacity. Treatment of lenses with MP and determinations of lenticular LPO, GSH, vitamin E, and GAP-DH indicated were conducted as described in 'Materials and Methods'. □ = Control lenses without opacity; ▨ = lenses with MP-induced grade II opacity. Each value is the mean ± SD from three experiments with 5–8 lenses each. * $p < 0.05$ (vs. control lenses).

an appropriate amount of 10% lens homogenates in 82.3 m*M* triethanolamine buffer (pH 7.6) (total volume, 1.0 ml) at 37°C. The decrease in absorbance at 340 nm following the oxidation of NADH was measured for 4 min after starting the reaction. One unit (U) of this enzyme is expressed as the amount of enzyme to cause the oxidation of NADH at an initial rate of 1 μmol/min. The amount of NADH oxidized was calculated using an extinction coefficient of 6.22 $mM^{-1} \cdot cm^{-1}$ at 340 nm for NADH. Na^+,K^+-ATPase in lenses was assayed by the method of Nakao et al. [16]. This enzyme activity is expressed in terms of the amount of inorganic phosphorus (P_i) released from ATP hydrolyzed by the enzyme at 37°C for the first 1 h incubation per lens. P_i was determined by the method of Goldenberg and Fernandez [17].

Statistical Analyses

Results obtained for lens components and enzymes are expressed as the means ± SD. The statistical analyses of these results were performed using the Student's t test. For results of the severity of lens opacity, statistical analyses were carried out using the Mann-Whitney test. Values of significance were set at $p < 0.05$ for both tests.

Results

Rat lenses with MP-induced early cortical cataract that were used throughout in this study had grade II opacity.

The contents of LPO, GSH, and vitamin E and the activity of GAP-DH were first examined in lenses with MP-induced grade II opacity. LPO content in the opaque lenses significantly increased and was 1.2-fold more than that in clear control lenses (fig. 2A). GSH content in the opaque lenses significantly decreased and was 56% of the control level (fig. 2B). There was no difference in vitamin E content between the opaque and clear control lenses (fig. 2C). GAP-DH activity in the opaque lenses significantly decreased and was 78% of the control level (fig. 2D).

Na^+/K^+ ratio, water content, and Na^+,K^+-ATPase activity were next examined in lenses with MP-induced grade II opacity. Na^+/K^+ ratio in the opaque lenses significantly increased and was 3.1-fold higher than that in clear control lenses (fig. 3A). There was no difference in water content between the opaque

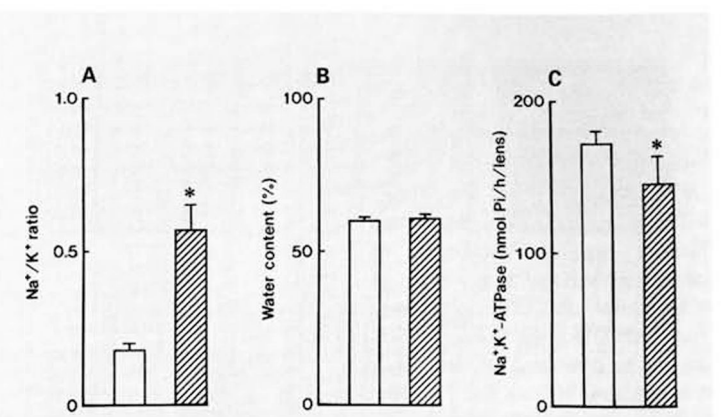

Fig. 3. Na^+/K^+ ratio (**A**), water content (**B**), and Na^+,K^+-ATPase activity (**C**) in rat lenses with MP-induced grade II opacity and without opacity. Treatment of lenses with MP and determinations of lenticular Na^+/K^+ ratio, water content, and Na^+,K^+-ATPase indicated were conducted as described in 'Materials and Methods'. □ = Control lenses without opacity; ▨ = lenses with MP-induced grade II opacity. Each value is the mean ± SD from three experiments with 5–11 lenses each. * $p < 0.05$ (vs. control lenses).

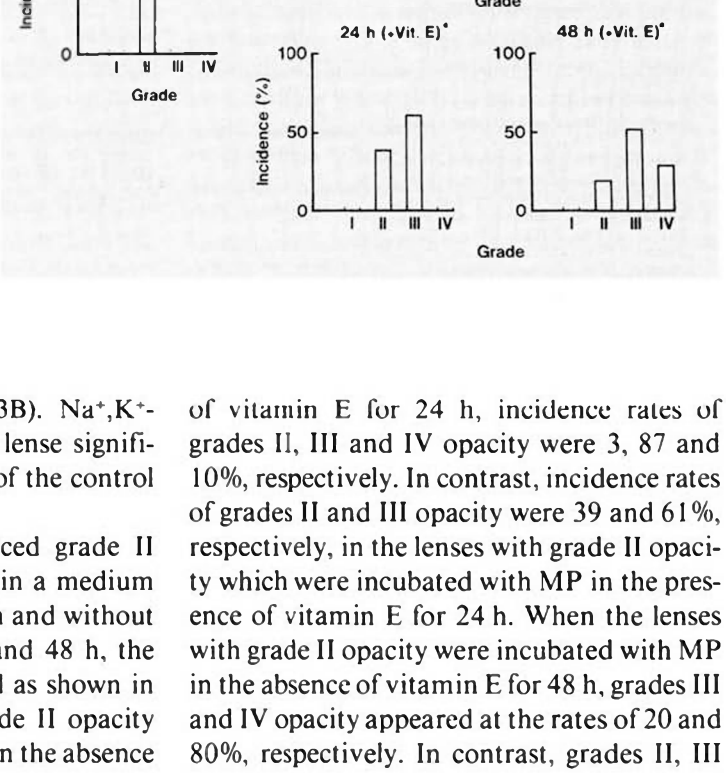

Fig. 4. Effect of vitamin E treatment on cataract progression in rat lenses with MP-induced grade II opacity following further incubation with MP for 24 and 48 h. Treatment of lenses with MP and vitamin E and estimation of cataract severity were conducted as described in 'Materials and Methods'. The number of lenses used is 30–31. * $p < 0.05$ (vs. vitamin E-untreated lenses).

and clear control lenses (fig. 3B). Na^+,K^+-ATPase activity in the opaque lense significantly decreased and was 84% of the control level (fig. 3C).

When lenses with MP-induced grade II opacity were further incubated in a medium containing MP (1.5 mg/ml) with and without vitamin E (250 μg/ml) for 24 and 48 h, the progression of cataract occurred as shown in figure 4. In the lenses with grade II opacity which were incubated with MP in the absence of vitamin E for 24 h, incidence rates of grades II, III and IV opacity were 3, 87 and 10%, respectively. In contrast, incidence rates of grades II and III opacity were 39 and 61%, respectively, in the lenses with grade II opacity which were incubated with MP in the presence of vitamin E for 24 h. When the lenses with grade II opacity were incubated with MP in the absence of vitamin E for 48 h, grades III and IV opacity appeared at the rates of 20 and 80%, respectively. In contrast, grades II, III

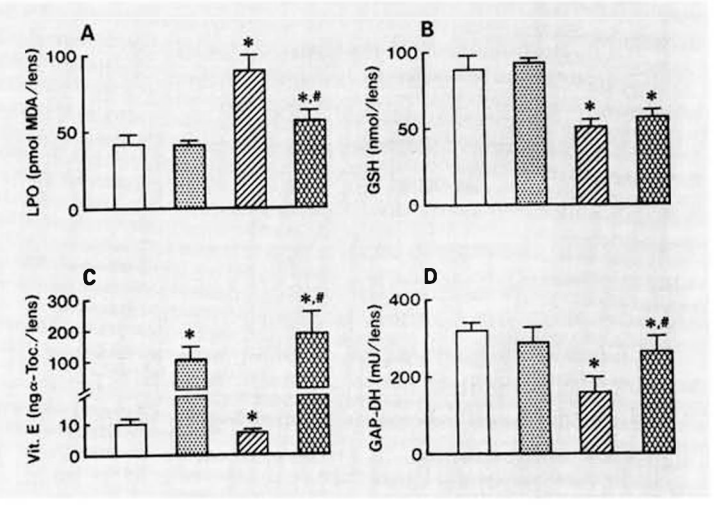

Fig. 5. Effect of vitamin E treatment on changes of LPO (**A**), GSH (**B**), and vitamin E (**C**) contents and GAP-DH activity (**D**) in rat lenses with MP-induced grade II opacity and without opacity following further incubation with and without MP for 48 h. Treatment of lenses with MP and/or vitamin E and determinations of lenticular LPO, GSH, vitamin E, and GAP-DH indicated were conducted as described in 'Materials and Methods'. □ = Control group (clear lenses with neither MP nor vitamin E treatments); ▩ = vitamin E-treated group (clear lenses with vitamin E treatment alone); ▨ = MP-treated group (opaque lenses with MP treatment alone); ▩ = MP + vitamine E-treated group (opaque lenses with MP and vitamin E treatments). Each value is a mean ± SD from three experiments with 4–5 lenses each. * $p < 0.05$ (vs. control group). # $p < 0.05$ (vs. MP-treated group).

and IV opacity appeared at the rates of 19, 52 and 29%, respectively, when the lenses with grade II opacity were incubated with MP in the presence of vitamin E for 48 h. Thus, vitamin E prevented the progression of MP-induced cataract and this preventive effect was significant.

When lenses with MP-induced grade II opacity were further incubated with MP (1.5 mg/ml) in the presence or absence of vitamin E (250 μg/ml) for 48 h, LPO, GSH, and vitamin E contents and GAP-DH activity in the tissues changed as shown in figure 5. The opaque lenses cultured with MP in the absence of vitamin E (MP-treated group) showed a significant increase in LPO content and significant decreases in GSH and vitamin E contents and GAP-DH activity. LPO content in the MP-treated group was 2.1-fold more than that in clear lenses cultured with neither MP nor vitamin E (control group) (fig. 5A). GSH content in the MP-treated group was 57% of that in the control group, while vitamin E content in the former group was 70% of that in the latter group (fig. 5B, C). GAP-DH activity in the MP-treated group was 50% of that in the control group (fig. 5D). LPO content in the opaque lenses cultured with MP in the presence of vitamin E (MP + vitamin E-treated group) was 1.3-fold more than that in the control group but was significantly less than that in the MP-treated group (fig. 5A). GSH content in the MP + vitamin E-treated group was 64% of that in the control group and this content was almost equivalent to that in the MP-treated group (fig. 5B). Vita-

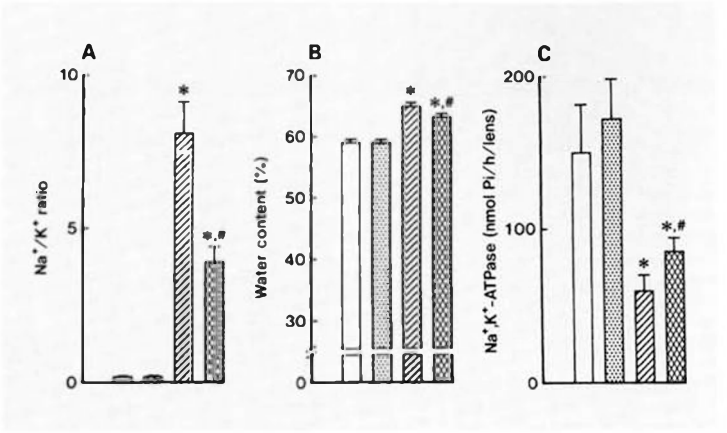

Fig. 6. Effect of vitamin E treatment on changes of Na^+/K^+ ratio (**A**), water content (**B**), and Na^+,K^+-ATPase activity (**C**) in rat lenses with MP-induced grade II opacity and without opacity following further incubation with and without MP for 48 h. Treatment of lenses with MP and/or vitamin E and determinations of lenticular Na^+/K^+ ratio, water content, and Na^+,K^+-ATPase indicated were conducted as described in 'Materials and Methods'. □ = Control group (clear lenses with neither MP nor vitamin E treatments); ▨ = vitamin E-treated group (clear lenses with vitamin E treatment alone); ▨ = MP-treated group (opaque lenses with MP treatment alone); ▨ = MP + vitamin E-treated group (opaque lenses with MP and vitamin E treatments). Each value is a mean ± SD from three experiments with 4–5 lenses each. * $p < 0.05$ (vs. control group). # $p < 0.05$ (vs. MP-treated group).

min E content in the MP + vitamin E-treated group was 20-fold more than that in the control group and was significantly higher than that in the MP-treated group (fig. 5C). GAP-DH activity in the MP + vitamin E-treated group was 83% of that in the control group but was significantly higher than that in the MP-treated group (fig. 5D). There were no significant changes in LPO and GSH contents and GAP-DH activity in clear lenses incubated with vitamin E (250 μg/ml) in the absence of MP for 48 h (vitamin E-treated group) (fig. 5A–C). Vitamin E content in the vitamin E-treated group was 11-fold more than that in the control group and was not significantly different from that in the MP + vitamin E-treated group (fig. 5C).

When lenses with MP-induced grade II opacity were further incubated with MP (1.5 mg/ml) in the presence or absence of vitamin E (250 μg/ml) for 48 h, Na^+/K^+ ratio, water content, Na^+/K^+-ATPase activity in the tissues changed as shown in figure 6. The opaque lenses cultured with MP in the absence of vitamin E (MP-treated group) showed significant increases in Na^+/K^+ ratio and water content and a significant decrease in Na^+,K^+-ATPase activity. Na^+/K^+ ratio in the MP-treated group was 40-fold higher than that in clear lenses cultured with neither MP nor vitamin E (control group) (fig. 6A). Water content in the MP-treated group was 1.12-fold more than that in the control group (fig. 6B). Na^+,K^+-ATPase activity in the MP-treated group was 39% of that in the control group (fig. 6C). Na^+/K^+ ratio in the opaque lenses cultured with MP in the presence of vitamin E (MP + vitamin E-treated group) was 19-fold higher than that in the control group but was significantly lower than that in the MP-

treated group (fig. 6A). Water content in the vitamin E-treated group was significantly more than that in the control group but was significantly less than that in the MP + vitamin E-treated group (fig. 6B). Na^+,K^+-ATPase activity in the vitamin E-treated group was 57% of that in the control group but was significantly higher than that in the MP + vitamin E-treated group (fig. 6C). No significant changes in Na^+/K^+ ratio, water content, and Na^+,K^+-ATPase activity occurred in clear lenses incubated with vitamin E (250 μg/ml) in the absence of MP for 48 h (vitamin E-treated group) (fig. 6A–C).

Discussion

The present study was undertaken to estimate the anticataract action of vitamin E on cataractogenesis in rat lenses cultured with MP. In this study, we examined the effect of vitamin E on the progression of cataract and changes in not only vitamin E, GSH, and LPO contents and GAP-DH activity, which are indices of oxidative stress, but also Na^+/K^+ ratio, water content, and Na^+,K^+-ATPase activity, which are indices of membrane function, in rat lenses with MP-induced early cortical cataract following further MP treatment.

Increases in LPO content and Na^+/K^+ ratio and decreases in GSH content and Na^+,K^+-ATPase activity were found in rat lenses with MP-induced early cortical cataract, indicating the occurrence of oxidative damage and membrane dysfunction with increased membrane permeability in the cataractous lenses. When such cataractous lenses with oxidative damage and membrane dysfunction were further cultured in a medium containing MP over a 48-hour period, cataract progressed and consequently the whole cortex was covered with opacity. In the lenses with such advanced cortical cataracts, oxidative damage was aggravated with a decrease in vitamin E content and an increase in water content occurred with further loss of the membrane function with markedly increased membrane permeability. We have already reported that vitamin E content decreases with an increase in LPO content and decreases in GSH content and GAP-DH activity at the late stage of cataractogenesis in cultured rat lenses with MP treatment [5]. Accordingly, these results indicate that not only oxidative damage but also membrane dysfunction should be linked to the progression of cataract in rat lenses cultured with MP. These results also support the mechanism by which damage of the cation pump in the lens membrane takes part in in vitro cataractogenesis induced by corticosteroids, which has been proposed by Mayman et al. [10].

We have reported that vitamin E can prevent cataract progression in rat lenses cultured with MP when the vitamin treatment is conducted at the early stage of the cataractogenesis [6]. Vitamin E is well known to function as a chain-breaking antioxidant and a membrane stabilizer [8, 9]. It is known that Na^+,K^+-ATPase, a component of the cation pump mechanism, in lens membranes requires lipids and sulfhydryl (-SH) group for expression of its enzymatic activity, and that this enzyme in lens is inactivated by changes of membrane lipid composition and membrane fluidity in the tissue and by oxidation of -SH group in the enzyme protein [18–21]. Takenaka et al. [22] have demonstrated that vitamin E functions as an antioxidant on the oxidation of membrane protein thiols induced by free radicals generated in different sites. When rat lenses with MP-induced early cortical cataract were further incubated in a medium containing MP with vitamin E over a 48-hour period, the progression of cataract was found to be prevented significantly. In

addition, this vitamin E treatment was found to depress increases in LPO and water content and Na^+/K^+ ratio and decreases in vitamin E content and GAP-DH and Na^+,K^+-ATPase activities in rat lenses with MP-induced early cataract following further incubation with MP. From these results, it is further defined that vitamin E is capable of preventing cataract progression in cultured rat lenses with MP-induced early cortical cataract following further MP treatment. These results also suggest that vitamin E can prevent the progression of cataract in the opaque rat lenses further cultured with MP by protecting the lenses against oxidative damage and loss of the membrane function. However, vitamin E treatment could not depress a decrease in GSH content in rat lenses with MP-induced early cataract following further incubation with MP, while the vitamin treatment had a preventive effect on the increase of LPO content and the decreases of vitamin E content and GAP-DH activity in the cataractous lenses following further incubation with MP as described above. Although it is not clear at present why vitamin E cannot depress this decrease in lenticular GSH content following further MP treatment, the reason may be explained as follows: In rat lenses with MP-induced early cortical cataract, not only oxidative damage but also membrane dysfunction with increased membrane permeability occurred as mentioned above. It has been reported that a similar membrane dysfunction with increased membrane permeability takes place in rat lenses with early cortical cataract induced by treatment with high concentrations of sugars such as glucose, galactose, and xylose in vitro [23–25]. Lou et al. [26] have shown that in in vitro xylose-treated rat lenses with early cortical cataract, a decrease in lenticular GSH levels may be due to its loss through the osmotically damaged leaky membrane. In addition, we have reported that in rat lenses cultured with a high concentration of glucose or xylose, vitamin E in a liposomal form cannot arrest a decrease in GSH content with the development of cortical cataract, although the vitamin prevents an increase in LPO content in the tissues [11, 27]. From these findings, it can be presumed that the decrease of GSH content found in rat lenses with MP-induced early cortical cataract may be due to not only oxidative damage but also its loss through the osmotically damaged leaky membrane, and that in lenses cultured with MP, vitamin E treatment after early cataract formation is unable to prevent the loss of GSH through the once oxidatively and osmotically damaged leaky membrane, resulting in no prevention of the decrease of lenticular GSH content.

In conclusion, the present results indicate that in rat lenses cultured with MP, oxidative damage and membrane dysfunction in the tissues should be closely related to the progression of cataract, and that vitamin E is able to prevent this cataract progression when the vitamin treatment is conducted at the early stage of the cataractogenesis. In addition, the present results lead us to estimate that vitamin E can prevent in vitro cataractogenesis in MP-treated rat lenses by protecting the lenses against oxidative damage and loss of the membrane function.

References

1 Gerster H: Antioxidant vitamins in cataract prevention. Z Ernährungswiss 1989;28:56–75.

2 Machlin LJ: Influence of antioxidant vitamins on cataract formation; in Hayaishi O, Niki E, Kondo M, Yoshikawa T (eds): Medical, Biochemical and Clinical Aspects of Free Radicals. Amsterdam, Elsevier, 1989, pp 351–359.

3 Ohta Y, Ishiguro I: Causative factors of cataract and its prevention. Bio Industry 1995;12:13–24.

4 Creighton MO, Sanwal M, Stewart-DeHaan PJ, Trevithick JR: Modelling cortical cataractogenesis. 5. Steroid cataracts induced by solumedrol partially prevented by vitamin E in vitro. Exp Eye Res 1983;37:65–75.

5 Okada H, Ito A, Majima Y, Ohta Y, Ishiguro I, Torii H: Changes in vitamin E and related compounds in rat lenses cultured with methylprednisolone following opacification. J Eye (Atarashii Ganka) 1993;10:1263–1266.

6 Okada H, Majima Y, Ohta Y, Ishiguro I, Ito A, Torii H: Preventive effect of vitamin E on cataractogenesis in cultured rat lenses with steroid treatment. J Eye (Atarashii Ganka) 1994;11:79–83.

7 Spector A, Huang R-RC, Wang G-M, Schmidt C, Yan G-Z, Chifflet S: Dose elevated glutathione protect the cell from H_2O_2 insult? Exp Eye Res 1987;45:453–465.

8 Liebler DC: The role of metabolism in the antioxidant function of vitamin E. Clin Rev Toxicol 1993;23:147–169.

9 Urano S: Membrane-stabilizing effect of vitamin E. Vitamins (Bitamin) 1989;63:75–85.

10 Mayman CI, Miller D, Tijerina ML: In vitro production of steroid cataract in bovine lens. 2. Measurement of sodium-potassium adenosine triphosphatase activity. Acta Ophthalmol 1979;57:1107–1116.

11 Ohta Y, Torri H, Okada H, Hattori H, Majima Y, Ishiguro I: Involvement of oxidative stress in *D*-xylose-induced cataractogenesis in cultured rat lenses. Curr Eye Res 1996;15:1–7.

12 Abe K, Yuguchi Y, Katsui G: Quantitative determination of tocopherols by high-speed liquid chromatography. J Nutr Sci Vitaminol 1975;21:183–188.

13 Sedlak J, Lindsay RH: Estimation of total, protein-bound and nonprotein sulfhydryl groups in tissue with Ellman's reagent. Anal Biochem 1968;25:192–205.

14 Ohkawa H, Ohishi N, Yagi K: Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979;95:351–358.

15 Bergmeyer HU, Grassl M, Walter G-E: Glyceraldehyde-3-phosphate dehydrogenase; in Bergmeyer HU (ed): Methods of Enzymatic Analysis. Weinheim, Verlag Chemie, 1983, vol II, pp 211–213.

16 Nakao T, Nagano K, Adachi K, Nakao M: Separation of two adenosine triphosphatases (ATPase) from erythrocyte membranes. Biochem Biophys Res Commun 1963;13:444–448.

17 Goldenberg H, Fernandez A: Simplified method for the estimation of inorganic phosphate in body fluids. Clin Chem 1966;12:871–992.

18 Baghieri S, Garner MH: Na/K-ATPase and phospholipid degradation in bovine and human lenses. Curr Eye Res 1992;11:459–467.

19 Mizuno GR, Chapman CJ, Chipault JR, Pfeiffer DR: Lipid composition and ($Na^+ + K^+$)-ATPase activity in rat lens during triparanol-induced cataractogenesis. Biochim Biophys Acta 1981;644:1–12.

20 Sen PC, Pfeiffer DR: Characterization of partially purified ($Na^+ + K^+$)-ATPase from porcine lens. Biochim Biophys Acta 1982;693:34–44.

21 Fukui HN, Merola LO, Kinoshita JH: The effects of oxidants on the membrane sulfhydryl groups of the lens. Doc Ophthalmol 1976;8:161–169.

22 Takenaka Y, Miki M, Yasuda H, Mino M: The effect of α-tocopherol as an antioxidant on the oxidation of membrane protein thiols induced by free radicals generated in different sites. Arch Biochem Biophys 1991;285:344–350.

23 Kinoshita JH, Merola LO, Dikmak E: Osmotic changes in experimental galactose cataracts. Exp Eye Res 1962;1:405–410.

24 Obazawa H, Merola LO, Kinoshita JH: The effect of xylose on the isolated lens. Invest Ophthalmol 1974;13:204–209.

25 Kinoshita JH: Mechanism initiating cataract formation. Proctor Lecture. Invest Ophthalmol 1974;13:713–724.

26 Lou MF, McKellar R, Chyan O: Quantitation of lens protein mixed disulfides by ion-exchange chromatography. Exp Eye Res 1986;42:607–616.

27 Hattori H, Majima Y, Nagamura Y, Ishiguro I: Effect of vitamin E-containing liposome on experimental sugar cataract. Acta Soc Ophthalmol Jpn (Nippon Ganka Gakkai Zasshi) 1989;93:97–102.