

Effects of Alpha Tocopherol and Probucol Supplements on Allergen-Induced Airway Inflammation and Hyperresponsiveness in a Mouse Model of Allergic Asthma

Nami Okamoto^a Takuji Murata^a Hiroshi Tamai^a Hiroyuki Tanaka^b Hiroichi Nagai^b

^aDepartment of Pediatrics, Osaka Medical College, Takatsuki City, and ^bDepartment of Pharmacology, Gifu Pharmaceutical University, Gifu, Japan

Key Words

Asthma model mouse · α -Tocopherol · Probucol · Airway hyperresponsiveness · Cytokine · Oxidative stress

Abstract

Objective: We investigated the role of antioxidants in airway hyperresponsiveness to acetylcholine using young asthma model mice, which were sensitized and stimulated with ovalbumin. **Methods:** The mice had been fed either a normal diet, an α -tocopherol-supplemented diet or a probucol-supplemented diet 14 days before the first sensitization. They were immunized with antigen at intervals of 12 days and, starting from 10 days after the second immunization, they were exposed to antigen 3 times every 4th day using an ultrasonic nebulizer. Twenty-four hours after the last antigen inhalation, airway responsiveness to acetylcholine was measured and bronchoalveolar lavage fluid (BALF) was collected. A blood and lung tissue study was also carried out. **Results:** Twenty-four hours after the last antigen challenge, both IL-4 and IL-5 in the BALF of α -tocopherol-supplemented mice were significantly decreased. The IL-5 level in probucol-supplemented mice was also decreased, but there was no difference in IL-4 levels. The serum IgE level was decreased in probucol-supplemented mice. Differential cell rates of the fluid revealed a significant decrease in eosinophils due to antioxidant supplementation. Airway hyperresponsiveness to acetylcholine was also repressed in antioxi-

dant-supplemented mice. In histological sections of lung tissue, inflammatory cells and mucus secretion were markedly reduced in antioxidant-supplemented mice. We investigated the antioxidant effect on our model mice by examining 8-isoprostane in BALF and lung tissue, and acrolein in BALF; however, our experiment gave us no evidence of the antioxidant properties of either α -tocopherol or probucol contributing to the reduction of airway inflammation. **Conclusion:** These findings indicate that α -tocopherol and probucol suppress allergic responses in asthma model mice, although these two drugs cause suppression in different ways that are unrelated to antioxidation.

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Introduction

Asthma is a chronic inflammatory disease of the airways. The inflammatory cells (neutrophils, eosinophils, and macrophages) infiltrating the airways exist even in patients with mild asthma [1–3]. These cells release H_2O_2 or NO, resulting in the formation of superoxide or peroxynitrite anion [4–11], which are highly reactive oxygen species (ROS) that likely play a major role in asthma and are involved in cellular injury [12]. Increased levels of ROS are shown to be associated with many pathophysiologic states and characteristics of asthma, such as increased production of lipid peroxidation, increased air-

Table 1. Composition of the normal diet

Ingredients	Concentration, %
Vitamin-free casein	20.0
DL-Methionine	0.3
Glucose	25.0
Sucrose	25.0
Alpha cornstarch	15.0
Cellulose	5.0
AIN-76 mineral mixture	1.0
AIN-76 vitamin mixture	3.5
Tartaric acid	0.2

The normal diet contains 20 mg α -TOC/kg diet. The α -TOC-supplemented diet was prepared by adding 480 mg α -TOC/kg diet to the normal diet and the 1% probucol diet was prepared by adding 1% v/w probucol to the normal diet. The composition is based on the AIN-76 diet.

way reactivity and secretion, increased production of chemoattractants, and increased vascular permeability [13]. Moreover, eosinophils, macrophages and neutrophils from asthmatic patients produce more ROS than those from normal subjects [14, 15]. On the other hand, changes in the antioxidant defenses of asthmatic patients have been reported, including decreased GSH-Px, selenium deficiency, decreased vitamins C and E, and decreased protein sulfhydryls and total antioxidant capacity in plasma [16–23]. As a result, the oxidant-antioxidant balance shifts toward increased oxidative stress in asthma. Some antioxidant therapies have previously been described as disappointing, but superoxide dismutases and N-acetylcysteine could attenuate the hyperresponsiveness of airways [24–26].

Vitamin E is a potent antioxidant that exists in nature and its relationship with immune functions is becoming clearer. Vitamin E can not only affect the metabolism of arachidonic acid via the 5-lipoxygenase pathway but also modulate the immune function directly through cellular and humoral immunity, and lymphoproliferative response at the thymus [27]. Concerning allergic diseases, it has been reported that vitamin E supplementation can reduce the symptoms and levels of serum immunoglobulin E (IgE) in a mouse model of nasal allergy [28], that there is a significant relationship between dietary vitamin E intake and serum IgE concentration in patients with atopic diseases [29], and that an injection of vitamin E reduced the airway hyperresponsiveness (AHR) of sensitized guinea pigs [30]. Vitamin E is therefore thought to

improve the allergic state both as an antioxidant and immune modulator.

Probucol [4,4'-isopropylidenedithio-bis(2,6-di-tert-butylphenol)] is a lipophilic agent effective for hyperlipidemia and atherosclerosis, and is structurally different from other hypocholesterolemic agents. The antiatherogenic effects of probucol are known to relate not only to the hypocholesterolemic effect but also to the antioxidative effect [31–33]. In plasma, it is transported with lipoproteins, mainly by LDL, and prevents the oxidative modification of LDL by its radical-scavenging property [34]. Furthermore, probucol has a direct effect on macrophage migration without major impairment of the ability of the cells to accumulate and metabolize modified LDL. To clarify the effect of this potent antioxidant on asthma, we examined the possible usefulness of probucol to improve the AHR in a murine model compared with one species of vitamin E, α -tocopherol (α -TOC).

Materials and Methods

Animals

Five-week-old female BALB/C mice, weighing 16–20 g, were purchased from Crea Japan and housed in plastic cages in an air-conditioned room at $23 \pm 2^\circ\text{C}$. The animals were divided into three groups (plus one control group) of 15 mice each. One group was fed a normal diet, one an α -TOC-supplemented diet and one a 1% probucol diet. Experiments were performed following the 1987 Japanese Association for Laboratory Animal Science guidelines for the care and use of experimental animals.

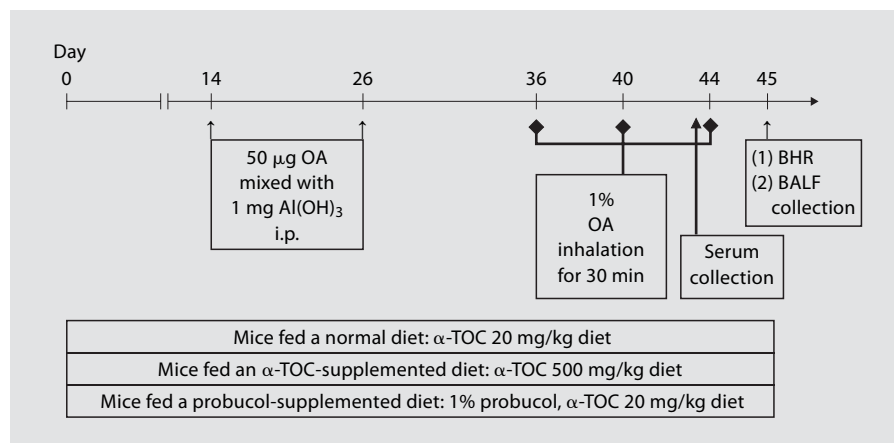
Diets

Normal and α -TOC-supplemented diets were purchased from Funabashi Farm (Chiba, Japan). The 1% probucol diet was made by mixing probucol (Daiichi Seiyaku, Tokyo, Japan) into a normal diet. The normal diet contains 20 mg α -TOC/kg diet and the α -TOC-supplemented diet originally contains 500 mg α -TOC/kg diet. All diets were based on AIN-76 diet (table 1) and prepared by being mixed with stripped corn oil (Tama Biochemical, Tokyo, Japan). The final concentration of corn oil was 9.1% v/w, which falls within the most recent recommended guidelines for the composition of AIN-93G. The diets were stored at -30°C before use. Fresh food was supplied each day.

Sensitization and Antigen Challenge

Figure 1 shows our experimental protocol. All three groups of mice were actively sensitized by intraperitoneal injections of 50 μg ovalbumin (OA; Nacalai Tesque, Kyoto, Japan) with 1 mg $\text{Al}(\text{OH})_3$: alum (LSL, Tokyo, Japan) on day 14 and day 26. Starting on day 36, they were exposed to OA (1% w/v diluted in sterile saline) for 30 min, 3 times every 4th day according to a previously reported method by Tanaka et al. [35]. Control animals fed a normal diet were injected with 1 mg alum and sterile saline into the peritoneal cavity, and exposed to saline in a similar manner (hereinafter, this group is referred to as 'nonsensitized mice'). The

Fig. 1. Experimental protocol. Mice were fed either a normal diet, an α -TOC-supplemented diet or a probucol-supplemented diet. They were sensitized by intraperitoneal injections (i.p.) of 50 μ g OA with 1 mg $\text{Al}(\text{OH})_3$ on day 14 and day 26, and were exposed to 1% OA for 30 min on day 36 and then twice every 4th day. Nonsensitized mice were injected with $\text{Al}(\text{OH})_3$ only and exposed to saline in a similar manner. Sera were obtained prior to the final antigen provocation. 24 h after the last antigen challenge, the mice were anesthetized and AHR to Ach was measured and BALF was obtained. BHR = Bronchial hyperresponsiveness.



aerosol (particle size: 1–8 μ m) was generated using an ultrasonic nebulizer (NE-U12; Omron, Tokyo, Japan), which filled a cylindrical chamber (diameter 12 cm, height 17 cm), in which the mice were exposed.

Measurement of IgE

Just before the final allergen challenge, blood was collected from amputated tail ends. Sera were obtained by centrifugation and stored at -80°C . Values of IgE in mice sera were measured using the Rbis-IgE-ELISA kit for mice (Shibayagi, Gunma, Japan). Briefly, serum IgE was measured by coating monoclonal rat anti-mouse IgE antibodies. After being blocked with 1% BSA, serum dilutions were incubated for 2 h, then biotinylated-anti-mouse IgE antibodies and peroxidase-conjugated streptavidin were added. Sequentially diluted monoclonal mouse IgE antibodies were used as a standard. Optical densities of the enzymatic reactions were read using an automatic ELISA plate reader (Immuno Mini NJ-2300, Nalge Nunc Int., Tokyo, Japan) at 450 nm.

Detection of α -TOC and Probucol

Concentrations of α -TOC and probucol were detected using an HPLC system with an electrochemical detector (Shiseido, Tokyo, Japan). An LC-18 column (Supelco, Tokyo, Japan) was used with an eluent of methanol/ NaClO_4 containing 50 mM NaClO_4 at a flow rate of 1 ml/min. The concentration of α -TOC was calculated by comparing the area under the curve (AUC) with that of an internal standard. The concentration of probucol was calculated from AUC using a standard curve [36].

Measurement of Airway Function

Measurement of bronchial responsiveness to acetylcholine chloride (Ach; Nacalai Tesque, Kyoto, Japan) was carried out according to a modified method previously described [35]. Ten of the 15 mice in each group were anesthetized with pentobarbital sodium salt (Tokyo Kasei, Tokyo, Japan; 80 mg/kg) and a 26-gauge needle was inserted into the jugular vein to inject several doses of Ach. Mice were injected with pancronium bromide (Sigma-Aldrich, St. Louis, Mo., USA; 0.1 mg/kg) intravenously to suppress spontaneous respiration. After disclosing the trachea, an 18-gauge cannula was inserted and the mice were ventilated with

a rodent ventilator (SN-480-7; Shinano, Tokyo, Japan) at 0.2-ml tidal volume and 120 strokes/min. Bronchoconstriction was measured directly using a respiratory amplifier (AR-601G; Nihon Kohden, Tokyo, Japan) connected to the tracheal cannula. Changes in bronchial responsiveness to Ach were measured with doses of 62.5, 125, 250, 500, 1,000 and 2,000 μ g/kg of Ach. We defined the area enclosed with the dose-response curve for Ach and each baseline of airway resistance as the AUC [35].

Bronchoalveolar Lavage Study

To evaluate the airway inflammation, we studied the accumulation of inflammatory cells and cytokines in the bronchoalveolar lavage fluid (BALF). After measuring airway function, the mice were anesthetized with intraperitoneal injections of pentobarbital sodium salt (150 mg/kg). We used calcium- and magnesium-free phosphate-buffered saline [PBS(-); Cosmo Bio Co., Tokyo, Japan] containing 0.1% bovine serum albumin (BSA; Biotecx Laboratories, Houston, Tex., USA) and 0.05 mM disodium ethylenediaminetetraacetic acid (EDTA-2Na ; Nacalai Tesque, Kyoto, Japan) as a solution to collect the BALF. A cannula was inserted into the trachea and 1 ml solution was pumped in and out 4 times, sending it back and forth through the air lumen. This procedure was repeated 3 times (total volume, 3 ml) and about 90% of the injected solution was collected. The BALF of each animal was centrifuged at 1,500 rpm for 10 min. Cell pellets were floated in a 20% BSA solution (20 μ l), then smeared onto slide glasses and stained with hematoxylin and eosin. Values of interleukin-4 (IL-4), interleukin-5 (IL-5) and interferon- γ (IFN- γ) in BALF were measured with ELISA kits (Endogen Mouse Interleukin-4 ELISA, Endogen Mouse Interleukin-5 ELISA, Mouse Interferon Gamma ELISA; Endogen, Cambridge, Mass., USA).

Histological Study

Five of 15 mice in each group were euthanized 24 h after the third antigen inhalation. Whole lungs were distended by 10% buffered formalin injected via the trachea. The tissues were then clamped at the trachea, excised from the bodies and immersed in the same fixative for 24 h. Tissues were sliced at 6- μ m thickness, embedded in paraffin, and stained with hematoxylin and eosin, and Luna.

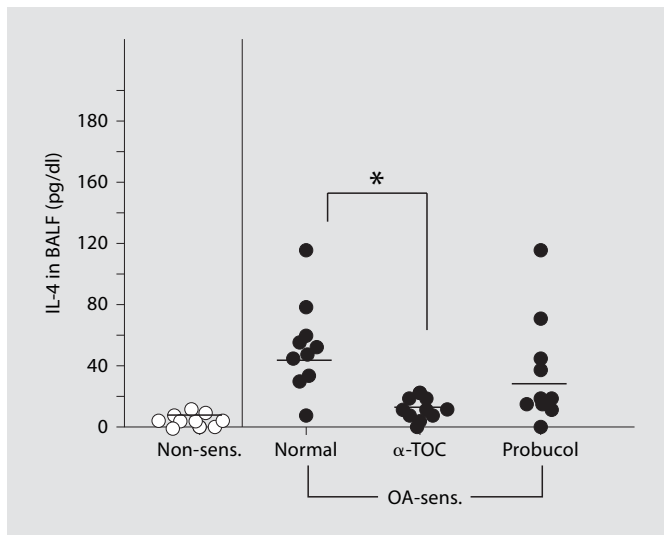


Fig. 2. IL-4 level in BALF was significantly increased by OA sensitization and exposure ($p < 0.005$). In mice fed an α -TOC diet, IL-4 level in BALF was decreased (* $p < 0.05$). Non-sens.: nonsensitized mice; normal: injected with saline and exposed to saline; OA-sens.: sensitized and exposed to OA.

Measurement of Oxidative Stress, 8-Isoprostane and Acrolein

Total 8-isoprostane values in BALF and lung tissue were quantified with an 8-isoprostane EIA kit (Cayman, Ann Arbor, Mich., USA). One milliliter of BALF or supernatant fluid of homogenized lung tissue in saline was incubated with KOH, and all 8-isoprostane was eluted to ethanol. The supernatant was diluted with ultrapure water and acidified with HCl. The samples were then extracted on C18 Sep-Pak cartridges (Waters, Milford, Mass., USA), which had been activated by rinsing first with methanol, then HCl of pH 3.0. After passing the samples through, the cartridges were rinsed first with HCl of pH 3.0, and then with heptane. The samples were eluted on anhydrous sodium sulfate (Wako, Osaka, Japan) with ethyl acetane-containing heptane, and extracted again on Silica Sep-Pak cartridges (Waters), which had been activated by rinsing first with methanol, then ethyl acetane. After the samples were passed through, the cartridges were rinsed again with ethyl acetane. Finally, purified 8-isoprostane was eluted with ethyl acetane-containing methanol. These solvents were evaporated and the samples were reconstituted with an assay buffer.

Acrolein ($\text{CH}_2=\text{CH}-\text{CHO}$; ACR) in BALF was measured with the ACR-lysine adduct ELISA system (NOF Medical Department, Tokyo, Japan).

Statistical Analyses

Data were presented as the mean $\pm$ SEM. Statistical analysis of data was performed by ANOVA. The significance of the difference between nonsensitized and OA-sensitized animals, and between animals on a normal diet and an antioxidant-supplemented diet sensitized by OA was estimated using Fisher's protected least significant difference. p values less than 0.05 were considered significant.

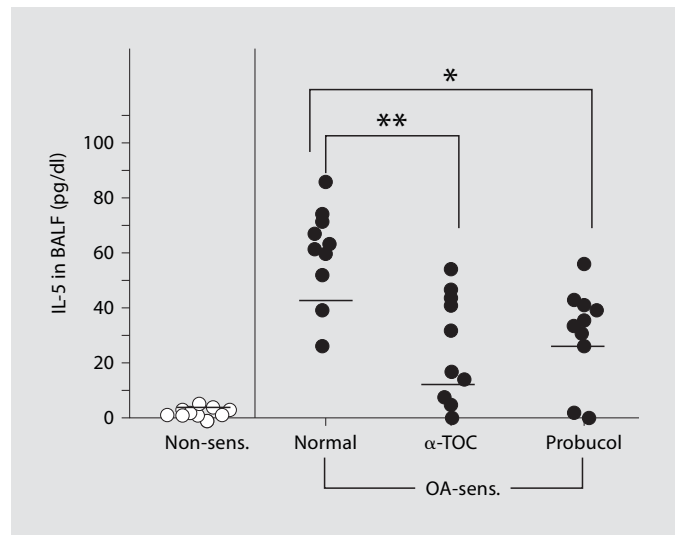


Fig. 3. IL-5 level in BALF was significantly increased by OA sensitization and exposure ($p < 0.05$). This level was decreased both in mice fed an α -TOC-supplemented and a probucol diet compared to mice fed a normal diet. * $p < 0.05$; ** $p < 0.001$.

Results

Serum Concentration of α -TOC and Probucol

The mean concentrations of serum α -TOC in mice with normal diet and mice with α -TOC-supplemented diet were 213 ± 73 and 651 ± 67 $\mu\text{g/ml}$, respectively ($p < 0.001$). The mean concentration of serum probucol in mice receiving the probucol-supplemented diet was 33.7 ± 9.8 $\mu\text{g/ml}$, which is almost the same as that of humans who take probucol as medication.

IL-4, IL-5 and IFN- γ Levels in BALF

IL-4 levels in BALF of mice fed a normal diet were significantly increased by OA sensitization and exposure compared with nonsensitized mice ($p < 0.05$). Those of α -TOC-fed mice were significantly decreased compared with normal diet mice ($p < 0.05$) and, conversely, those of probucol-fed mice were not different from mice fed a normal diet and significantly increased compared with nonsensitized mice ($p < 0.05$) (fig. 2). IL-5 levels in BALF of mice fed a normal diet were significantly increased compared with nonsensitized mice ($p < 0.005$). Those of α -TOC-fed mice and probucol-fed mice were both significantly decreased compared with mice fed a normal diet ($p < 0.05$) (fig. 3). IFN- γ levels of mice fed a normal diet were significantly decreased compared with nonsensitized mice ($p < 0.0005$). There was no difference in the

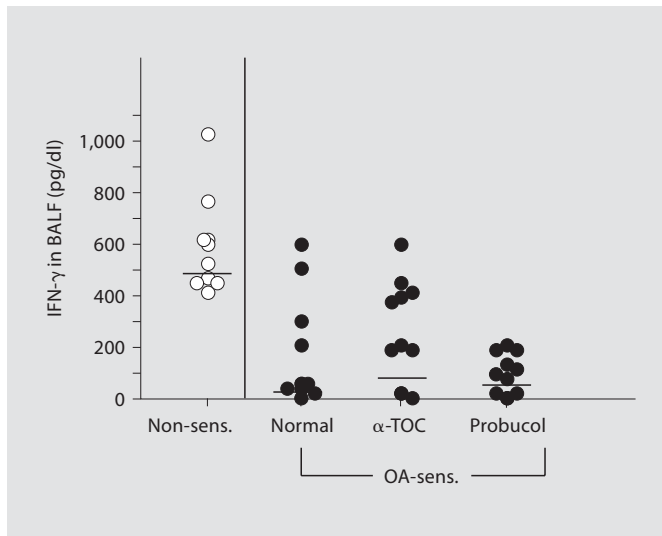


Fig. 4. IFN- γ level in BALF was decreased by OA sensitization and exposure ($p < 0.005$). There was no difference between levels in mice fed a normal, an α -TOC and a probucol diet.

IFN- γ levels of each diet group sensitized and exposed to OA and these were significantly decreased compared with nonsensitized mice (fig. 4).

Serum IgE Level

Serum IgE levels in mice fed a normal diet were significantly elevated by sensitization and exposure to OA compared with nonsensitized mice ($p < 0.0005$). Those of probucol-fed mice were significantly decreased ($p < 0.05$). There was no difference in the levels of mice fed a normal diet and α -TOC-fed mice (fig. 5).

Eosinophil Rates in BALF

Eosinophil rates in BALF of mice fed a normal diet were significantly increased by sensitization and exposure to OA compared with nonsensitized mice ($p < 0.0005$). Those of α -TOC-fed mice and probucol-fed mice were significantly decreased compared with mice fed a normal diet ($p < 0.005$) (fig. 6).

Airway Responsiveness to Ach

Figure 7 shows the dose-response curve of airway responsiveness to Ach after repeated antigen inhalation in each group. A curve of nonsensitized mice is also presented as a control and AHR was not found in these animals. The dose-response curve of airway responsiveness to Ach was shifted leftward in mice fed a normal diet af-

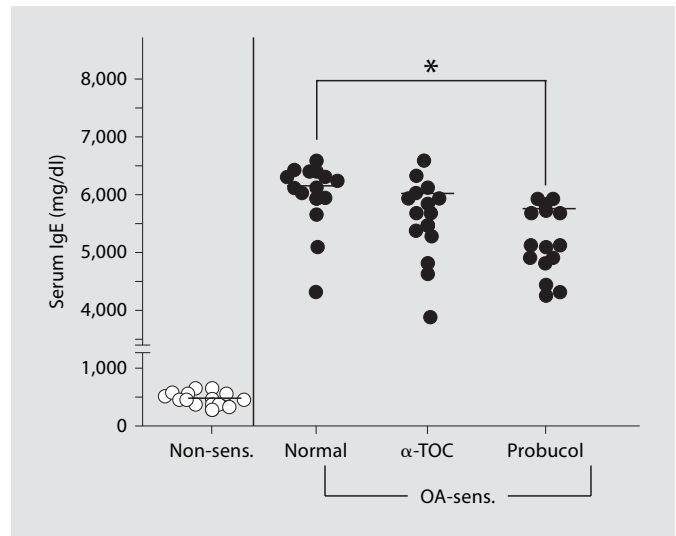


Fig. 5. Serum IgE level was increased by OA sensitization and exposure ($p < 0.005$). That of mice fed a probucol diet was significantly decreased compared with mice fed a normal diet. * $p < 0.05$.

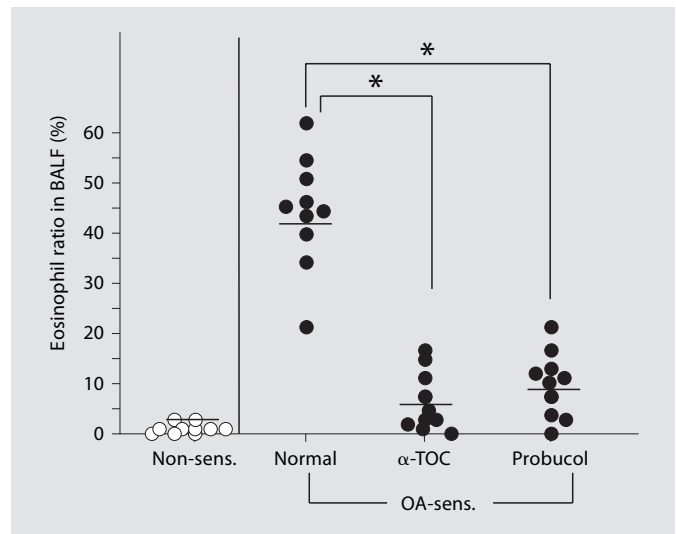


Fig. 6. Ratio of eosinophils to whole cells in BALF was significantly elevated by OA sensitization and exposure ($p < 0.005$). This ratio was decreased in both mice fed an α -TOC and a probucol diet. * $p < 0.05$.

ter repeated allergen provocation and AUC was significantly increased compared with that of nonsensitized mice (943.61 ± 58.3 vs. 655.52 ± 17.4). The degree of airway responsiveness to Ach was smaller both in

Fig. 7. Airway responsiveness to Ach 24 h after the final antigen challenge (mean $\pm$ SD). The AUC was calculated from the dose-response curve for Ach. AUC of mice fed a normal diet was significantly larger than that of nonsensitized mice ($p < 0.05$). AUC of mice fed an α -TOC-supplemented diet and mice fed a probucol diet was significantly smaller than that of mice fed a normal diet ($p < 0.05$).

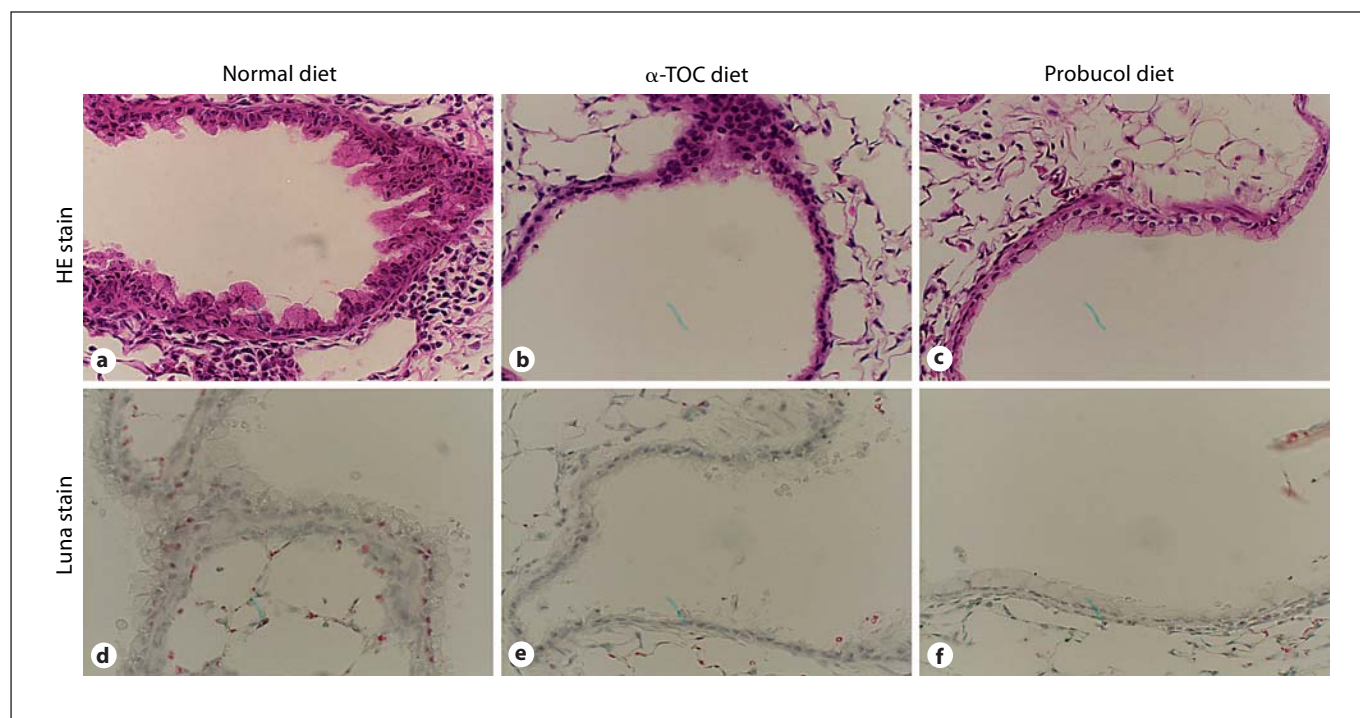
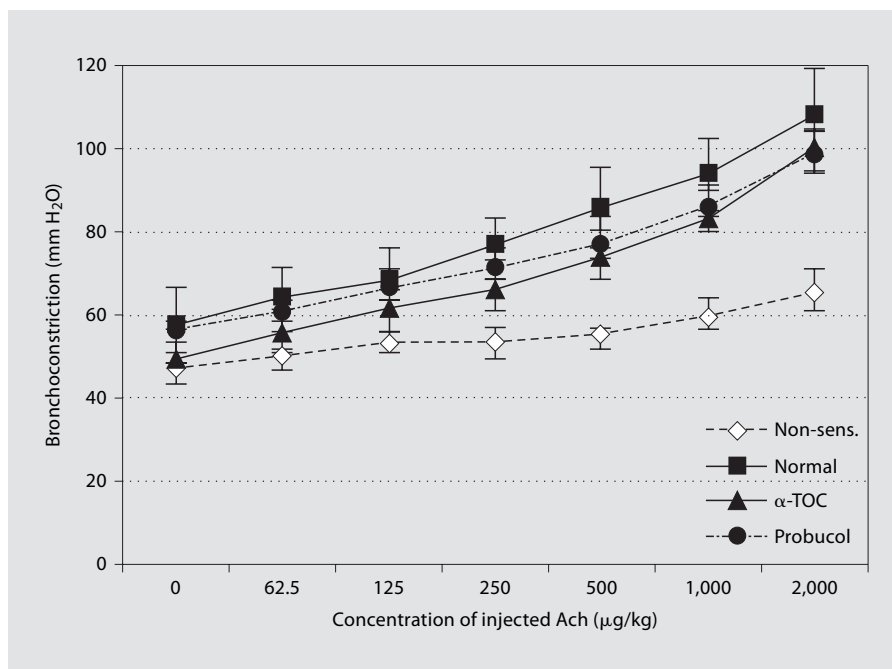


Fig. 8. Histological analysis of lung sections from mice fed a normal diet, an α -TOC diet and a probucol diet. Tissues were stained with hematoxylin and eosin (a–c) and with Luna (d–f). a, d Airway mucus hypersecretion and the infiltration of eosinophils were seen in mice fed a normal diet. b, c, e, f These findings were not detected in mice fed an α -TOC diet and a probucol diet. $\times 400$.

α -TOC-fed mice and in probucol-fed mice than in mice fed a normal diet, and the AUC of α -TOC-fed mice and probucol-fed mice (827.89 ± 23.9 and 890.94 ± 38.2 , respectively) was significantly smaller than that of mice fed a normal diet ($p < 0.05$).

Histological Analysis

We examined the histology of 5 of the 15 mice in each group and made 5 slices from each mouse. The lungs of nonsensitized mice showed normal histology (data not shown). Similar to the results in the BALF study, histological sections of lung tissue from mice fed a normal diet sensitized and provoked with OA exhibited obvious eosinophilic inflammation and airway mucus hypersecretion (fig. 8a, d). α -TOC-fed mice and probucol-fed mice had a marked reduction in airway inflammation and mucus secretion in their bronchiolus (fig. 8b, c, e, f).

8-Isoprostane and Acrolein

The levels of 8-isoprostane in BALF of nonsensitized, normal-diet, α -TOC and probucol group were 366.69 ± 107.44 , 248.24 ± 177.32 , 276.54 ± 61.21 and 264.61 ± 51.99 pg/ml, respectively. There was no difference between sensitized mice groups. The levels of 8-isoprostane in lung tissue of the nonsensitized, normal-diet, α -TOC and probucol group were 447.77 ± 44.00 , $1,629.37 \pm 164.44$, $1,716.75 \pm 395.80$ and $1,843.904 \pm 272.88$ pg/mg, respectively. Those of sensitized mice were significantly increased compared with that of nonsensitized mice ($p < 0.05$), but there was no difference between all three sensitized groups. The levels of acrolein in BALF of the nonsensitized, normal-diet, α -TOC and probucol group were 27.74 ± 5.88 , 48.93 ± 8.54 , 50.48 ± 16.10 and 52.49 ± 14.42 nmol/ml, respectively. These results are statistically almost identical between the three sensitized groups, though it was significantly increased by OA sensitization ($p < 0.05$).

Discussion

In our study, we found that repeated exposure of a sensitized animal to antigen caused a significant increase in several markers of airway inflammation and bronchial responsiveness to Ach, and that these effects were inhibited by both α -TOC and probucol supplements; however, some factors were different in each group. Eosinophil infiltration in BALF and lungs, the values of IL-5 in BALF and serum IgE levels after the final antigen provocation were all significantly decreased in both α -TOC-fed mice

and probucol-fed mice in comparison with mice fed a normal diet. On the other hand, the values of IL-4 in BALF after the final antigen inhalation were significantly decreased in α -TOC-fed mice, and were significantly increased in probucol-fed mice in comparison with mice fed a normal diet. These results indicate that both α -TOC and probucol, which are known to relieve oxidative stress, could also decrease airway inflammation and AHR in sensitized animals. However, we were unable to find any difference in 8-isoprostane and acrolein levels between the three groups. 8-Isoprostanes are arachidonate products formed on membrane phospholipids by the activity of ROS and represent a quantitative measurement of oxidant stress in vivo [37]. Acrolein has a strong cytotoxicity and is also increased by lipid oxidation. We expected the effects of α -TOC and probucol as antioxidants on asthma model mice; however, our experiment gave us no evidence of the antioxidant properties of these drugs contributing to the reduction of airway inflammation. This fact suggests that these drugs could influence the immune system directly (although in different ways), and not merely through the oxidative pathway. Further studies into the usefulness of these products for asthma model mice should be performed.

There have been a few recent reports indicating that α -TOC has a modulating effect on the immune system. Zheng et al. [28] reported that higher doses of vitamin E supplementation may suppress both nasal allergic responses and the increase of serum IgE in nasal allergy model mice sensitized by TDI. They investigated splenocyte proliferation and cytokine production from splenocytes, and found that vitamin E could influence the immune responses directly. Fogarty et al. [29] investigated the relationship between dietary vitamin E intake and serum IgE concentrations and atopy in a random sample of 2,633 adults. They reported that higher concentrations of vitamin E intake were associated with lower serum IgE concentrations and a lower frequency of allergen sensitization. Tsourei-Nikita et al. [38] investigated the dietary intake of vitamin E in patients with atopic dermatitis and found that there were negative correlations between vitamin E intake, and both serum IgE levels and clinical manifestations. Initially, vitamin E, which is a potent antioxidant, exhibited the ability to enhance both cellular and humoral immunity in vitro and in vivo. The results of our study and former findings suggest that vitamin E can play an important role in IgE-mediated atopic responses to allergic stimuli by decreasing the serum IgE levels. For this reason, vitamin E is now thought to be an immune regulator.

Although serum IgE levels in mice fed an α -TOC diet after the final antigen challenge did not differ from those in mice fed a normal diet, other markers (such as IL-4 and IL-5 levels in BALF, eosinophil count in BALF, inflammation of lung tissue and airway responsiveness to Ach) were decreased significantly compared to those in mice fed a normal diet. This discrepancy is possibly due to OA provocation being stronger than the inhibitory effect of α -TOC and/or the fact that α -TOC affects the allergic pathways further from the IgE-mediated response. Another possibility is that α -TOC and probucol each affect a different point of the allergic pathway from Th2 cells to eosinophilic inflammation. For example, IL-5 is produced by both Th2 cells and eosinophils, but IL-4 is produced only by Th2 cells. This might explain why IL-4 levels in BALF after the final antigen challenge differ between α -TOC-fed mice and probucol-fed mice.

There is increasing evidence that inflammation from asthma results in increased oxidative stress in the airways. The H_2O_2 concentration and NO levels in exhaled air condensate are increased in stable asthmatics and they may contribute to airway edema and inflammation [39–41]. The inflammatory and immune cells in the airways (such as macrophages, neutrophils and eosinophils) release increased amounts of ROS in asthmatic patients [15, 42], and their ability to produce O_2^- correlates with the degree of bronchial responsiveness to inhaled methacholine [43]. The direct oxidative damage can result in the characteristic features of asthma [44, 45] and it may evoke AHR [46]. Leukotrienes (LT) are now thought to play an important role in AHR in asthma [1] and oxidative stress leads to AHR via LT production [47]. Centanni et al. [48] studied the effect of TOC supplementation and the results obtained showed a significant inhibition of LT production by α -TOC supplementation. These studies pro-

vide support for the hypothesis of a potential effect of α -TOC supplementation in asthma patients. As the results of our experiment, we confirmed that allergic response leads to oxidative stress in the airways of mice. However, there was no evidence of the antioxidant properties of α -TOC. We suggest that the correlation of the antioxidant dose with inflammatory intensity may contribute to this discrepancy between previous reports and our results; however, the details of these mechanisms remain unknown.

On the other hand, the effect of probucol on allergic diseases has not yet been clarified. Probuco is a cholesterol-lowering drug, and serum cholesterol increases in asthmatic patients [49]. Yeh and Huang [50] reported that dietary cholesterol enhances bronchial inflammation in a murine model of asthma. While probucol is used as an antioxidant in various diseases [51], a recent study showed that the immunomodulatory effect of probucol predicts a role in chronic inflammatory diseases [52]. This immunomodulatory effect is believed to be independent of the cholesterol-lowering effect. We believe that the pleiotropic effects of probucol outlined above are responsible for the differences in results between the α -TOC and probucol groups. From our study of AHR in animal models, we concluded that probucol may also have a potent effect on IgE-mediated atopic responses and reduces serum IgE levels.

In our study, α -TOC and probucol also suppressed AHR in asthma model mice, although they used different immunological pathways against Th2 activity and/or the IgE-mediated allergic response. Additional studies are needed to clarify the mechanism of each activity and its potential role for the treatment of asthmatic patients. The optimal dose of each drug should also be determined.

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