Effect of Food Reductones, 2,5-Dimethyl-4-hydroxy-3(2*H*)-furanone (DMHF) and Hydroxyhydroquinone (HHQ), on Lipid Peroxidation and Type IV and I Allergy Responses of Mouse

Hongbin Mi,[†] Kazuyuki Hiramoto,[†] Kaori Kujirai,[†] Ken Ando,[†] Yoshiaki Ikarashi,[‡] and Kiyomi Kikugawa*[,†]

School of Pharmacy, Tokyo University of Pharmacy and Life Science, Hachioji, Tokyo 192-0392, Japan, and Division of Medical Devices, National Institute of Health Sciences, Setagaya-ku, Tokyo 158-8501, Japan

The effect of long-term supplementation of food reductones, 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone (DMHF) (2%, w/w), detected in many foodstuffs including soy sauce, and hydroxyhydroquinone (1,2,4-benzenetriol) (HHQ) (1.2%, w/w), detected in coffee, on mouse lipid peroxidation and type IV and I allergy responses was investigated. The effect of supplementation of these reductones combined with NO_2 inhalation (5−6 ppm) was also investigated. Levels of thiobarbituric acid-reactive substances in lung were remarkably increased, and those in kidney and liver were slightly decreased by supplementation of DMHF or HHQ. The degree of 2,4-dinitrochlorobenzene (DNCB)-sensitized lymph node cell proliferation as assessed by lymph node assay was remarkably enhanced by supplementation of DMHF or HHQ. Both the DNCB-sensitized and the trimellitic anhydride-sensitized increases in IgE levels of mice were enhanced to greater extent by supplementation of DMHF or HHQ. In no cases were additive effects of NO_2 inhalation observable. Allergen-sensitized type IV and I allergy responses of mice may be enhanced by supplementation of food reductones, DMHF or HHQ.

Keywords: *2,5-Dimethyl-4-hydroxy-3(2H)-furanone; hydroxyhydroquinone; nitrogen dioxide; type I allergy; type IV allergy*

INTRODUCTION

A variety of reductones are present in foodstuffs. The Maillard reaction-derived reductones, hydroxyfuranone and dihydroxypyranone derivatives, are widely distributed as fragrant components in processed foods (*1*). 2,5-Dimethyl-4-hydroxy-3(2*H*)-furanone (DMHF) is a most popular item among these fragrant furanones, and it is detected in a wide variety of foods including soy sauce. These fragrant reductones are present in stable forms in rather acidic foods, but they degrade in neutral media to generate reactive oxygen species that break DNA single strands in the presence of atmospheric oxygen (*2*−*7*). An adverse effect of the reductones is also shown: one of the reductones prevents Fe(III)-induced lipid peroxidation of red blood cell membranes and low-density lipoprotein in vitro (*8*). Another kind of reductone, hydroxyhydroquinone (1,2,4-benzenetriol) (HHQ), is detected in coffee (*9*). This reductone is stable in rather acidic brewed coffee but decomposes in neutral media to generate reactive oxygen species that break DNA single strands (*9*−*11*). Coffee drinking increases levels of urinary hydrogen peroxide, suggesting that oxidative stress is induced by HHQ in human body (*12*).

Nitrogen dioxide (NO_2) is a free radical toxin usually present in polluted urban and room air (0.02−0.2 ppm) and in fresh smoke or cigarette smoke (up to 100 ppm). Long-term inhalation of a low level of NO_2 causes oxidative damage, especially to the lung. Inhalation of NO_2 causes pulmonary edema, pulmonary fibrosis, bronchitis (*13*−*15*), and cancer (*16*). Inhalation of NO_2 by rats causes lung lipid peroxidation as assessed by the increase in conjugated diene formation (*17*) and hydrocarbon exhalation (*18*).

It is worthwhile to investigate whether intake of the food reductones or the intake in combination with NO_2 inhalation causes tissue lipid peroxidation and undesirable effects on allergen-sensitized type IV (contact sensitization) and type I (respiratory sensitization) allergy responses. The aim of the present study was to find the effect of reductones, DMHF and HHQ, on oxidative stress, and on type IV and type I allergy responses of mice. In this study, mice were supplemented with a diet containing DMHF or HHQ in an atmosphere of purified air or air containing NO_2. For investigation of the effect on the oxidative stress, the levels of thiobarbituric acid-reactive substances (TBARS) of mouse tissues were measured. For investigation of the effect on type IV allergy responses, contact sensitization responses induced by 2,4-dinitrochlorobenzene (DNCB), a potent sensitizer used for many years to induce contact sensitization (*19*, *20*), were examined. For investigation of the effect on type I allergy responses, serum IgE levels were measured using mice sensitized by DNCB or trimellitic anhydride (TMA) (*21*, *22*).

MATERIALS AND METHODS

Materials. Purified air and air containing 10 ppm of NO_2 were prepared by Nihonsanso Ltd. (Tochigi, Japan). Hanks' balanced salt solution (HBSS) and *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES) were obtained from Dojindo Laboratories (Kumamoto, Japan). RPMI 1640 medium

* Author to whom correspondence should be addressed (e-mail kikugawa@ps.toyaku.ac.jp; fax +81-426-76-4508).
[†] School of Pharmacy.
[‡] Division of Medical Devices.

10.1021/jf010556l CCC: $20.00
Published on Web 09/06/2001

and penicillin−streptomycin solution were obtained from Gibco Laboratories (Grand Island, NY). Fetal calf serum (FCS) was obtained from Bio Whittaker (Walkersville, MD). Thiobarbituric acid (TBA) was obtained from Merck KGaA (Darmstadt, Germany). Hydroxyhydroquinone (HHQ), 2,4-dinitrochlorobenzene (DNCB), trimellitic anhydride (TMA), olive oil, and normal goat serum (NGS) were obtained from Wako Pure Chemical Industries (Osaka, Japan). 2,5-Dimethyl-4-hydroxy-3(2*H*)-furanone (DMHF) was obtained from Tokyo Chemical Industry (Tokyo, Japan). Standard bovine serum albumin (BSA) (γ-globulin-free and fatty acid-free), mouse monoclonal IgE anti-dinitrophenol (DNP) antibody (clone SPE-7), and *o*-phenylediamine dihydrochloride tablet kits were obtained from Sigma Chemical Co. (St. Louis, MO). [^{3}H]Methylthymidine ([^{3}H]TdR) was obtained from Amersham Pharmacia Biotech U.K., Ltd. (Buckingham, U.K.). Rat monoclonal anti-mouse IgE antibody was from Southern Biotechnology Associates Inc. (Birmingham, AL), and goat anti-mouse IgE conjugated with horseradish peroxidase was from Nordic Immunology (Tilberg, The Netherlands).

Animals and Diets. The protocol of animal preparations for the present experiment was approved by the Ethics Committee of our institute. Five-week-old male BALB/c mice weighing 10−15 g obtained from Japan Laboratory Animals Inc. (Tokyo, Japan) were used. Six mice were housed together in a plastic cage in a room of controlled temperature at 23 ± 1 °C, humidity at 55 ± 5%, and lighting on a 12 h dark−daylight cycle. The animals were allowed free access to diet. The animals were fed a normal (vitamin E adequate) powdered diet MF containing protein at 254 g/kg, energy at 3422 kcal/kg, fat at 44 g/kg, fiber at 41 g/kg, and vitamin E at 70×10^{-3} g/kg (Oriental Yeast Co., Tokyo, Japan) solidified by mixing 400 g of diet and 210 mL of water (C group) or the diet solidified by mixing 400 g of diet and 210 mL of water containing 12 g of DMHF (2.0%, w/w) (DMHF group) or 8 g of HHQ (1.3%, w/w) (HHQ group) for 1 week. The DMHF and HHQ contents in the diets at maximum gave no observable toxic effects to the mice.

Each mouse was moved to a special gastight glass cage Metabolica MM-AP type prepared by Sugiyama-Gen Iriki Co. (Tokyo, Japan) of controlled temperature at 23 ± 1 °C and lighting on a 12 h dark−daylight cycle. Air (C, DMHF, and HHQ groups) or air containing 10 ppm of NO_2 (C + NO_2, DMHF + NO_2, and HHQ + NO_2 groups) was introduced into the glass cage at 100 or 200 mL/min, respectively. Under the conditions, NO_2 content as assessed by the Saltzman method (*23*) in the cage was maintained at 5−6 ppm when the animal and diet were introduced in the cage. Mice of the C and C + NO_2 groups were continuously supplied with a normal diet, mice of the DMHF and DMHF + NO_2 groups were continuously supplied with a diet containing DMHF, and mice of the HHQ and HHQ + NO_2 groups were continuously supplied with a diet containing HHQ for 1−2 weeks. Weights of mice after 2-weeks of housing were 19−21 g.

TBARS in Mouse Tissues. A mouse was anesthetized with an air/diethyl ether mixture, and blood was collected over heparin by cardiac puncture. The mouse was then sacrificed, and heart, kidney, liver, and lung were quickly isolated and washed with physiological saline.

TBARS of red cell membranes were determined as described elsewhere (*24*). Briefly, a red cell pellet was collected and washed. The hemoglobin content in the pellet was determined. The pellet was lysed, and the lipid fraction of the lysate was extracted with 2-propanol/chloroform. TBA assay (−EDTA assay) was performed according to the method described elsewhere (*25*) in the presence of butylated hydroxytoluene (BHT). TBARS were expressed as nanomoles per gram of hemoglobin.

TBARS of tissues were determined as described elsewhere (*26*). To the tissue preparation were added 10 equivalent amounts (v/w) of phosphate-buffered saline (pH 7.4) (PBS), and the mixture was homogenized using a Potter type Teflon homogenizer on an ice bath. The protein content of the homogenate was determined according to the Lowry method (*27*) using BSA as a reference standard. TBARS in the homogenate was determined as described (*25*) in the presence of BHT and in the absence (−EDTA assay) and presence of EDTA (+EDTA assay). TBARS (nanomoles per milligram of tissue protein) was calculated using the protein content of the homogenate.

Local Lymph Node Assay. Local lymph node assay was performed according to the method previously described (*19*, *20*). A set of mice ($n = 3$) was treated on both ears by painting 25 μL of a 1% (w/v) DNCB solution in acetone/olive oil (4:1, v/v) daily for three consecutive days starting on the 11th day from the beginning. On the 14th day from the beginning, mice were sacrificed after they were anesthetized with an air/diethyl ether mixture, and draining auricular lymph nodes were excised. They were combined for each experimental set and weighed.

LNC suspensions were prepared by mechanical disaggregation through a steile 200-mesh gauze. The cells were transferred through a nylon mesh into a 15 mL centrifuge tube, and the tube was centrifuged at 1200 rpm for 5 min. The LNC suspensions were washed once with HBSS, and total LNCs were resuspended in an RPMI-1640 culture medium supplemented with 2.5 mmol/L HEPES, 100 units/mL pecinillin, 100 μg/mL streptomycin, and 10% FCS at a cell concentration of 5×10^6 cells/mL. The LNC suspensions (200 μL) were seeded into 96-well culture plates (4 wells per group) and cultured with 18.5 kBq [^{3}H]TdR at 37 °C for 24 h in a humidified atmosphere of 5% CO_2 in air. Culturing was terminated by a semiautomatic cell harvester, and [^{3}H]TdR incorporation was determined using a liquid scintillation counter. A mean value of four culture wells was obtained.

Eight sets of experiments were done for mice of C and C + NO_2 groups, and four sets of experiments for mice of DMHF, HHQ, DMHF + NO_2, and HHQ + NO_2 groups were performed.

Mouse IgE Test. The mouse IgE test was performed as described elsewhere (*21*, *22*). On the eighth day of housing, three mice of each C, DMHF, HHQ, C + NO_2, DMHF + NO_2, and HHQ + NO_2 group received 50 μL of 1% (w/v) DNCB or 25% (w/v) TMA solution in acetone/olive oil on both shaved flanks; 7 days later, the mice received 25 μL of the same solution on both ears. On the 7th day after the challenge, the mice were anesthetized with an air/diethyl ether mixture, and blood was collected by cadiac puncture. After standing at room temperature for 12 h, serum was prepared by centrifugation at 4 °C and 2000 rpm for 20 min and stored at −20 °C.

Serum IgE was measured using a sandwich enzyme-linked immunosorbent assay (ELISA). To a Costar EIA/RIA 96-well strip (Corning Inc. Corning, NY) was added 200 μL of rat monoclonal anti-mouse IgE antibody solution (2.5 μg/mL in 0.1 M carbonate buffer, pH 9.6), and the plate was kept at 4 °C overnight. The plate was blocked with 120 μL of 5% normal goat serum (NGS) in PBS at 37 °C for 30 min. One hundred microliters of test samples or mouse monoclonal IgE anti-dinitrophenol (DNP) antibody solution (standard) diluted to various extents in 0.5% NGS−PBS was added to the wells of the plate, and the plate was incubated at 37 °C for 2 h. Subsequently, plates were incubated at 37 °C for 2 h with 100 μL of a 1:2000 diluted solution of goat anti-mouse IgE conjugated with horseradish peroxidase in 0.5% NGS−PBS. The plate was washed with 0.05% Tween 20 in PBS three times. *o*-Phenylenediamine and urea hydrogen peroxide solution (100 μL) were placed in the wells, and the plate was incubated at 37 °C for 10 min. The enzyme reaction was terminated by the addition of 0.5 M citric acid. Absorbance at 450 nm of the product of the enzyme reaction was determined using a microplate reader, Benchmark (Bio-Rad Laboratories, Tokyo, Japan). The concentration of serum IgE was obtained from the standard curve for the mouse monoclonal IgE.

Statistical Analysis. Data of TBARS and serum IgE were analyzed by Student's *t* test.

RESULTS

Levels of TBARS in Tissues of Mice Supplemented with DMHF or HHQ. Mice of C, DMHF, and HHQ groups, six mice for each group, were fed a normal diet, a diet containing DMHF (2%, w/w), or a diet containing HHQ (1.3%, w/w), respectively, for 1 week. The mice were then housed in an atmosphere of purified

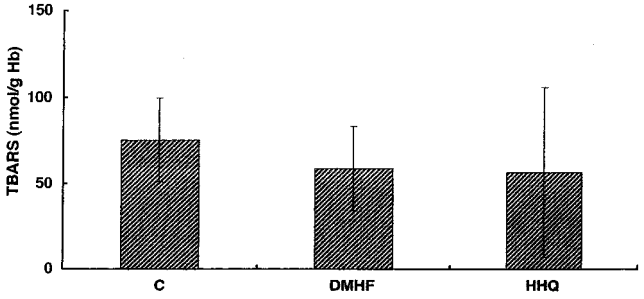

Figure 1. Levels of TBARS obtained by −EDTA assay in red cell membranes of mice supplemented with a normal diet (C), a diet containing DMHF (DMHF), and a diet containing HHQ (HHQ) in an atmosphere of air for 2 weeks. Results are expressed as TBARS (nmol/g of hemoglobin) (mean value ± SD) of six mice for each group.

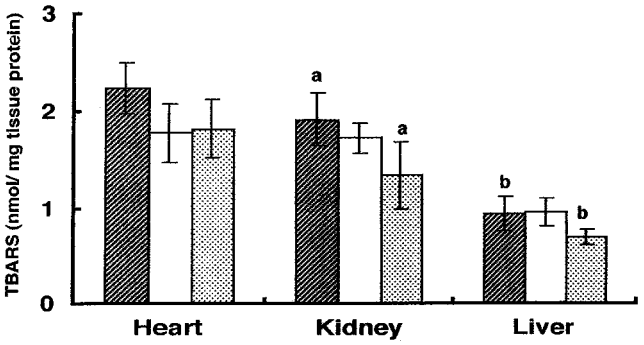

Figure 2. Levels of TBARS obtained by −EDTA assay in heart, kidney, and liver of mice supplemented with a normal diet (slashed bars), a diet containing DMHF (white bars), and a diet containing HHQ (dotted bars) in an atmosphere of air for 2 weeks. Results are expressed as TBARS (nmol/mg of tissue protein) (mean value ± SD) of six mice for each group. a, $p < 0.007$; b, $p < 0.007$.

air or air containing NO_2 (C + NO_2, DMHF + NO_2, and HHQ + NO_2 groups) by supplying the corresponding diets, for a further 1 week. To know the effect of DMHF and HHQ, and combined NO_2 inhalation, on the degree of lipid peroxidation, TBA assay of red cell membranes, heart, kidney, liver, and lung tissues was performed. TBA assay was conducted according to a method that could discriminate TBARS derived from malonaldehyde derivatives plus alkadienal/alkenal derivatives (−EDTA assay) and TBARS derived from malonaldehyde derivatives alone (+EDTA assay) in the presence of BHT as an antioxidant (*25*, *28*, *29*). Figure 1 shows TBARS of red cell membranes obtained by −EDTA assay. There were no significant differences in the levels of TBARS among the red cell membranes from C, DMHF, and HHQ groups. Figure 2 shows the levels of TBARS of heart, kidney, and liver obtained by −EDTA assay. Here, the levels of TBARS of the heart were slightly lowered by supplementation of DMHF, and those of kidney and liver were significantly decreased by HHQ. Figure 3 shows the levels of TBARS of lung obtained by both −EDTA and +EDTA assays. The left three bars show the results of mice of DMHF and HHQ groups. The levels of TBARS from malonaldehyde derivatives plus alkadienal/alkenal derivatives were remarkably increased by supplementation of DMHF or HHQ, whereas those from malonaldehyde derivatives were not. The right three bars show the results of mice of DMHF + NO_2 and HHQ + NO_2 groups, which were similar to those unexposed to NO_2, indicating that there wee no additional effects of NO_2 inhalation on the lung lipid peroxidation. Hence, lung lipid peroxidation was

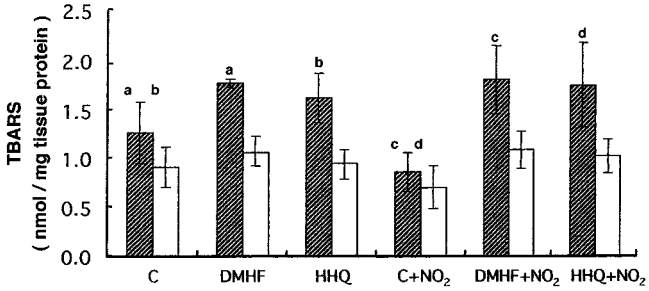

Figure 3. Levels of TBARS obtained by -EDTA assay (slashed bars) and +EDTA assay (white bars) in lung of mice supplemented with a normal diet (C), a diet containing DMHF (DMHF), and a diet containing HHQ (HHQ) in an atmosphere of air for 2 weeks or air (for 1 week) and then air containing NO_2 (for 1 week). Results are expressed as TBARS (nmol/mg of tissue protein) (mean value ± SD) of six mice for each group. a, $p < 0.02$; b, $p < 0.05$; c, $p < 0.01$; d, $p < 0.01$.

Table 1. Typical Profile of the Effect of DMHF and HHQ Supplementation with and without NO_2 Inhalation on Draining Auricular Lymph Node Responses Induced by DNCB in a Single Set of Samples[a]

group	lymph node wt (mg/set)	total LNC no. (× 10^{-6}/set)	local lymph node assay, [^{3}H]TdR incorporation (dpm)
C	60.1	71	8.12 ± 0.76
DMHF	49.0	52.5	30.11 ± 1.07
HHQ	53.5	90	33.25 ± 1.25
C + NO_2	70.9	74	8.31 ± 0.56
DMHF + NO_2	42.3	57.7	23.85 ± 2.92
HHQ + NO_2	50.2	70.8	45.79 ± 3.41

[a] Mice were supplemented with a normal diet (C), a diet containing DMHF (DMHF), and a diet containing HHQ (HHQ) in an atmosphere of air for 2 weeks or air (for 1 week) and then air containing NO_2 (for 1 week) (C + NO_2, DMHF + NO_2, and HHQ + NO_2). A set of mice ($n = 3$) was treated on both ears by painting DNCB solution daily for 3 consecutive days on the 11th day from the beginning. On the 14th day from the beginning, draining auricular lymph nodes were excised, pooled for each experimental set, and weighed. The LNC suspensions were cultured with [^{3}H]TdR, and [^{3}H]TdR incorporation was determined. An average of four culture wells for the set was obtained.

enhanced by supplementation of DMHF or HHQ, whereas lipid peroxidation of other tissues were not affected or slightly decreased by these reductones.

Effect of DMHF and HHQ Supplementation on Type IV Allergy Responses of Mice Sensitized by DNCB. Mice of C, DMHF, HHQ, C + NO_2, DMHF + NO_2, and HHQ + NO_2 groups were housed for 1 week and then 1 week in air or air containing NO_2. Three mice of each group were treated on both ears by the DNCB solution, daily for three consecutive days, on the 11th day from the beginning. On the 14th day from the beginning, draining auricular lymph nodes were excised.

A typical profile of one set of the experiment on lymph node weight, LNC number, and local lymph node assay induced by DNCB sensitization is shown in Table 1. The mean values of lymph node weight and total LNC number of more than four sets of experiments are listed in Table 2. The mean values of local lymph node assay of more than four sets of experiments are shown in Figure 4. Lymph node weight was significantly lowered by supplementation of DMHF or HHQ, whereas total LNC number was unaffected. There were no differences in the weight and LNC number between NO_2-unexposed groups and NO_2-exposed groups (Tables 1 and 2). The degree of DNCB-sensitized cell proliferation was increased ~2-fold by supplementation of DMHF or HHQ in both NO_2-unexposed groups and NO_2-exposed groups (Table 1 and Figure 4).

Table 2. Averaged Effect of DMHF and HHQ Supplementation with and without NO_2 Inhalation on Draining Auricular Lymph Node Weight and LNC Number Induced by DNCB[a]

group	set no.	lymph node wt (mg ± SD/group)	total LNC no. [(no. ± SD) × 10^{-6}/group)]
C	8	60.05 ± 25.10	62.61 ± 11.90
DMHF	4	46.50 ± 5.61	72.06 ± 8.37
HHQ	4	45.78 ± 9.21	72.52 ± 12.77
C + NO_2	8	65.11 ± 18.10	65.23 ± 16.90
DMHF + NO_2	4	43.93 ± 9.13	58.11 ± 15.38
HHQ + NO_2	4	44.40 ± 17.28	58.83 ± 13.58

[a] Mean values ± SD of four or eight sets of the experiments as estimated in Table 1 are shown.

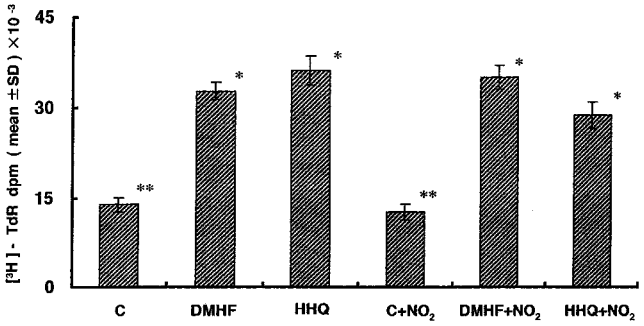

Figure 4. Average effect of DMHF and HHQ supplementation and average effect of the supplementation combined with NO_2 inhalation on draining auricular LNC proliferation induced by painting DNCB on ears. Mean values ± SD of four (*) or 8 (**) sets of the experiments of [^{3}H]TdR incorporation in local lymph node assay as estimated in Table 1 are shown.

Effect of Supplementation of DMHF and HHQ on Type I Allergy Responses of Mice Sensitized by DNCB and TMA. Mice of C, DMHF, HHQ, C + NO_2, DMHF + NO_2, and HHQ + NO_2 groups were housed for 1 week and then for 2 weeks in air or air containing NO_2. Three mice of each group were treated on both flanks by the DNCB solution or the TMA solution, on the eighth day from the beginning. Seven days later, the same solution was challenged to both ears. On the seventh day after the challenge, blood serum was obtained, and the serum IgE levels were determined by sandwich ELISA. The IgE levels of the three individual serum samples of each group are listed in Table 3, and the mean IgE levels of three serum samples of each group are shown in Figure 5. In the NO_2-unexposed groups, supplementation of DMHF or HHQ strongly enhanced the serum IgE levels induced by DNCB sensitization and TMA sensitization. In the NO_2-exposed control groups (C and C + NO_2 groups), NO_2 inhalation alone enhanced the IgE levels sensitized by TMA. There were no significant differences in the IgE levels in the NO_2-unexposed and NO_2-exposed groups supplemented with DMHF or HHQ.

DISCUSSION

Absorption of orally supplied fragrant furanones including DMHF by digestive tract has been studied (*30*). DMHF rapidly appears in plasma after a single oral administration to rats, reaches a maximum within 1 h, and gradually disappears after 2 h, indicating that the compound is rapidly absorbed and metabolized. The compound induces micronucleated peripheral reticulocytes after a single oral administration. Coffee drinking increases levels of urinary hydrogen peroxide, suggesting that HHQ is absorbed and induces oxidative stress in the human body (*12*). In the present study, because long-term oral supplemetation of DMHF (2%, w/w) and HHQ (1.3%, w/w) with diet was conducted for more than 1 week, the effects of these reductones may be accumulated in the animal body.

The degree of lipid peroxidaion of lung of mice as assessed by levels of TBARS was enhanced by supplementation of DMHF or HHQ. A similar increase in the levels of TBARS of lung has been observed when plant polyphenolics are supplied to rats (*31*). In contrast, levels of TBARS of heart were slightly decreased by supplementtion of DMHF, and those of kidney and liver were significantly decreased by supplementation of HHQ. Quite adverse effects, increasing effect and decreasing effect on lipid oxidation, were observed depending on the tissues in situ. The increased susceptibility of lung to oxidative stress induced by the reductones may be derived from the prooxidant activity of the reductones owing to the higher partial oxygen pressure. In contrast, the reductones may protect other tissues at the lower partial oxygen pressure from oxidative damages by maintaining the reducing conditions.

Adverse activities of fragrant furanones including DMHF have been demonstrated in other in vitro studies: the activity providing oxidative conditions and the activity providing reducing conditions. The reductones generate reactive oxygen species to break DNA single strands in vitro in the presence of atmospheric oxygen (*2*−*7*), and they prevent Fe(III)-induced lipid peroxidation of red blood cell membranes and low-density lipoprotein in vitro (*31*). It is likely that which activity prevails depends on the target components and also on the environmental conditions.

In the lymph node assay for type IV allergy (contact sensitization) responses, the degree of DNCB-sensitized LNC proliferation was enhanced by supplementation of DMHF or HHQ. No additional effects were observed in combination with NO_2 inhalation. In the present study DNCB sensitization did not increase serum IgE levels in control mice in type I allergy (respiratory sensitization), which is consistent with the earlier observation by Hilton et al. (*22*). The serum IgE levels in mice in type I allergy responses after DNCB sensitization were

Table 3. Effect of DMHF and HHQ Supplementation with and without NO_2 Inhalation on IgE Concentration (Micrograms per Milliliter) of Three Individual Serum Samples of Mice Treated with DNCB or TMA[a]

	DNCB			TMA			DNCB			TMA		
mouse	C	DMHF	HHQ	C	DMHF	HHQ	C + NO_2	DMHF + NO_2	HHQ + NO_2	C + NO_2	DMHF + NO_2	HHQ + NO_2
1	0.260	2.310	2.240	0.543	2.135	2.170	0.175	2.870	1.015	1.978	2.170	3.752
2	0.125	2.856	3.752	0.800	3.500	2.949	0.225	2.870	2.156	1.633	2.940	2.450
3	0.175	1.540	1.295	1.275	1.750	1.750	0.275	3.290	2.170	1.900	2.548	2.870

[a] Mice were supplemented with a normal diet (C), a diet containing DMHF (DMHF), and a diet containing HHQ (HHQ) in an atmosphere of air for 3 weeks or air (for 1 week) and then air containing NO_2 (for 2 weeks) (C + NO_2, DMHF + NO_2, and HHQ + NO_2). Three mice for each group were treated on both shaved flanks by painting DNCB solution or TMA solution on the eighth day after beginning; 7 days later the same solution was painted on both ears. Seven days after the challenge blood was collected, serum IgE was determined by ELISA. The serum IgE levels of three individual mice samples are shown.

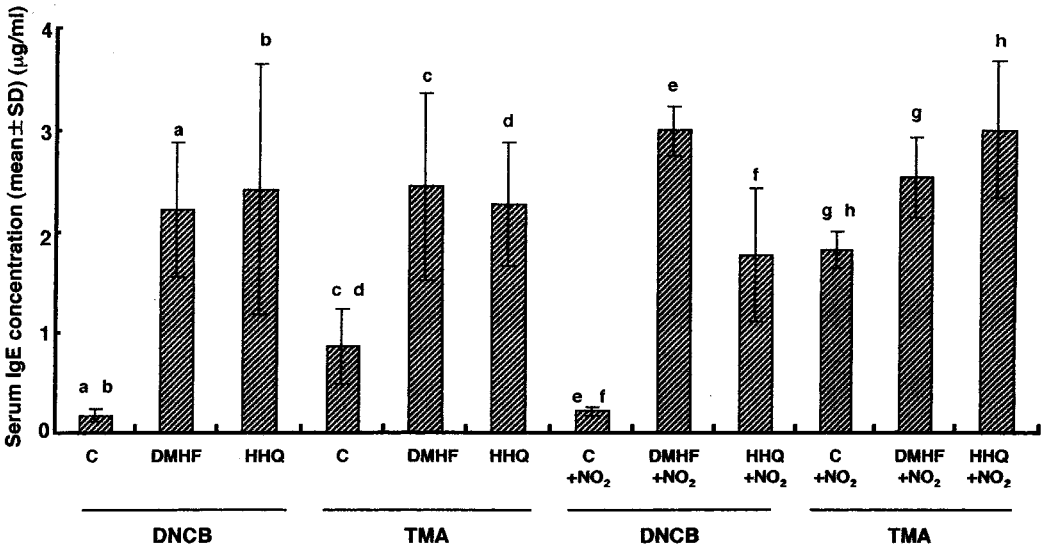

Figure 5. Average effect of DMHF and HHQ supplementation and average effect of the supplementation combined with NO_2 inhalation on IgE concentrations of serum samples of mice ($n = 3$) treated with DNCB or TMA. Mean values of serum IgE concentration ± SD of three individual serum samples shown in Table 3 are shown. a, $p < 0.01$; b, $p < 0.04$; c, $p < 0.05$; d, $p < 0.03$; e, $p < 0.01$; f, $p < 0.01$; g, $p < 0.05$; h, $p < 0.05$.

increased to a greater extent by supplementation of DMHF or HHQ. TMA sensitization increased serum IgE levels in control mice, which is consistent with the earlier observation (*22*). The increased serum IgE levels in mice after TMA sensitization were enhanced to a greater extent by supplementation of DMHF or HHQ. Effects of combination with NO_2 inhalation were observable only in the control mice with TMA sensitization, but not in DMHF or HHQ mice group. Hence, oral supplementation of DMHF and HHQ caused increase in type IV allergy responses in DNCB sensitization and caused increase in type I allergy responses in DNCB and TMA sensitization. The results may indicate that intake of DMHF and HHQ has an undesirable effect on both allergen-sensitized type IV and I allergy responses.

It is interesting to note that DMHF and HHQ, having no related structure, equally show properties affecting lipid peroxidation and allergen-sensitized type IV and I allergy responses. Common reducing activity of the reductones may participate in the properties. However, it is not clear what factors induced by the reductones participated in enhancement of the allergen-sensitized type IV and I allergy responses. It has been demonstrated that chemical allergens of different types provoke divergent immune responses by selective activation of discrete $CD4^+$ Th cell populations (*32*−*35*) of two main populations, Th1 and Th2, that differ with respect to cytokine secretion patterns (*36*, *37*). In type IV allergy responses, contact allergens induce immune responses characterized by Th1 T cell activation (*38*), which releases cytokine IFN-γ, to antagonize the production of IgE (*39*). In contrast, in type I allergy responses, respiratory allergens induce immune responses characterized by Th2 cell activation, which produce cytokine IL-4 initiating and maintaining IgE production (*40*). What factors provided by the reductones stimulate and augment release of cytokines from Th1 and Th2 cells, and thus enhance the allergy responses, must await further investigation. Oxidative stress induced by the reductones is more likely to participate in enhancement of allergen-sensitized type IV and I allergy responses, because vitamin E deficiency combined with NO_2 inhalation gave similar enhancement of allergen-sensitized type IV and I allergies (unpublished data).

In conclusion, supplementation of DMHF or HHQ enhanced DNCB-sensitized LNC proliferation increase and DNCB- or TMA-sensitized serum IgE increase of mice. Intake of a large amount of DMHF and HHQ may give an undesirable effect on both allergen-sensitized type IV and type I allergy responses.

ABBREVIATIONS USED

BHT, butylated hydroxytoluene; BSA, bovine serum albumin; DMHF, 2,5-dimethyl-4-hyroxy-3(2*H*)-furanone; ELISA, enzyme-linked immunosorbent assay; HEPES, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid; HBSS, Hanks' balanced salt solution; HHQ, hydroxyhydroquinone; LNC, lymph node cell; NGS, normal goat serum; PBS, phosphate-buffered saline; TBA, thiobarbituric acid; TBARS, thiobarbituric acid-reactive substances; TMA, trimellitic anhydride.

NOTE ADDED AFTER ASAP POSTING

Several errors in the caption of Figure 2, the text immediately below it, and the data in Tables 2 and 3 were detected after initial Web posting on September 6, 2001. These errors have been corrected in this posting.

LITERATURE CITED

(1) Kikugawa, K.; Hiramoto, K.; Kato, T. Generation of oxygen-derived radicals in the Maillard reaction that induce DNA strand cleavage. *Recent Res. Dev. Agric. Food Chem.* **1998**, *2*, 237−257.

(2) Hiramoto, K.; Sekiguchi, K.; Aso-o, R.; Ayuha, K.; Niiyama, H.; Kato, T.; Kikugawa, K. DNA strand breaks induced through active oxygen radicals by fragrant component 4-hydroxy-2-hydroxymethyl-5-methyl-3(2*H*)-furanone in Maillard reaction of hexose/amino acid. *Food Chem. Toxicol.* **1995**, *33*, 803−814.

(3) Hiramoto, K.; Aso-o, R.; Ni-iyama, H.; Hikage, S.; Kato, T.; Kikugawa, K. DNA strand break by 2,5-dimethyl-4-hydroxy-3(2*H*)-furnaone, a fragrant compound in various foodstuffs. *Mutat. Res.* **1996**, *359*, 17−24.

(4) Hiramoto, K.; Sekiguchi, K.; Ayuha, K.; Aso-o, R.; Moriya, N.; Kato, T.; Kikugawa, K. DNA breaking activity and mutagenicity of soy sauce: characterization of the active components and identification of 4-hydroxy-5-methyl-3(2*H*)-furanone. *Mutat. Res.* **1996**, *359*, 119−132.

(5) Hiramoto, K.; Nasuhara, A.; Michikoshi, K.; Kato, T.; Kikugawa, K. DNA strand-breaking activity and mutagenicity of 2,3-dihydro-3,5-dihydroxy-6-methyl-4*H*-pyran-4-one (DDMP), a Maillard reaction product of glucose and glycine. *Mutat. Res*. **1997**, *395*, 47−56.
(6) Li, X.; Hiramoto, K.; Yoshida, M.; Kato, T.; Kikugawa, K. Identification of 2,5-dimethyl-4-hydroxy-3(2*H*) furanone (DMHF) and 4-hydroxy-2(or 5)-ethyl-5(or 2)-methyl-3(2*H*)-furanone (HEMF) with DNA breaking activity in soy sauce. *Food Chem. Toxicol*. **1998**, *36*, 305−314.
(7) Yamashita, N.; Murata, M.; Inoue, S.; Hiraku, Y.; Yoshinaga, T.; Kawanishi, S. Superoxide formation and DNA damage induced by a fragrant furanone in the presence of copper(II). *Mutat. Res*. **1998**, *397*, 191−201.
(8) Ando, K.; Ogawa, K.; Li, X.; Kikugawa, K. Inhibition of iron ion-induced oxidative damage of erythrocyte membranes and low density lipoprotein by a Maillard product, 4-hydroxy-2(or 5)-ethyl-5-(or 2)-methyl-3(2*H*)-furanone (HEMF). *Biol. Pharm. Bull*. **2000**, *23*, 689−694.
(9) Hiramoto, K.; Li, X.; Makino, M.; Kato, T.; Kikugawa, K. Identification of hydroxyhydroquinone in coffee as a generator of reactive oxygen species that break DNA single strands. *Mutat. Res*. **1998**, *419*, 43−51.
(10) Kawanishi, S.; Inoue, S.; Kawanishi, M. Human DNA damage induced by 1,2,4-benzenetriol, a benzene metabolite. *Cancer Res*. **1989**, *48*, 4762−4765.
(11) Hiramoto, K.; Mochizuki, R.; Kikugawa, K. Generation of hydrogen peroxide from hydroxyhydroquinone and its inhibition by superoxide dismutase. *J. Oleo Sci*. **2001**, *50*, 21−28.
(12) Long, L. H.; Halliwell, B. Coffee drinking increases levels of urinary hydrogen peroxide detected in health human volunteers. *Free Radical Res*. **2000**, *32*, 463−467.
(13) Dawson, S. V.; Schenker, M. B. Health effects of inhalation of ambient concentrations of nitrogen dioxide. *Am. Rev. Respir. Dis*. **1979**, *120*, 281−292.
(14) Guidotti, T. L. The higher oxides of nitrogen: Inhalation toxicology. *Environ. Res*. **1972**, *15*, 443−472.
(15) Stephen, R. J.; Freeman, G.; Evans, M. Early response of lungs to low levels of nitrogen dioxide. *Arch. Environ. Health* **1972**, *24*, 160−179.
(16) Richter, A. Effects of nitrogen dioxide and ozone on blood-born cancer cell colonization of the lungs. *J. Toxicol. Environ. Health* **1988**, *25*, 383−390.
(17) Thomas, H. V.; Mueller, P. K.; Lyman, R. L. Lipoperoxidation of lung lipids in rats exposed to nitrogen dioxide. *Science* **1968**, *159*, 532−534.
(18) Sagai, M.; Ichinose, T.; Oda, H.; Kubota, K. Studies on biochemical effects of nitrogen dioxide: I. Lipid peroxidation as measured by ethane exhalation of rats exposed to nitrogen dioxide. *Lipids* **1981**, *16*, 64−67.
(19) Ikarashi, Y.; Tsuchiya, T.; Nakamura, A. Evaluation of contact sensitivity to rubber chemicals using the murine local lymph node assay. *Contact Dermatitis* **1993**, *2*, 77−80.
(20) Kimber, I.; Dearman, R. J.; Sholes, E. W.; Basketter, D. A. The local lymph node assay: Developments and applications. *Toxicology* **1994**, *93*, 13−31.
(21) Dearman, R. J.; Basketterr, D. A.; Kimber, I. Variable effects of chemical allergens on serum IgE concentration in mice. Preliminary evaluation of a novel approach to the identification of respiratory sensitizers. *J. Appl. Toxicol*. **1992**, *12*, 317−323.
(22) Hilton, J.; Dearman, R. J.; Basketter, D. A.; Scholes, E. W.; Kimber, I. Experimental assessment of the sensitizing properties of formaldehyde. *Food Chem. Toxicol*. **1996**, *34*, 571−578.
(23) Saltzman, B. E. Colorimetric microdetermination of nitrogen dioxide in the atmosphere. *Anal. Chem*. **1960**, *32*, 135−136.
(24) Ando, K.; Nagata, K.; Beppu, M.; Kikugawa, K.; Kawabata, T.; Hasegawa, K.; Suzuki, M. Effect of n-3 fatty acid supplementation on lipid peroxidation and protein aggregation in rat erythrocyte membranes. *Lipids* **1998**, *33*, 505−512.
(25) Kosugi, H.; Kojima, T.; Yamaki, S.; Kikugawa, K. Interpretation of the thiobarbituric acid reactivity of rat liver and brain homogenates in the presence of ferric ion and ethylenediaminetetraacetic acid. *Anal. Biochem*. **1992**, *202*, 249−255.
(26) Ando, K.; Nagata, K.; Yoshida, R.; Kikugawa, K.; Suzuki, M. Effect of n-3 polyunsaturated fatty acid supplementation on lipid peroxidation of rat organs. *Lipids* **2000**, *35*, 401−407.
(27) Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J. Protein measurement with the Folin phenol reagent. *J. Biol. Chem*. **1951**, *193*, 265−275.
(28) Kosugi, H.; Kato, T.; Kikugawa, K. Formation of red pigment by a two-step 2-thiobarbituric acid reaction of alka-2,4-dienals. Potential products of lipid peroxidation. *Lipids* **1988**, *23*, 1024−1031.
(29) Kosugi, H.; Kojima, T.; Kikugawa, K. Characteristics of the thiobarbituric acid reactivity of oxidized fats and oils. *J. Am. Oil Chem. Soc*. **1991**, *68*, 51−55.
(30) Hiramoto, K.; Kato, T.; Takahashi, Y.; Yugi, K.; Kikugawa, K. Absorption and induction of micronucleated peripheral reticulocytes in mice after oral administration of fragrant hydroxyfuranones generated in the Maillard reaction. *Mutat. Res*. **1998**, *41*, 79−83.
(31) Ando, K.; Nagata, K.; Yoshida, R.; Kikugawa, K.; Suzuki, M. Effect of feeding plant polyphenolics on fatty acid composition, vitamin E level and degree of lipid peroxidation in rat organs. *J. Oleo Sci*. **2000**, *50*, 13−19.
(32) Dearman, R. J.; Kimber, I. Differential stimulation of immune function by respiratory and contact chemical allergens. *J. Immunol*. **1991**, *72*, 563−570.
(33) Dearman, R. J.; Kimber, I. Divergent immune responsed to respiratory and contact chemical allegens: antibody elicited by phthalic anhydride and oxazolone. *Clin. Exp. Allergy* **1992**, *22*, 241−250.
(34) Dearman, R. J.; Spence, L. M.; Kimber, I. Characterization of murine immune responses to allergenic diisocyanates. *Toxicol. Appl. Pharmacol*. **1992**, *112*, 190−197.
(35) Dearman, R. J.; Ramdin, L. S. P.; Basketter, D. A.; Kimber, I. Inducible interleukin-4-secreting cells provoked in mice during chemical sensitization. *Immunology* **1994**, *81*, 551−557.
(36) Mosmann, T. R.; Cherwinski, H.; Bond, M. W.; Giedlin, M. A.; Coffman, R. L. Two types of murine helper T cell clon. I. Definition according to profiles of lymphokine activities and secreted proteins. *J. Immunol*. **1986**, *136*, 2348−2357.
(37) Mosmann, T. R.; Coffman, R. L. Heterogeneity of cytokine secretion patterns and functions of T helper cells. *Adv. Immunol*. **1989**, *46*, 111−147.
(38) Cher, D. J.; Mosmann, T. R. Two types of murine helper T cell clones. II. Delayed type hypersensitivity is mediated by Th 1 clones. *J. Immunol*. **1987**, *13*, 3688−3694.
(39) Finkelman, F. D.; Katona, I. M.; Mosmann, T. R.; Coffman, R. L. IFN-γ regulates the isotypes of Ig secreted during in vivo humoral immune responses. *J. Immunol*. **1988**, *140*, 1022−1027.
(40) Finkelman, F. D.; Katona, I. M.; Urban, J.F., Jr.; Holmes, J.; Ohara, J.; Tung, A. S.; Sample, J. G.; Paul, W. E. IL-4 is reqired to generate and sustain in vivo IgE responses. *J. Immunol*. **1988**, *141*, 2335−2341.

Received for review April 30, 2001. Revised manuscript received July 17, 2001. Accepted July 17, 2001. This work was performed through Special Coordination Funds of the Ministry of Education, Culture, Sports, Science and Technology of the Japanese Government.

JF010556L