韓國營養學會誌 24(1) : 12~19, 1991
Korean J Nutrition 24(1) : 12~19, 1991

Effects of Long-Term Ozone Exposure and Dietary Factors on Lipid Peroxidation in Lung & Liver Tissues of Mice

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장기간의 오존조사와 식이요인이 생쥐의 폐와 간조직의 지질과산화에 미치는 영향

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국문초록

장기간의 연속적 오존조사와 식이항산화제 및 식이지방의 불포화도가 생쥐의 폐와 간조직의 지질산화에 미치는 영향을 조사하였다. 실험동물들은 각각 0.25ppm, 0.50ppm의 오존 또는 정상적 대기환경에서 18개월간 식이항산화제의 수준과 식이지방의 불포화도를 달리한 실험식이들에 의해 사육되었으며 조직의 지질과산화의 정도는 Thiobarbituric acid 반응물질의 농도와 free malonaldehyde의 농도에 의해 측정되었다. 본 연구의 결과, 장기간의 오존조사는 실험식이의 종류에 관계없이 폐조직과 간조직의 지질과산화를 유도하지 않음이 발견되었으며 식이중 비타민 E의 함량과 조직의 지질과산화생성물의 농도와는 역의 관계를 나타냈고 적절한 식이비타민 E 의 첨가(30ppm)는 식이지방의 불포화도에 관계없이 조직의 지질과산화를 억제하는데 효과적임에 관찰되었다.

Abstract

The chronic effects of long-term ozone exposure and dietary factors on the lipid peroxidation were investigated in mouse lung and liver tissues. Eighteen groups of mice were exposed to ozone(0.25 or 0.50 ppm) or ambient air over an 18-month period. Within each esposure regimen, animals were fed diets containing different levels of antioxidants and unsaturated fat. Ozone exposure did not have an effect on the production of thiobarbituric acid-reactive substances in lung and liver or free malondialdehyde in the liver at all levels of dietary vitamin E. An inverse relationship between the level of vitamin E supplementation and the concentration of lipid peroxidation products was observed. Results indicate the possible adaptation of animals to long-term continuous ozone exposure by unknown mechanism and the effectiveness of dietary vitamin E at sufficient level(30

접수일자 : 1991년 1월 30일

ppm) to protect against tissue lipid peroxidation regardless of the degree of unsaturation of the dietary fat.

KEY WORDS : lipid peroxidation · ozone exposure · vitamin E.

Introduction

Ozone(O_3) is a common environmental pollutant in industrialized urban areas, and a key component of smog[1]. This gas is capable of forming free radicals which are highly significant health hazards[2]. Ozone is produced in the upper atmosphere by solar irradiation and also formed when hydrocarbons like gasoline react with nitrogen oxides in the presence of sunlight or at high temperature. Normal air contains 0.01 to 0.04 ppm ozone[3]. Typical ranges for ozone in Los Angeles on a non-smoggy day are 0.05 to 0.30 ppm and on a smoggy day 0.20 to 0.65 ppm[3]. New health studies show that one out of every three Americans is exposed to ozone levels above the standard (0.12 ppm by the Clean Air Act), especially in the summertime[4].

The primary target of ozone is the lung and respiratory tract. Exposure to ozone has been reported to result in a deep-lung injury, and the pathological changes in the small airways have been well described by many investigators[5][6][7]. Ozone exposure also damages the structural and functional integrity of the trachea and main bronchi. Thus, both cellular damage[8] and increased permeability of small substances through the trachea have been reported to occur after exposure to ozone[9]. The threshold dose of ozone for ventilatory function decrement in normal human subject has been found to be between 0.20 and 0.30 ppm[10].

The biochemical mechanism by which exposure to ozone damages biological systems has been the subject of considerable speculation and investigation ; however, there is still no generally accepted explanation. Several investigators suggested that the toxicity of ozone might be attributed to its oxidative nature, including the initiation of lipid peroxidation[11][12]. Some lines of evidence supported this hypothesis. Various antioxidants such as α-tocopherol[13][14] BHT[14] and p-aminobenzoic acid[15] have been reported to exert a protective effect against ozone toxicity. The protective effect of dietary vitamin E supports the theory of free radical mechanism as a part of the causes of ozone toxicity. Therefore, the nutritional status could be a factor influencing the effects of ozone.

Most of the ozone studies, to date, investigated the acute toxicity on the pulmonary pathophysilolgical aspects with the length of exposure varying from a few hours to 90 days[16]. The consequences of continuous life-long exposure to low levels of ozone are relatively unknown. The purpose of the present study was to investigate the chronic effect of long-term exposure(18-months) to low concentrations of ozone(0.25 or 0.50 ppm) on the in vivo lipid peroxidation in lung and liver tissues of mice. Animals were fed diets containing different levels of antioxidants and unsaturated fat. Thiobarbituric acid-reactive substances(TBA-RS) and free malondialdehyde (MDA) were measured as an indicator of tissue lipid peroxidation.

Materials and Methods

1. Animals and exposure

Two hundred sixteen weanling mice of the C57 BL/6J strain(The Jackson Lab., Bar Harbor, Maine) were housed in groups of four animals per stainless steel cage. Animal cages were placed in one of three stainless steel exposure chambers designed after the model of Hinners et al.[17] with a capacity of 2.165 m^3, and animals were exposed

for 18 months to either 0.25 ppm, 0.50 ppm ozone, or filtered air. Ozone was generated with an Airox Ozonator(Pollution Control Industries, Inc., Stanford, Connecticut) and was admitted tangentially to the top of two chambers at approximately 0.0212 m^3 filtered air per sec. (45 ft^3/min.). As a result of a mild, swirling motion, the air and gas were mixed, and the final concentrations were 0.25 ppm in Chamber II and 0.50 ppm in Chamber III. Chamber I(control) was identical to the ozone exposure chambers except that there was no introduction of ozone into the system. The gas mixture and room air were thermostatically maintained at 22± 2°C Concentrations of ozone in the chambers were monitored continuously throughout the experimental period using a Dasibi pc-1008 ozone monitor(Dasibi Environmental Corp., Glendale, California). The chambers were opened two times per week for approximately four hours to feed the mice. They were also opened a third time each week for approximately six to eight hours in order to feed and weigh the animals and clean the chambers and the cages. Over a period of one week, the mice were gradually introduced to the ozone as follows ; Day 1, one-half hour of exposure ; Day 2, 1 hour ; Day 3, 2 hours ; Day 4, 4 hours ; Day 5, 8 hours ; and Day 6, 12 hours. Thereafter, the exposure was continuous. Without a period of gradual adaptation, immediate, continuous exposure to these concentrations of ozone would have been fatal.

2. Diets

Groups of 9 to 14 mice were fed one of the following six diets ; (1) a basal vitamin E-deficient diet(shown in Table 1) adequate in all respects except for vitamin E, containing 8% stripped corn oil ; (2) a basal diet supplemented with 30 ppm vitamin E ; (3) a basal diet supplemented with 300 ppm vitamin E ; (4) a basal diet supplemented with 30 ppm vitamin E, containing 8% stripped lard(Eastman Kodak Co.) instead of 8% corn oil ; (5) a basal diet supplemented with 30 ppm vitamin E, containing 5% cod liver oil and 3% stripped corn oil instead of 8% corn oil ; (6) a basal diet supplemented with 30 ppm N, N'-diphenyl-p-phenylenediamine(DPPD), a synthetic antioxidant. Vitamin E was supplied as RRR-α-tocopheryl acetate(Type III, 1.36 IU/mg, Sigma Chemical Co., St. Louis, MO). These diets will be designated as follows : (1)-E.CO, (2) NE. CO, (3) HE.CO, (4) NE. LD (5) NE. CL and (6) DPPD.CO. Diets and water were provided

Table 1. Composition of Basal, Vitamin E-Deficient Diet for Mice

Ingredients	Percent(%)
Anhydrous d(+)-dextrose[1]	67.1
Vitamin-free casein[1]	20.0
Stripped corn oil[2]	8.0
Salt mix[1,3]	4.0
Vitamin mix[4]	0.5
Choline chloride[1]	0.1
DL-methionine	0.3
	100.0
Vitamin A[5] 10,000 IU/kg diet	
Vitamin D[6] 1,000 IU/kg diet	

1 : Purchased from ICN Pharmaceuticals, Inc., Cleveland, OH.
2 : Eastman Kodak Co., Rochester, NY.
3 : Salt Mix No. 4164 by Draper, H.H. et al.
4 : Vitamin mix composition(g/kg mix) ; riboflavin 1.0(Merck and Co. Inc., Rahway, NJ), thiamin hydrochloride 2.0, nicotinic acid 5.0, pyridoxine hydrochloride 1.0, calcium pantothenate 2.0(all from Baker Chem., Phillipsburg, NJ), menadione 0.2, folic acid 0.2, biotin 0.02(Calbiochem, La Jolla, CA), vitamin B_{12}(0.1% trituration with mannitol) 20.0(Gibco, Grand Island, NY), and d (+)-Dextrose 968.58.
5 : Retinyl palmitate(water dispersable) from Gibco, Grand Island, NY.
6 : Aqueous ergocalciferol solution(5,000 IU/ml) from Endo Laboratories, Inc., Garden City, NY.

ad libitum. Sodium salt of sulfamerazine (Sigma Chemical Co.) was added to the drinking water(1 g/3.8 l) to prevent pulmonary infection. Each of these dietary groups were placed in an environmental chamber described previously and exposed continuously to control ambient air, 0.25 ppm ozone, or 0.50 ppm ozone.

3. Measurement of lipid peroxidation products in tissues

After 18 months, the animals were killed by decapitation. Liver and lung tissues were removed from the animals, dipped in cold physiological saline, blotted dry with cheese-cloth, wrapped in parafilm and frozen at −70C. Free malondialdehyde was determined by the HPLC method of Csallany et al.[18] Thiobarbituric acid-reactive substances were measured by the modified method of Uchiyama.[19]

4. Statistical analysis of data

Data were analyzed using one way analysis of variance and Honestly Significant Difference(Tukey method) for comparison of means.[20] In the liver tissue assay, sample sizes were unequal but the balance between sample sizes was fairly well maintained. Therefore, the Tukey method was employed using for the average sample size. Significance was accepted when p-value was less than 0.05.

Results and Discussion

The major biochemical mechanism of ozone toxicity has been attributed to its oxidative nature including free radical formation and initiation of lipid peroxidation.[11][12] Accordingly, increased lipid peroxidation in vivo might be generally expected as a result of ozone exposure. However, a number of factors such as animal species, age, weight, and nutritional status have been reported to influence the effects of ozone.[5][21][22] In particular, the level of antioxidant and the degree of unsaturation of dietary fat could be important factors influencing the effects of ozone as far as lipid peroxidation is concerned.

The levels of TBA-RS in lungs and livers of

Table 2. TBA-reactive substances levels of lungs from ozone-and control air-exposed mice fed various diets for 18 months

Diet[2]	Filtered Air	No. of Animals	0.25ppm Ozone	No. of Animals	0.50ppm Ozone	No. of Animals
−E.CO	$14.30\pm 1.45^{1\ aA3}$	7	$17.81\pm 2.51^{a,A}$	7	$10.36\pm 2.76^{a,A}$	7
NE.CO	$6.28\pm 0.47^{a,B}$	7	$8.27\pm 0.67^{b,B}$	7	$6.53\pm 0.47^{a,A}$	7
HE.CO	$4.87\pm 0.58^{a,B}$	7	$7.42\pm 0.75^{b,B}$	7	$5.36\pm 0.71^{a,A}$	7
NE.LD	$7.09\pm 1.34^{a,B}$	7	$4.82\pm 0.42^{b,B}$	7	$6.56\pm 0.64^{a,A}$	7
NE.CL	$4.96\pm 0.58^{a,B}$	7	$5.58\pm 0.65^{b,B}$	7	$7.06\pm 0.78^{a,A}$	7
DPPD.CO	$4.70\pm 0.51^{a,B}$	7	$5.98\pm 0.99^{b,B}$	7	$9.35\pm 2.61^{a,A}$	7

1 : Mean± SEM(ug/g lung).

2 : Diet Key : −E.CO=basal vitamin E deficient diet containing 8% stripped corn oil ; NE.CO=basal diet supplemented with 30 ppm vitamin E containing 8% stripped corn oil ; HE.CO=basal diet supplemented with 300 ppm vitamin E containing 8% stripped corn oil ; NE.LD=basal diet supplemented with 30 ppm vitamin E containing 8% stripped lard ; NE.CL=basal diet supplemented with 30 ppm vitamin E containing 5% cod liver oil and 3% stripped corn oil ; DPPD.CO=a basal diet supplemented with 30 ppm N,N'-diphenyl-p-phenylenediamine containing 8% stripped corn oil.

3 : Capital letter superscripts in the same column and samll letter superscripts in the same line that are different indicate significant differences between groups($p<0.05$).

ozone-exposed and control air mice fed various diets are shown in Tables 2 and 3. Ozone exposure had no effect on the production of TBA-RS in both lung and liver tissues under the various dietary conditions after 18-month exposure. Lung is the primary target of ozone toxicity, therefore, increased lipid peroxidation in this tissue was anticipated following long-term exposure to ozone. Liver tissue is known to be the most sensitive organ to lipid peroxidation in animals, but no positive responses to the long-term ozone exposure were found in this study. Free MDA was found in this laboratory to be even a more sensitive index for lipid peroxidation in the liver than TBA-RS[19] Nevertheless, the levels of free MDA in the liver of mice exposed to ozone(Table 4) were not significantly increased compared to the liver levels of control air mice. This further confirms no effect of chronic ozone exposure on the tissue lipid peroxidation.

An early study by Goldstein[11] reported the presence of conjugated dienes in lung lipids from mice exposed to ozone, indicating the occurrence of lipid peroxidation. Studies done by Chow and Tappel[23] on rats continuously exposed to 0.7 to 0.8 ppm ozone for 5 to 7 days have shown that ozone exposure significantly raised the concentrations of lung TBA reactants produced by lipid

Table 3. TAB-reactive substances levels of livers from ozone- and control air-exposed mice fed various diets for 18 months

Diet[2]	Filtered Air	No. of Animals	0.25ppm Ozone	No. of Animals	0.50ppm Ozone	No. of Animals
-E.CO	$18.37\pm 2.19^{1\ a}A^{3}$	9	$16.46\pm 1.86^{a,A}$	8	$17.35\pm 2.17^{a,A}$	7
NE.CO	$8.04\pm 0.93^{a,B}$	7	$8.96\pm 1.30^{b,BCD}$	7	$7.10\pm 1.03^{a,B,C}$	7
HE.CO	$6.49\pm 0.75^{a,B}$	8	$4.22\pm 0.42^{b,D}$	7	$5.51\pm 0.36^{a,b,C}$	6
NE.LD	$5.77\pm 1.19^{a,B}$	8	$4.75\pm 0.54^{a,D}$	8	$7.79\pm 1.67^{a,B,C}$	7
NE.CL	$10.96\pm 0.81^{a,B}$	7	$12.36\pm 2.14^{a,A,B,C}$	7	$15.40\pm 2.24^{a,A,B}$	8
DPPD.CO	$9.10\pm 2.70^{a,B}$	7	$14.60\pm 2.77^{a,A,B}$	6	$11.53\pm 3.42^{a,A,B,C}$	8

1 : Mean± SEM(ug/g liver).
2 : Diet Key : see Table 2.
3 : Capital letter superscripts in the same column and samll letter superscripts in the same line that are different indicate significant differences between groups($p<0.05$).

Table 4. Free malondialdehyde levels of livers from ozone- and control air-exposed mice fed various diets for 18 months.

Diet[2]	Filtered Air	No. of Animals	0.25ppm Ozone	No. of Animals	0.50ppm Ozone	No. of Animals
-E.CO	$3.34\pm 0.26^{1\ aA3}$	9	$3.09\pm 0.43^{a,A}$	7	$2.87\pm 0.18^{a,A}$	8
NE.CO	$0.62\pm 0.01^{a,B,C}$	8	$0.68\pm 0.16^{a,B}$	7	$0.68\pm 0.19^{a,B,C}$	8
HE.CO	$0.12\pm 0.08^{a,C}$	8	$0.12\pm 0.10^{a,B}$	8	$0.14\pm 0.11^{a,C}$	8
NE.LD	$0.86\pm 0.12^{a,B,C}$	9	$0.79\pm 0.17^{a,B}$	9	$0.62\pm 0.11^{a,B,C}$	9
NE.CL	$0.59\pm 0.12^{a,B,C}$	7	$0.57\pm 0.01^{a,B}$	7	$0.67\pm 0.12^{a,B,C}$	6
DPPD.CO	$1.16\pm 0.13^{a,B}$	6	$0.64\pm 0.13^{b,B}$	6	$0.96\pm 0.01^{a,b,B}$	8

1 : Mean± SEM(ug/g liver).
2 : Diet Key : see Table 2.
3 : Capital letter superscripts in the same column and samll letter superscripts in the same line that are different indicate significant differences between groups($p<0.05$).

peroxidation. Dumelin et al[24] also demonstrated that, using exhaled pentane as an index of lipid peroxidation, a 60-min. exposure of rats to 1 ppm ozone resulted in a significant increase in pentane in rats fed a vitamin E-deficient diet but not in rats fed a vitamin E-supplemented diet. From the above reports, it appears that the reaction responsible for acute ozone toxicity involves free radical-mediated lipid peroxidation. However, the signs of increased lipid peroxidation were not observed after long-term continuous exposure of mice in the present study when TBA-RS and free MDA were measured. These results suggest an adaptability to continuous long-term ozone exposure by the experimental anaimals. Adaptation to repeated exposures has been reported by several investigators[25][26][27] in the past. Repeated daily exposure of human subjects to ozone resulted in marked attenuation of the response of lung function. Adaptation to ozone has been attributed to a variety of mechanisms. They include enzymatic responses[28] and possible changes in the content of unsaturated fatty acids in the lung[11]. In particular, the elevation of palmitic acid content of lung lipids in ozone-treated animals reported by Bartov et al[29] is of specific interest. Palmitic acid is a major component of lung surfactant. Therefore, one of possible adaptation mechanisms to ozone exposure might be that elevation of palmitic acid for the increased production of lung surfactant would decrease the relative amount of membrane unsaturated fatty acids and thereby reduce the substrates of lipid peroxidation and the resulting toxicity of peroxidation. However, the exact mechanism for the adaptation phenomenon still remains to be elucidated.

Even though the ozone effects were not found, dietary effects were found when TBA-RS and free MDA were measaured in this study. Vitamin E-deficient diets significantly increased lung tissue lipid peroxidation in the filtered air control and low ozone environments(Table 2). When adequate amounts of vitamin E were provided, the degree of unsaturation of dietary fat did not affect tissue lipid peroxidation. Synthetic antioxidant, DPPD, exhibited a protective effect similar to vitamin E against lung tissue lipid peroxidation. Vitamin E deficient diets also resulted in significantly increased production of TBA-RS and free MDA in the liver tissue regardless of the different environmental atmospheres(Table 3 and 4). The cod liver oil diet increased liver TBA-RS significantly in the ozone-exposed animals, indicating that enrichment of diet with highly unsaturated fat may have a strong effect on the induction of lipid peroxidation in the liver under ozone exposure. The DPPD diet also showed a tendency to increase both TBA-RS and free MDA in the liver tissues of mice exposed to ozone, indicating this synthetic antioxidant is less effective in liver tissue than in lung. Relatively low levels of free MDA compared to the high levels of TBA-RS were shown in the liver tissues of mice fed cod liver oil diet or corn oil diet supplemented with vitamin E and DPPD respectively. The reason might be explained by the finding[19] that free MDA is the major form of MDA in vitamin E deficient tissues but bound form of MDA is predominant in antioxidant-supplemented tissues.

In conclusion, it appears that in long-term continuous ozone exposure animals are capable of adapting to increased oxidative stress by some unknown mechanism. A strong inverse relationship between the dietary vitamin E levels and the extent of tissue lipid peroxidation was found in this longterm experiment.

Summary

The chronic effect of low levels of ozone on

the in vivo lipid peroxidation was investigated in mouse lung and liver by measuring TBA-reactive substances and free MDA. Eighteen groups of weanling mice were exposed to ozone(0.25 or 0.50 ppm) or ambient air over an 18-month period. Within each exposure regimen, groups of mice were fed a vitamin E-deficient diet, a vitamin E-sufficient diet(30 ppm), a high vitamin E diet (300 ppm), or a DPPD-supplemented diet(30 ppm), each containing 8% stripped corn oil. Additional groups of mice were fed 8% lard or 5% corn oil and 3% cod liver oil at a sufficient level of vitamin E and exposed as the animals above. Ozone exposure did not have an effect on the production of TBA-reactive substances in lung and liver or free malondialdehyde in the liver at all levels of dietary vitamin E. An inverse relationship between the level of vitamin E supplementation and the concentration of lipid peroxidation products was observed. There was no apparent effect of unsaturation of dietary fat on tissue lipid peroxidation under the sufficient level of vitamin E when the above mentioned two parameters were measured. Overall results indicate the possible adaptation of animals to long-term exposure of ozone and the effectiveness of dietary vitamin E at sufficient levels to protect against tissue lipid peroxidation.

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