0163–4984/95/5002–0167 $05.20

Lead-Catalyzed Peroxidation of Essential Unsaturated Fatty Acid

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Received July 28, 1994; Accepted August 18, 1994

ABSTRACT

In the present study, the reaction mixtures (lead compounds with essential unsaturated fatty acids) were preincubated at 37°C for 24 h prior to the measurement of malondialdehyde (MDA) by HPLC. The metal-catalyzed reactions were also compared in the presence of butylated hydroxytoluene (BHT), a free radical scavenger.

Our results showed that according to the difference in the number of double bonds of essential unsaturated fatty acids, the kinds of lead compounds, and the concentrations of lead compounds, the extent of lipid peroxidation was different. The addition of BHT to the reaction mixtures significantly reduced the production of MDA ($P < 0.01$). These in vitro studies support prior in vivo reports that the important mechanism of the acute toxic effects of the lead compounds is owing at least in part, to metal-catalyzed peroxidation of polyunsaturated fatty acids.

Index Entries: Peroxidation; essential unsaturated fatty acid; lead; malondialdehyde; in vitro.

INTRODUCTION

Lipid peroxidation causes not only deterioration of foods, but also damage to tissues in vivo. It has been implicated in many pathological conditions, for example, rheumatoid arthritis, various liver diseases, acute myocardial infarction, and toxicity induced by certain metals *(1–4)*.

Metal ions result in many oxidation-reduction reactions within the organism, including increased lipid peroxide in alveolar macrophages of rats administered $NiCl_2$ *(5)*. They can contribute to the production of free radicals, including oxygen-centered radicals. Some animal studies indi-

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cated that indices of lipid peroxidation were enhanced in target tissues of rodents exposed to lead compounds and lipid peroxidation as a molecular mechanism for cell damage induced by lead compounds. For example, Levander et al. showed that erythrocyte TBA-chromogens and deformability were increased in lead-poisoned rats *(6,8)* and Shafiq-Ur-Rehman observed a twofold increase in TBA-chromogen production in brain homogenates of adult rats exposed to lead compound for 10 d *(7,8)*.

Increased lipid peroxide in vitro owing to the catalytic peroxidation of linoleic acid by various transition metal ions was reported *(9)*. There are three essential unsaturated fatty acids, linoleic acid, linolenic acid, and arachidonic acid, which are of importance in organisms. The present study will discuss the metal-catalyzed reactions of these three essential unsaturated fatty acids with three lead compounds and compared them with those to which butylated hydroxytoluene (BHT), a well-known free radical scavenger, has been added.

MATERIALS AND METHODS

Apparatus

The high-performance liquid chromatographic equipment used has been recently described at our previous study *(10)*.

Reagent

The 1,1,3,3-tetra-ethoxypropane (TEP), phosphoric acid, thiobarbituric acid (TBA), methanol-NaOH, phosphate buffer, and mobile-phase solutions were prepared as described by Wong et al. *(11)* with minor modification in the potassium phosphate buffer and mobile-phase mixture *(12)*. Linoleic acid, linolenic acid, arachidonic acid, and BHT were of the highest purity obtainable (Sigma Chemical Co., St. Louis, MO). Unsaturated fatty acids (10 mmol/L) and BHT solutions (10 mmol/L) were prepared in 95% ethanol. The reagent-grade chemicals of lead oxide mono, lead oxide di, and trilead tetra oxide were prepared in deionized water, precipitates were removed and then prepared as three levels of lead concentration at 20, 40, 60 μg/dL (normal level, preclinical toxic level, clinical toxic level).

Procedure

Linoleic acid, linolenic acid, and arachidonic acid solution (0.05 mL) were pipeted into test tubes. To the control tubes (linoleic acid, linolenic acid, arachidonic acid) were added 0.95 mL of deionized water, 0.20 mL of the metal solutions, and 0.75 mL of deionized water. In the experiments containing BHT, 0.60 mL water and 0.15 mL BHT were added. The final volume was 1.0 mL. The tubes were then capped and incubated in a 37°C water bath for 24 h. Then 3.0 mL of phosphoric acid and 1.0 mL

of TBA solutions were added. The tubes were placed in a boiling water bath for 60 min, and then they were cooled in a cold water bath. Precisely 0.5 mL each of the boiled solutions was added to polypropylene microcentrifuge tubes and neutralized with 0.5 mL of methanol-NaOH. The tubes were thoroughly mixed, centrifuged at 9500 *g* for 90 s, and 50 µL of each were sequentially injected into the HPLC system. Peak areas were measured at 532 nm.

Data Analysis

Statistical analysis was performed with SAS software. Statistical methods included the mean, standard deviation, and Wilcoxon two-sample test.

RESULTS

Tables 1–3 summarized the PbO-, PbO_2, and Pb_3O_4-catalyzed peroxidation of linoleic acid, linolenic acid and arachidonic acid after preincubation of the reaction mixtures at 37°C for 24 h. In each control tube for the linoleic acid, linolenic acid, and arachidonic acid, 20 analyses were done. In each metal test tube, 10 analyses were done. As indicated, 60 µg/dL PbO_2 and Pb_3O_4 significantly increased the production of MDA by the linoleic acid, linolenic acid, and arachidonic acid over that of the controls (Wilcoxon two-sample test). Forty µg/dL PbO_2 significantly increased the production of MDA by the three polyunsaturated fatty acids over that of the controls, and as low as 20 µg/dL PbO_2 can significantly increase the production of MDA. Tables 4–6 illustrated that BHT, a well-known free radical scavenger, significantly decreased the quantities of MDA produced by the polyunsaturated fatty acids that is catalyzed by PbO, PbO_2, and Pb_3O_4.

DISCUSSION

Previously, increased lipid peroxide was reported when linolenic acid was mixed with a variety of transition metal ions *(9)*. However, in that study, the reaction mixtures contained phosphoric acid and TBA, and were heated to form the colored TBA adduct with generated MDA. In the present study, in order to mimic more closely in vivo studies, linoleic acid, linolenic acid, and arachidonic acid were preincubated with the metal ions at 37°C for 24 h. This resulted in marked enhancement of MDA (Tables 1–3) over that produced in the previous study, which showed relatively little MDA produced during the MDA–TBA reaction phase *(13)*. When the reaction mixture was incubated with the free radical scavenger BHT, a marked reduction in MDA was noted (Table 4–6).

It should be noted that the more double bonds the unsaturated fatty acid has, the more lipid peroxide it may produce. For example, the

Table 1
Lead in PbO-Catalyzed Peroxidation of Linoleic Acid, Linolenic Acid, and Arachidonic Acid at 37°C Measurement of MDA (μmol/L) by High-Performance Liquid Chromatography

Fatty acid	Control, $N = 20$	PbO,[b] μg/dL, $N = 10$		
		4	8	12
Linoleic acid	0.37 ± 0.06	0.43 ± 0.06	0.50 ± 0.09[a]	0.51 ± 0.08[a]
Linolenic acid	0.49 ± 0.08	0.53 ± 0.06	0.54 ± 0.07	0.60 ± 0.13[a]
Arachidonic acid	1.37 ± 0.23	1.47 ± 0.38	2.32 ± 0.72	2.38 ± 0.78

[a]$P < 0.05$ (by Wilcoxon two-sample test).
[b]Lead concentration in PbO solution.

Table 2
Lead in PbO_2-Catalyzed Peroxidation of Linoleic Acid, Linolenic Acid, and Arachidonic Acid at 37°C Measurement of MDA (μmol/L) by High-Performance Liquid Chromatography

Fatty acid	Control, $N = 20$	PbO_2,[b] μg/dL, $N = 10$		
		4	8	12
Linoleic acid	0.37 ± 0.06	0.49 ± 0.09[a]	0.49 ± 0.07[a]	0.50 ± 0.11[a]
Linolenic acid	0.49 ± 0.08	0.53 ± 0.02[a]	0.64 ± 0.11[a]	0.67 ± 0.08[a]
Arachidonic acid	1.37 ± 0.23	1.16 ± 0.43	2.06 ± 0.10[a]	2.09 ± 0.48[a]

[a]$P < 0.05$ (by Wilcoxon two-sample test).
[b]Lead concentration in PbO_2.

Table 3
Lead in Pb_3O_4-Catalyzed Peroxidation of Linoleic Acid, Linolenic Acid, and Arachidonic Acid at 37°C Measurement of MDA (μmol/L) by High-Performance Liquid Chromatography

Fatty acid	Control, $N = 20$	Pb_3O_4,[b] μg/dL, $N = 10$		
		4	8	12
Linoleic acid	0.37 ± 0.06	0.37 ± 0.12	0.41 ± 0.09	0.56 ± 0.10[a]
Linolenic acid	0.49 ± 0.08	0.50 ± 0.06	0.55 ± 0.06	0.87 ± 0.05[a]
Arachidonic acid	1.37 ± 0.23	1.62 ± 0.28	1.69 ± 0.36	1.90 ± 0.30[a]

[a]$P < 0.05$ (by Wilcoxon two-sample test).
[b]Lead concentration in Pb_3O_4.

arachidonic acid (four double bonds) produced much heigher concentrations of MDA than that of linolenic acid (three double bonds). The linolenic acid, on the other hands, produced much higher concentrations of MDA than that of linoleic acid (two double bonds). Therefore, the con-

Table 4
Effect of BHT on Lead in PbO-Catalyzed Peroxidation of Linoleic Acid, Linolenic Acid, and Arachidonic Acid at 37°C

Fatty acid	*N*	Without BHT, mean ± SD	With BHT, mean ± SD	Statistical significance[a]
PbO,[b] 4 μg/dL				
Linoleic acid	10	0.43 ± 0.06	0.34 ± 0.02	0.0010
Linolenic acid	10	0.53 ± 0.06	0.33 ± 0.06	0.0002
Arachidonic acid	10	1.47 ± 0.38	1.03 ± 0.13	0.1505
PbO,[b] 8 μg/dL				
Linoleic acid	10	0.50 ± 0.09	0.31 ± 0.02	0.0002
Linolenic acid	10	0.54 ± 0.07	0.24 ± 0.02	0.0002
Arachidonic acid	10	2.32 ± 0.72	1.12 ± 0.33	0.0046
PbO,[b] 12 μg/dL				
Linoleic acid	10	0.51 ± 0.08	0.34 ± 0.03	0.0002
Linolenic acid	10	0.60 ± 0.13	0.42 ± 0.08	0.0011
Arachidonic acid	10	2.38 ± 0.78	1.67 ± 0.23	0.0257

[a]By Wilcoxon two-sample test.
[b]Lead concentration in PbO.

Table 5
Effect of BHT on Lead in PbO_2-Catalyzed Peroxidation of Linoleic Acid, Linolenic Acid, and Arachidonic Acid at 37°C

Fatty acid	*N*	Without BHT, mean ± SD	With BHT, mean ± SD	Statistical significance[a]
PbO_2,[b] 4 μg/dL				
Linoleic acid	10	0.49 ± 0.09	0.30 ± 0.05	0.0004
Linolenic acid	10	0.53 ± 0.02	0.43 ± 0.03	0.0002
Arachidonic acid	10	1.16 ± 0.43	0.88 ± 0.14	0.0257
PbO_2,[b] 8 μg/dL				
Linoleic acid	10	0.49 ± 0.07	0.36 ± 0.03	0.0007
Linolenic acid	10	0.64 ± 0.11	0.39 ± 0.09	0.0002
Arachidonic acid	10	2.06 ± 0.10	0.56 ± 0.14	0.0002
PbO_2,[b] 12 μg/dL				
Linoleic acid	10	0.50 ± 0.11	0.29 ± 0.05	0.0002
Linolenic acid	10	0.67 ± 0.08	0.41 ± 0.15	0.0002
Arachidonic acid	10	2.09 ± 0.48	0.69 ± 0.22	0.0002

[a]By Wilcoxon two-sample test.
[b]Lead concentration in PbO_2.

centrations of lipid peroxides are different because of the difference in the numbers of double bonds of the unsaturated fatty acid. Lead dioxide is the much more toxic than the others, and it can significantly increase the production of MDA in the lowest concentration (20 μg/dL).

Table 6
Effect of BHT on Lead in Pb_3O_4Catalyzed Peroxidation of Linoleic Acid, Linolenic Acid, and Arachidonic Acid at 37°C

Fatty acid	*N*	Without BHT, mean ± SD	With BHT, mean ± SD	Statistical significance[a]
Pb_3O_4,[b] 4 μg/dL				
Linoleic acid	10	0.37 ± 0.12	0.25 ± 0.07	0.0134
Linolenic acid	10	0.50 ± 0.06	0.31 ± 0.02	0.0002
Arachidonic acid	10	1.62 ± 0.28	0.67 ± 0.23	0.0002
Pb_3O_4,[b] 8 μg/dL				
Linoleic acid	10	0.50 ± 0.09	0.31 ± 0.02	0.0002
Linolenic acid	10	0.55 ± 0.06	0.35 ± 0.05	0.0002
Arachidonic acid	10	1.69 ± 0.36	0.80 ± 0.16	0.0002
Pb_3O_4,[b] 12 μg/dL				
Linoleic acid	10	0.56 ± 0.10	0.31 ± 0.04	0.0002
Linolenic acid	10	0.87 ± 0.05	0.74 ± 0.03	0.0002
Arachidonic acid	10	1.90 ± 0.30	0.80 ± 0.13	0.0002

[a]By Wilcoxon two-sample test.
[b]Lead concentration in Pb_3O_4.

In conclusion, the lipid peroxidation is also a probable mechanism of the toxic effects of leads causing damage to the polyunsaturated fatty acid on the cell membranes. The administration of free radical scavenger might be very helpful in the emergency treatment of individuals who have ingested toxic doses of these metal ions.

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