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Serum lipid peroxide level and blood superoxide dismutase activity in workers with occupational exposure to lead

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Summary. We studied whether lead exposure increased the serum lipid peroxide (LPO) level and inhibited blood superoxide dismutase (SOD) activity in workers with occupational exposure to lead and rats injected with lead. We examined the following subjects: (1) manual workers (712 males) from 18 to 59-years-old in steel production with occupational exposure to lead, (2) office workers (155 males) without exposure to lead, (3) rats subcutaneously injected with lead in concentrations of 10 or 20 mg/kg as lead acetate. The nutritional intakes of manual workers and office workers were approximately equal. Serum LPO and high-density lipoprotein cholesterol (HDL-CL) levels in manual workers (LPO: 4.4 ± 1.9 nmol/ml, HDL-CL: 55.6 ± 14.2 mg/dl) were significantly higher than those in office workers (LPO: 4.0 ± 1.4 nmol/ml, HDL-CL: 53.0 ± 13.9 mg/dl). Serum LPO level in the manual workers increased with an increase of the lead concentration in the blood, while blood SOD activity decreased. Similar phenomena were observed in rats subcutaneously injected with lead acetate. Furthermore, the addition of lead at higher than 20-μ*M* concentrations to non-treated rat liver microsomes increased NADPH-dependent lipid peroxidation, and these lead concentrations inhibited bovine erythrocyte SOD activity in vitro assay system. In conclusion, the present results seem to indicate that the increase of serum LPO level in workers with occupational exposure to lead is due not only to the stimulation of lipid peroxidation, but also to the inhibition of SOD activity by exposure to lead in the manufacturing processes.

Key words: Blood lead – Lipid peroxide – Superoxide dismutase

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Introduction

It is well known that lead affects heme metabolism, since the determinations of red cell count (RBC), hemoglobin content, lead concentration, δ-aminolevulinic acid dehydratase (ALA-D) activity in the blood are commonly undertaken in screening health conditions of workers exposed to lead (Chisolm 1978; Granick et al. 1978).

Recently, lipid peroxides (LPO) have been shown to induce the denaturation of various cell and tissue membranes (Tappel 1973; Mead 1976; Kellogg and Fridovich 1977), and to be a risk factor for vascular diseases (Demopoulos et al. 1982). Heavy metals, such as iron (Wills 1969) and mercury (Stacey and Kappus 1982), have already been reported to increase lipid peroxidation in rat liver microsomes. On the other hand, superoxide dismutase (SOD) catalyzes the reaction of superoxide anions to hydroperoxide and oxygen and suppresses lipid peroxidation (McCord and Fridovich 1969).

It has been found that lead stimulates hemoglobin-catalyzed lipid peroxidation in vitro (Ribarov et al. 1981), and that the activities of blood and lung SOD in rats are inhibited by inhalation of lead (Minami et al. 1982).

We, therefore, examined the increase of serum LPO level and the inhibition of blood SOD activity in workers exposed to lead. We found that serum LPO level increased and blood SOD activity decreased, depending on the lead concentration in the blood of workers occupationally exposed to lead.

Materials and methods

Subjects

The subjects in this study were 712 male manual workers from 18- to 59-years-old who were exposed to lead in various manufacturing processes, such as smelting and casting, etc. The controls were 155 male office workers from 40- to 59-years-old in the same factory who were not exposed to lead. These workers were healthy as diagnosed by the following medical examinations: liver function tests (GOT, GPT and γ-GTP activity, etc.), kidney function tests (blood urea nitrogen, urinary tests) and blood tests (RBC, hemoglobin, specific gravity, etc.).

In the animal experiments, three groups of four male Wistar rats, aged 9 weeks (260 ± 5 g), were subcutaneously injected with 10 or 20 mg/kg of lead (as lead acetate) once a week for five weeks. The control rats were injected with sodium acetate solution by the same method. They were fed with laboratory rat chow (MF, Oriental Yeast Co. Ltd.) and deionized water ad libitum in the aluminium cages.

Analysis

Serum LPO was fluorometrically measured by the thiobarbituric acid reaction method (Yagi 1976). Blood SOD activity was determined by the method of Shinohara et al. (1976), using superoxide anions produced by NADPH cytochrome c reductase in rat microscomes as a substrate and neotetrazolium salt as a color reagent, after removal of hemoglobin as described by Minami and Yoshikawa (1979).

Blood ALA-D was assayed by the modified European standardized method (Berlin and Schaller 1974). Lead concentrations in the blood and liver were determined by anodic stripping voltammetry (Searle et al. 1973) after ashing blood and liver cytosol with a mixed acid solution (HNO_3:H_2SO_4:$HClO_4$ = 24:1:24, v/v).

Table 1. Comparison of serum lipid values between manual workers and office workers in a steel factory

Serum component		Age 40–49		Age 50–59		Overall	
		Manual workers	Office workers	Manual workers	Office workers	Manual workers	Office workers
Total cholesterol	(mg/dl)	202 (40)[a]	195 (31)	211 (44)	204 (36)	206 (42)	199 (33)
HDL-cholesterol	(mg/dl)	55.6 (14.3)*	51.7 (12.7)	55.2 (13.9)	54.6 (14.8)	55.6 (14.2)*	53.0 (13.9)
Triglyceride	(mg/dl)	118 (65)	111 (50)	123 (69)	130 (76)	120 (67)	122 (69)
Phospholipid	(mg/dl)	196 (31)*	187 (26)	201 (32)	196 (26)	198 (32)	192 (26)
Lipid peroxides	(nmol/ml)	4.3 (2.0)*	3.7 (1.3)	4.5 (1.9)	4.3 (1.5)	4.4 (1.9)*	4.0 (1.4)
Albumin	(g/dl)	5.4 (0.4)	5.4 (0.2)	5.3 (0.3)	5.3 (0.4)	5.4 (0.3)	5.3 (0.3)
GOT activity	(mU/ml)	15 (11)	15 (9)	19 (9)*	14 (6)	15 (9)	16 (9)
γ-GTP activity	(mU/ml)	22 (31)	21 (21)	23 (30)	20 (24)	23 (31)	20 (23)
n		92	321	63	132	155	453

[a] Mean value (Standard deviation)

* $P < 0.05$ (difference of serum component values between manual workers and office workers, Student's *t*-test)

Results

Serum lipid values and some clinical data of manual workers and office workers in a steel factory, aged from 40- to 59-years-old, are summarized in Table 1. In this study, precision of the present LPO determinations was C.V. 3–6%. Serum high-density lipoprotein cholesterol and serum LPO levels in manual workers were significantly higher than those in office workers in both the 40- to 49-year old group and the overall group, but there was no significant difference in the other component values between manual and office workers in the overall group.

In the manual workers exposed to lead, the increase of serum LPO was higher correlatively to the duration of exposure to lead than to aging as shown in Fig. 1. In the manual workers, not only ALA-D activity, but also serum and blood SOD activities decreased, whereas the serum LPO level increased with the increase of lead concentration in the blood, though age and RBC count in the manual workers were approximately constant (Table 2). Serum LPO level was only slightly raised when lead concentration in the blood was increased up to 1.45 nmol/ml, but at lead concentrations higher than 1.45 nmol/ml, the serum LPO level was significantly raised and blood SOD activity was decreased (Fig. 2).

When rats were subcutaneously injected with lead acetate solution once a week for five weeks, serum LOP level was significantly correlated with lead concentration in the blood as shown in Fig. 3. In the lead-treated rats, furthermore, LPO levels in serum and liver cytosol fraction increased with the increase in lead concentration, while SOD acitivities in the blood and liver cytosol fractions decreased (Table 3).

Lipid peroxidation in non-treated rat liver microsomes with NADPH was enhanced by addition of iron or lead at concentrations higher than 10 or 20 μM, respectively (Fig. 4). The activity of pure bovine erythrocyte SOD was inhibited by the addition of lead at concentrations of higher than 5 μM to the xanthine oxidase system and 15-μM concentrations to the NADPH cytochrome c reductase system (Fig. 5).

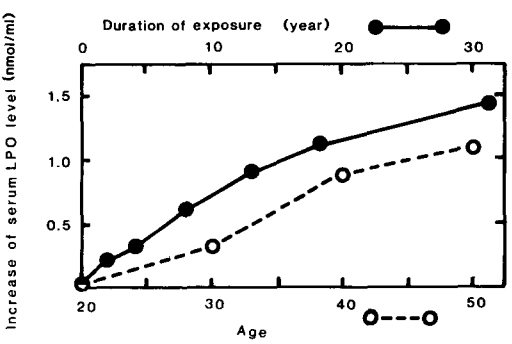

Fig. 1. The increasing ratio of serum LPO level depending on the duration of exposure to lead or aging in manual workers. ●——●: Duration of exposure to lead (years). O---O: Age

Table 2. Comparison of blood component values according to blood-lead concentrations in manual workers

Component		Blood-lead concentration (nmol/ml)							
		0.00–0.24	0.25–0.48	0.49–0.72	0.73–0.97	0.98–1.21	1.22–1.45	1.46–1.93	1.94–3.00
Serum LPO	(nmol/ml)	4.1 (1.2)[a]	4.3 (1.0)	4.3 (1.1)	4.0 (1.1)	4.3 (1.2)	4.2 (1.3)	4.7 (1.7)	7.3 (2.4)
Serum SOD	(unit/ml)	3.9 (1.8)	3.9 (2.3)	4.1 (3.5)	3.6 (1.9)	3.6 (2.0)	3.8 (2.2)	3.7 (1.0)	1.9 (1.2)
Blood SOD	(unit/ml)	603 (79)	571 (88)	559 (108)	567 (114)	547 (105)	574 (91)	521 (99)	504 (145)
Blood ALA-D	(unit)	40.1 (10.5)	36.3 (8.2)	33.3 (7.8)	30.7 (7.2)	25.8 (7.8)	26.9 (8.0)	23.4 (6.7)	16.5 (11.8)
RBC count	($\times 10^4/mm^3$)	468 (37)	465 (39)	470 (38)	477 (38)	480 (37)	483 (34)	474 (27)	485 (41)
Age		36.1 (9.4)	37.0 (9.9)	35.5 (9.3)	36.0 (9.3)	36.4 (8.9)	36.4 (8.9)	38.8 (8.5)	35.6 (12.7)
n		63	170	190	154	73	37	25	3

[a] Mean value (Standard deviation)

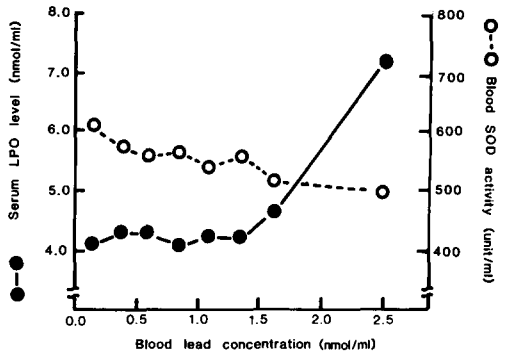

Fig. 2. Relationships between blood-lead concentration and serum LPO level or blood SOD activity in manual workers. ●——●: Serum LPO level (nmol/ml). O---O: Blood SOD activity (unit/ml)

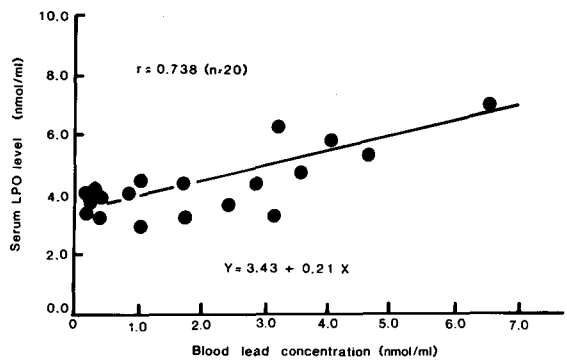

Fig. 3. Correlation between blood-lead concentration and serum LPO level in rats subcutaneously injected with lead acetate

Table 3. Lipid peroxides and superoxide dismutase activity in rats

Subject	Serum LPO (nmol/ml)	Blood		Liver cytosol[b]		
		SOD (unit/ml)	Lead (nmol/ml)	LPO (nmol/ml)	SOD (unit/ml)	Lead (nmol/ml)
Control	4.1 (0.2)[a]	1542 (14)	0.18 (0.05)	3.4	950	0.11
10 mg Pb/kg	4.4 (0.1)	1424 (56)	2.42 (0.39)	3.5	900	0.42
20 mg Pb/kg	6.2 (0.1)	1258 (84)	3.82 (0.29)	6.8	500	0.69

[a] Mean value (Standard deviation)
[b] Liver cytosol fraction produced with 10% liver homogenate in 0.25 *M* sucrose solution

Discussion

It is generally known that serum LPO level in humans increases with physical factors such as aging (Rothstein 1982). Lipid peroxidation in rat liver microsomes is accelerated with heavy metals such as iron, copper and zinc (Wills 1969; Aust and Svingen 1976), and mercury (Stacey and Kappus 1982). LPO is produced by the reaction of various unsaturated fatty acids contained in tissues with activated oxygens such as hydroxy radicals and superoxide anions, etc. (Tappel 1973; Demopoulos 1982). On the other hand, superoxide anions are dismutated to oxygen and hydroperoxide by SOD (McCord and Fridovich

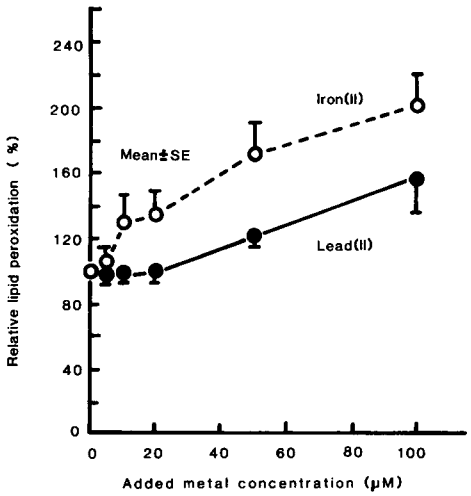

Fig. 4. Effect of lead on lipid peroxidation in vitro assay system using NADPH cytochrome c reductase in rat liver microsomes

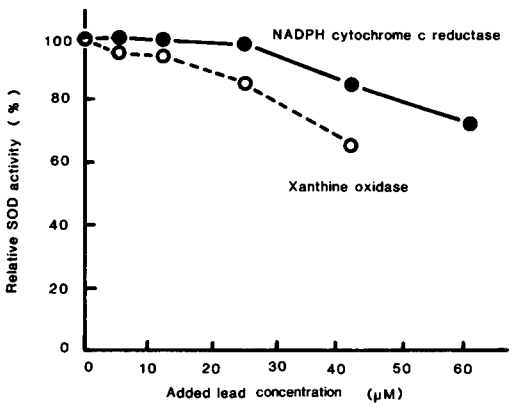

Fig. 5. Effect of lead on SOD activity in vitro assay system using NADPH cytochrome c reductase in rat liver microsomes or xanthine oxidase

1969; Fridovich 1975). Thus, serum LPO level is controlled by oxygen radical-scavenging enzymes such as SOD under healthy medical conditions (Demopoulos 1982; Vladimirov et al. 1980).

In general, aging decreases the biological functions metabolizing the activated oxygens produced in some enzymatic or non-enzymatic reactions in various tissues (Rothstein 1982) and the compounds having the electron-transferring function, such as iron, promote the reaction of activated oxygens with unsaturated fatty acids (Wills 1969; Aust and Svingen 1976).

In this study, we obtained the following results: (1) serum LPO level in manual workers occupationally exposed to lead is higher than that of office workers not exposed to lead, but that of manual workers, from 50- to 59-years old who had been exposed for shorter durations to lead, were not significantly different from that of office workers; (2) The increase of serum LPO level is higher correlatively to the duration of exposure to lead than to aging; (3) Serum LPO level increases significantly in manual workers with blood-lead concentrations higher than 1.45 nmol/ml.

These results show that there are factors, such as lead-exposure, other than aging which effect lipid peroxidation in workers occupationally exposed to lead.

In rats injected with lead, the serum LPO level increased by 7 and 51% at the blood-lead concentrations of 2.4 and 3.8 μM, respectively, while blood SOD activity decreased by 8 and 19%. We also obtained similar results on LPO level and SOD activity in liver cytosol fractions injected with lead.

In addition, lipid peroxidation in non-treated rat liver microsomes in vitro is accelerated by the addition of lead at concentrations higher than 20 μM, and the activity of pure bovine erythrocyte SOD is inhibited by lead in concentrations above 5 μM.

Accordingly, it is suggested that the increase of serum LPO level in lead-exposed workers is connected with the induction of lipid peroxidation in tissues by disruption of the normal biological metabolism of activated oxygens. This might be caused by the inhibition of SOD activity induced by exposure to lead in the manufacturing processes.

The results in this study seem to indicate that the increase of serum LPO level is one of the useful indicators in the evaluation of medical conditions in workers who are occupationally exposed to lead.

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