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Lead Effect on the Oxidation Resistance of Erythrocyte Membrane in Rat Triton-Induced Hyperlipidemia

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ABSTRACT

The anemia observed in severe chronic lead poisoning is in part attributable to alterations in the erythrocyte physicochemical properties. Since they are partly related to the membrane lipid composition, the aim of the present study was to determine the effects of a triton-induced hyperlipidemia on the resistance to oxidation of erythrocyte membranes in lead-treated Wistar rats.

Our results showed that triton administration to lead-treated rats induced an increase in erythrocyte choline phospholipid levels together with a significant decrease in the erythrocyte membrane lipid resistance to oxidation. These results led us to suggest that anemia in lead poisoning is linked to interactions between lead present in the membrane and plasma phospholipids. Their increase in rat hyperlipidemia induced by triton resulted in a decrease in the membrane resistance to oxidation and finally in an erythrocyte fragility leading to their destruction.

Index Entries: Triton-induced hyperlipidemia; lead-treated rat; erythrocyte membrane; lipid peroxidation.

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INTRODUCTION

Anemia is frequently observed in lead poisoning (*1–3*). It is probably related to the inhibition of the hemoglobin synthesis and to various alterations of the erythrocyte membrane biological properties, leading to red blood cell increased fragility, and finally to an erythrocyte destruction in capillaries and spleen (*4–6*). In agreement with this concept, alterations of erythrocyte membrane enzyme activities and salt exchanges have been reported in lead-intoxicated workers (*7,8*).

However, the decrease in erythrocyte filterability observed in vitamin-E-deficient rats (*9*) and its enhancement by lead poisoning suggest that anemia in lead poisoning could also be the result of an alteration of the erythrocyte resistance to oxidation (*10*).

In erythrocyte membranes, as in other biological samples, the resistance to oxidation is determined by the balance between the polyunsaturated fatty acid lipid content (PUFA index) and the antioxidants (*11,12*). Since the plasma lipid concentration influences the lipid composition of the erythrocyte membrane, triton-induced hyperlipidemia will alter the erythrocyte membrane resistance to oxidation. The present experiments were conducted in order to assess the lead-induced alterations of rat erythrocyte lipid composition and of oxidation resistance in posttriton hyperlipidemia.

MATERIAL AND METHODS

Chemicals and Reagents

Triton WR 1339 (Tyloxapol) and 1,1,3,3, tetra ethoxy propane were purchased from Sigma Chemical Co. (St. Louis, MO), and Phenylhydrazine and Thiobarbituric acid (TBA) from Fluka Chemie (Buchs, Switzerland). Acetic acid, metaphosphoric acid, and methanol were purchased from Carlo Erba (Turin, Italy). Dipotassium phosphate, citric acid, and sodium hydroxide were from Prolabo (Paris, France). Diagnostic kits for the assay of cholesterol, phospholipids, and triglycerides were purchased from Biomérieux (Charbonnières les Bains, France).

Animals

Twelve Wistar rats were exposed to lead *in utero* and up to 4 mo after birth through free access to 0.4% lead acetate as drinking water. Another group of 12 Wistar rats were similarly treated with 0.4% sodium acetate. They were housed in a room of controlled lighting (10:00 AM–10:00 PM) at constant temperature (21 ± 1°C) and were fed *ad libitum* with a standard diet from Iffa Credo (St Germain/l'Arbresles, France).

After a fasting period of 18 h and under a light ether anesthesia, six animals from each group were given iv 0.5 mL of saline. They represent control (C) or lead (L) groups. The six other animals of each group were given iv 0.5 mL of a 20% triton WR 1339 solution in saline, and represent the triton control group (T) or the triton lead-treated group (TL).

They were killed 6 h later under a slight ether anesthesia. The blood was drawn by aortic puncture and collected into heparinized tubes. The plasma was obtained by immediate centrifugation at 2000 × *g* for 10 min and kept at + 4°C until assayed.

Erythrocyte Membrane Preparation

One hundred microliters of the erythrocyte pellet were lysed by adding 90 vol of cold water. The erythrocyte ghosts were then precipitated by addition of 1 mL of cold 0.8 m*M* citric acid to the hemolysate. After centrifugation (30 min, 2000 x *g*), the supernatants were removed and the pellet of erythrocyte ghosts was solubilized in 1 mL of 0.015*N* NaOH in saline. This preparation was suitable for lipid and oxidation resistance assays.

Assays

Trace Element Analysis

Blood lead levels were determined by atomic absorption spectrophotometry (Perkin Elmer atomic spectrometer, model 2380), on samples collected in EDTA lead-free vacutainers.

Plasma Lipids

Cholesterol (TC), phospholipids (PL), and triglycerides (TG) were determined by enzymatic and colorimetric procedures (*13–15*) according to the instructions of the diagnostic kits in plasma and erythrocyte membrane preparation samples.

Fatty Acid Analysis

Total lipids were extracted by the Folch procedure (*16*). The lipid extract dried under a stream of N_2 was transmethylated by heating for 2 h at 80°C, in the presence of methanol and HCl (*17*). The methyl esters of long chain fatty acids were then extracted by heptane, and vigorously shaken, sampled, and dried under N_2. The dried material was chloroform extracted and analyzed by gas chromatography (Becker-Packard) on CP Sil 88 column using an isothermal column temperature of 195°C. The injector and detector temperatures were 240 and 250°C, respectively. Retention times, peak areas, and peak relative areas were determined using a Hewlett-Packard integrator. Identification of fatty acid methyl esters was performed by comparing relative retention times with that of authentic standards.

Plasma and Erythrocyte Membranes Oxidation Resistance

TBA Reactive Substances (TBARS) and oxidation resistance were determined by a modification of a previously reported procedure (*18*): on 200 μL of plasma samples, 1/10 diluted in NaOH 0.015*N* (C and L groups) (nevertheless, the plasma samples from triton-treated rats [TC and TL groups] were 1/100 diluted) and on 200 μL of the membrane preparations.

In Vitro Oxidation Step

Lipid oxidation was achieved by adding 10 μL of phenylhydrazine (0.3 m*M* in 35% methanol) to the above preparations. The mixtures were vigorously shaken and then incubated for 45 min at 37°C.

TBARS Assay

The TBARS formed during the previous step were assayed by addition of 1 mL of 1% TBA in 1% acetic acid (pH 3.5) and heating for 45 min at 95°C. After cooling, samples were centrifuged at 3000 rpm, for 10 min at 20°C, and the pink coloration of the supernatants was measured at 532 nm. Since any effect of the Triton WR 1339 was observed on the spectral characteristics and the intensity of the coloration, the amount of TBARS was derived from a curve using malondialdehyde (obtained by the hydrolysis of 1,1,3,3, tetra ethoxy propane) as standard. The lipid oxidation resistance of the sample was inversely related to the difference between TBARS levels in plasma incubated with and without phenylhydrazine.

Statistical Analysis

Results were expressed as mean ± SEM of six determinations. Statistical significance was calculated by a one-way analysis of variance. The difference between the different groups was calculated using the multiple comparisons Scheffe's F-test.

RESULTS

Body Weight Gain and Lead Blood Levels

Lead intoxication in rats resulted in a significant decrease ($p < 0.01$) of the body wt (280 ± 55 vs 460 ± 20 g in controls) and in a very large increase of the lead blood levels (1.040 ± 0.140 vs 0.075 ± 0.029 mg/L in controls).

Erythrocyte Membranes

Lipid Composition

Triglycerides were absent in all the preparations assayed. In lead-treated rats (Table 1), we observed an increase in the cholesterol (ns) and

Table 1
Cholesterol (C), Phospholipids (PL), and Triglycerides (TG) in mmol/L and C/PL Molar Ratio in Erythrocyte Membranes from Controls and Lead-Treated Rats Before (C and L groups) and Six Hours After Triton Injection (TC and TL Groups)[a]

Groups	C	L	TC	TL
CH	2.55 ± 0.10	2.90 ± 0.05 ns	2.40 ± 0.15 ns	3.50 ± 0.15°*a*
PL	1.65 ± 0.05	2.15 ± 0.05**	1.65 ± 0.10 ns	2.60 ± 0.10°*b*
TG	0	0	0	0
C/PL	1.55 ± 0.05	1.36 ± 0.01**	1.47 ± 0.01 ns	1.33 ± 0.02°*NS*

[a] Values are mean ± SEM (n = 6 for each group): vs C group: ns = not significant, ** = $p < 0.01$: vs TC group: ° = $p < 0.01$: vs L group: *NS* = not significant, *a* = $p < 0.05$, *b* = $p < 0.01$.

in the choline lipid content ($p < 0.01$), but the cholesterol/choline lipid molar ratio was significantly reduced (−12%).

After Triton injection, the membrane cholesterol and choline lipid levels increased significantly again, whereas they were not affected in control rats. The cholesterol/choline lipid molar ratio remained significantly reduced and lower than in controls.

Fatty Acid Composition

It was slightly changed after lead treatment. An increase by 27% of the polyunsaturation (PUFA) index was observed (Table 2). It was accentuated 6 h after Triton administration by an enrichment of the erythrocyte membrane lipids in ω-6 fatty acid.

Oxidation Resistance

It was not significantly modified in lead-treated as compared to control rats (Fig. 1). However 6 h after Triton administration, the oxidation resistance of the erythrocyte membranes from control or lead-treated rats was strongly reduced. The decrease was more important in membranes from lead-treated rats, since the TBARS-induced formation was higher (+53%) than in preparations from control rats.

Plasma

Lipid Composition

Cholesterol, choline lipid, and triglyceride plasma levels were not significantly modified by lead intoxication (Table 3). They were similarly increased in control and lead-treated rats 6 h after the Triton injection. The characteristics of the induced hyperlipidemia were unmodified by lead treatment, since the cholesterol/phospholipid, cholesterol/triglycerides, and phospholipid/triglycerides ratios were similar.

Oxidation Resistance

The TBARS levels in the plasma from control as well as lead-treated rats were initially undetectable and remained undectectable after in vitro

Table 2
Fatty Acid Composition of Erythrocyte Lipid Membranes (in Percent) from Control and Lead-Treated Rats Without Triton and Six Hours After Triton Administration

	C	L	TC	TL
C14:0	0.50 ± 0.20	0.40 ± 0.25	0.60 ± 0.32	0.37 ± 0.08
C16:0	32.25 ± 4.45	27.85 ± 5.70	30.65 ± 3.40	26.50 ± 2.50
C16:1	0.80 ± 0.15	0.52 ± 0.24	0.90 ± 0.30	0.52 ± 0.11
C18:0	7.80 ± 2.70	18.70 ± 2.85	16.35 ± 2.35	17.35 ± 2.65
C18:1	11.70 ± 0.70	9.62 ± 1.42	11.60 ± 1.35	10.50 ± 0.30
C18:2 (ω-6)	9.37 ± 1.35	8.24 ± 1.22	9.90 ± 0.75	9.05 ± 1.20
C20:0	0.30 ± 0.06	0.37 ± 0.20	0.25 ± 0.07	0.18 ± 0.04
C20:4 (ω-6)	13.75 ± 3.70	19.55 ± 5.32	15.40 ± 2.50	20.60 ± 1.90
C20:5 (ω-3)	0.53 ± 0.15	0.65 ± 0.20	0.45 ± 0.15	0.60 ± 0.07
C22:0	1.03 ± 0.35	0.95 ± 0.31	0.90 ± 0.20	0.75 ± 0.13
C22:4 (ω-6)	1.13 ± 0.20	1.20 ± 0.40	1.20 ± 0.30	1.52 ± 0.32
C22:5 (ω-3)	1.02 ± 0.30	1.43 ± 0.50	1.50 ± 0.65	1.75 ± 0.27
C24:0	2.90 ± 0.60	2.80 ± 0.35	3.60 ± 0.35	2.95 ± 0.30
C22:6 (ω-3)	3.40 ± 1.40	5.95 ± 1.30	3.60 ± 1.05	5.60 ± 0.60
C24:1 (ω-9)	2.10 ± 0.65	2.10 ± 0.75	2.70 ± 0.55	1.50 ± 0.36

oxidation (Table 4). Six hours after Triton administration, they were still undetectable but the lipid resistance to in vitro oxidation was reduced in the two groups as reflected by a similar increase in TBARS-induced formation.

DISCUSSION

The chronic lead treatment used in the present study resulted in blood lead levels in treated animals largely over the upper limit of safety. These values are well known to induce, beyond biological alterations, severe clinical damage, such as anemia, renal tubule dysfunction, and neurological effects.

The observed increase induced by lead of the erythrocyte membrane cholesterol and choline phospholipids content agrees with other reports (*19,20*). It is owing neither to a plasma lipoprotein adsorption on nor to a coprecipitation with erythrocyte membrane, since triglycerides were totally absent from our samples.

However, in our lead-treated rats, a relative enrichment in plasma choline lipids of erythrocyte membranes is observed as suggested by the C/PL decrease. It is not related to the LCAT inhibition reported in lead workers, since in these subjects, the erythrocyte membranes C/PL was increased. In agreement with this interpretation, we observed a slight diminution of C/PL in triton-treated rats, whereas LCAT and LPL were totally inhibited (*21,22*). Then we could assume that the net decrease by lead treatment of the C/PL is more related to the interactions between plasma phospholipids and lead-enriched membrane than to LCAT inhibition (*23*).

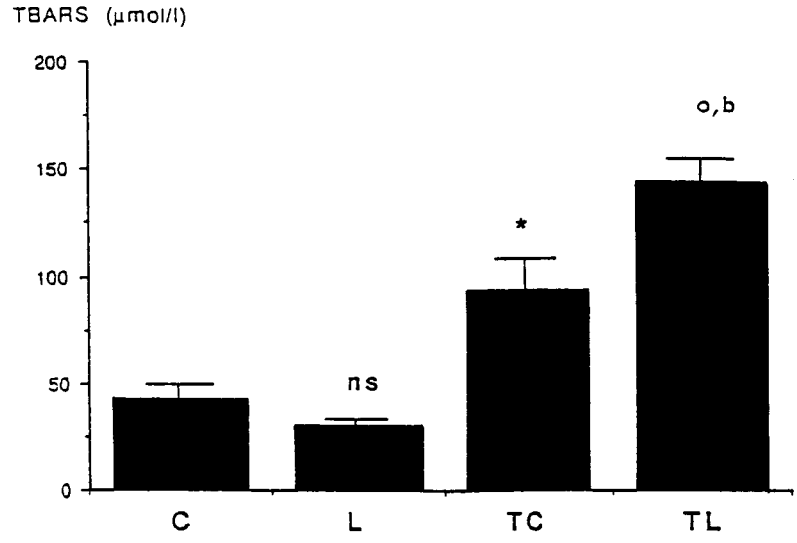

Fig. 1. TBARS formed (µmol/L) after an in vitro oxidative stress in erythrocyte membrane from control and lead-treated rats before (C and L groups) and 6 h after triton injection (TC and TL groups). Values are mean ± SEM (n = 6 for each group); vs C group: ns = not significant,* = $p < 0.05$, vs TC group: ° = $p < 0.05$, vs L group: b = $p < 0.01$.

Table 3
Plasma Cholesterol (C), Phospholipids (PL), and Triglycerides (TG) (in mmol/L) C/PL and C/TG in Control and Lead-Treated Rats Before (C and L Groups) and Six Hours After Triton Injection (TC and TL Groups)[a]

	C	L	TC	TL
CH	1.90 ± 0.10	1.92 ± 0.05 ns	5.30 ± 0.25**	5.47 ± 0.40 *NS***
PL	1.45 ± 0.05	1.45 ± 0.12 ns	5.85 ± 0.57**	6.60 ± 0.70 *NS***
TG	0.87 ± 0.17	1.17 ± 0.12 ns	28.15 ± 2.40**	31.55 ± 4.10 *NS***
C/PL	1.32 ± 0.08	1.35 ± 0.11 ns	0.93 ± 0.06**	0.85 ± 0.05 *NS***
C/TG	2.55 ± 0.45	1.72 ± 0.17 ns	0.20 ± 0.01**	0.18 ± 0.02 *NS***

[a] vs C group: ns = not significant, ** = $p < 0.01$: vs TC group: ° = $p < 0.01$: vs L group: *NS* = not significant, a = $p < 0.05$, b = $p < 0.01$.

Table 4
TBARS Formed (µmol/L) after an In Vitro Oxidative Stress in Plasma from Control and Lead-Treated Rats Before (C and L Groups) and Six Hours After Triton Injection (TC and TL Groups)[a]

Groups	C	L	TC	TL
TBARS	0	0	2650 ± 335**	2905 ± 385 *NS***

[a] Values are mean ± SEM (n = 6 for each group); vs C group:** = $p < 0.01$: vs L group: *NS* = not significant.

Finally, all these results shed light on enrichment in choline lipids and then on the alteration of the antioxidant to polyunsaturated lipid balance that determines oxidation resistance of erythrocyte membranes. Our results are consistent with this interpretation, since we observed in triton-administered rats a decrease of the erythrocyte membrane oxidation resistance. This decrease and the phospholipid enrichment were more marked in the lead-treated rat groups than in controls, although hyperlipidemia was the same in both groups.

We can therefore conclude that there is an impairment of the erythrocyte oxidation resistance by lead-induced membrane enrichment in choline phospholipids. Its possible accentuation in physiological hyperlipidemia during postabsorptive periods could explain erythrocyte fragility, and destruction in the capillaries and in the spleen of lead workers.

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