Arch Toxicol (2002) 76: 437–441
DOI 10.1007/s00204-002-0350-x

INORGANIC COMPOUNDS

Rajagopalan Sivaprasad · Manickam Nagaraj
Palaninathan Varalakshmi

Lipoic acid in combination with a chelator ameliorates lead-induced peroxidative damages in rat kidney

Received: 10 January 2002 / Accepted: 27 March 2002 / Published online: 4 July 2002

Abstract The deleterious effect of lead has been attributed to lead-induced oxidative stress with the consequence of lipid peroxidation. The present study was designed to investigate the combined effect of DL-α-lipoic acid (LA) and *meso*-2,3-dimercaptosuccinic acid (DMSA) on lead-induced peroxidative damages in rat kidney. The increase in peroxidated lipids in lead-poisoned rats was accompanied by alterations in antioxidant defence systems. Lead acetate (Pb, 0.2%) was administered in drinking water for 5 weeks to induce lead toxicity. LA (25 mg/kg body weight per day i.p) and DMSA (20 mg/kg body weight per day i.p) were administered individually and also in combination during the sixth week. Nephrotoxic damage was evident from decreases in the activities of γ-glutamyl transferase and *N*-acetyl β-D-glucosaminidase, which were reversed upon combined treatment with LA and DMSA. Rats subjected to lead intoxication showed a decline in the thiol capacity of the cell, accompanied by high malondialdehyde levels along with lowered activities of catalase, superoxide dismutase, glutathione peroxidase and glutathione metabolizing enzymes (glutathione reductase, glucose-6-phosphate dehydrogenase, glutathione-*S*-transferase). Supplementation with LA as a sole agent showed considerable changes over oxidative stress parameters. The study has highlighted the combined effect of both drugs as being more effective in reversing oxidative damage by bringing about an improvement in the reductive status of the cell.

Keywords Lead acetate · DL-α-Lipoic acid, *Meso*-2,3-dimercaptosuccinic acid · Lipid peroxidation · Antioxidants

R. Sivaprasad · M. Nagaraj · P. Varalakshmi (✉)
Department of Medical Biochemistry,
Dr. A.L. Mudaliar Post Graduate Institute
of Basic Medical Sciences, University of Madras (Taramani),
Chennai 600 113, India
E-mail: drvlakshmi@yahoo.com
Tel.: +91-44-4925548
Fax: +91-44-4926709 or +91-44-4916316

Introduction

Occupational and environmental exposure to lead has been associated with significant impairment in the renal function (Payton et al. 1994). Several researchers have reported nephrotoxic effects in animals fed various doses of lead (Vyskocil et al. 1989), and studies conducted in humans (Bennet 1985) and children (Hu 1991) have indicated that high levels of lead exposure may impair renal function. The renal toxicity of lead is closely related to its selective accumulation in the kidneys (Choie and Richter 1980). Some of the deleterious effects of lead have been attributed to induction of oxidative stress (Soliway et al. 1996), and lead is known to disrupt the prooxidant/antioxidant balance of tissues that leads to biochemical and physiological dysfunction (Neal et al. 1998). Peroxidation of lipids, implicated in a wide variety of physiological events, is a complex process that occurs during innumerable pathological conditions (Kornbrust and Mavis 1979). Peroxide formation in vivo may produce serious consequences in the tissues such as oxidative destruction of thiol groups of amino acids and proteins (Lewis and Wills 1962). Lipids, particularly unsaturated fatty acids, are damaged directly by free radical oxidation (Mill 1980). Lead-induced enhancement of lipid peroxidation has been suggested as a possible mechanism for some of its toxic effects (Villeda-Hernández et al. 2001). Peroxidation of lipids due to elevated production of reactive oxygen species (ROS) has been implicated in many cases of lead poisoning both in vitro (Ribarov and Bochev 1982) and in vivo (Sandhir et al. 1994). Evidence for the involvement of free radicals in lead poisoning has also been reported (Jendryczko 1998).

Peroxidative damage induced in the cell are encountered by the elaborate defence mechanisms including enzymic and non-enzymic antioxidants (Jansen et al. 1993).

Lipoic acid (LA), a potent antioxidant (Kagan et al. 1990), has been identified as an effective antidote in mitigating heavy metal-induced toxicities (Sumathi et al. 1994; Anuradha and Varalakshmi 1999; Gurer et al.

1999) and *meso*-2,3 dimercaptosuccinic acid (DMSA) has been identified as a potentially useful drug for the treatment of lead poisoning (Friedheim et al. 1976). The antioxidant potentials of LA and DMSA prompted us to study the combined effects of both the drugs in lead-intoxicated conditions.

The present study was designed with the objective of investigating the combined effect of LA and DMSA on lead-induced lipid peroxidative damage and on the reduced antioxidant status of the cell.

Materials and methods

Chemicals

DL-α-Lipoic acid and *meso*-2,3-dimercaptosuccinic acid were procured from Sigma Chemicals (St. Louis, Mo., USA). DMSA was prepared immediately before use to a concentration of 57 mM in 5% w/v $NaHCO_3$. All other chemicals were of analytical grade, procured from local commercial sources.

Animal model

Male albino rats (Wistar strain, 10–12 weeks old), weighing 120 ± 20 g, were used throughout the study. The rats were fed with a standard rat pelleted diet (M/s. Hindustan Lever Ltd., India) and drinking water was available ad libitum. The rats were maintained in well-ventilated animal quarters with 12-h light and 12-h dark exposure.

Experimental conditions

The animals were assigned into eight groups of six animals each. Group I served as control receiving saline during week 6 only (0.5 ml/day per rat i.p.). Group II received lead acetate (Pb 0.2%) in drinking water for 5 weeks and distilled water during week 6. Group III received LA (25 mg/kg body weight per day per rat i.p.) during week 6 only. Group IV received DMSA (20 mg/kg body weight per day per rat i.p.) during week 6 only. Group V received LA plus DMSA at the same dose levels during week 6 only. Group VI received Pb (0.2%) in drinking water for 5 weeks and LA during week 6. Group VII received Pb (0.2%) in drinking water for 5 weeks and DMSA during week 6. Group VIII received Pb for 5 weeks; LA plus DMSA was administered during week 6.

After week 6, the animals were killed by cervical decapitation and the kidneys were excised immediately and washed in ice-cold saline. The tissues (whole kidney) were then homogenized in ice-cold Tris-HCl buffer (0.1 M, pH 7.4) to give a 10% homogenate.

Analyses

Lipid peroxidation (LPO) was assayed by the method of Devasagayam (1986), in which the malondialdehyde (MDA) released served as the index of LPO. The activity of catalase (CAT) was assayed by the method of Sinha (1972). In this method, dichromate in acetic acid was reduced to chromic acetate when heated in the presence of hydrogen peroxide (H_2O_2), with the formation of perchloric acid as an unstable intermediate. The chromic acetate thus produced was measured colourimetrically at 610 nm.

Superoxide dismutase (SOD) was assayed according to the method of Marklund and Marklund (1974). The unit of enzyme activity is defined as the enzyme required to give 50% inhibition of pyrogallol auto-oxidation. Glutathione peroxidase (GPx) was assayed by the method of Rotruck et al. (1973), which is based on the reaction between glutathione remaining after the action of GPx and 5,5'-dithio-*bis*(2-nitro benzoic acid) to form a complex that absorbs maximally at 412 nm. Total glutathione (GSH) was determined by the method of Moron et al. (1979).

Glutathione reductase (GR), which utilizes NADPH to convert oxidized glutathione (GSSG) to the reduced form, was assayed by the method of Staal et al. (1969). The estimation of glucose 6-phosphate dehydrogenase (G6PD) was assayed by the method of Beutler (1983), whereby the increase in absorbance was measured when the reaction was started by the addition of glucose-6-phosphate. Glutathione-*S*-transferase (GST) was assayed by the method of Habig et al. (1973).

γ-Glutamyl transferase (γ-GT) was estimated according to the method of Orlowski and Meister (1965), and *N*-acetyl-β-D-glucosaminidase (NAG) was assayed by the method of Marhun (1976).

Protein content in the rat renal tissue was determined by the method of Lowry et al. (1951).

Statistical analysis

The values are expressed as means ± SD. The results were computed statistically (SPSS software package) using one-way Analysis of Variance. Post hoc testing was performed for inter-group comparisons using the Least Significance Difference (LSD) test; A *P*-value < 0.05 was considered significant.

Results

Lead administration induced extensive damage to the proximal tubule. As a result there was a decline in the activities of brush border enzyme γ-GT ($P < 0.05$) and lysosomal enzyme NAG ($P < 0.05$) (Table 1). Among the treatment groups, LA in combination with DMSA restored the activities of kidney marker enzymes close to control values, indicating nephroprotection.

Table 2 shows the effects of LA and DMSA on lead-induced LPO and antioxidant status. Significant ($P < 0.05$) increase in lipid peroxides with a concomitant falls in CAT, SOD and GPx activities are notable manifestations of lead poisoning. Co-administration of LA plus DMSA (Group VIII) had an additive effect in reducing MDA formation by significantly increasing CAT, SOD and GPx activities, whereas LA alone (Group VI) and DMSA alone (Group VII) showed a moderate effect over LPO and enzymic antioxidants.

GSH was markedly depleted in the kidney of lead-intoxicated rats as shown in Table 3. Evaluation of the GSH metabolizing enzymes namely GR, G6PD and GST also showed depressed activities in the kidneys of lead-poisoned rats. Administration of DMSA showed only a marginal increase in the activities of GSH and GSH metabolizing enzymes when compared with combined treatment with LA and DMSA (Group VIII).

The combined effect of LA along with DMSA paved the way for an improved reductive status of the cell by significantly ($P < 0.05$) increasing the GSH levels and the activities of GR, G6PD and GST in the rat kidney (Table 3).

Discussion

Lead, a ubiquitous metal, is known to have toxic effects on several biological systems with the kidneys as one of

Table 1. Effects of lipoic acid (*LA*), *meso*-2,3-dimercaptosuccinic acid (*DMSA*) and lead (*Pb*) on the activities of kidney enzymes, Values represent the mean ±SD for six rats. *NAG N*-acetyl-*β*-D-glucosaminidase activity (as nmoles of *p*-nitrophenol released per min at 37°C), *γ-GT* γ-glutamyl transferase activity (as nmoles of *p*-nitroaniline released per min at 37°C). Enzyme activities are expressed in terms of units/mg protein

Parameter	Group I (Control)	Group II (Pb)	Group III (LA)	Group IV (DMSA)	Group V (LA + DMSA)	Group VI (Pb + LA)	Group VII (Pb + DMSA)	Group VIII (Pb + LA + DMSA)
NAG	0.45 ± 0.04	0.21 ± 0.02*	0.47 ± 0.04	0.46 ± 0.03	0.46 ± 0.04	0.38 ± 0.03†	0.32 ± 0.04†	0.43 ± 0.03†,‡,§
γ-GT	1.54 ± 0.15	0.73 ± 0.09*	1.57 ± 0.14	1.59 ± 0.13	1.55 ± 0.14	1.35 ± 0.16†	1.33 ± 0.11†	1.53 ± 0.11†,‡,§

*$P < 0.05$, for comparisons between groups I and II
$^{\dagger}P < 0.05$, for comparisons of group II with groups VI, VII, VIII
$^{\ddagger}P < 0.05$, for comparisons of group VI with group VIII
$^{\S}P < 0.05$, for comparisons of group VII with group VIII

Table 2. Effects of lipoic acid (*LA*) and *meso*-2,3-dimercaptosuccinic acid (*DMSA*) on lead (*Pb*) -induced lipid peroxidation and antioxidant status of rat kidney. Values represent the mean ±SD for six rats, *LPO* lipid peroxidation (as nmoles of malodialdehyde released per mg protein per incubation period), *CAT* catalase activity (as μmoles of H_2O_2 consumed per min per mg protein), *SOD* superoxide dismutase activity (as units per min, where one unit is equal to the amount of enzyme required to inhibit auto-oxidation of pyrogallol by 50%); *GPx* glutathione peroxidase activity (as μg of reduced glutathione utilized per min per mg protein)

Parameter	Group I (Control)	Group II (Pb)	Group III (LA)	Group IV (DMSA)	Group V (LA + DMSA)	Group VI (Pb + LA)	Group VII (Pb + DMSA)	Group VIII (Pb + LA + DMSA)
LPO	1.27 ± 0.11	1.89 ± 0.19*	1.30 ± 0.12	1.29 ± 0.13	1.29 ± 0.09	1.46 ± 0.12†	1.48 ± 0.14†	1.30 ± 0.10†,‡,§
CAT	121.10 ± 10.89	75.57 ± 8.31*	123.25 ± 13.55	123.96 ± 9.92	122.30 ± 9.78	112.17 ± 11.65†	110.89 ± 10.67†	125.55 ± 8.78†,‡,§
SOD	4.58 ± 0.46	2.97 ± 0.29*	4.63 ± 0.42	4.60 ± 0.51	4.61 ± 0.37	4.00 ± 0.36†	3.87 ± 0.46†	4.60 ± 0.32†,‡,§
GPx	4.12 ± 0.37	2.66 ± 0.21*	4.10 ± 0.45	4.14 ± 0.49	4.15 ± 0.37	3.63 ± 0.29†	3.49 ± 0.31†	4.09 ± 0.32†,‡,§

*$P < 0.05$, for comparisons between groups I and II
$^{\dagger}P < 0.05$, for comparisons of group II with groups VI, VII, VIII
$^{\ddagger}P < 0.05$, for comparisons of group VI with group VIII
$^{\S}P < 0.05$, for comparisons of group VII with group VIII

Table 3. Effects of lipoic acid (*LA*), *meso*-2,3-dimercaptosuccinic acid (*DMSA*) and lead (*Pb*) on glutathione and glutathione metabolizing enzymes. Values represent the mean ±SD for six rats. *GSH* glutathione activity (as μg per min per mg protein), *GR* glutathione reductase activity (as nmoles NADPH oxidized per min per mg protein), *G6PD* glucose 6-phosphate dehydrogenase activity (as nmoles NADPH formed per min per mg protein), *GST* glutathione-*S*-transferase activity (as nmoles 1-chloro-2,4-dinitrobezene–GSH conjugate formed per min per mg protein)

Parameter	Group I (Control)	Group II (Pb)	Group III (LA)	Group IV (DMSA)	Group V (LA + DMSA)	Group VI (Pb + LA)	Group VII (Pb + DMSA)	Group VIII (Pb + LA + DMSA)
GSH	2.46 ± 0.25	1.57 ± 0.14*	2.44 ± 0.26	2.49 ± 0.19	2.47 ± 0.19	1.88 ± 0.13†	1.79 ± 0.14†	2.41 ± 0.23†,‡,§
GR	0.25 ± 0.02	0.10 ± 0.01*	0.24 ± 0.02	0.25 ± 0.03	0.27 ± 0.03	0.22 ± 0.02†	0.20 ± 0.02†	0.28 ± 0.03†,‡,§
G6PD	1.75 ± 0.15	1.24 ± 0.12*	1.76 ± 0.15	1.74 ± 0.19	1.78 ± 0.16	1.46 ± 0.13†	1.47 ± 0.14†	1.70 ± 0.16†,‡,§
GST	0.75 ± 0.06	0.54 ± 0.04*	0.75 ± 0.05	0.73 ± 0.06	0.74 ± 0.07	0.63 ± 0.08†	0.60 ± 0.03†	0.72 ± 0.06†,‡,§

*$P < 0.05$, for comparisons between groups I and II
$^{\dagger}P < 0.05$, for comparisons of group II with groups VI, VII, VIII
$^{\ddagger}P < 0.05$, for comparisons of group VI with group VIII
$^{\S}P < 0.05$, for comparisons of group VII with group VIII

its main targets. Quantifying the excretion of the lysosomal enzyme NAG may provide a sensitive and specific index of renal damage. Apostolov et al. (1976) apparently reported the damage of the lysosomal membrane during lead poisoning. The decrease in NAG activity in the tissue could be a result of lead-induced damage to the lysosomal membrane and a possible leakage of the enzyme leading to its excretion in the urine.

Renal γ-GT is an extrinsic brush border membrane protein. The decrease in γ-GT activity might be due to increased tubular lesion since the brush borders of proximal tubule remain the toxic target of lead. A similar decrease in the activity of the brush border enzyme γ-GT has been reported following oral exposure to lead (Teichert-Kuliszewska and Nicholls 1985).

Lead-induced oxidative stress has been referred to as a possible contributor to the pathogenesis of lead poisoning. The elevated levels of lipid peroxides, resulting from an increase in ROS, have been implicated in various cases of lead poisoning (Yiin and Lin 1995). Peroxidation of membrane phospholipids eventually leads to loss of membrane integrity and finally to cell death. The increase in lipid peroxides in the lead-intoxicated group might result from increased production of free radicals and a decrease in antioxidant status.

Analogous to the lead-induced oxidative stress observed in our study, the transition metal has been shown to be implicated in tissue LPO activity, as evidenced by many reports (Sandhir et al. 1994; Villeda Hernández et al. 2001). The combined effect of both the drugs, LA and DMSA, significantly reversed the effects of lead on oxidative parameters, suggesting ROS as a possible contributor to the damage that occurred. Several studies have shown that both LA (Sumathi et al. 1994) and DMSA (Neal et al. 1998) chelate transition metals, and therefore it is plausible to assume that they inhibit LPO that is triggered by the production of ROS.

GSH is an important oxidant defence, which exhibits its antioxidant effect by reacting with superoxide, hydroxyl radical and singlet oxygen following the formation of GSSG and other disulphides. It provides the cells with their reducing milieu and deficiency of GSH results in tissue damage. Increased oxidative stress increases the formation and efflux of GSSG (Eklow et al. 1984).

As the first enzyme of the pentose phosphate pathway, G6PD supplies cells with most of the extra mitochondrial NADPH through the oxidation of glucose-6-phosphate to 6-phosphogluconate. This NADPH maintains GSH at a constant level by providing reducing equivalents for GR, which mediates reduction of GSSG to GSH. G6PD is known to contain many SH-groups, which play a crucial role in maintaining its tertiary structure (Yoshida and Huang 1986). In some in vitro studies, G6PD was reported to be inhibited by lead (Lachant et al. 1984). Therefore, a decrease in NADPH production due to G6PD inhibition, and hence a decrease in GSH, may be responsible for the impaired functioning of GPx in the tissues of lead-treated rats.

NADPH serves to prevent and partially reverse the inactivation of CAT by its toxic substrate, H_2O_2 (Nicholls and Schonbaum 1963). Hence, the observed decrease in G6PD activity might have been the ultimate cause for the inactivation of CAT recorded in our study.

Detoxification of electrophilic species is mediated via a spontaneous enzyme catalysed conjugation reaction by GST. Inhibition of GST observed in the present study may partly be due to lack of substrate (GSH) and also because of inhibition of their protein structures.

SOD constitutes an important link in the biological defence mechanism through disposition of endogenous cytotoxic superoxide radicals that are deleterious to polyunsaturated fatty acids (PUFA) and structural proteins of plasma membrane (Fridovich 1975). The inhibition of CAT and GPx results in excessive accumulation of H_2O_2 in the mitochondria and peroxisomes/cystosol or both. The excessive accumulation of H_2O_2 might subsequently decrease the activity of SOD as in the case of lead intoxication. Minami et al. (1982) showed decreases of 15–40% in the activity of SOD in erythrocytes of rats exposed to fumes of heavy metals such as lead, cadmium and antimony.

H_2O_2 formed by SOD and flavin-linked oxidases is acted upon by CAT. Rister and Bachner (1976) speculated that during oxidative stress CAT activity decreases, H_2O_2 accumulates and, thereby, more peroxidation of lipids is favoured. This could be the mechanism for decreased CAT activity and increased LPO in lead-intoxicated rats.

The ''universal antioxidant'' LA and its reduced form dihydrolipoic acid (DHLA) have the ability to scavenge ROS. They regenerate other antioxidants (i.e. vitamin E, vitamin C and GSH) from their radical or inactive forms and also have metal chelating activity (Devasagayam et al. 1993; Müller and Menzel 1990). On the other hand, the dithiol DMSA has specific affinity for lead as a chelator with antioxidative properties (Gurer and Ercal 2000).

In the event that oxidative stress can be partially implicated in lead toxicity, a therapeutic strategy to increase the antioxidant capacity of the cells may benefit the long-term effective treatment of lead poisoning. Both LA and DMSA, when administered individually, resulted in significant improvement of the oxidative stress parameters, but the combined treatment elicits an additive response in restoring altered lead-sensitive biochemical variables. The therapeutic efficacy of both the drugs in mitigating lead-induced toxic damage has thus been highlighted.

References

Anuradha B, Varalakshmi P (1999) Protective role of DL-α-lipoic acid against mercury-induced neural lipid peroxidation. Pharmacol Res 39:67–80

Apostolov I, Zaprianov Z, Gylybova V (1976) Lysosomal enzyme activity in the serum of rats with experimental lead poisoning. Biull Eksp Biol Med 82:1070–1071

Bennet WM (1985) Lead nephropathy. Kidney Int 28:212–220

Beutler E (1983) Active transport of glutathione disulfide from erythrocytes. In: Larson A, Orrenius S, Holmgren A, Mannerwik B (eds) Functions of glutathione – biochemical, physiological, toxicological and clinical aspects. Raven Press, New York, pp 65

Choie DD, Richter GW (1980) Effects of lead on the kidney. In: Singhal RL, Thomas JA (eds) Lead toxicity. Urban & Schwarzenberg, Baltimore, pp 187–211

Devasagayam TPA (1986) Lipid peroxidation in rat uterus. Biochim Biophys Acta 876:507–514

Devasagayam TPA, Subramanian M, Pradhan DS, Sies H (1993) Prevention of singlet oxygen-induced DNA damage by lipoate. Chem Biol Interact 86:79–92

Eklow L, Moldeus P, Orrenius S (1984) Oxidation of glutathione during hydroperoxide metabolism. A study using isolated hepatocytes and the glutathione reductase inhibitor 1,3-*bis*(2-chloroethyl)-1-nitrosourea. Eur J Biochem 135:151–157

Fridovich I (1975) Superoxide dismutases. Annu Rev Biochem 44:147–159

Friedheim E, Corvi C, Walker CJ (1976) *Meso*-dimercaptosuccinic acid – a chelating agent for the treatment of mercury and lead poisoning. J Pharm Pharmacol 28:711–712

Gurer H, Ercal N (2000) Can antioxidants be beneficial in the treatment of lead poisoning? Free Radic Biol Med 29:927–945

Gurer H, Ozgunes H, Oztezcan S, Ercal N (1999) Antioxidant role of α-lipoic acid in lead toxicity. Free Radic Biol Med 26:75–81

Habig WH, Pabst UJ, Jacob WB (1973) Glutathione-*S*-transferase. J Biol Chem 249:7130–7139

Hu H (1991) A 50-year follow up of childhood plumbism. Hypertension, renal function, and hemoglobin levels among survivors. Ann J Dis Child 145:681–687

Jansen YMW, Houten BV, Borm PJA, Mossman BT (1993) Biology of disease. Cell and tissue responses to oxidative damages. Lab Invest 69:261–274

Jendryczko A (1998) Involvement of free radicals in lead poisoning. Med Pr 49:1

Kagan V, Khan S, Swanson C, Shvedova A, Serbinova E, Packer L (1990) Antioxidant action of thioctic acid and dihydrolipoic acid. Free Radic Biol Med 9 [Suppl]:15

Kornbrust DJ, Mavis RD (1979) Relative susceptibility of microsomes from lung, heart, liver, kidney, brain and testis to lipid peroxidation-correction with vitamin E content. Lipids 15:315–322

Lachant NA, Tomoda A, Tanaka KR (1984) Inhibition of the pentose phosphate shunt by lead: a potential mechanism for hemolysis in lead poisoning. Blood 63:518–524

Lewis SE, Wills ED (1962) Destruction of sulphydrl groups of proteins and amino acids by peroxides of unsaturated fatty acids. Biochem Pharmacol 11:901–912

Lowry OH, Rosebrough NJ, Farr AI, Randall RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193:265–275

Marhun D (1976) Rapid colorimetric assay of β-galactosidase and *N*-acetyl-β-D-glucosaminidase in human urine. Clin Chim Acta 73:453–461

Marklund S, Marklund G (1974) Involvement of superoxide anion radical in autooxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur J Biochem 47:469–474

Mill T (1980) Liquid phase oxidation In: Bamford GH, Tipper CFH, (eds) Comprehensive chemical kinetics. Elsevier, Amsterdam, pp 1–87

Minami M, Koshi K, Homma K, Suzuki Y (1982) Changes of the activities of superoxide dismutase after exposure to the fume of heavy metals and the significance of zinc in the tissue. Arch Toxicol 49:215–225

Moron MS, Despierre JW, Minnervik B (1979) Levels of glutathione, glutathione reductase and glutathione-*S*-transferase activities in rat lung and liver. Biochim Biophys Acta 582:67–78

Müller L, Menzel H (1990) Studies on the efficacy of lipoate and dihydrolipoate in the alteration of cadmium^{2+} toxicity in isolated hepatocytes. Biochim Biophys Acta 1052:385–391

Neal R, Cooper K, Gurer H, Ercal N (1998) Effects of *N*-acetyl cysteine and 2,3-dimercaptosuccinic acid on lead-induced oxidative stress in rat lenses. Toxicology 130:167–174

Nicholls P, Schonbaum GR (1963) In: Boyer PD, Lardy H, Myrback K (eds) The enzymes, vol VIII. Academic Press, New York, pp 147–225

Orlowski M, Meister A (1965) Isolation of γ-glutamyl transpeptidase from hog kidney. J Biol Chem 240:338–347

Payton M, Hu H, Sparrow D, Weiss ST (1994) Low-level lead exposure and renal function in the normative aging study. Am J Epidemiol 140:821–829

Ribarov SR, Bochev PG (1982) Lead-hemoglobin interaction as a possible source of reactive oxygen species – a chemiluminescent study. Arch Biochem Biophys 213:288–292

Rister M, Bachner RL (1976) The alteration of superoxide dismutase, catalase, glutathione peroxidase and NAD(P)H cytochrome-C reductase in guinea pig polymorphonuclear leukocytes and alveolar macrophages during hypoxia. J Clin Invest 58:1174–1184

Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hajeman DG, Hoekstra WG (1973) Selenium: biochemical role as a component of glutathione purification and assay. Science 179:588–590

Sandhir R, Julka D, Gill KD (1994) Lipoperoxidative damage on lead treatment in rat brain and its implications on membrane bound enzymes. Pharmacol Toxicol 74:66–71

Sinha AK (1972) Colorimetric assay of catalase. Anal Biochem 47:389–394

Solliway BM, Schaffer A, Pratt H, Yannai S (1996) Effects of treatment to lead on selected biochemical and hematological variables. Pharmacol Toxicol 78:18–22

Staal GEJ, Visser J, Veeger C (1969) Purification and properties of glutathione reductase of human erythrocytes. Biochim Biophys Acta 185:39–48

Sumathi R, Kalpana Devi V, Varalakshmi P (1994) DL-α-lipoic acid protection against cadmium induced tissue lipid peroxidation. Med Sci Res 22:23–25

Teichert-Kuliszewska K, Nicholls DM (1985) Rat kidney brush border enzyme activity following subcronic oral lead exposure. Toxicol Appl Pharmacol 77:211–218

Villeda-Hernández J, Barroso-Moguel R, Méndez-Armenta M, Nava-Ruíz C, Huerta-Romero R, Ríos C (2001) Enhanced brain regional lipid peroxidation in developing rats exposed to low level lead acetate. Brain Res Bull 55:247–251

Vyskocil A, Pancl J, Tusl M, Ettlerova E, Semecky V, Kasparova L, Lauwerys R, Bernard A (1989) Dose-related proximal tubular dysfunction in male rats chronically exposed to lead. J Appl Toxicol 9:395–399

Yiin SJ, Lin TH (1995) Lead catalysed peroxidation of essential unsaturated fatty acids. Biol Trace Elem Res 50:167–172

Yoshida A, Huang IY (1986) Structure of human glucose-6-phosphate dehydrogenase. In: Yoshida A, Beutler E (eds) Glucose-6-phosphate dehydrogenase. Academic Press, New York, pp 473–482