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Combined efficacies of lipoic acid and 2,3-dimercaptosuccinic acid against lead-induced lipid peroxidation in rat liver

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

Abstract

Oxidative stress with subsequent lipid peroxidation has been postulated as one mechanism for lead toxicity. Hence in assessing the protective effects of lipoic acid (LA) and meso 2,3-dimercaptosuccinic acid (DMSA) on lead toxicity, they were tested either separately or in combination for their effects on selected indices of hepatic oxidative stress. Elevated levels of lipid peroxides were accompanied by altered antioxidant defense systems. Lead acetate (Pb - 0.2%) was administered in drinking water for five weeks to induce toxicity. LA (25 mg kg^{-1} body wt. day^{-1} i.p) and DMSA (20 mg kg^{-1} body wt. day^{-1} i.p) were administered individually and also in combination during the sixth week. Lead damage to the liver was evident in the decreases in hepatic enzymes alanine transaminase (−38%), aspartate transaminase (−42%) and alkaline phosphatase (−43%); increases in lipid peroxidation (+38%); decreases in the antioxidant enzymes catalase (−45%), superoxide dismutase (−40%), glutathione peroxidase (−46%) and decreases in glutathione (−43%) and decreases in glutathione metabolizing enzymes, glutathione reductase (−59%), glucose-6-phosphate dehydrogenase (−27%) and glutathione-S-transferase (−42%). In combination LA and DMSA completely ameliorated the lead induced oxidative damage. Either compound alone was however only partially protective against lead damage.

Keywords: Lead acetate; DL-α-lipoic acid; Meso 2,3-dimercaptosuccinic acid; Lipid peroxidation; Antioxidants

1. Introduction

Lead is a non-essential toxic heavy metal widely distributed in the environment and chronic exposure to low levels of lead has been a matter of public health concern in many countries. Lead induces a broad range of physiological, biochemical and behavioral dysfunctions. Many studies have explored the mechanisms and symptoms of this toxicity through the years but recent studies have reported lead as a potential agent for inducing oxidative stress by the production of reactive oxygen species (ROS) [1].

Approximately 90% of the total body lead is contained within bones [2]. Blood accounts for 4% and the remaining lead resides mainly in the liver and the kidneys [3]. The liver and the kidneys are also known to play a major role in the elimination of lead [4] and hence account for the toxic actions [5].

Lead is known to produce oxidative damage in the liver tissues by enhancing peroxidation of membrane lipids [6], a deleterious process solely carried out by free radicals [7]. Many studies have investigated possible relationship between lipid peroxidation (LPO) and cellular damage in hepatic tissues under various pathological conditions [8]. Lewis and Wills [9], have suggested that peroxide formation may lead to oxidative destruction of thiol groups of amino acids and proteins. Reports on lead-induced oxidative stress dates back to 1965 [10]. Lead can cause derangement of several hepatic biochemical pathways and energy metabolism [11]. In particular lead causes transient, but marked hypercalaemia, which may contribute to hepatotoxicity [12].

Cell membranes are targets for oxidative damage produced by xenobiotics including heavy metals [7]. Peroxidative decomposition of membrane lipids is catastrophic for living system. Antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione-S-transferase (GST) and glutathione reductase (GR) scavenge free radicals and lipid peroxides and detoxify them [13]. Previous reports by Dwivedi et al. [13] and

* Corresponding author. Tel.: +91-44-24925548; fax: +91-44-24926709.

E-mail address: drvlakshmi@yahoo.com (P. Varalakshmi).

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Tan et al. [14] have shown that LPO is enhanced by disturbances such as depletion of cellular antioxidants.

Biological compounds with antioxidant properties contribute to the protection of cells and tissues against deleterious effects of ROS and other free radicals [15]. Lipoic acid (LA), a potent antioxidant [16,17] was identified as an effective antidote against heavy metal induced toxicities [18–20]. Meso 2,3-dimercaptosuccinic acid (DMSA) has a high therapeutic index, which scavenges ROS [21] and has been identified as a potentially useful drug for the treatment of lead poisoning [22,23]. Yet little is known about the combined effect of LA and DMSA on lead toxicity. Hence the current study was designed to investigate the combined effects of the two compounds on free radical toxicity during lead exposure.

2. Materials and methods

2.1. Materials

DL-α-lipoic acid, meso 2,3-dimercaptosuccinic acid, nicotinamide adenine dinucleotide phosphate and bovine serum albumin 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) $NaHCO_3$. Sodium pyruvate was purchased from L. H. Boehringer, Ingelhelm, Germany and glutathione was obtained from BDH Chemicals Ltd., Poole, England. Thiobarbituric acid and glucose-6-phosphate were procured from Loba-Chemie, Mumbai and Sisco Research Laboratories Ltd., Mumbai, India, respectively. Lead in the form of lead II acetate (Central Drug House Pvt. Ltd., Mumbai, India) was prepared in double distilled water prior to use. All other chemicals and solvents used were of highest purity and analytical grade.

2.2. Animal model

Male albino rats (Wistar strain) procured from Tamilnadu University for Veterinary and Animal Sciences, Chennai, India, weighing 120 $\pm$ 20g (10 to 12 weeks old) were used throughout the study. The rats were fed with a standard rat pellet diet (M/s. Pranav Agro Industries Ltd., India) under the trade name Amrut rat/mice feed and had free access to water. The rats were maintained in a well-ventilated animal quarter with 12-hr light and 12 hr dark exposure.

2.3. Experimental design

The animals were assigned into eight groups of six animals each. Group I (Control) received isotonic saline (0.5 mL day^{-1}i.p) during week 6 only. Group II (Pb) received Pb (0.2%) in drinking water for 5 weeks and isotonic saline during week 6. Group III (LA) received LA (25 mg kg^{-1} body wt day^{-1}i.p) during week 6 only. Group IV (DMSA) received DMSA (20 mg kg^{-1} body wt day^{-1}i.p) during week 6 only. Group V (LA + DMSA) received LA (25 mg kg^{-1} body wt day^{-1}i.p) plus DMSA (20 mg kg^{-1} body wt day^{-1}i.p) at the same doses during week 6 only. Group VI (Pb + LA) received Pb (0.2%) in drinking water for 5 weeks and LA during week 6. Group VII (Pb + DMSA) received Pb (0.2%) in drinking water for 5 weeks and DMSA during week 6. Group VIII (Pb + LA + DMSA) received Pb (0.2%) for 5 weeks; LA and DMSA combined were administered during the sixth week. Dosing regime for the test animals were fixed based from a previous study [24, 25]. Solutions were prepared fresh daily and the concentration was adjusted such that a rat would receive 0.5 mL/day.

After the sixth week the animals were sacrificed by cervical decapitation and the liver was excised immediately and washed in ice-cold saline. The tissue (liver) was then homogenized in ice-cold Tris-HCl buffer (0.1 M, pH 7.4) to give a 10% homogenate.

2.4. Measurements

Alkaline phosphatase (ALP) [26] and the transaminases; alanine transaminase (ALT) and aspartate transaminase (AST) were estimated by the method of King [27]. Protein content in the rat hepatic tissue was determined by the method of Lowry et al. [28]. LPO was assayed by the method of Devasagayam [29], in which malondialdehyde (MDA) released served as the index of LPO. The activity of CAT was assayed by the method of Sinha [30]. 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. Chromic acetate thus produced was measured colorimetrically at 610 nm.

SOD was assayed according to the method of Marklund and Marklund [31]. The unit of enzyme activity is defined, as the enzyme required giving 50% inhibition of pyrogallol autooxidation. GPx was assayed by the method of Rotruck et al. [32] 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.

GSH was determined by the method of Moron et al. [33]. GR that utilizes NADPH to convert oxidized glutathione (GSSG) to the reduced form was assayed by the method of Staal et al. [34]. The estimation of glucose-6-phosphate dehydrogenase (G6PD) was assayed by the method of Beutler [35], where the increase in absorbance was measured when the reaction was started by the addition of glucose-6-phosphate. GST was assayed by the method of Habig et al. [36].

2.5. Statistical analysis

The values are expressed as mean$\pm$SE. The results were computed statistically (SPSS software package, version 7.5)

Table 1
Effect of lipoic acid, meso 2,3-dimercaptosuccinic acid and lead on the activities of hepatic enzymes

Parameters	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)
ALT	1.32 ± 0.05	0.82 ± 0.04[a*]	1.34 ± 0.06	1.30 ± 0.05	1.31 ± 0.05	1.13 ± 0.05[b*]	1.09 ± 0.04[b*]	1.30 ± 0.05[b*c*d*]
AST	1.10 ± 0.03	0.64 ± 0.04[a*]	1.12 ± 0.05	1.10 ± 0.04	1.12 ± 0.05	0.89 ± 0.04[b*]	0.76 ± 0.03[b*]	1.06 ± 0.04[b*c*d*]
ALP	0.28 ± 0.008	0.16 ± 0.004[a*]	0.29 ± 0.012	0.27 ± 0.004	0.29 ± 0.008	0.23 ± 0.004[b*]	0.21 ± 0.008[b*]	0.25 ± 0.008[b*c*d*]

Values represent the [mean ± SE] for six rats
Enzyme activities are expressed in terms of unit's mg^{-1} protein
One unit of enzyme activity is expressed as: ALT and AST - μ moles $\times 10^{-1}$ of pyruvate; ALP - μmoles $\times 10^{-1}$ of phenol liberated min^{-1} mg^{-1} protein at 37° C
Comparisons are made between: [a]groups I and II, [b]group II and VI, VII, VIII, [c]group VI and VIII, [d]groups VII and VIII
Values are statistically significant at $^{*}P < 0.05$, $^{@}P < 0.01$, $^{\#}P < 0.001$
Standard errors have been calculated and incorporated in all tables.

using one-way Analysis of Variance. Post hoc testing was performed for inter-group comparison using the LSD. $P < 0.001$, < 0.01, < 0.05 was considered significant.

3. Results

Lead administration caused a significant ($P < 0.05$) decrease in the activities of ALT (−38%), AST (−42%) and ALP (−43%) (Table 1). Lead administration also caused a significant ($P < 0.05$) increase in LPO as measured by the levels of MDA (+38%) as well as significant ($P < 0.05$) decreases in the antioxidant enzymes CAT (−45%), SOD (−40%), GPx (−46%) (Table 2). Furthermore, lead administration caused significant ($P < 0.05$) decreases in GSH (−43%) and the glutathione metabolizing enzymes, GR (−59%), G6PD (−27%) and GST (−42%) (Table 3). Treatment with either LA or DMSA ameliorated the changes induced by lead administration but treatment with both compounds completely restored all measured values back to normal (Tables 1 to 3).

4. Discussion

Redox disturbances are known to negatively impact body system through generation of ROS, which modify proteins, lipids, and DNA [7]. Liver being one of the targets for lead accumulation has witnessed the toxic insult of lead [37] by way of decrease in the activities of transaminases and ALP.

Aminotransferases (ALT and AST) being an important class of enzymes linking carbohydrate and aminoacid metabolism, have established a relationship between the intermediates of the citric acid cycle. These enzymes are regarded as markers of liver injury since; liver is the major site of metabolism [38]. ALP is membrane bound and its alteration is likely to affect the membrane permeability and produce derangement in the transport of metabolites. Moreover Plaa and Hewitt [39] have reported that ALP acts as an indicator of cholestatic changes. Several researchers have reported decreased activities of ALT, AST and ALP in liver during lead poisoning, which corroborates our study [40, 41]. LA in combination with DMSA restored the activities of these enzymes suggesting the regulatory influence of the dithiols.

Lead is known to produce oxidative damage in the liver by enhancing LPO [42,43]. All products of LPO inactivate cell constituents by oxidation or cause oxidative stress by undergoing radical chain reaction ultimately leading to loss of membrane integrity [44,45]. In analogy lead has shown to implicate hepatic LPO as evidenced by many reports [46–48].

The stimulation of LPO observed as a result of lead

Table 2
Effect of lipoic acid and meso 2,3-dimercaptosuccinic acid on lead induced lipid peroxidation and antioxidant status of rat liver

Parameters	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	1.56 ± 0.06	2.15 ± 0.08[a*]	1.53 ± 0.06	1.58 ± 0.05	1.57 ± 0.05	1.79 ± 0.07[b*]	1.80 ± 0.06[b*]	1.59 ± 0.053[b*c*d*]
CAT	326.48 ± 12.66	180.15 ± 8.27[a*]	332.16 ± 14.02	328.36 ± 11.61	334.65 ± 10.55	287.57 ± 10.96[b*]	280.15 ± 12.39[b*]	330.64 ± 14.78[b*c*d*]
SOD	8.49 ± 0.28	5.08 ± 0.20[a*]	8.57 ± 0.38	8.47 ± 0.30	8.58 ± 0.32	7.56 ± 0.29[b*]	7.50 ± 0.26[b*]	8.52 ± 0.30[b*c*d*]
GPx	5.44 ± 0.16	2.96 ± 0.09[a*]	5.48 ± 0.25	5.45 ± 0.18	5.50 ± 0.22	4.76 ± 0.18[b*]	4.60 ± 0.16[b*]	5.39 ± 0.20[b*c*d*]

Values represent the [mean ± SE] for six rats
LPO-nmoles of MDA released mg^{-1} protein per incubation period
Enzyme activities are expressed as: CAT - μg of H_2O_2 consumed min^{-1} mg^{-1} protein; SOD-units mg^{-1} protein (one unit is equal to the amount of enzyme required to inhibit autooxidation of pyrogallol by 50%); GPx-μg of GSH utilized min^{-1} 1 mg^{-1} protein
Comparisons are made between: [a]groups I and II, [b]group II and VI, VII, VIII, [c]group VI and VIII, [d]groups VII and VIII
Values are statistically significant at $^{*}P < 0.05$, $^{@}P < 0.01$, $^{\#}P < 0.001$

Table 3
Effect of lipoic acid, meso 2,3-dimercaptosuccinic acid and lead on glutathione and glutathione metabolizing enzymes in rat liver

Parameters	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	4.13 ± 0.15	$2.34 \pm 0.11^{a*}$	4.16 ± 0.12	4.14 ± 0.15	4.16 ± 0.16	$3.65 \pm 0.14^{b*}$	$3.61 \pm 0.15^{b*}$	$4.09 \pm 0.12^{b*c*d*}$
GR	0.29 ± 0.012	$0.12 \pm 0.004^{a*}$	0.27 ± 0.008	0.28 ± 0.008	0.28 ± 0.012	$0.25 \pm 0.012^{b*}$	$0.23 \pm 0.008^{b*}$	$0.31 \pm 0.012^{b*c*d*}$
G6PD	2.02 ± 0.08	$1.48 \pm 0.05^{a*}$	2.04 ± 0.07	2.00 ± 0.09	2.03 ± 0.07	$1.72 \pm 0.07^{b*}$	$1.69 \pm 0.07^{b*}$	$1.97 \pm 0.08^{b*c*d*}$
GST	1.15 ± 0.04	$0.67 \pm 0.02^{a*}$	1.18 ± 0.05	1.14 ± 0.04	1.17 ± 0.03	$0.96 \pm 0.04^{b*}$	$0.86 \pm 0.05^{b*}$	$1.09 \pm 0.03^{b*c*d*}$

Values represent the [mean $\pm$ SE] for six rats

Enzyme activities are expressed as: GSH - μg mg^{-1} protein; GR - nmoles of NADPH oxidized min^{-1} mg^{-1} protein; G6PD - nmoles of NADPH formed min^{-1} mg^{-1} protein; GST - nmoles of CDNB - GSH conjugate formed min^{-1} mg^{-1} protein

Comparisons are made between: [a]groups I and II, [b]group II and VI, VII, VIII, [c]group VI and VIII, [d]groups VII and VIII

Values are statistically significant at *P $<$ 0.05, @P $<$ 0.01, #P $<$ 0.001

administration could be due to the formation of free radicals [49] through an exhaustion of antioxidants [14] leading to oxidative stress [50]. However, as lead does not undergo oxidation - reduction cycle, the effect of lead on LPO is not a direct effect but these changes could rather be due to an indirect effect of lead on free-radical scavenging enzymes and GSH status [51]. Several studies have shown that both LA [52] and DMSA [53] chelates transition metal ions thereby inhibiting LPO triggered by ROS. Our reports are also consistent with the above findings. Sumathi et al. [54] has shown that LA affords protection against cadmium-induced hepatotoxicity. LA has displayed protection against LPO in combination with several antioxidants such as ascorbic acid and α-tocopherol [55] and oxidized GSH [56].

Antioxidant enzymes like CAT, SOD and GPx form the first line of defense against ROS and the decrease in their activities contribute to the oxidative insult on the tissue. CAT is a major antioxidant enzyme having heme as the prosthetic group. Lead is known to reduce the absorption of iron in the gastrointestinal tract and to inhibit the heme biosynthesis [57]. Decreased CAT activity observed in lead exposed animals was attributed to the interference of lead by both processes [58]. Rister and Bachner [59] speculated that during oxidative stress CAT activity decreases, H_2O_2 accumulates and thereby the peroxidation of lipids is favored.

SOD plays an important role in protecting the toxic effects of superoxide radical by catalyzing its dismutation reactions. SOD, which requires copper and zinc for its activity was found to be decreased in lead administered rats. Mylorie et al. [60] suggested that this could be due to the lead induced copper deficiency. Minami et al. [61] showed a 15% to 40% decrease in the activity of SOD in erythrocytes of rats exposed to fumes of heavy metals like lead, cadmium and antimony. Ito et al. [62] have suggested a partial inhibition of SOD activity in workers exposed to lead.

On the other hand GPx, a hydroperoxide-degrading enzyme, which requires selenium for activity was decreased in lead, poisoned rats. Loss in GPx activity can be correlated to the antagonistic effects between lead and selenium that has been suggested by Schrouzer [63]. Somashekaraiah et al. [43] apparently reported a decrease in GPx activity at 72 hr of lead injection in developing chick embryo. The overall inhibitory effects of lead on various enzymes would probably result in impaired antioxidant defense by cells and would render them more prone to oxidative attack.

The usefulness of the antioxidant LA in conjunction with DMSA was effective in rebalancing the impaired prooxidant/antioxidant ratio. Exogenous administration of LA [16] and DMSA separately brought about an improvement in the cells antioxidant defense armory due to their antioxidative property [1].

GSH is a tripeptide containing cysteine that has a reactive SH group with reductive potency. It can act as a non-enzymatic antioxidant by direct interaction of SH group with ROS, or it can be involved in the enzymatic detoxification reaction for ROS, as a cofactor or a coenzyme [64]. Lead binds exclusively to the SH groups [65] which decreases the GSH levels [66] thereby interfering with the antioxidant activity. Gurer et al. [42] has apparently reported a decline in GSH levels upon lead intoxication.

GR is responsible for the reduction of GSSG to GSH. The decrease in GR activity could possibly be due to the interaction of lead with the sulfydryl group present at the active site of this enzyme, which in turn prevents the enzyme from participating in the reaction [67]. Jindall and Gill [51] have reported a decline in GR activity after lead exposure.

G6PD supplies the cells with most of the extra mitochondrial NADPH through oxidation of glucose-6-phosphate. This NADPH keeps GSH at a constant level by providing NADPH for GR, which mediates the reduction of GSSG to GSH. G6PD is known to contain many SH groups, which play a crucial role in maintaining its tertiary structure [68]. The formation of lead sulfydryl complex was suggested as a plausible mechanism behind G6PD inhibition [69].

GST detoxifies electrophilic species via a spontaneous enzyme catalyzed conjugation reaction. Decreased activity of GST observed in the present study might be due to inhibition of protein structure by lead and due to want of substrate. Evidence for the inhibition of GST, GR by lead along with a fall in GSH comes from the report made by Neal et al. [70].

Previous report from our laboratory has shown the beneficial effects of the combined administration of LA and DMSA in reversing the oxidative damage to the kidney of lead poisoned rats [24]. Moreover the beneficial effects of coadministering LA and DMSA in bringing down the blood lead levels have been recently reported [25].

It is plausible that impaired oxidant / antioxidant balance can be partially responsible for the toxic effects of lead. Restoration of the cells antioxidant capacity appears to provide a partial remedy against lead-induced oxidative stress. Despite the preponderance of both the drugs as sole agents for lead toxicity, the combinatorial aid of both drugs seems to abate the oxidative insult by restoring the altered lead sensitive biochemical variables to an appreciable extent. Further studies need to be focused on exploring the dual benefits of these drugs and mechanisms underlying these beneficial effects.

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