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Effect of DL α-lipoic acid on tissue redox state in acute cadmium-challenged tissues

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Cadmium as an environmental pollutant has aroused great concern due to its toxic effects on various body tissues. Supplementation of thiol compounds has been suggested to protect against the toxic effects of reduced oxygen species by contributing to the thiol pool of the cell. The present study was designed to determine whether dietary supplementation of DL α-lipoic acid (15 and 30 mg/kg), a "meta-vitamin," to cadmium-intoxicated rats (3 mg/kg) affords protection against the oxidative stress caused by the metal. The liver and kidney of the metal-administered rats showed elevated levels of hydroxyl radicals and malondialdehyde (basal and induced), a decreased level of antioxidants-reduced glutathione, total thiols, protein thiols, nonprotein thiols, ascorbate, α-tocopherol and retinol and antioxidizing enzymes-superoxide dismutase, catalase, τ-glutamyl transpeptidase, glutathione peroxidase, glucose-6-phosphate dehydrogenase, glutathione reductase, and glutathione-S-transferase. Lipoate supplementation changed the tissue redox state directly by scavenging the free radicals and indirectly by bolstering the antioxidants and antioxidizing enzyme defenses. In vitro studies revealed that, among the mono and dithiols (glutathione, cysteine, dithiothreitol, and lipoic acid), lipoic acid was the most potent scavenger of free radicals produced during cadmium-induced hepatotoxicity. The drug contributes its thiol groups to detoxify the divalent metal and subsequently ameliorates the cell membrane integrity. (J. Nutr. Biochem. 7:85–92, 1996.)

Keywords: DL α-lipoic acid; cadmium chloride; lipid peroxidation; antioxidants; antioxidizing enzymes

Introduction

The trace elemental pollutant, cadmium, is in a class by itself and ranks close to other heavy metals in toxicologic importance due to its increasing levels in the environment as a result of past and present practices. One of the current theories by which cadmium exerts its toxic effect relates to a specific cell membrane lesion. It has been suggested that a possible mechanism for the cell membrane lesion and subsequent altered membrane permeability may involve lipid peroxidation.[1]

A report states that cadmium preferentially binds to membrane sulfhydryl groups and affects cellular thiols.[2] Hence, chelating agents possessing –SH moieties are speculated to be the most effective antidotes against heavy metal poisoning.[3]

The role of DL α-lipoic acid, a dithiol, as a cofactor in multienzyme complexes that catalyze the oxidative decarboxylation of α-keto acids (pyruvate, α-keto glutarate, and branched chain α-keto acids) has been established for some time.[4] Since lipoic acid can be synthesized by the animals,[5] little attention has been given to a dietary requirement for lipoic acid or its content in dietary constituents, which in any case is relatively low.

Patients diagnosed with hepatitis,[6] diabetes,[7] atherosclerosis,[8] urolithiasis,[9] and HIV infection[10] have been found to have a reduced level of endogenous lipoic acid. A report states that lipoic acid administration alleviates the symptoms of ascorbate and tocopherol deficiencies.[11]

DL α-lipoic acid, a cyclic disulfide, has been reported to exert its therapeutic effectiveness in pathologies where free radicals are involved.[12,13] Our earlier studies on the effect of lipoate in cadmium toxicity showed reduction in metal-induced peroxidation of rat heart, brain, and testis.[14] This

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provoked interest in us to study at length the cadmium-induced cytotoxicity (liver and kidney) manifested in terms of free radical production and the protection rendered by DL α-lipoic acid supplementation.

Methods and materials

Male albino Wistar rats weighing about 150 to 180 g were divided into six groups.

Group I – Normal rats, injected with 0.5 mL of physiological saline, formed the control group.
Group II – Administered cadmium chloride (3 mg/kg of body weight) subcutaneously.
Group III – Administered lipoic acid (15 mg/kg of body weight) intraperitoneally.
Group IV – Administered lipoic acid (30 mg/kg of body weight) intraperitoneally.
Group V – Administered cadmium chloride (3 mg/kg of body weight) subcutaneously followed by lipoic acid (15 mg/kg of body weight) intraperitoneally after 30 min.
Group VI – Administered cadmium chloride (3 mg/kg of body weight) subcutaneously followed by lipoic acid (30 mg/kg of body weight) intraperitoneally after 30 min.

Because time- and concentration-dependent studies carried out by us with cadmium revealed sufficient cytotoxic effects, both biochemically and histopathologically, within 24 hr, this time point was taken up for the study. Twenty-four hours after cadmium chloride injection, the animals were fasted overnight and killed by cervical dislocation. The liver and kidneys were dissected out, and a 10% homogenate was prepared using Tris-HCl buffer, 0.01 M, pH 7.4.

The activity of lipid peroxidation was determined by measuring the content of the thiobarbituric acid–reactive substances (TBARS) in the tissue homogenates following the procedure of Hogberg et al.[15] The hydrogen peroxide–induced system in a total volume of 2 mL contained 0.2 mL of tissue homogenate, 1 mM KH_2PO_4, and 7.5 mM H_2O_2 in 0.15 M Tris-HCl buffer, pH 7.4. For a ferrous sulphate–induced system, the assay system (2.0 mL) contained 0.2 mL of tissue homogenate, 1 mM KH_2PO_4, and 10 mM $FeSO_4$ in a 0.15 M Tris-HCl buffer, pH 7.4. An ascorbate-induced system contained 0.4 mM ascorbic acid, 50 μM $FeCl_3$, 5 mM ADP, and 1 mM KH_2PO_4 in 0.15 M Tris-HCl buffer, pH 7.4. The system without inducers consisted of 0.2 mL homogenate, 1 mM KH_2PO_4 in 0.15 M Tris-HCl buffer, pH 7.4. The basal lipid peroxidation system contained 0.2 mL of homogenate, 1 mM KH_2PO_4, and 1 mL of 10% trichloroacetic acid (TCA) in 0.15 M Tris-HCl buffer, pH 7.4. After incubation at 37°C in a mechanical shaker for 20 min, the reaction was arrested with 10% TCA, 1.5 mL of 1% thiobarbituric acid was added, the solution was boiled for 10 min, cooled, and read at 540 nm. The formation of a hydroxyl radical ion in the homogenate was assayed by measuring the generation of formaldehyde from dimethyl sulfoxide (DMSO) as described by Puntarulo and Cederbaum.[16] To 1.0 mL of tissue homogenate, 0.2 mL of 0.05 M phosphate buffer, pH 7.4, 0.1 mL each of 0.1 M $MgCl_2$ and 0.004 M $NADPH_2$, 0.01 M sodium azide, and 0.33 M dimethyl sulfoxide were added and incubated for 10 min at 37°C and then terminated by adding 0.5 mL of 20% TCA. After centrifuging, to the supernatant 5.0 mL of chromotropic acid (0.2% in 19 N H_2SO_4) was added and boiled for 30 min, cooled and read at 570 nm.

Reduced glutathione (GSH) in the homogenate was determined by the method of Moron et al.[17] based on the reaction with 2,2′-dithiobis (2-nitrobenzoic acid) to produce a compound with maximum absorption at 412 nm. Total protein and nonprotein thiol (PSH and NPSH) contents were determined following the method of Sedlack and Lindsay.[18] The other antioxidants, namely ascorbate,[19] α-tocopherol[20] and retinol[21] were determined in the tissue homogenates.

The superoxide dismutase (SOD) activity was measured as the degree of inhibition of autoxidation of pyrogallol at an alkaline pH.[22] The activity of catalase was measured as the amount of hydrogen peroxide consumed per minute per milligram of protein.[23] Enzymes involved in glutathione metabolism-glutathione peroxidase (GPX),[24] glucose-6-phosphate dehydrogenase (G-6-PD),[25] glutathione reductase (GR),[26] glutathione-S-transferase (GST),[27] and τ-glutamyl transpeptidase (τ-GT)[28] were also estimated in the homogenate. Protein was estimated by the method of Lowry et al.[29]

In vitro experiments were carried out by the method of Gelman and Michaelson (1979) using 20% liver homogenate.[30] Time- and dose-dependent study of varying concentrations of cadmium was carried out (0.1 to 3.0 mM). Cadmium-induced malondialdehyde (MDA) production in the presence of mono (GSH and cysteine) and dithiols (dithiothreitol [DTT] and lipoate) was assessed.

For statistical analysis, one-way analysis of variance (ANOVA) was used followed by the Newman-Keuls multiple comparison test.

Results

Table 1 depicts the activity of lipid peroxidation in the liver and kidney of control, cadmium-intoxicated, and lipoic acid treated rats. The hepatic cells show more susceptibility to free radical damage both under basal and induced conditions. The kidneys produced more MDA only in the presence of ascorbate and iron. A 2 fold increase in hydroxyl radical ion concentrations was observed in the liver of cadmium-challenged rats. These observations suggest that acute cadmium exposure causes hepatotoxicity rather than nephrotoxicity.

Lipoic acid administration was dose-dependently effective in clearing the free radicals. The hydroxyl radical production was also effectively suppressed by the drug in the cadmium-administered groups.

The nonenzymatic antioxidant status of the tissues of control and metal-exposed animals is shown in *Table 2*. Cadmium-administered liver showed a significant fall in the antioxidant status. The kidneys showed a marginal decrease in GSH, total sulfhydryls (TSH), ascorbate, and α-tocopherol levels. Lipoic acid treatments restored the level of the thiols and vitamins in a concentration-dependent manner.

Table 3 presents the antioxidant enzyme activities in the liver and kidney of control and experimental animals. Cadmium intoxication caused a significant decline in the activities of all enzymes other than GR and GST in the liver tissue. In the kidney, τ-GT, G-6-PD, and catalase were found to be inhibited. A fifteen milligram concentration of lipoic acid was effective in boosting the enzyme activities in the cadmium-administered tissues, and the 30 mg concentration restored the enzyme activities to that of the controls.

Figure 2 represents the MDA formation (nmol of MDA/mg of protein) in the presence of varying concentrations of cadmium (0.1 to 3.0 mM). At 0.6 mM concentration of the metal, maximum amount of MDA (4.8 nmol/mg of protein) was produced.

Time-dependent study revealed that inclusion of the

Table 1 Effect of cadmium and lipoic acid on tissue lipid peroxidation

Parameters	Group I control, saline	Group II $CdCl_2$	Group III saline + LPA (15 mg)	Group IV saline + LPA (30 mg)	Group V $CdCl_2$ + LPA (15 mg)	Group VI $CdCl_2$ + LPA (30 mg)
Liver						
Lipid peroxidation						
basal (0′)	1.32 ± 0.10	2.59 ± 0.22[a*]	1.29 ± 0.14	1.26 ± 0.16	1.88 ± 0.18[b*c*d*]	1.26 ± 0.14[b*e*]
Without inducers	2.34 ± 0.18	4.76 ± 0.46[a*]	2.30 ± 0.22	2.28 ± 0.20	3.81 ± 0.38[b*c*d*]	2.30 ± 0.21[b*e*]
H_2O_2-induced	3.13 ± 0.27	5.81 ± 0.64[a*]	3.13 ± 0.32	3.10 ± 0.34	4.73 ± 0.40[b*c*d*]	3.14 ± 0.31[b*e*]
Ascorbate-induced	4.20 ± 0.32	6.99 ± 0.70[a*]	4.19 ± 0.45	4.16 ± 0.43	5.57 ± 0.57[b*c*d*]	4.27 ± 0.42[b*e*]
$FeSo_4$-induced	7.30 ± 0.47	10.37 ± 1.05[a*]	7.30 ± 0.78	7.26 ± 0.76	9.00 ± 0.91[b*c*d*]	7.39 ± 0.72[b*e*]
Hydroxyl free						
radical	4.06 ± 0.14	7.83 ± 0.75[a*]	4.03 ± 0.43	4.03 ± 0.37	6.02 ± 0.59[b*c*d*]	4.10 ± 0.38[b*e*]
Kidney						
Lipid peroxidation						
basal (0′)	1.01 ± 0.10	1.13 ± 0.12	0.96 ± 0.12	0.94 ± 0.09	1.07 ± 0.10	0.97 ± 0.08
Without inducers	1.54 ± 0.10	1.67 ± 0.17	1.51 ± 0.09	1.49 ± 0.15	1.60 ± 0.13	1.50 ± 0.13
H_2O_2-induced	3.28 ± 0.43	3.69 ± 0.34	3.25 ± 0.33	3.22 ± 0.34	3.41 ± 0.31	3.26 ± 0.32
Ascorbate-induced	4.31 ± 0.11	5.59 ± 0.54[a*]	4.30 ± 0.47	4.28 ± 0.45	4.80 ± 0.47[b*]	4.34 ± 0.43[b*]
$FeSo_4$-induced	7.58 ± 0.37	9.10 ± 0.84[a*]	7.58 ± 0.81	7.56 ± 0.78	8.11 ± 0.84[b*]	7.62 ± 0.80[b*]
Hydroxyl free						
radical	2.31 ± 0.15	2.47 ± 0.24	2.28 ± 0.21	2.27 ± 0.22	2.36 ± 0.22	2.26 ± 0.22

Values are mean ± SD for six rats in each group. Lipid peroxidation: nmol/mg of protein; Hydroxyl free radical: nmol/min/mg of protein.
[a]As compared with Group I; [b]as compared with Group II; [c]as compared with Group III; [d]as compared with Group IV; [e]as compared with Group V.
The symbol represents statistical significance: $*P < 0.05$.

metal in the liver suspension triggers MDA production within 10 min and it progresses to form a peak at the end of the incubation period (90 min) (*Figure 3*). Addition of 2 mM lipoate abrogates the metal-induced free radical production from the start of the incubation period and renders effective protection thereafter. Lipoate added to the liver homogenate in the absence of the metal did not show any significant change with regard to the control.

The effect of a range (0.02 to 5.0 mM) of thiols on the metal-induced lipid peroxidation is depicted in *Figure 4*. The observations made in this study suggest that the dithiols (DTT and lipoate) are much more effective than the monothiols (GSH and cysteine) in scavenging the free radicals. At lower concentrations (0.02 to 0.5 mM), both GSH and cysteine manifested a similar trend. At higher concentrations (1.0 to 5.0 mM), GSH was comparatively better than cysteine. With reference to the dithiols, the difference observed between lipoate and DTT was narrowed down with increas-

Table 2 Effect of cadmium and lipoic acid on tissue antioxidants

Parameters	Group I control, saline	Group II $CdCl_2$	Group III saline + LPA (15 mg)	Group IV saline + LPA (30 mg)	Group V $CdCl_2$ + LPA (15 mg)	Group VI $CdCl_2$ + LPA (30 mg)
Liver						
GSH	4.06 ± 0.41	2.13 ± 0.15[a*]	4.10 ± 0.41	4.12 ± 0.42	3.81 ± 0.37[b*]	4.10 ± 0.38[b*]
TSH	15.17 ± 1.37	11.86 ± 1.07[a*]	15.19 ± 1.32	15.23 ± 1.38	13.94 ± 1.18[b*]	15.22 ± 1.48[b*]
PSH	10.63 ± 0.95	8.49 ± 0.92[a*]	10.64 ± 1.04	10.65 ± 0.86	9.03 ± 1.01[c*d*]	10.61 ± 1.02[b*e*]
NPSH	4.51 ± 0.16	2.32 ± 0.20[a*]	4.54 ± 0.43	4.57 ± 0.43	3.98 ± 0.34[b*c*d*]	4.55 ± 0.40[b*e*]
Vitamin C	2.83 ± 0.11	1.10 ± 0.09[a*]	2.85 ± 0.06	2.88 ± 0.27	2.10 ± 0.17[b*c*d*]	2.87 ± 0.23[b*e*]
Vitamin E	140.51 ± 11.73	104.73 ± 10.56[a*]	143.04 ± 14.48	145.18 ± 15.22	121.13 ± 12.47[b*c*d*]	139.42 ± 13.90[b*e*]
Vitamin A	13.86 ± 1.19	10.20 ± 1.16[a*]	13.93 ± 1.25	14.28 ± 1.41	12.77 ± 1.24[b*]	13.80 ± 1.38[b*]
Kidney						
GSH	2.33 ± 0.15	1.85 ± 0.17[a*]	2.35 ± 0.20	2.37 ± 0.26	2.26 ± 0.17[b*]	2.36 ± 0.20[b*]
TSH	9.92 ± 0.65	8.71 ± 0.56[a*]	9.95 ± 0.59	9.98 ± 0.61	9.26 ± 0.60[b*c*d*]	9.99 ± 0.59[b*e*]
PSH	7.43 ± 0.11	7.37 ± 0.33	7.44 ± 0.34	7.44 ± 0.38	7.40 ± 0.37	7.44 ± 0.33
NPSH	2.41 ± 0.11	2.23 ± 0.23	2.43 ± 0.21	2.46 ± 0.21	2.37 ± 0.20	2.45 ± 0.25
Vitamin C	1.03 ± 0.06	0.93 ± 0.08[a*]	1.05 ± 0.04	1.07 ± 0.08	1.01 ± 0.07	1.05 ± 0.07[b*]
Vitamin E	104.30 ± 10.69	87.02 ± 8.69[a*]	106.04 ± 10.65	107.12 ± 10.81	98.86 ± 10.12	105.17 ± 10.95[b*]
Vitamin A	6.08 ± 0.60	5.46 ± 0.54	6.11 ± 0.60	6.14 ± 0.63	5.83 ± 0.56	6.14 ± 0.62

Values are mean ± SD for six rats in each group.
Thiols and vitamin C: μg/mg/protein; Vitamins E and A: nmol/g of tissue.
Statistical comparisons as in *Table 1*.
The symbol represents statistical significance: $*P < 0.05$.

Table 3 Effect of cadmium and lipoic acid on tissue antioxidizing enzymes

Parameters	Group I control, saline	Group II $CdCl_2$	Group III saline + LPA (15 mg)	Group IV saline + LPA (30 mg)	Group V $CdCl_2$ + LPA (15 mg)	Group VI $CdCl_2$ + LPA (30 mg)
Liver						
SOD	8.78 ± 0.48	6.92 ± 0.61^{a*}	8.81 ± 0.90	8.83 ± 0.86	7.43 ± 0.67^{c*d*}	8.65 ± 0.87^{b*c*}
Catalase	337.29 ± 22.96	210.03 ± 19.91^{a*}	340.13 ± 35.14	344.18 ± 35.84	295.68 ± 29.12^{b*}	333.89 ± 32.11^{b*c*}
γ-GT	0.81 ± 0.07	0.56 ± 0.06^{a*}	0.84 ± 0.08	0.88 ± 1.00	0.64 ± 0.06^{c*d*}	0.76 ± 0.06^{b*d*e*}
GPX	5.38 ± 0.10	3.46 ± 0.29^{a*}	5.41 ± 0.59	5.44 ± 0.56	4.55 ± 0.44^{b*c*d*}	5.21 ± 0.53^{b*e*}
G-6-PD	2.04 ± 0.15	0.97 ± 0.07^{a*}	2.09 ± 0.17	2.11 ± 0.21	1.66 ± 0.16^{b*c*d*}	2.06 ± 0.16^{b*d*e*}
GR	0.30 ± 0.02	0.16 ± 0.07^{a*}	0.31 ± 0.03	0.30 ± 0.13	0.21 ± 0.02^{c*d*}	0.28 ± 0.03^{b*}
GST	1.04 ± 0.06	0.91 ± 0.07^{a*}	1.05 ± 0.10	1.06 ± 0.09	0.99 ± 0.07	1.05 ± 0.08^{b*}
Kidney						
SOD	4.94 ± 0.28	4.81 ± 0.33	4.96 ± 0.41	4.97 ± 0.51	4.86 ± 0.50	4.93 ± 0.42
Catalase	101.22 ± 9.47	80.79 ± 7.63^{a*}	104.33 ± 10.28	107.04 ± 11.31	90.35 ± 8.75^{b*d*}	102.88 ± 9.66^{b*}
γ-GT	1.37 ± 0.02	1.01 ± 0.11^{a*}	1.37 ± 0.14	1.39 ± 0.13	1.26 ± 0.11^{b*}	1.38 ± 0.11^{b*}
GPX	4.19 ± 0.43	4.10 ± 0.27	4.18 ± 0.40	4.25 ± 0.40	4.21 ± 0.40	4.24 ± 0.41
G-6-PD	1.65 ± 0.08	1.51 ± 0.13^{a*}	1.67 ± 0.14	1.69 ± 0.13	1.59 ± 0.12	1.66 ± 0.12^{b*}
GR	0.19 ± 0.02	0.23 ± 0.02	0.23 ± 0.04	0.28 ± 0.02	0.25 ± 0.05	0.27 ± 0.06
GST	0.66 ± 0.01	0.63 ± 0.03	0.69 ± 0.06	0.69 ± 0.07	0.65 ± 0.05	0.68 ± 0.06

Values are mean ± SD for six rats in each group.
SOD, G-6-PD, and GST: U/min/mg of protein; Catalase and γ-GT: μmol/min/mg of protein; GR: nmol/min/mg of protein; GPX: μg/min/mg of protein.
Statistical comparisons as in *Table 1*.
The symbol represents statistical significance: $^*P < 0.05$.

ing drug concentrations. Lipoate established its efficacy as a potent scavenger even at low concentrations in comparison with other thiols. Addition of thiols to the control tube did not bring about any remarkable change.

Discussion

Cadmium induces oxidative damage in different tissues by enhancing the peroxidation of membrane lipids and by inhibiting the antioxidants and enzymes involved in the utilization of some activated oxygen species.[31] As seen in *Table 1*, cadmium administration has caused profound lipid peroxidation in the presence of inducers. This obviously indicates the oxidative stress created by the divalent cation. Cadmium-induced free radical toxicity is manifested in terms of hydroxyl radicals, superoxide radicals, and peroxide production. The metal produced only marginal elevation in free radical production in the renal tissue. Lipid peroxidation is known to occur in biological membranes with potential injurious consequences, and it is possible that peroxidation of membrane lipids, especially that of the hepatic cells compared with kidney cells, is associated with acute cadmium toxicity. This observation is in agreement with earlier reports on cadmium-induced MDA production in the liver and kidney.[32] Free radicals are involved at the early stages of cadmium intoxication, and lipid peroxidation preceded extensive tissue damage.[33] Hence, it can serve as a potential marker of susceptibility or of early and reversible tissue damage.[31]

Thiols are thought to play a pivotal role in protecting cells against lipid peroxidation.[34] Lipoic acid has been reported to be effective in reducing the amount of hydroxyl radicals generated by Fenton-type reaction[35] and also as a scavenger of peroxide and superoxide radicals.[36] In the present study, a concentration-dependent protection is rendered by lipoic acid against cadmium-induced MDA production and this can be attributed to the two sulfhydryl moieties of lipoic acid.

Regarding the antioxidant status during metal exposure, remarkable depletion of total thiol, which includes non-

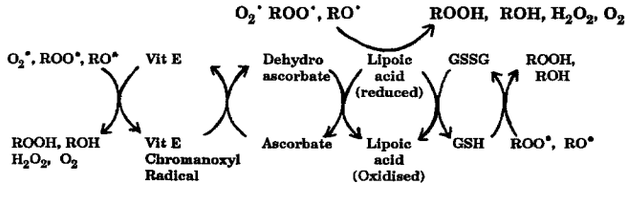

Figure 1 A scheme illustrating the functioning of the GSH cycle and its interaction with the vitamin E and C cycle and the dihydrolipoic acid/lipoic acid redox couple.[47]

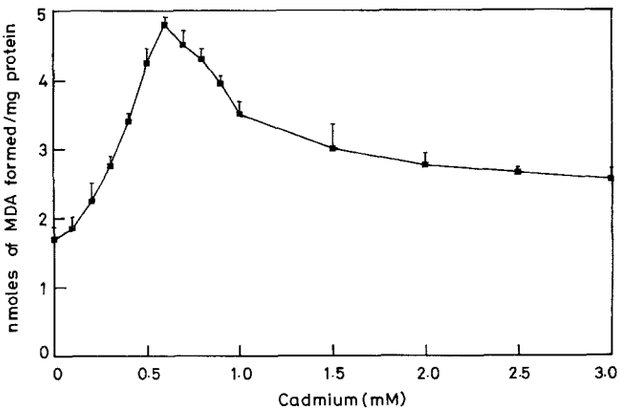

Figure 2 Effect of cadmium chloride on MDA production.

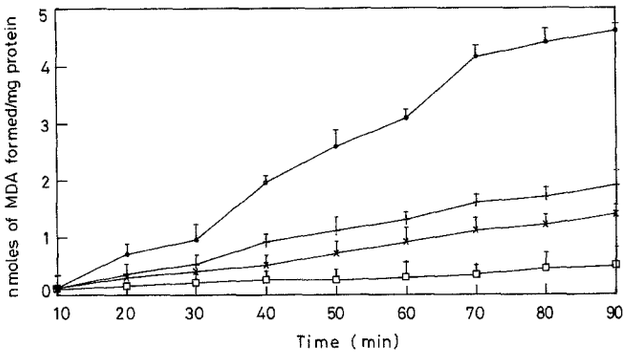

Figure 3 Time-dependent study on the effect of lipoic acid on cadmium-induced lipid peroxidation. -●- 0.6 mM Cd, -|- 0.6 mM Cd + 2 mM LPA, -x- control, -□- control + 2 mM LPA.

protein sulfhydryls (NPSH) (predominantly GSH) and PSH, and vitamins E, A, and C have been observed. Lowered levels of GSH in the tissues represent increased utilization due to oxidative stress. GSH, a nonprotein reservoir in the liver, is involved in many cellular processes including the detoxification of endogenous and exogenous compounds. GSH effectively binds with the divalent metal cadmium, because of a free sulfhydryl group in its tripeptide, and eventually gets oxidized to oxidized glutathione (GSSG), which accumulates in the system.[37] The levels of TSH and NPSH appear to decline more profoundly in the liver.

Vitamin E, a chain-breaking antioxidant, is the first antioxidant present in the cell membrane that counteracts the entry of the metal-induced free radicals into the cell and ultimately gets transformed into a tocopheroxyl radical.[38] Carotenoids are also reported to be effective free radical

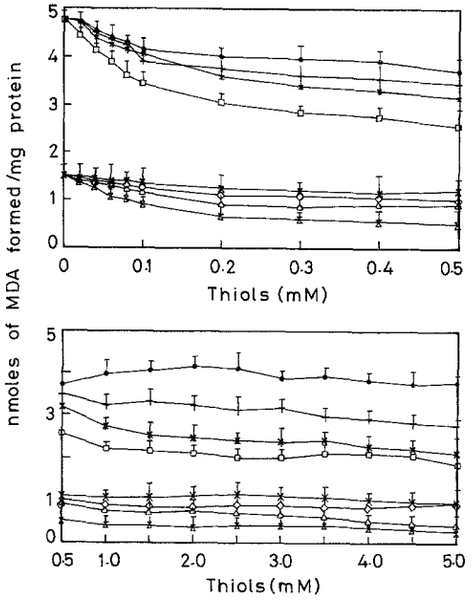

Figure 4 Effect of mono- and dithiols on cadmium-induced lipid peroxidation. -●- Cd + Cys, -|- Cd + GSH, -*- Cd + DTT, -□- Cd + LPA, -x- control + Cys, -◇- control + GSH, -△- control + DTT, -⧖- control + LPA.

scavengers.[39] It is a quencher of singlet oxygen and has the ability to react directly with the peroxyl radicals involved in lipid peroxidation and trap them from further propagation.[40] Vitamins E and A, the two vital lipophilic antioxidants, play a critical role in detoxifying cadmium.[41]

Vitamin C, the major water-soluble and nonenzymatic primary preventive antioxidant in the cells and body fluids, scavenges the free radicals produced by cadmium and serves as a metabolic marker of cadmium toxicity.[42] It interacts with the tocopheroxyl radical, resulting in the formation of dehydroascorbic acid and α-tocopherol.[43] GSH is required for the reduction of dehydroascorbate back to ascorbate.[44] When there is a reduction in the level of GSH, this conversion is affected and hence the vitamin C level is lowered. The cadmium-induced depletion of water-soluble and lipid-soluble antioxidants has led to the increased susceptibility of the tissues to free radical damage.

Administration of lipoic acid, a nonprotein thiol, reveals its effectiveness in affording protection to cell membranes by a possible interaction with the nonenzymatic antioxidants GSH, α-tocopherol, and ascorbate. DL α-lipoic acid and dihydrolipoic acid, a derivative obtained by the intracellular conversion of lipoic acid, act as antioxidants for ascorbic acid and tocopherol.[45] It is also possible that the protective action of lipoic acid is based on its ability to react with heavy metals that are pro-oxidants for ascorbic acid and tocopherol.[11] It can be stated that the dithiol, apart from effectively clearing the hydroxyl, peroxyl, and superoxide radicals,[46] effectively enhances the level of these antioxidants by providing the reducing milieu and regenerating them via the reduction of their radicals (*Figure 1*). The observations made in the present study indicate the antioxidant property of lipoic acid.

Subcellular membranes and associated thiol-bearing enzymes represent sensitive sites for cadmium, causing perturbation of cellular functions.[48] Reactive oxygen radicals can themselves reduce the activity of the enzymes.[49] Significant elevation in MDA production during metal exposure may in part be attributed to the effective inhibition of free radical scavenging enzymes. Inhibition of SOD and catalase results in the accumulation of superoxides and peroxides.

Inhibition of G-6-PD causes a decreased supply of reducing equivalents (NADPH) for the conversion of oxidized glutathione to its reduced form in the presence of GR. Under conditions of oxidative assault, the NADP/NADPH ratio will switch in favor of NADP, indicating decreased G-6-PD activity. The paucity of NADPH production will in turn decrease the catalase activity.[50] This statement proves true in the metal-administered rats. Precursors for GSH synthesis are transported into the cell by an enzyme present on the cell membrane, namely τ-GT. The decline in GSH levels due to cadmium ingestion may be partly due to inhibition of τ-GT. GST, the enzyme responsible for the conjugation of GSH to foreign compounds, is inhibited by cadmium which binds to the nonhistone protein part of the enzyme.[51]

An elevation in the activities of the antioxidizing enzymes has been observed during the disulfide supplementation. The concomitant increase in SOD and catalase activity effectively eliminates the superoxides and peroxides produced by cadmium chloride. Lipoic acid interacts with GSH and clears the free radicals in the presence of GPX.[52]

Lipoate elicits the G-6-PD activity producing more reducing equivalents and subsequently regenerating GSH from GSSG. Lipoic acid administration also favors the clearing of the divalent metal ion by its increased conjugation with GST. The increased activity of τ-GT observed during drug administration subsequently increases GSH synthesis from its precursors. Cadmium reportedly interacts with the thiol bearing enzymes of the cell membrane and renders membrane permeability.[53] During such membrane disturbances, a thiol/disulfide exchange has been observed in the presence of a dihydrolipoic acid/lipoic acid redox couple. Lipoic acid may thereby stabilize membraneous enzymes and contribute to the amelioration of membrane integrity in cadmium-challenged cells.[54]

The concurrent presence of glutathione and lipoate in the cell may be responsible for the regulation of intracellular level lipid peroxidation observed during metal exposure. Lipoate operates nonenzymatically unlike GSH, which requires enzymes (GPX, G-6-PD, GR, GST, and τ-GT). The meta-vitamin, endowed with the favorable capacity to pass membranes, can become accommodated both in a hydrophilic and hydrophobic environment and can thus be shifted to sites where reduced –SH compounds are actually required.

Cadmium preferentially binds to the membrane-bound sulfhydryl groups and disrupts the redox state of the cell. This concept proves true in the in vitro study (*Figure 2*), where profound free radical production is observed in the presence of the metal, which eventually leads to cellular disintegration.

Ochi et al.[55] stated that intracellular GSH functions in protection against cadmium toxicity and that this tripeptide provides a first line of defense against cadmium before induction of metallothionein synthesis occurs. Cysteine has established its protection against cadmium toxicity in rat brain microsomes[56] and kidney cells.[57] Bruggeman et al.[57] hypothesized that complex formation between cadmium and GSH or cysteine is reversible, and the toxicity found in the presence of GSH and cysteine at higher concentrations or larger exposure times may very well be due to cadmium ions released from the extracellular complex with these thiols. A similar picture is observed in the present study, where cysteine exhibits a pro-oxidant effect at higher concentrations. This observation confirms an earlier report by Murphy et al.[58]

The use of DTT in brain[59] and liver[60] establishes its role in protecting cells against cadmium intoxication. DTT is a permeable thiol and rendered better protection than GSH, a nonpermeable thiol, in this study. Muller[54] reported the role of lipoic acid in reducing cadmium toxicity in isolated hepatocytes. Comparatively, lipoate rendered better protection at low concentrations, and as the concentration increased, its protection was equivalent to that of DTT.

It has long been established that chelating agents possessing sulfhydryl groups are most effective antidotes of heavy metal poisoning.[61] The number and the arrangement of –SH groups are the vital factors deciding the efficacy of the drugs.[62] Cherian[62] reported that all dithiol compounds containing vicinal –SH groups mobilized far more cadmium from hepatic metallothionein and more effectively increased biliary excretion of cadmium than the compounds with nonvicinal –SH groups or a single –SH group subject to their lipophilicity.

The protective effect of lipoate and DTT is mediated by direct chemical reactions, unlike GSH which requires enzymes. Haenen et al.[35] reported that lipoate and DTT delayed lipid peroxidation and protected protein thiols but cysteine did not. Nagasaka et al.[63] stated that under certain conditions DTT fails to inhibit lipid peroxidation. In contrast to other antioxidants, lipoate functions as a universal free radical quencher scavenging free radicals in the cytosol and in the hydrophobic membrane domains. Lipoate readily penetrates the cell membrane, reaching a high cellular concentration within 30 s.[64] Intracellularly, mitochondrial reduction of lipoate to dihydrolipoic acid by ketoacid dehydrogenases may constitute the source of physiologically important direct chain breaking antioxidants.[65]

Lipoate has a high singlet oxygen quenching constant which highlights its antioxidant property in comparison with other sulfur compounds.[66] The S-S bond of a five-membered ring structure is prone to a much faster reductive and/or nucleophilic attack than the open-chain analogs.[67] A smaller torsional angle in the cyclic structure, which results in a comparatively higher electron density of the disulfide bridge of lipoate, enhances its free radical scavenging effect.

In brief, this study has shown that acute cadmium exposure results in profound hepatotoxicity and mild renal toxicity. During lipoate supplementation, it acts as a "double-edged sword," in that it appears to interact directly with peroxyl radicals or indirectly affords protection by bolstering the antioxidants and antioxidizing enzyme systems via a cascade mechanism. Both the in vivo and in vitro data reported here clearly indicate the antioxidant property of DL α-lipoic acid against cadmium-induced oxidative stress.

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