Prevention of chromate induced oxidative stress by alpha-lipoic acid*

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The parenteral administration of alpha-lipoic acid (LA) protected against chromate induced oxidative stress in mouse liver. A shift in Cr induced pro-oxidant state to antioxidant-state by LA was noteworthy. The degree of protection was significant and similar in different LA administration regimens (prior-, co- and post- parenteral Cr exposure) explored. An improved status of the tissue antioxidants by LA appeared to be the mechanism of mitigation. The results are of chemopreventive value and suggest a possible alternative to ascorbic acid for abrogation of Cr toxicity.

Keywords: Chemoprevention, Lipoic acid, Oxidative stress, Potassium dichromate

Chromate compounds are toxic and carcinogenic to humans. A variety of adverse health effects viz. dermatitis, ulcer formation; nasal-septum perforation, inflammation and carcinoma of larynx and carcinoma of paranasal sinuses etc are reported following a chronic exposure to Cr in humans. Oxidative-stress plays an important role in Cr toxicity. Reactive oxygen species (ROS) are formed during Cr VI metabolism and these result in a spectrum of cellular or genetic lesions in tissues. ROS are formed through Fenton/Haber-Weiss chemistry and generate DNA damaging OH⁺ (ref, 2). Cellular macromolecules viz. lipid, protein and DNA are the targets. ROS modify DNA bases and protein carboxyl groups, depurate nucleic acid or induce thymine glycol resulting in Cr induced strand scissions, genotoxicity and carcinogenesis.

In studies aimed at abrogation of Cr toxicity, oxidative stress has been targeted for chemopreventive/therapeutic research and ROS-scavengers have been explored. Various antioxidants (e.g. ascorbic acid, vitamin E, melatonin, pyrroolidine-dithiocarbamate and epilgallo-catechin-3-gallate) have been evaluated and their chemopreventive effect confirmed both in vitro and in vivo.

Alpha-lipoic acid (LA), a naturally occurring metabolic antioxidant, has never been studied in this context. The natural sources of LA are spinach, broccoli and tomato etc. It is a vicinal-dithiol containing compound and is different from other antioxidants studied so far. LA contains an intramolecular disulfide bond, which in reduced state makes vicinal dithiols. It is reduced to di-hydro-lipoic acid (DHLA) enzymatical ly. However, due to low redox potential, it is re-oxidized to LA by interactions with oxidants. Of late, LA/DHLA couple has gained considerable attention as biological thiol antioxidant and is being explored for its therapeutic implications in diseases associated with oxidative stress. At sub-millimolar concentration, LA/DHLA couple scavenges a variety of ROS and transition metals directly.

In the present study, LA has been evaluated for its potential to protect against chromate induced oxidative stress in Swiss albino mice. The measure of oxidative stress was the status of oxidative damage and the level of antioxidant enzymes in mouse liver. Results are of chemopreventive value for abrogation of Cr toxicity.

Materials and Methods
Potassium dichromate, LA, 5, 5-dithiobis-2-nitrobenzoic acid (DTNB), glutathione (GSH), malondialdehyde (MDA), 2-thiobarbituric acid (TBA), 2,4-dinitrophenyl hydrazine (DNPH), epinephrine, NADPH, oxidized-glutathione (GSSG) were purchased from Sigma, USA. Other chemicals used in the assays were of analytical grade and procured locally.
Swiss male albino mice (20-25 g), maintained on standard pellet diet and water ad libitum, were used. Animals were randomized in various groups (four animals in each) and housed at 25°C with 12 hr L: D cycle. Cr and LA were administered as per the treatment schedule (Fig. 1). Potassium dichromate (Cr VI) was administered orally in drinking water at the dose of 25 mg/kg body weight. LA was administered i.p at the dose of 25 mg/kg body weight in ethanol: saline (1:9) mixture.

Animals were sacrificed 24 h after the last treatment by cervical dislocation. Liver was excised and homogenized in ice cold 0.15 M KCl-O.1 M phosphate buffer pH 7.4. A 10% homogenate was used for biochemical assays. Enzyme and biochemical assays were done using S-10 fraction (10,000 g, 10 min, 4°C) of liver homogenate, unless and otherwise specified.

Biochemical assays

**GSH—**Liver homogenate was de-proteinized by 10% TCA and the extract (~100 mg tissue) was mixed with 0.1 M phosphate buffer pH 8.0 and 0.1 M DTNB in a final volume of 3 ml. A412 was recorded. GSH was quantified using a standard curve drawn with GSH and DTNB. Mean values were expressed as μmol GSH/g tissue.

**Lipid peroxidation (MDA contents)—**The rate of lipid peroxidation was determined by estimating MDA content with TBA. A535 was read for the MDA-TBA colored complex, which was quantified using molar extinction coefficient of 1.56×10^5 M^-1 cm^-1. MDA content was expressed as μmol malondialdehyde formed hr^-1 g^-1 tissue.

**Protein carbonyl group—**The protein carbonyl groups were allowed to react with di-nitrophenyl hydrazine. The colored complex was measured at 380 nm in 6M guanidine hydrochloride after de-proteinization. The protein carbonyl content was calculated using molar coefficient of 22,000 M^-1 cm^-1 and expressed as nmol mg^-1 protein.

**Protein—**Protein was assayed by Lowry’s method using bovine serum albumin as a standard.

**Superoxide dismutase—**Enzyme activity was determined by following the inhibition of auto-catalyzed adrenochrome formation in presence of liver homogenate (fractionated with chloroform and ethanol, containing ~12 mg protein/ml) at 480 nm. The reaction medium contained 45 mM epinephrine and 30 mM carbonate-bicarbonate buffer (pH 10.2). The activity was expressed as amount of protein (required to inhibit epinephrine oxidation to 50% of its control value) equal to 1 Unit.

**GSH peroxidase—**The assay was based on the reaction of exogenously added GSH with H2O2 and estimation of the unutilized GSH using Ellman’s reagent. The reaction medium consisted of 100 mM Tris buffer (pH 7.4), 2 mM sodium azide, 10 mM H2O2 and S-10 fraction (1 mg protein). The enzyme activity was expressed as nmol GSH oxidized.min^-1 mg^-1 protein (ref. 10).

**Catalase—**The activity was determined by method of Sinha. The method is based on the fact that the dichromate in acetic acid is reduced to chromic acetate when heated in presence of unutilized H2O2 and measured at 570 nm. Standard curve was drawn using 20-50 μmol H2O2. The reaction mixture consisted of 10 mM phosphate buffer pH 7, 20 mM H2O2 and S-10 fraction (2 mg protein). The activity has been expressed as the μmol of H2O2 utilized min^-1 mg^-1 protein.

**GSSG reductase—**The enzyme activity was measured in S-10 fraction by following the oxidation of NADPH in GSSG reduction at 340 nm. The reaction mixture consisted of 67 mM phosphate buffer pH 6.6, 7.5 mM oxidized-glutathione, NADPH and S-10 fraction (0.5 mg protein). The enzyme activity was expressed as nmol NADPH oxidized min^-1 mg^-1 protein.
Results

LA administration alleviated the chromate induced oxidative stress in mouse liver (Table 1). It mitigated oxidative damage and restored the normal levels of antioxidants in tissue significantly. The substantial increase in MDA contents and protein carbonyl groups, following exposure to Cr, reversed to normal levels (Table 1). Similarly, Cr-induced changes in tissue GSH content and activities of SOD, Catalase and GSSG-reductase also reversed to normal (Table 1). Three regimens of parenteral LA administration viz. pre-, co- and post to oral administration of the toxicant were studied for their protective efficacy. A similar degree of protection was observed in all the regimens.

Oral administration of potassium dichromate (Cr VI) induced oxidative stress in mouse liver (Table 1). Oxidative damage was evident from the significant increase in tissue MDA content and protein carbonyl groups (Table 1). The Cr influenced status of antioxidants was evident from 2X increase in hepatic SOD and Catalase activities, and 30% decrease in oxidized-glutathione reductase activity and GSH content. Glutathione peroxidase activity remained unchanged (data not shown).

Administration (ip) of LA or the vehicle (ethanol: saline mixture) alone did not influence oxidative stress in the liver tissue (Table 1).

Discussion

Various studies in animals or in cell culture have shown mitigation of oxidative stress by LA. Chemoprevention of lead toxicity by LA has been reported in CHO cells10. Alleviation of oxidative injury and restoration of antioxidant levels by LA has been shown in rat cardiac tissue21. LA mediated abatement of ROS-mediated cisplatin-induced nephrotoxicity22 and re-perfusion injury following cerebral ischemia in rat are also documented23. The present study has also demonstrated a remarkable protection against Cr induced oxidative stress by LA.

Several mechanisms are proposed for mitigating effect of LA. These include its anti-oxidant activity in vivo, redox interactions with tissue antioxidants, metal chelating property and most importantly its ability to increase GSH content, the first line of defense, in tissues9. Elevation in GSH levels following LA administration both in vivo and in vitro has been reported24-26. Studies in human or murine cells also confirmed this observation10,27. Increase in tissue GSH concentration following LA administration is attributed to increase in its biosynthesis. The

<table>
<thead>
<tr>
<th>Group &amp; Treatment</th>
<th>Level of oxidative damage</th>
<th>Level of antioxidants</th>
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<tbody>
<tr>
<td></td>
<td>MDA (μmol/h/g tissue)</td>
<td>Protein Carbonyl Group (nmol/mg protein)</td>
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<tr>
<td>-------------------</td>
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</tr>
<tr>
<td>Gr. 1</td>
<td>No treatment</td>
<td>0.44±0.09</td>
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<tr>
<td>Gr. 2</td>
<td>Cr VI</td>
<td>0.57±0.02</td>
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<td></td>
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</tr>
<tr>
<td>Gr. 3</td>
<td>Cr + LA Pre-treatment</td>
<td>0.45±0.05</td>
</tr>
<tr>
<td>Gr. 4</td>
<td>LA Pre-treatment</td>
<td>0.40±0.01</td>
</tr>
<tr>
<td>Gr. 5</td>
<td>Vehicle (pre-treatment)</td>
<td>0.42±0.02</td>
</tr>
<tr>
<td>Gr. 6</td>
<td>Cr and LA Co-treatment</td>
<td>0.33±0.10</td>
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<tr>
<td>Gr. 7</td>
<td>Cr + LA Post-treatment</td>
<td>0.30±0.05</td>
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<tr>
<td>Gr. 8</td>
<td>Co- &amp; Post-treatment (LA)</td>
<td>0.39±0.01</td>
</tr>
<tr>
<td>Gr. 9</td>
<td>Vehicle (co and post-treatment)</td>
<td>0.42±0.02</td>
</tr>
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P values: "<0.001 compared to the corresponding value in group 1; "<0.001 compared to the corresponding value in group 2; "<0.05 compared to corresponding value in group 1; "<0.01 compared to corresponding value in group 1; "≤0.05 compared to corresponding value in group 2; "<0.01 compared to corresponding value in group 2.
effectiveness of LA to protect against Cr induced oxidative stress, in our study, could also be an endresult of these mechanisms. Mattagajasingh & Misra have shown that protective effects of LA may not be attributable to its metal chelating property.28

Chromate compounds cause severe irritation, corrosion and ulcer formation in tissues. These target both skin as well as the respiratory tract. High risks of skin ulcer and lung cancer among workers handling chromate compounds are reported. Cr VI compounds are used commonly and in a variety of industrial activities e.g. chromeplating, welding, painting, metal finishes, steel manufacturing, alloy, cast iron and wood treatment. It is a proven toxin, mutagen and carcinogen. In literature, DNA damage, apoptotic cell death and altered gene expression following Cr VI treatment is also reported.29 Such biochemical changes result in tissue damage, which ultimately contributes to toxicity and carcinogenicity of Cr VI.30

Sufficient evidence is available for its carcinogenic potential in human.30

The administration of ascorbic acid has been advocated for use in systemic Cr poisoning.31 However, there is no confirmed clinical report on the subject. In animal studies, parenteral administration of ascorbic acid has been shown to reduce the mortality. Nonetheless, demerit of ascorbic-acid use is also described. The administration of parenteral ascorbic acid after parenteral chromate either failed to protect against the renal damage or aggravated the toxicity. Ascorbic acid is a metabolic precursor of oxalate. The administration of ascorbic acid in high doses could lead to acute oxalate nephropathy, particularly in the presence of renal failure.31

In conclusion, the present study has demonstrated that parenteral administration of LA protected against Cr induced oxidative stress. It advocates the potential of LA in therapeutics of diseases associated with oxidative stress and in particular the abrogation of Cr-carcinogenesis. More studies are needed to suggest the chemopreventive/therapeutic potential of LA. Nevertheless, the nutritional/therapeutic use of alpha-lipoic acid in prevention or in management of systemic Cr poisoning or cancer is appealing.

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