Effect of vitamin E and C supplementation on oxidative damage and total antioxidant capacity in lead-exposed workers

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\textbf{A B S T R A C T}

The molecular response of the antioxidant system and the effects of antioxidant supplementation against oxidative insult in lead-exposed workers has not been sufficiently studied. In this work, antioxidants (vitamin E 400 IU + vitamin C 1 g/daily) were supplemented for one year to 15 workers exposed to lead (73 µg of lead/dl of blood) and the results were compared with those on 19 non-lead exposed workers (6.7 µg of lead/dl). Lead intoxication was accompanied by a high oxidative damage and an increment in the erythrocyte antioxidant response due to increased activity of catalase and superoxide dismutase. Antioxidant supplemenations decreased significantly the oxidative damage as well as the total antioxidant capacity induced by lead intoxication with reduction of the antioxidant enzyme activities. We conclude that antioxidant supplementation is effective in reducing oxidative damage and induces modifications in the physiopathological status of the antioxidant response in lead-exposed workers.

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\textsuperscript{b} Bleecker et al., 1995; Calderón-Salinas et al., 1996; Ehrlich et al., 1998; Levin and Goldberg, 2000; Patrick, 2006a,b; Quintanar-Escorza et al., 2007). Exposure to lead can result in significant alterations in various organs, the hematological system being an important target (Garçon et al., 2004; Gurer-Orhan et al., 2004). Erythrocytes have high affinity for lead and typically contain the majority of the lead found in

1. Introduction

Lead is one of the metals most commonly used in industry; its toxicity is a public health problem due mainly to its environmental persistence, inadequate labor security conditions and low lead excretion in lead-exposed workers (Nilsson et al., 1991; Bleecker et al., 1995; Calderón-Salinas et al., 1996; Ehrlich et al., 1998; Levin and Goldberg, 2000; Patrick, 2006a,b; Quintanar-Escorza et al., 2007). Exposure to lead can result in significant alterations in various organs, the hematological system being an important target (Garçon et al., 2004; Gurer-Orhan et al., 2004). Erythrocytes have high affinity for lead and typically contain the majority of the lead found in
the blood stream (Leggett, 1993). Several studies suggest that lead toxicity involves oxidative damage (Ercal et al., 2001; Patrick, 2006a,b; Rendón-Ramírez et al., 2007; Flajs et al., 2009; Hamed et al. 2010; Quintanar-Escoza et al., 2010; Jomova and Valko, 2011). Lead intoxication induces inhibition of hem synthesis and accumulation of pro-oxidant and autoxidizability substrates as δ-aminolevulinic acid (δ-ALA), free hem groups and free iron ions in erythrocytes (Ercal et al., 2001; Rendón-Ramírez et al., 2007); in turn, accumulation of oxidizable substrates can result in superoxide and hydrogen peroxide formation (Patrick, 2006b; Flora et al., 2008). Additionally, under lead poisoning, high oxygen concentrations, autoxidizability of hemoglobin and vulnerable membrane components to lipid peroxidation make erythrocytes extremely sensitive to oxidative damage (Cimen, 2008; Ercal et al., 2001; Rendón-Ramírez et al., 2007; Jomova and Valko, 2011).

Organisms have diverse and highly efficient antioxidant mechanisms, but few and divergent results have been found in antioxidant responses to lead exposure; differences seem to depend on the experimental model, concentration of lead and exposure time (Kasperczyk et al., 2004a,b; Mishra and Acharva, 2004; Haleagrahara et al., 2010; Hamed et al., 2010). Different insults induce complex responses in antioxidant enzymatic systems (Dröge, 2002; Valko et al., 2007; Auten and Davis, 2009), and little has been done to evaluate the effects of exogenous antioxidant treatments in lead intoxicated workers. Non-enzymatic exogenous antioxidants such as vitamins C, E, and B6, taurine, methionine, selenium, zinc, alpha-lipoic acid or N-acetylcysteine have been used as antioxidant treatment in animal models and shown to lower reactive oxygen species (ROS) that generate cellular damage in different cell types of lead exposed rats (Simon and Hudes, 1999; Flora et al., 2003; Shalan et al., 2005; Patrick, 2006b; Rendón-Ramírez et al., 2007; Wang et al., 2007; Flora et al., 2008). Simultaneous administration of vitamins E – a lipid-soluble non-enzymatic antioxidant and C – an aqueous antioxidant – is particularly interesting since it produces a more effective regeneration of the enzymatic system reducing the oxidation manifestation (Traber and Stevens, 2011). Other studies have demonstrated the synergistic protective action of vitamins C and E against genotoxicity, spermatozoona and hippocampus damage (Mishra and Acharva, 2004; Li et al., 2008). In lead-exposed workers, treatment with vitamin C, vitamin E, beta-carotene, selenium, zinc and chromium prevented the inhibition of δ-aminolevulinic acid dehydratase (δ-ALAD) activity in blood (Tandon et al., 2001), increased the total antioxidant capacity (TAC) as well as superoxide dismutase (SOD) and glutathione peroxidase (GPx) activity (Machartová et al., 2000), while abating the toxic effects associated with inhibition of the calcium pump (Abam et al., 2008). These results suggest investigating the effects of antioxidant supplements in lead-exposed workers. In this work we study the antioxidant benefits of vitamin E and C supplementation as well its effects on the antioxidant enzymatic system in these subjects.

## 2. Materials and methods

A group of lead exposed workers was supplied with vitamins C and E for one year. The effects on blood lead concentration, δ-ALAD, SOD, glutathione reductase (GRx) and GPx activities, as well as on lipid peroxidation and erythrocyte TAC was determined. In healthy people, both significant effects and not effects at all of these vitamins on the antioxidant systems have been reported (Traber and Stevens, 2011). To clarify matters, a control group of non-exposed workers was subjected to the same treatment for comparison.

The studied group consisted of male workers occupationally exposed to lead in a recycling battery factory, particularly to dust of a mixture of lead oxides; only fifteen workers, with exposure time ranged from 5 to 10 years (mean = 6.8 years), out of twenty subjected to exposure conditions, accepted to participate and concluded the treatment for this study. We remark the difficulty of finding a plant industry willing to allow its employees being studied, much less to be treated in its premises. Lead exposed workers did not present renal damage albuminuria or signs or a history of severe neurological damage (seizures or severe disturbances of balance), nor did they receive chelation treatment during this study.

As a control group we used 19 (initially 20, one dropped) male workers clinically healthy and without a history of occupational lead exposure. They all showed normal parameters in blood and urine. None of them presented a history of, or current, physical symptoms of serious neurological, cardiovascular, renal, hepatic, endocrine, metabolic or gastrointestinal disease, nor were they subjected to previous pharmacological treatment. Both groups had similar age and social position score. Non-lead exposed workers ranged from 29 to 42 years old (mean = 34.5), whereas the exposed group was from 25 to 41 years old (mean = 32.9). Ranges of social score (Hollingshed, 2011) for non-lead exposed and lead exposed groups were from 7.0 to 10.5 (mean = 9.2) and from 7.8 to 9.6 (mean = 8.6), respectively. All subjects provided written, informed consent, and participation was voluntary. The Medical Center-Bajío, IMSS-Mexico, approved the study.

Each subject received 400 IU Alpha Tocopherol Acetate vitamin E (capsules) and 1 g tablet vitamin C per day during the morning for a period of 12 months. These doses were used in previous studies for different illness associated with oxidative damage (Plantinga et al., 2007; Castillo et al., 2011), and no unwanted side effects are mentioned in the literature. We remark that these dosages are within the FDA recommended dosage for supplementation (maximum of 1000 mg of vitamin C and 1500 IU for alpha tocopherol). No worker was reported to have missed his takings, and no worker presented lateral effects that affected his participation in this study or that would lead to suspend the use of these antioxidants. Patients were instructed to take them with breakfast at their workplace. Also, used dosages are not known to produce pro-oxidant effects, nor did we find such effects in subjects of the non-exposed group. Besides vitamin supplementation, better safety procedures and hygienic and dietetic practices were promoted for all workers at this site. The use of masks and uniforms to reduce lead exposure, as well as a diet with higher content of fiber and reduced calories were recommended to comply with ethics procedures, even though such practices might reduce lead exposure and its effects. Venous blood was obtained by venous puncture in heparinized tubes. Each sample was centrifuged (700 × g, 10 min) and plasma was collected; white cells were carefully removed by aspiration to avoid loss.
of erythrocytes. To obtain serum, venous blood was collected in non-heparinized tubes and centrifuged as above.

2.1. Blood lead concentration

Lead concentration in blood was determined with a Lead Analyzer, model 3010B (ESA), as previously described (Quintanar-Escorza et al., 2007). Concentration is reported in micrograms per deciliter of blood. Lead determinations were run in triplicates. The standard curves were calculated with the method of addition to minimize the matrix effect on the absorption peak (recovery of 85–113%). Precision of analyses was around 87–104% (using ESA Hi and Lo calibrators) and the detection limit set at 1 μg/dl.

2.2. δ-ALAD activity

δ-ALAD activity in erythrocytes was determined spectrophotometrically (US/VIS DU 650) (Beckman) and expressed as nanomol of porphobilinogen (PBG) per milliliter of erythrocytes per hour (nmol/ml/h) (Rendón-Ramírez et al., 2007).

2.3. Vitamins E and C

Serum tocoferol and ascorbate were determined by high-performance liquid chromatography in a single chromatographic run with an internal standard (Merzouk et al., 2003; Lenton et al., 2003).

2.4. Lipid peroxidation

The amount of lipid peroxidation in erythrocytes was estimated as reported in Quintanar-Escorza et al. (2007) by measuring the thiobarbituric acid-reactive substances (TBARS). The absorbance was measured at 532 nm and 600 nm using a spectrophotometer US/VIS DU 650 (Beckman). TBARS are expressed as nmol of malondialdehyde equivalents per ml of erythrocyte (Quintanar-Escorza et al., 2010).

2.5. Erythrocytes TAC

The antioxidant capacity was measured by the formation of ROS in red blood cell samples. ROS levels were determined by fluorescence, as described in Oyama et al. (1994). Briefly, DCF-DA was used as a detector of ROS by flow cytometry. This dye is not fluorescent in reduced state, but it becomes fluorescent once cellular oxidation and removal of acetate groups by cellular esterases take place (Karlsson et al., 2010). Samples were excited at 480 nm and the emission detected at 520 nm. The intensity of fluorescence in presence of fluorophore can be correlated with the cellular content of free radicals. Fluorescence is measured in arbitrary units per 500,000 cells and expressed as the inverse of these units (1/FUAV × 105 cells).

2.6. CAT activity assays

CAT activity was determined in erythrocytes by spectrophotometry and is expressed in U/mg protein. This method measures the exponential disappearance of H2O2 (3 mM) at 240 nm in presence of cellular homogenates (5 Hb/dl) with addition of buffer phosphates pH 7.3. The decay of H2O2 depends on the CAT activity (Aebi, 1984). The hemoglobin concentration (Hb) was estimated using the reagent described by Van Kampen and Zijlstra (1961).

2.7. SOD activity assays

SOD in erythrocytes inhibits the production of superoxide radicals. The xanthine–xanthine oxidase system, in presence of oxygen, induces the formation of superoxide radicals; this radical reduces the nitro blue tetrazolium and gives rise to a colored compound. Since SOD inhibits this reaction, its activity is proportional to the degree of inhibition of nitro blue tetrazolium reduction to superoxide (Sun et al., 1988). Results are expressed in units of SOD/g of hemoglobin.

2.8. GRx activity assays

The activity of the enzyme GRx in erythrocytes is based on the formation of reduced glutathione from glutathione disulfide in the presence of the enzyme. Glutathione disulfide formed in one minute reacts with NADPH and the product is measured spectrophotometrically at 340 nm and 37 °C, (UV/VIS modelo 30 Beckman-USA). Results of activity of all enzymes were expressed in U (μM/min)/mg of Hb (Vives-Bauza et al., 2007).

2.9. GPx activity assays

The activity of the enzyme GPx in erythrocytes is based on the formation of glutathione disulfide from reduced glutathione in the presence of the enzyme. Enzyme activity was measured by modifying a procedure of Paglia and Valentine (1967). Solution A consisted of 50 mM potassium phosphate buffer (pH 7), 1 mM EDTA, 1 mM NaN3, 0.2 mM NADPH, 1 IU/ml GS-GSSG-reductase, 1 mM GSH, 1.5 mM cumene hydroperoxide or 0.25 mM H2O2 in a total volume of 1 ml. Erythrocytes lysated (0.1 ml) was added to 0.8 ml of Solution A and allowed to incubate 5 min at room temperature before initiation of the reaction by the addition of 0.1 ml peroxide solution. Absorbance at 340 nm was recorded for 5 min and the activity was calculated from the slope of these lines as μmoles NADPH oxidized per minute.

2.10. Statistical analysis

Comparisons between non-lead and lead exposed groups were done through two-tailed unpaired t-tests. To compare each group with itself before and after the treatment, we used two-tailed paired t-tests; in this case, proportions, instead of mean differences, were estimated. In all cases the level of significance was established at P < 0.05. 95% CI were computed to evaluate the biological significance besides the statistical significance. For these tests we used GraphPad Prism version 5.03 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com. Other, supplementary statistics methods are described where used.
Table 1 – Number of individuals presenting clinical symptoms and signs associated to lead intoxication in lead and non-lead exposed workers.

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Non-lead exposed (n = 19)</th>
<th>Lead exposed (n = 15)</th>
<th>% in lead exposed (95% confidence interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dizziness</td>
<td>4</td>
<td>7†</td>
<td>46.6 (21–73)</td>
</tr>
<tr>
<td>Headache</td>
<td>3</td>
<td>11†</td>
<td>73.3 (45–92)</td>
</tr>
<tr>
<td>Paresthesia</td>
<td>1</td>
<td>6†</td>
<td>40.0 (16–68)</td>
</tr>
<tr>
<td>Paresis</td>
<td>0</td>
<td>3†</td>
<td>20.0 (4–48)</td>
</tr>
<tr>
<td>Abdominal colic</td>
<td>2</td>
<td>5†</td>
<td>33.3 (12–62)</td>
</tr>
<tr>
<td>Myalgia</td>
<td>2</td>
<td>8†</td>
<td>53.3 (27–79)</td>
</tr>
<tr>
<td>Motor coordination alteration</td>
<td>1</td>
<td>14</td>
<td>93.3 (68–100)</td>
</tr>
</tbody>
</table>

* Significant difference ($p < 0.05$) between lead and non-lead exposed workers, according to $X^2$ test. In the third column, percent (lower 95% CI – upper 95% CI) of individuals with a sign or symptom in the lead-exposed group, assuming binomial distribution, ‘exact method’, computed following (Blair and Taylor, 2008).

Table 2 – Biological indices (mean ± SD) of lead intoxication in blood of non-lead exposed and lead exposed workers.

<table>
<thead>
<tr>
<th>Index</th>
<th>Non-lead exposed (n = 19)</th>
<th>Lead exposed (n = 15)</th>
<th>Difference between means ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>PbB (µg/dl)</td>
<td>6.7 ± 2.2</td>
<td>73 ± 11</td>
<td>65.8 ± 2.5†</td>
</tr>
<tr>
<td>δ-ALAD activity (nmol PBG/h/ml)</td>
<td>683 ± 61</td>
<td>138 ± 34</td>
<td>545 ± 17.6†</td>
</tr>
</tbody>
</table>

*** $p < 0.0001$.

3. Results

3.1. Conditions before the treatment

Clinical symptoms and signs associated with chronic lead intoxication as dizziness, headache, paresthesia, paresis, abdominal colic, myalgia and motor coordination alteration were frequently found in the lead-exposed workers. Percentage of people with these symptoms was significantly higher than that of the control group (Table 1). Frequency from zero to seven symptoms and signs for both lead exposed and non-lead exposed workers follow a binomial distribution (Fig. 1). For non-lead exposed workers, the probability of presenting any sign or symptom ranges from 0.05 to 0.16, whereas for exposed workers the probability of showing any sign or symptom goes from 0.38 to 0.64 ($\alpha = 0.05$, df = 6, Pearson’s Chi-square test). Blood lead concentration was higher in lead exposed with respect to non-lead exposed workers ($P < 0.0001$) indicative of a chronic and significant lead exposure. Erythrocyte δ-ALAD activity was significantly lower in lead exposed than in non-lead-exposed workers ($P < 0.0001$) also suggesting a severe chronic lead intoxication (Table 2). The oxidative damage (lipid peroxidation) and TAC in erythrocytes were higher in lead exposed with respect to non-lead exposed workers ($P < 0.0001$ and $P < 0.0001$, respectively) denoting an oxidative stress characterized by an increase in the antioxidant response, but not enough to contain oxidative insult (Table 3). A major enzymatic antioxidant response was present in lead exposed workers, as both CAT and SOD activities in erythrocytes were much higher in this group than in the control group ($P < 0.0001$ and $P < 0.0001$, respectively). However, lower

Table 3 – Lipid peroxidation and antioxidant defense system status at the beginning of vitamin E/vitamin C supplementation. Thiobarbituric acid reactive species concentration (TBARS), total antioxidant capacity (TAC), CAT activity, SOD activity, GPx activity, GRx activity and serum concentration of vitamins E and C, in blood of non-lead exposed and lead exposed workers (mean ± SD).

<table>
<thead>
<tr>
<th>Index</th>
<th>Non-lead exposed (n = 19)</th>
<th>Lead exposed (n = 15)</th>
<th>Difference between means ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBARS (nmol MDA/ml PG)</td>
<td>0.7 ± 0.2</td>
<td>1.3 ± 0.4</td>
<td>0.57 ± 0.01†</td>
</tr>
<tr>
<td>TAC (U/[FUΑ/5 × 10⁹ cells])</td>
<td>66 ± 3.7</td>
<td>110 ± 8.1</td>
<td>44.4 ± 2.08†</td>
</tr>
<tr>
<td>Enzymatic activity (U/g Hb)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CAT</td>
<td>11 ± 1.5</td>
<td>37 ± 7.5†</td>
<td>26.0 ± 1.8†</td>
</tr>
<tr>
<td>SOD</td>
<td>209 ± 45</td>
<td>534 ± 55†</td>
<td>324 ± 17.1†</td>
</tr>
<tr>
<td>GPx</td>
<td>12 ± 1.8</td>
<td>10 ± 2.2</td>
<td>2.0 ± 0.7†</td>
</tr>
<tr>
<td>GRx</td>
<td>9.7 ± 2.2</td>
<td>3.1 ± 1.2†</td>
<td>6.6 ± 0.6†</td>
</tr>
<tr>
<td>Serum concentration (µmol/l)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin E</td>
<td>24 ± 5.4</td>
<td>22 ± 4.3</td>
<td>1.1 ± 1.7</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>69 ± 9.5</td>
<td>72 ± 7.8</td>
<td>3.7 ± 3.0</td>
</tr>
</tbody>
</table>

* 0.01 <$p$ < 0.05.
** 0.001 <$p$ < 0.01.
*** $p < 0.001$. 
activity of GRx in erythrocytes was found in lead exposed as compared to non-lead exposed workers \( (P < 0.0001) \), indicating a low antioxidant response at the glutathione system (Table 3). Erythrocytes GPx activity (95% CI from 0.6149 to 3.441) and serum vitamins E and C concentrations were not different between lead exposed and non-exposed workers (Table 4).

3.2. Effects of vitamins C and E supplementation

Administration of Vitamins E and C for 12 months did not induce a reduction in blood lead concentration of both worker groups (Table 4); clinical symptoms and signs of lead-exposed workers were not reduced either (Table 4). Although it is not possible to state an increment in GPx and GRx activities (Table 5), δ-ALAD activity increased significantly, twice as much its initial value, in lead-exposed workers (Table 4). Lipid peroxidation was reduced to values between 47.1 and 69.4% (95% CI) in lead-exposed workers after supplementation with antioxidant vitamins, attaining values non-statistically different from those of the non-lead exposed workers \( (P = 0.2119) \) (Fig. 1a). Additionally, TAC was reduced to values between 58.9 to 67.7% (95% CI) in lead-exposed workers after supplementation with vitamins E and C, restoring the TAC level up to a similar value of the non-lead exposed workers \( (P = 0.1298) \) (Fig. 1b). CAT and SOD activities decreased to values between 33.8% and 48.9% (95% CI), and from 44.2 to 53.2% (95% CI), respectively, after treatment in lead-exposed workers. Despite this reduction, the activity of CAT was still between 31.8% and 79.3% higher in lead exposed than in non-lead exposed individuals. SOD activity became after treatment statistically non different between lead and non-exposed groups \( (P = 0.0921) \) (Fig. 2). Finally, although in both groups vitamins C and E increased significantly their concentrations, non-led exposed workers were sensibly more affected: between 44 and 69% for vitamin C, and between 101 and 153% for vitamin E (95% CI) (Table 5).

4. Discussion

Lead exposed workers presented very high blood lead concentration and δ-ALAD activity severely inhibited, which indicates that these workers had been extensively exposed to lead. Additionally, they showed clinical symptoms and signs frequently associated to lead intoxication (Calderón-Salinas et al., 1996; Levin and Goldberg, 2000; Patrick, 2006a; Quintanar-Escorza et al., 2007). They also presented elevated oxidative damage, similar to that described in other lead exposure populations (Gurer-Orhan et al., 2004; Quintanar-Escorza et al., 2007; Costa et al., 1997; Flajs et al., 2009) (Fig. 3).

The lead-exposed group showed an adaptive response to oxidative insult – high TAC – due to lead intoxication;
Table 4 – Effects of vitamin E/vitamin C supplementation on levels of biological indices of lead intoxication in blood of non-lead exposed and lead exposed workers. Blood lead concentration (PbB) and α-aminolevulinic acid dehydratase activity (δ-ALAD), before and after supplementation (mean ± SD).

<table>
<thead>
<tr>
<th></th>
<th>Non-lead exposed (n = 19)</th>
<th>Lead exposed (n = 15)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td>PbB (µg/dl)</td>
<td>6.7 ± 2.2</td>
<td>7.1 ± 1.6</td>
</tr>
<tr>
<td>δ-ALAD activity</td>
<td>683 ± 61</td>
<td>696 ± 54</td>
</tr>
</tbody>
</table>

* 0.01 < p < 0.05.
** p < 0.001.

however, this antioxidant response was insufficient to abate severe oxidative damage. In some oxidative stress conditions (systemic sclerosis and physical fitness, for example) the increment in oxidative damage is accompanied of increments in antioxidant global response (Ogawa et al., 2011; Traustadóttir et al., 2012). However, Haleagrarahra et al. (2010) found a significant increment of lipid hydroperoxide and protein carbonyl content, but a significant decrement of total antioxidants in bone marrow of rats after lead acetate exposure. Our results show that high TAC in lead-exposed workers depended on elevated CAT and SOD activities, since GPx activity and serum vitamin E and C concentrations were not different – GRx activity was in fact reduced – in lead-exposed workers. Studies about lead influence on CAT activity have produced divergent results in different lead-exposure models. It has been proposed that lead intoxication induces and increment of synthesis of CAT in the erythroblastic system, but an inhibition of its activity has been found instead (Kasperczyk et al., 2004a).

In some studies, erythrocytes in lead-exposed workers presented several times higher CAT activity above non-exposed ones (Gurer et al., 1999; Gurer and Ercal, 2000); in at least one study (Sugawara et al., 1991), a decrease in activity of the CAT was observed, while other authors found no influence of lead exposure on CAT activity (Monteiro et al., 1985). Our work found an increase in CAT activity indicating that it is part of the general antioxidant response induced by the oxidative insult. With respect to SOD activity in blood of lead intoxicated people, results of clinical research are divergent as well. Comparing lead exposed populations to non-lead exposed ones different authors have found lower, higher and similar SOD activities (Ribarov and Benov, 1981; Michiels et al., 1994; Ye et al., 1999; Kasperczyk et al., 2004a). In our work, SOD activity in erythrocytes was higher in lead-exposed workers, which might be interpreted as a probable response to an increased production of superoxide anion radicals caused by lead exposure (Ribarov and Benov, 1981).

Several enzymes in antioxidant defense systems may protect against the imbalance between pro-oxidants and antioxidants, however, most of these enzymes contain sulphydryl groups at their active site and might become inactive due to a direct binding of lead to the sulphydryl group (Flora et al., 2008). The effect of the activation induced by the oxidative insult could overcome the inhibitor effect of lead. Augmentation in antioxidant activity of both enzymes, SOD and CAT, is frequently found in the presence of different oxidative insults, as observed in these lead-exposed workers. The antioxidant enzymes act in a cooperative or synergistic way to ensure global protection, for they frequently act on different radicals. Efficient protection is attained when there exists an appropriate balance between the activities of these enzymes (Michiels et al., 1994; Valko et al., 2006).

Vitamins C and E supplementation caused no reduction in the blood lead concentration, in spite of the improvement

Table 5 – Effect of vitamin E/vitamin C supplementation on the antioxidant status in blood of non-lead exposed and lead exposed workers: GPx activity, GRx activity, serum vitamin E concentration, serum vitamin C concentration in blood of lead and non-lead exposed workers. Values are mean ± SD.

<table>
<thead>
<tr>
<th></th>
<th>Non-lead exposed (n = 19)</th>
<th>Lead exposed (n = 15)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td>Serum vitamin E</td>
<td>24 ± 5.4</td>
<td>52 ± 5.0</td>
</tr>
<tr>
<td>(µmol/ml)</td>
<td></td>
<td></td>
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<tr>
<td>Serum vitamin C</td>
<td>69 ± 9.5</td>
<td>106 ± 9.8</td>
</tr>
<tr>
<td>(µmol/l)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GPx activity (U/g Hb)</td>
<td>12 ± 1.8</td>
<td>14 ± 1.3</td>
</tr>
<tr>
<td>GRx activity (U/g Hb)</td>
<td>9.7 ± 2.2</td>
<td>11 ± 2.1</td>
</tr>
</tbody>
</table>

* 0.01 < p < 0.05.
** p < 0.001.
*** p < 0.0001.
of safety and hygienic-dietetic lines for these workers. Some authors have observed a reduction in blood lead concentration, mostly in animal models, suggesting a chelate effect of vitamin C (Flora and Tandon, 1986; Patrick, 2006b; Flora et al., 2008). In contrast, another report stated that rats treated with ascorbic acid did not reduce the lead burden in liver, kidney, brain, and blood (Patra et al., 2001). Although it is biologically plausible that vitamin C may affect lead absorption, the effect is more obvious when lead is provided via drinking water at low concentrations combined with high supplementation of this vitamin (Flora et al., 2008). In this study the chelate effect of vitamin C was not observed; however, it is important to note that lead-exposed workers kept continually being exposed to lead via respiratory pathway during vitamin E and C supplementation. Supplementation with vitamin E and C was very effective in reducing lipid peroxidation in erythrocytes of lead-exposed workers; simultaneous administration of vitamin E and C generate a synergistic antioxidant effect through the enzymatic regeneration system in a redox cycle (Traber and Stevens, 2011). Our results show that the enhanced antioxidant activity was able to compensate the oxidative insult, reducing the oxidative damage and preventing macro-molecular alterations in lead-exposed workers. Studies in vivo and in vitro on the antioxidant effect of vitamin E and/or vitamin C in erythrocytes of lead intoxicated rats suggest that antioxidant supplementation prevents the inhibition of δ-aminolevulinic dehydratase activity and lipid peroxidation (Tandon et al., 2001; Patrick, 2006b; Rendón-Ramírez et al., 2007). Vitamin E alone or in combination with CaNa₂EDTA was found to be effective in decreasing the lead-induced lipid peroxide levels of liver and brain in rats (Patra et al., 2001). Although many investigators have shown that lead-induced oxidative damage and some antioxidant nutrients reduce lead toxicity, the mechanisms of dietary supplementation of antioxidants remain to be further clarified in lead exposed humans or animals (Hsu and Guo, 2002; Hsu et al., 1988). In this work, supplementation with antioxidants produced a significant increment of δ-ALAD activity; the direct effects of lead on the enzyme may explain the non-total recovery of δ-ALAD activity. Several models prove that δ-ALAD is a protein susceptible to oxidation. Our results suggest that vitamin E and C are effective to prevent protein oxidative damage while preserving their activity in lead-exposed workers. Remarkably, the TAC after vitamin E and C supplementation was reduced in lead-exposed workers. This TAC reduction was accompanied by lowering SOD and CAT enzymatic activity. One possible explanation is that supplemented antioxidants counteract the oxidative insult with the consequent relaxation of the antioxidant enzymatic system. This response may be useful in saving energy and reducing the expenditure of endogenous antioxidants metabolites. An increment in oxidative damage and antioxidant response with high activity of SOD and CAT in red cells was found in workers exposed to particles derived from a coal electric-power; after daily supplementation with vitamins C (500 mg) and E (800 mg) during six months, antioxidant enzymatic activity reached control levels (Possamai et al., 2010). A similar result was obtained in subjects exposed to the airborne contamination from coal dusts and solid residues of incineration health services (Wilhelm et al., 2010). In contrast, in animal studies (in vivo and in vitro) lead exposure induces a reduction in the TAC, and supplementation with exogenous antioxidants (epigallocatechin-3-gallate and eltingera elatior or green tea extracts) increased the enzymatic antioxidant activity and, as consequence, the TAC (Yin et al., 2008; Haleagrahara et al., 2010; Hamed et al., 2010). In humans chronically exposed, the organism may be generating a very complex adaptive process which causes an energy saving that reduces enzymatic activities when it uses exogenous antioxidants; besides, we found (see also Niki and Noguchi, 2004; Choi et al., 2004; Traber and Stevens, 2011) that the antioxidant used in this work is physiologically integrated in the antioxidant response and have a regeneration enzymatic system included in the redox cycle. Evidently, the antioxidant enzymatic system contributes primarily to the TAC. On the other hand, vitamins E and C are spent during their scavenger function, generating oxidized metabolites which are removed and eliminated (Choi et al., 2004; Niki and Noguchi, 2004); consistent with this observation, vitamins E and C concentrations showed no substantial increment in lead-exposed workers after vitamins supplementation took place. On the other hand, a quick distribution and redistribution in cells could cause that concentration of vitamin E and C in serum change negligibly after vitamins supplementation in lead-exposed workers (Traber and Stevens, 2011). The activity of GPx and GRx basically changed nothing after vitamins supplementation indicating that these antioxidant enzymes do no

Fig. 3 – Effects of vitamin E/vitamin C supplementation on the antioxidant status in erythrocytes of non-lead exposed and lead exposed workers: (A) CAT activity and (B) SOD activity, before (−) and after (□) supplementation. ***(p < 0.001) for paired test before and after supplementation.
participate in the reduction of the TAC in lead-exposed workers.

Different studies have shown that lead can induce oxidative stress and damage by producing reactive oxidative species such as superoxide anion (O$_2^-$), hydrogen peroxide (H$_2$O$_2$), hydroxyl radical (•OH) and singlet oxygen (O$_2^*$) (Patrick, 2006a), which might account be responsible of the damage found in this study. On the other hand, both alpha-tocopherol and vitamin C can remove superoxide anion and others free radicals (Traber and Stevens, 2011; Patrick, 2006b), which might accounts for the beneficial effects of its supplementation in lead-exposed workers: protecting enzymatic activity, reducing oxidative damage, improving the efficiency of antioxidant systems, allowing the reduction of antioxidant enzymatic activity and, as consequence, the TAC. Treatment with chelates and antioxidant vitamins are currently being carried out in lead-exposed workers with severe neurological damage.

**Conflict of interest statement**

The authors of this article expressed no conflict of interest.

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**Appendix A. Supplementary data**

Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.etap.2013.10.016.

**References**


Kasperczyk, S., Birkner, E., Kasperczyk, A., Zalejska-Fiolka, J., 2004a. Activity of superoxide dismutase and catalase in...


