Original Paper

The Effect of Ascorbic Acid Supplementation on the Blood Lead Levels of Smokers

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Background: The study subjects were 75 adult men (20 to 30 years of age), who smoked one pack of cigarettes per day (minimum) and had no clinical signs of ascorbic acid deficiency or lead toxicity. None had a history of industrial exposure to lead, and the blood-lead levels were anticipated to be below 1.45 μmol/L, the minimum blood level associated with toxicity symptoms.

Methods: The men were randomly assigned to three study groups of 25, and each group was provided a four-week supply of one level of daily ascorbic acid supplements (placebo, 200 mg or 1000 mg of ascorbic acid). We measured baseline and weekly serum and urine ascorbic-acid levels as well as blood and urine lead levels. The weekly group means and variations of the measured data were statistically compared by means of ANOVA and Pearson's correlation.

Results: The serum ascorbic-acid levels of the groups receiving ascorbic acid increased significantly after one week ($p \leq .001$). There was no effect of placebo or 200 mg ascorbic-acid supplementation on the blood or urine lead levels. However, there was a 81% decrease in blood-lead levels in the 1000 mg ascorbic acid group after one week of supplementation ($p \leq .001$).

Conclusions: Daily supplementation with 1000 mg of ascorbic acid results in a significant decrease of blood-lead levels associated with the general population. Ascorbic acid supplementation may provide an economical and convenient method of reducing blood-lead levels, possibly by reducing the intestinal absorption of lead.

INTRODUCTION

Lead toxicity was recognized in antiquity [1]. The metal is widely used in industry and disseminated via air, industrial pollution, agricultural technology and food processing [2]. Numerous studies have shown that 15 to 30% of lead exposure for the general population comes from inhalation and 70 to 85% from ingestion [3–6]. Other studies have demonstrated that cigarette smoking or secondary exposure to cigarette smoke results in an elevation of blood-lead levels [7–12]. Consequently, all individuals have a body burden of lead, whether they are exposed in urban, rural or occupational environments. The average whole-blood lead level of healthy adults in the United States has been reported to range between 0.72 and 1.93 μmol/L and is slightly higher in urban populations [13].

A study of the influence of ascorbic acid on the tissue deposition of lead in rats suggested that ascorbic acid might be useful as a prophylactic agent for lead poisoning [14]. Later studies in rats demonstrated that ascorbic acid decreases the intestinal absorption of lead [15] and increases the renal clearance of lead [16]. Further studies indicated that ascorbic acid reduced ferric iron in the duodenum to the ferrous state, which competes with lead for intestinal transport [17,18]. Early clinical trials have shown that the orally administered ascorbic acid alleviated the clinical symptoms of lead poisoning and increased the urinary excretion of lead [19–21]. However, these studies were not carefully monitored, and the body levels and intake of ascorbic acid were not estimated. While calcium disodium ethylene diamine tetra-acetic acid ($CaNa_2EDTH$) is the most commonly employed antidote for lead poisoning, it must be administered by intravenous infusion and has serious side effects [22]. In contrast, ascorbic acid supplementation is readily available and inexpensive. The purpose of this study was to conduct a carefully monitored investigation of the effect of ascorbic acid supplementation on blood-lead levels.

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MATERIALS AND METHODS

The study subjects were 75 adult men between 20 and 35 years of age who said they smoked a minimum of one pack of cigarettes per day. In compliance with the regulations of the Institutional Human Research Committee, the research protocol was explained to them, and they signed human consent forms. Each received a physical examination, and none showed clinical signs of inflammatory or chronic disease. All of them resided in a resort community, and none was employed in a toxic work environment or was using nutrient supplements.

Next, 10-mL samples of venous blood from the right antecubital vein and 30 mL samples of spontaneous urine were obtained to determine the prestudy baseline levels of ascorbic acid. The men were randomly assigned to one of three groups of 25 subjects each, and each group was provided with a four-week supply of daily supplements of either 200 mg of ascorbic acid, 1000 mg of ascorbic acid or a placebo. The placebo group served as a control for comparison with the groups receiving ascorbic acid supplementation. The subjects returned weekly for four weeks to provide 10 mL of blood and 30 mL of spontaneous urine samples for study. The blood samples were collected in lead-free Vacutainers (Becton-Dickinson 367734, Franklin Lakes, NJ) and the urine samples were collected in 50 mL sterile polypropylene containers (Fisher 14-372-30, Fisher Scientific, Pittsburgh, PA). Immediately after each sampling, aliquots of the whole blood for lead analysis were stored frozen (−60°C), and the blood samples were centrifuged (3000 rpm at 4°C) for ten minutes to obtain particulate-free serum. Aliquots of the serum and urine were diluted with 5% trichloroacetic acid for deproteinization and the stabilization of ascorbic acid and then stored frozen at −60°C until assayed. The ascorbic acid levels were determined by the 2,4-dinitrophenylhydrazene method of Lowry et al. [23] and expressed in μmol/L. Whole-blood lead, commonly used as an index of body-lead levels [24] and urine-lead levels, was measured by an atomic absorption spectrophotometer (Model 303, Perkin-Elmer Corp, Norwalk, CT), and the concentration was expressed in μmol/L. The creatinine level in each urine sample was determined by the picric acid method of Folin and Wu, and the concentration of urine ascorbic acid and lead calculated per gram of creatinine [25].

After biochemical analysis, the data were grouped according to the week of study within each level of ascorbic acid supplementation. The weekly group mean ± SD of all measurements was calculated and the analysis of variance (ANOVA) used to determine the statistical significance of the weekly group-mean changes from the baseline. In addition, we used Pearson's correlation coefficient to determine the association of the weekly group-mean levels of ascorbic acid and lead levels. We performed all calculations and statistical analysis on an IBM AT computer equipped with the Epistat (Dallas, Texas) statistical-analysis software program.

RESULTS

The prestudy information supplied by the men indicated a homogeneous population. All of them were Caucasian, and five were married with a total of eight children. The individual baseline serum ascorbic acid levels of the subjects ranged between 19.9 and 85.2 μmol/L, and none exhibited any clinical signs of ascorbic acid deficiency. The individual serum levels of supplemented subjects showed a progressive increase in serum ascorbic acid throughout the study, confirming individual compliance with the study protocol. The weekly group-mean levels (mean ± SD) of the serum ascorbic acid are listed in Table 1. The group-mean serum levels of ascorbic acid in the placebo group were higher at baseline (45.4 ± 5.6 μmol/L) than those of the supplementation groups: 34.1 ± 5.6 μmol/L for the 200 mg ascorbic acid group and 39.7 ± 5.6 μmol/L for the 1000 mg ascorbic acid group. The weekly group serum ascorbic-acid levels showed a slight decline after two weeks from 45.4 ± 5.6 μmol/L to 39.7 ± 5.6, and they decreased an average of 10% per week throughout the study. The 200 mg ascorbic acid group showed an average 133% linear increase in weekly mean serum ascorbic-acid levels from 34.1 ± 5.6 μmol/L at the baseline to 90.8 ± 5.6 μmol/L at the end of four weeks, a 166% increase ($p \leq .001$). The average 133% increase was significant ($p \leq .001$) after one week when the group mean was 68.1 ± 5.6 μmol/L, a 100% increase. Similarly, there was a weekly average increase of 100% in the mean serum ascorbic acid levels of the 1000 mg ascorbic acid group after one week of supplementation ($p \leq .001$) and a 143% increase after four weeks.

The weekly group mean ± SD of urine ascorbic-acid levels (μmol/g of creatinine) are listed in Table 2. The weekly mean of ascorbic acid increased each week in all three study groups.

Table 1. Weekly Group-Mean Changes (Mean ± SD) in Serum Ascorbic-Acid Levels (μmol/L)

Dosage Groups	Weeks				
	0	1	2	3	4
0	45.4 ± 5.6	45.4 ± 5.6	39.7 ± 5.6	39.7 ± 5.6	39.7 ± 5.6
200	34.1 ± 5.6	68.1 ± 5.6*	79.4 ± 5.6*	79.4 ± 5.6*	90.8 ± 5.6*
1000	39.7 ± 5.6	68.1 ± 5.6*	73.8 ± 5.6*	79.4 ± 5.6*	96.5 ± 5.6*

* Significantly higher than prestudy level; $p \leq .001$.

Table 2. Weekly Group Mean Changes in Urine Ascorbic-Acid Levels (μmol/g creatinine) During Supplementation (Mean ± SD)

Dosage Groups	Weeks				
	0	1	2	3	4
0	409 ± 73	517 ± 198	698 ± 153	925 ± 357	573 ± 193
200	233 ± 28	784 ± 198†	619 ± 91*	1215 ± 363‡	2339 ± 584*
1000	460 ± 158	943 ± 278	551 ± 108	5394 ± 1953‡	6978 ± 1732*

* Significantly higher than prestudy level; $p \leq .001$.
† Significantly higher than prestudy level; $p \leq .01$.
‡ Significantly higher than prestudy level; $p \leq .02$.

The average weekly increase in ascorbic acid excretion was 66% for the placebo group, 432% for the 200 mg ascorbic acid group and 653% for the 1000 mg ascorbic acid group. The weekly increases from the baseline in the supplemented groups were statistically significant ($p \leq .02$ to $\leq .001$). The highest level of ascorbic-acid excretion above the baseline (1417%) occurred after four weeks of supplementation in the 1000-mg ascorbic-acid group (460 ± 158 *vs.* 697 ± 1732 μmol/g of creatinine, $p \leq .001$).

The baseline blood-lead levels of the total study population ranged from 1.5 to 3.3 μmol/L, and none of the levels exhibited any clinical manifestation of lead toxicity. The weekly group mean ± SD of the whole-blood lead levels are listed in Table 3. The blood-lead levels decreased from the baseline in all study groups. They decreased an average of 9% per week in the placebo group, 3% in the 200 mg ascorbic acid group and 81% in the 1000 mg ascorbic acid group. The decrease from the baseline in the 1000 mg ascorbic acid group was statistically significant after one week of supplementation (1.8 ± 0.05 *vs.* 0.4 ± 0.05 μmol/L, $p \leq .001$) and remained at this level throughout the study.

The weekly group-mean ± SD levels of urine lead excretion are listed in Table 4. The average weekly lead excretion increased in all three study groups. The lead excretion increased an average of 16% in the placebo group, 41% in the 200 mg ascorbic acid group and 12% in the 1000 mg ascorbic acid group. The highest weekly increase in lead excretion from the baseline was observed in the 200 mg ascorbic acid group after four weeks of supplementation (0.13 ± 0.02 *vs.* 0.24 ± 0.06 μmol/g of creatinine). None of the observed differences from baseline levels were statistically significant.

The results of Pearson's correlation between weekly group-mean ascorbic acid and lead levels are listed in Table 5. A statistically significant inverse correlation was observed between the excretion of ascorbic acid and whole-blood lead levels ($r = -0.6055$, $p \leq .016$).

DISCUSSION

The higher baseline level of the serum ascorbic acid of the placebo group indicated a higher prestudy dietary intake. The increase in serum levels of ascorbic acid during the study in the supplemented groups agrees with previous supplementation studies lasting five to ten days [26]. The study group (200 mg of ascorbic acid a day supplementation) with the lowest baseline group-mean serum ascorbic acid level demonstrated the greatest percentage increase during the entire study, although this group did not receive the highest level of supplementation. In contrast, the 1000 mg ascorbic acid group, with a 16% higher baseline group-mean ascorbic acid level, demonstrated a 33% lower average weekly increase during the study.

The group-mean baseline blood-lead levels of the placebo and the 200 mg ascorbic acid groups were slightly higher than the range of the general population: 0.72–1.93 μmol/L [13]. Previous studies have shown the lowest observed blood-lead-toxicity level associated with adverse effects is 1.45 μmol/L in the adult male. Estimates indicate that this represents an ingestion of 750 μg of lead per day [27]. The work environment of the subjects was not associated with industrial pollution, and their body-lead levels before the study probably resulted from both dietary intake and heavy smoking [3–12]. The results of this study illustrate that the daily supplementation of 1000 mg of ascorbic acid results in the reduction of blood-lead levels associated with the general population to nil within one week, corresponding to a serum ascorbic acid level of 68.1 ± 5.6

Table 3. Weekly Group Mean Changes (Mean ± SD) in Whole-Blood Lead Levels (μmol/L)

Dosage Groups	Weeks				
	0	1	2	3	4
0	2.0 ± 0.2	2.1 ± 0.2	2.2 ± 0.2	1.4 ± 0.2	1.6 ± 0.2
200	2.0 ± 0.1	1.9 ± 0.2	2.0 ± 0.3	2.0 ± 0.1	1.9 ± 0.9
1000	1.8 ± 0.05	0.4 ± 0.05*	0.3 ± 0.05*	0.4 ± 0.05*	0.3 ± 0.05*

* Significantly lower than prestudy level; $p \leq .001$.

Table 4. Weekly Group Mean Changes (Mean ± SD) in Urine Lead Levels (μmol/g Creatinine)

Dosage Groups	Weeks				
	0	1	2	3	4
0	0.14 ± 0.02	0.14 ± 0.03	0.16 ± 0.04	0.13 ± 0.03	0.22 ± 0.10
200	0.13 ± 0.02	0.16 ± 0.04	0.17 ± 0.03	0.16 ± 0.04	0.24 ± 0.06
1000	0.13 ± 0.03	0.11 ± 0.02	0.15 ± 0.02	0.14 ± 0.03	0.14 ± 0.03

Table 5. Pearson's Correlation between Weekly Group Mean Ascorbic Acid and Lead Levels

Lead Level	Ascorbic Acid			
	Serum		Urine	
	"r"	p	"r"	p
Blood	−0.4839	0.067	−0.6055	0.016*
Urine	+0.2907	0.293	+0.0124	0.964

* Statistically significant at $p \leq .05$.

μmol/L. In contrast, the urine-lead excretion showed little change, contrary to the study with rats [16], suggesting that the protective effect of ascorbic acid is due to the inhibition of intestinal absorption of lead [15]. However, previous studies have demonstrated that 99% of blood lead was contained in the erythrocytes and the major excretory route for erythrocyte lead (and iron) is the catabolism of erythrocytes by the liver and discharge through the spleen with possible intestinal resorption [28]. Furthermore, the correlation observed between the urinary excretion of ascorbic acid and blood-lead levels indicates that the intestinal absorption of ascorbic acid is the metabolic process that inhibits the absorption of lead. The results of this study confirm previous reports that the intestinal ascorbic acid reduces lead absorption by reducing ferric iron to the ferrous state in which it actively competes with lead for absorption [17,18]. It is logical to assume that the body levels of other toxic metals, e.g., cadmium or mercury, might be similarly reduced. However, early studies did not show a beneficial effect of ascorbic acid supplementation (500 and 1000 mg/day) for three months on the blood and hair levels of cadmium, lead and mercury [29]. This may be attributed to differences in methodology and lack of monitoring. This study demonstrates the effectiveness of ascorbic acid supplementation and shows it to be a convenient, inexpensive prophylactic treatment for subclinical chronic lead exposure.

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