

# EDTA Chelation Therapy, without Added Vitamin C, Decreases Oxidative DNA Damage and Lipid Peroxidation

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## Abstract

Chelation therapy is thought to not only remove contaminating metals but also to decrease free radical production. However, in standard ethylene diamine tetraacetic acid (EDTA) chelation therapy, high doses of vitamin C with potential pro-oxidant effects are often added to the chelation solution. The authors demonstrated previously that the intravenous administration of the standard chelation cocktail, containing high amounts of vitamin C, resulted in an acute transitory pro-oxidant burst that should be avoided in the treatment of pathologies at risk of increased oxidative stress such as diabetes and cardiovascular disease. The current study was designed to determine the acute and chronic biochemical effects of chelation therapy on accepted clinical, antioxidant variables. An EDTA chelation cocktail not containing ascorbic acid was administered to six adult patients for five weeks (10 sessions of chelation therapy); antioxidant indicators were monitored. Immediately after the initial chelation session, in contrast with the data previously reported with the standard cocktail containing high doses of vitamin C, none of the oxidative stress markers were adversely modified. After five weeks, plasma peroxide levels, monitored by malondialdehyde, decreased by 20 percent, and DNA damage, monitored by formamidopyrimidine-DNA glycosylase (Fpg) sensitive sites, decreased by 22 percent. Remaining antioxidant-related variables did not change. In summary, this study demonstrates that multiple sessions of EDTA chelation therapy in combination with vitamins and minerals, but without added ascorbic acid, decreases oxidative stress. These results should be beneficial in the treatment of diseases associated with increased oxidative stress such as diabetes and cardiovascular diseases.

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## Introduction

Antioxidants that decrease cardiovascular risk factors and improve endothelial function<sup>1</sup> are recommended to prevent and treat cardiovascular disease (CVD) and diabetes.<sup>2,3</sup> Ethylene diamine tetraacetic acid (EDTA) chelation therapy, in combination with vitamins and minerals, is proposed to have antioxidant properties<sup>4</sup> and considered to be a complementary therapy for patients with coronary artery disease.<sup>5</sup> However, this claim is controversial.<sup>6,7</sup> A recent review evaluating EDTA chelation therapy in treating cardiovascular diseases did not support the evidence of beneficial effects.<sup>8</sup> No clinical trials with a large enough study group have been completed to provide sufficient statistical power to determine the clinical effectiveness of EDTA chelation therapy.

EDTA might act to prevent metal-induced free radical production, but the direct *in vivo* effects of EDTA chelation therapy on oxidative stress markers

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have not been documented. In a previous study,<sup>9</sup> the authors demonstrated transitory pro-oxidant effects of sodium ascorbate (5 g, the amount often administered during chelation therapy).<sup>10</sup> Those results strongly suggest caution in administering ascorbic acid in EDTA chelation therapy for patients at high risk of increased oxidative stress.

The objective of the present study was to modify the composition of the standard EDTA chelation therapy cocktail and investigate its effectiveness in reducing oxidative variables in patients undergoing chelation therapy. The primary markers for lipids, proteins, and DNA oxidative damage and antioxidant enzyme activities were measured before and after one session and before and after 10 sessions (5 weeks) in patients receiving an EDTA cocktail without added ascorbic acid. This study was not designed to test the curative effects of chelation therapy but to determine the biochemical effects on antioxidant variables, since the previous study<sup>9</sup> demonstrated pro-oxidant effects when high amounts of vitamin C were added to the chelation cocktail. Previous studies also document the effects of chelation therapy on metal losses.<sup>11-13</sup>

## Materials and Methods

### Patients

Six patients, four women ranging in age from 50-76 and two men ages 56 and 57, participated in this study. The study was approved by the Clinic Human Studies Review Board, Wisconsin Dells, WI. Patients signed an informed consent form and were not reimbursed for participation. Data are available to the patients upon request.

### Chelation

The EDTA cocktail contained the following: EDTA 3 g, 2,500 units heparin, 3 mL 2% procaine, 100 mg pyridoxine HCL, 4 meq KCL, 7 mL Mg sulfate (equal to 686 mg elemental magnesium), 1 mL B-complex vitamins, and 1 mL sodium bicarbonate. The intravenous infusion in sterile water was given in an arm vein over a two-hour period.<sup>14</sup> The dose of EDTA was 3 g since all patients had good kidney function.

### Blood Sampling

Blood samples were collected from the antecubital vein before and after a two-hour chelation treatment at the beginning and end of the study (5 weeks, 10 sessions). Blood was collected in heparinized tubes protected from light and centrifuged at room temperature for 10 minutes at 3,000 g. Plasma and erythrocyte pellets were immediately isolated, aliquoted, and stored at -80° C and analyzed within six months. For glutathione (GSH) measurements, within 20 min of venipuncture, 400 µL whole blood was added to 3,600 µL aqueous solution of metaphosphoric acid (6% w/v). The mixture was centrifuged for 10 minutes at 4° C. The acidic protein-free supernatant fractions were stored at -80° C until analyses. For the comet assay and DNA damage determinations,<sup>15</sup> 500 µL of blood was stabilized with 500 µL of a 20/80 (v/v) mixture of dimethylsulfoxide (DMSO) and RPMI 1640 cell culture medium. Aliquots of this mixture were progressively frozen to -80° C by use of cryopreservation vessels (Bicell, Fisher Bioblock Scientific; Lyon, France) in dry ice overnight. After one night, samples were transferred from the cryopreservation vessels to storage at -80° C until analyses within four months.

### Biological Variables

#### Erythrocyte Antioxidant Metalloenzymes

Erythrocyte Cu-Zn superoxide dismutase (SOD) activity was measured after hemoglobin precipitation by monitoring the auto-oxidation of pyrogallol by the method of Marklund and Marklund.<sup>16</sup> Erythrocyte glutathione peroxidase (GSH-Px) activity was evaluated by the modified method of Gunzler et al<sup>17</sup> using tertbutyl hydroperoxide (Sigma Chemical Co., Via Coger; Paris, France) as a substrate instead of hydrogen peroxide. Results are expressed as µmoles of nicotinamide adenine dinucleotide phosphate (NADPH) (Boehringer-Mannheim, Germany) oxidized per minute per g hemoglobin.

Erythrocyte catalase activity was determined as described by Beers and Sizer<sup>18</sup> following the decomposition of hydrogen peroxide by catalase at 240 nm. The reaction rate was related to the amount of catalase present in the mixture. Enzymatic activity was expressed as international units per g of hemoglobin (U/g Hb).

## Original Research

### Protein Oxidation

Total glutathione (GSHt) was determined according to the method of Akerboom and Sies<sup>19</sup> using enzymatic cycling of GSH by means of NADPH and glutathione reductase coupled with 5,5'-dithiobis-2-nitrobenzoic acid (DTNB). To assay oxidized glutathione (GSSG), GSH was masked by adding 10  $\mu$ L of 2-vinylpyridine to 500  $\mu$ L deproteinized extract adjusted to pH 6 with triethanolamine.

Plasma thiol groups were assayed as described by Faure and Lafond.<sup>20</sup> The calibration was obtained from a stock solution of 100 mM N-acetylcysteine in the range of 0.125-1.0 mM. Standards and plasma samples diluted into 0.05 M phosphate buffer, EDTA 1mM, pH 8, and DTNB, 2.5 mM, and absorbance measured at 412 nm.

### Lipid Oxidation

Plasma malondialdehyde (MDA) concentrations were assessed using high-performance liquid chromatography (HPLC) as previously described by Richard et al.<sup>21</sup>

### DNA Damage

The evaluation of DNA damage was achieved by the comet assay (single-cell gel electrophoresis) on total blood following the method of Singh et al,<sup>22</sup> modified by Hininger et al.<sup>15</sup> Results were expressed as tail moment (TEM). Three samples per subject were assayed implicating 50 cells/sample. The mean of these three determinations was calculated for each subject.

The *Escherichia coli* formamidopyrimidine-DNA glycosylase (Fpg) was used separately to convert oxidized base into single strand break as previously described by Pouget et al.<sup>23</sup> *E. coli* Fpg was provided by Dr. Serge Boiteux (CEA/Fontenay-aux Roses, France). Analyses were performed using an epifluorescence microscope (Zeiss, microscope division; Oberkochen, Germany).

### Statistical Analysis

Data are expressed as means  $\pm$  SD. Individual means comparison were identified using Student's t-test (Statistica Program, Statistical Software; Paris, France). Statistical significance was set at  $p < 0.05$ .

### Results

No acute effects of chelation therapy were observed at the beginning and end of the study for the variables tested (Table 1). Chelation therapy for five weeks led to a 20-percent decrease in plasma MDA levels ( $p < 0.02$ ). Fpg-sensitive sites in DNA decreased 22 percent in the treated patients, indicating a lower level of oxidized DNA bases (8-OHdG) after treatment. Improvements in other measures of antioxidant status were not statistically significant.

### Discussion

EDTA chelation therapy is often used as a form of alternative medicine in the treatment of cardiovascular diseases and diabetes. This treatment aims to remove heavy metals, to reduce metal-induced free radical production, and to control oxidative complications. However, there is not strong evidence of *in vivo* antioxidant effects due to EDTA chelation therapy. In a previous study, the authors reported long-term beneficial effects of EDTA chelation therapy on MDA plasma levels as markers of lipid peroxidation, which were decreased after five weeks of standard treatment, but no improvement in other markers of oxidative stress.<sup>9</sup> Moreover, the study demonstrated the adjuvant supplementation with megadoses of ascorbic acid (5 g) in patients undergoing standard EDTA chelation therapy resulted in marked transitory free radical generation and oxidative damage at the end of each session of chelation therapy. These data strongly suggest that, for patients at high risk of increased oxidative stress, the composition of the standard chelation therapy cocktail should be modified to remove high doses of vitamin C.

In this study, pro-oxidant effects of the modified cocktail were not observed following chelation sessions. This result indicates that high doses of vitamin C were involved in the pro-oxidant effects reported previously using the standard chelation therapy cocktail. In this study, none of the markers measured indicated increased oxidative stress.

In the present study, markers of oxidative stress as percent of Fpg-sensitive sites and plasma MDA improved at the end of the treatment. This demonstrates a long-term antioxidant impact on important targets of free radical attack, including DNA, glutathione, and lipids, since increased Fpg-sensitive sites, higher MDA, and decreased total blood glutathione have been reported to

**Table 1. Effects of Standard EDTA Chelation Therapy on Oxidative Stress Markers and Antioxidant Enzyme Activities after Five Weeks**

Parameters	Before	After 1 session	Before 10th session	After 10 sessions
Plasma MDA, (µmol/L)	2.54 ± 0.44 <sup>a</sup>	2.20 ± 0.42 <sup>a</sup>	2.08 ± 0.23 <sup>b</sup>	2.02 ± 0.26 <sup>b</sup>
Plasma SH*, (µmol/g protein)	5.98 ± 0.56	6.02 ± 0.61	6.44 ± 1.94	6.02 ± 1.65
Blood GSHT, (µmol/L)	986 ± 121	1016 ± 143	1073 ± 93	1195 ± 310
Blood GSSG, (µmol/L)	57.20 ± 38.95	54.83 ± 36.64	56.33 ± 32.03	53.67 ± 29.37
RBC GSH-Px, (U/g Hb)	57.18 ± 13.32	57.43 ± 12.22	57.70 ± 13.62	58.22 ± 13.56
RBC SOD, (U/mg Hb)	1.31 ± 0.12	1.30 ± 0.11	1.30 ± 0.10	1.28 ± 0.09
RBC catalase (U/g Hb)	229.60 ± 26.34	236.88 ± 27.66	215.80 ± 35.91	233.90 ± 28.55
Comet, TEM <sup>1</sup>	3.05 ± 0.24	2.91 ± 0.29	3.29 ± 0.24	3.17 ± 0.15
% Δ Fpg	62.25 ± 18.45 <sup>a</sup>	46.55 ± 18.90 <sup>a</sup>	47.78 ± 3.30 <sup>b</sup>	43.38 ± 13.88 <sup>b</sup>

Values (means ± SD) are for six patients before and after the first session and before and after 10 sessions of chelation therapy over five weeks.

<sup>1</sup>Arbitrary units

<sup>a/b</sup>Values with different superscripts are significantly different at p<0.05.

\*SH = sulfhydryl groups

correlate with free radical production.<sup>24</sup> In this study, improvements in glutathione were not significant.

To monitor changes in DNA, the comet assay was used in combination with the repair-specific enzyme Fpg protein as a sensitive biomarker to assess oxidative DNA damage.<sup>24</sup> After EDTA chelation treatment there was a significant decrease (22%) in Fpg-sensitive sites in DNA, indicating a lower number of oxidized bases as 8-OHdG, which is the main substrate of Fpg and the

most common biomarker for oxidative DNA damage. Fpg-sensitive sites are described as more sensitive markers of DNA damage than the comet assay.<sup>25</sup> Furthermore, a positive correlation between 8-OHdG analysis by HPLC-EC and comet assay indicates the modified comet assay using Fpg appears to be an appropriate tool to estimate oxidative DNA damage.<sup>23</sup> The data from this study are predictive of long-term protective effects of EDTA chelation treatment against DNA oxidation.

GSH/GSSG ratio is considered to be the most important redox couple that determines the antioxidant capacity of cells.<sup>26</sup> A decreased GSH level might be a contributory factor for enhanced oxidative DNA damage since, in patients with type 2 diabetes, Fpg-sensitive sites were reported to be correlated with GSH.<sup>27</sup> In this study, EDTA chelation treatment resulted in an enhanced GSH/GSSG ratio, suggesting strong antioxidant effects and a predictive benefit for patients with type 2 diabetes.

EDTA chelation therapy also resulted in protective effects against lipid oxidation, monitored by lowered plasma MDA levels ( $p < 0.02$ ). For patients suffering from cardiovascular diseases and diabetes, decreasing lipid peroxidation is essential to avoid oxidative damage of the arterial walls and oxidative complications such as retinopathies or glomerulopathies.<sup>12</sup>

There were no changes in RBC antioxidant GSH-Px and SOD activities induced by the chelation treatments. These data rule out potential deleterious effects of chelation on metalloenzymes related to increased losses of selenium, zinc, and copper during EDTA chelation treatment.<sup>28</sup> Similarly, the iron-dependent antioxidant activity of RBC catalase was not altered, indicating the enzyme is not iron-depleted due to the chelation treatment.

It is not possible to determine the antioxidant involvement of each component of the chelation cocktail. B vitamins combined with EDTA chelation have been reported to be involved in nitric oxide-related endothelial vasodilator function.<sup>29</sup> In addition to the antioxidant effects of EDTA through the chelation of metals, the absence of high doses of vitamin C in the chelation cocktail seems essential to avoid a Fenton reaction, which would lead to the generation of hydroxyl radicals.

The benefits or harmful effects of high doses of ascorbic acid in chelation therapy may be related to individual patient pathology. High doses of vitamin C have been reported to be beneficial in cancers,<sup>30</sup> effects that could be related to the pro-oxidant and apoptotic effects of vitamin C.<sup>31</sup> Sodium ascorbate induced apoptosis in B16F10 murine melanoma cells by acting as a pro-oxidant, and the induction of a pro-oxidant state by sodium ascorbate with a subsequent reduction in mitochondrial membrane potential are involved in the

apoptotic pathway.<sup>32</sup> Nutrients acting as chemopreventives inhibit the continual growth of transformed clones of cells through pro-oxidant activity. In contrast, when antioxidant activity occurs in transformed cells an enhanced growth may result.<sup>33</sup> In addition, when undesirable pro-oxidant activity develops in normal cells the reactive oxygen metabolites generated may damage DNA and cellular membranes. Thus, in patients with increased risk of oxidative stress, strong evidence exists for the removal of high doses of ascorbic acid from the cocktail because of its potential pro-oxidant effects. In contrast, in cancer the efficacy of combined EDTA therapy with megadoses of ascorbic acid should be considered.

This study demonstrates that EDTA chelation therapy, in combination with vitamins other than vitamin C, exerts *in vivo* antioxidant protection of DNA and lipids, which should provide additional benefits in patients with CVD or diabetes. Further clinical trials are needed to demonstrate clinical outcomes in patients treated with EDTA chelation therapy.

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