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EDTA chelation therapy in the treatment of toxic metals exposure

Toksik metallere maruz kalım tedavisinde EDTA şelasyon terapisi

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SUMMARY

BACKGROUND: Metal induced toxicity with wide range of physiological, biochemical and behavioral dysfunctions was reported in many studies. The chelation has been used for treatment to toxic metals' exposure for many years. In our current clinical study, we compared different chelation protocols and two forms of EDTA (sodium calcium edetate and sodium edetate) in treatments of toxic metal exposure.
METHODS: A 24 h urine samples were collected from each subject before and after treatment by Ca-EDTA or Na-EDTA. The levels of toxic and essential metals were measured by Atomic Absorption Spectrometer (AAS) with graphite furnace and by Inductively Coupled Plasma Atomic Emission Spectrometer (ICP-AES). The cellular levels of ATP were determined by ATP-bioluminescence assay. Mitochondrial potential was measured by fluorometer and flow-cytometer after cells' staining by mitochondrial dye.
RESULTS: Data from over 600 patients with a variety of complaints, but not acute toxic mineral exposure, given chelation therapy with sodium EDTA or calcium EDTA, were analyzed. Ca-EDTA and Na-EDTA at intravenous infusions of 3 g per treatment were equally effective in removing lead from the body, while Ca-EDTA was more effective in aluminum removal. The removal of lead was dose dependent but non-linear. Chelation by different doses of Na-EDTA (3 g and 1 g) resulted in mean difference in lead urine less than 50%. In addition, deficiencies in essential minerals correlated with greater pre-treatment lead and aluminum levels. The intrinsic toxicity of EDTA on cells was investigated. EDTA concentrations above 600 uM reduced cellular energy metabolism.
CONCLUSION: Based on our data, we proposed that low dosages of chelating agents might be preferential for patients with no–occupational exposure, as the benefit of chelation is not linear with dosage, the risk of exposure to antidotes is increasing with increased dosages, and excretion of essential metals significantly increases with increased antidote dosage.
Keywords: NADH, chelation, lead, essential metals

ÖZET

GİRİŞ: Birçok çalışmada, metal zehirlenmelerine fizyolojik, biyokimyasal ve davranışsal fonksiyon bozukluklarının eşlik ettiği rapor edilmişitir. Toksik metallere maruz kalım tedavisi için yıllardır şelasyon kullanılmaktadır. Şimdiki klinik çalışmamızda, toksik metallere maruz kalımın tedavisinde, farklı şelasyon protokollerini ve iki EDTA formunu (sodyum kalsiyum EDTA ve sodyum EDTA) karşılaştırdık.
YÖNTEM: Ca-EDTA veya Na-EDTA ile tedavi öncesinde ve sonrasında, her denekten 24 saatlik idrar örnekleri toplandı. Toksik ve esansiyel metallerin düzeyleri, grafit fırınlı atomik absorbsiyon spektrometresi (AAS) ve indüktif olarak eşleştirilmiş plazma atomik emisyon spektrometresi (ICP-AES) ile ölçüldü. Hücresel ATP düzeyleri, ATP-biyolüminesans analizi ile saptandı. Mitokondriyal potansiyel, hücrelerin mitokondri boyası ile boyanmasından sonra florimetre ve akış sitometresi ile ölçüldü.
BULGULAR: Çeşitli şikayetleri olan fakat akut toksik mineral maruz kalımı olmayan, Na-EDTA ve Ca-EDTA ile şelasyon tedavisi alan 600'ün üzerindeki hastadan elde edilen veriler değerlendirildi. Ca-EDTA ve Na-EDTA'nın tedavi başına 3 g intravenöz infüzyonu, kurşunun vücuttan uzaklaştırılmasında eşit derecede etkinken; Ca-EDTA, alüminyumun uzaklaştırılmasında daha etkindi. Kurşunun uzaklaştırılması, doz bağımlıydı; fakat, doğrusal değildi. Farklı Na-EDTA dozları (3g ve 1g) ile şelasyon, idrar kurşununda %50'den daha az ortalama farklılıkla sonuçlandı. Ayrıca, esansiyel minerallerdeki eksiklikler, tedavi öncesi daha fazla kurşun ve alüminyum düzeyleri ile orantılıydı. Hücreler üzerine EDTA'nın intrensek toksisitesi araştırıldı. EDTA, 600 µM üzerindeki konsantrasyonlarda hücresel enerji metabolizmasını azalttı.
SONUÇ: Verilere göre; şelasyonun faydası dozla orantılı olmadığından, antidotlara maruziyet riski yüksek dozlarda arttığından ve esansiyel metallerin atılımı yüksek antidot dozları ile arttığından dolayı, şelasyon ajanlarının düşük dozları mesleki olmayan maruz kalımlar için öncelikli olabilir.
Anahtar Kelimeler: NADH, şelasyon, kurşun, esansiyel metaller

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INTRODUCTION

The build-up of heavy metals in the body can lead to a variety of health problems [1-3]. Poisonous or nonessential metals such as lead (Pb), mercury (Hg), cadmium (Cd), and aluminium (Al) have been associated with pathologies in the nervous, cardiovascular, haemotopoietic, gastrointestinal and immunological systems as well as renal dysfunction, anaemia, liver problems, cancer, and Alzheimer's disease [4-15]. In addition, nutritional trace elements can be toxic at concentrations outside their physiological range. Excessive levels of iron (Fe), manganese (Mn), copper (Cu), chromium (Cr) and zinc have been associated with conditions such as degenerative brain diseases, oxidative damage and heart problems [16-20]. Thus, it is important to regulate essential minerals while limiting toxic metal build-up in the body, and a means of clearing toxic or excess metals from the body is needed.

Chelation therapy has been proposed for removing poisonous metals such as Pb, Hg, Cd, and Al, as well as reducing abnormal accumulations of trace nutrients such as Fe, Cu, and Zn [21-24]. Chelation therapy employs anionic chelating agents such as ethylene diaminetetraacetic acid (EDTA) to bind heavy metal cations found in the blood. Once EDTA bound, these metals can be removed through the kidneys.

There is evidence that EDTA chelation benefits patients by removing toxic and trace elements from diseased organs and blood vessel walls [25-29]. The US Food and Drug administration has approved intravenous use of sodium EDTA (Na-EDTA) for treatment of hypercalcaemia, but its broader use in improving cardiovascular health is still considered controversial [30-34]. Because Na-EDTA therapy requires very slow (3 hr for a 3g IV dose) infusion, calcium EDTA (Ca-EDTA) has been employed in its place. Ca-EDTA is FDA approved for treating serious cases of lead poisoning.

The purpose of our study is to compare the effects Na-EDTA and Ca-EDTA on toxic and essential mineral levels in the body. We analyzed data collected over a ten-year period in over six-hundred patients given chelation therapy, measuring levels of Al, Pb, Cd, Mn, Ca, Mg, Fe, Cu, and Zn. Over this time period two chelation regiments were employed for each chelating agent: a "normal" regiment employing three grams of the chelating agent along with 15–25 grams of intravenous vitamin C; and a "mini" regiment employing lower chelating agent doses (1 g for Na-EDTA, 0.05-0.1 g for Ca-EDTA) without vitamin C. We also conducted in vitro experiments examining effects of each chelating agent on cellular lead toxicity, mitochondrial potential, and ATP production.

MATERIALS AND METHODS

Clinical Studies

Data from 600 patients treated with chelation therapy over a ten-year period (1999-2010) were analyzed. Each patient exhibited long-term effects of toxic metal exposure. Prior to the onset of treatment, each patient was screened for adequate kidney functioning by assaying fasting serum creatinine (levels <1.7mg/dL were considered acceptable). Four different chelation protocols were used over this time period, each involving infusions in either sterile water or Ringers solution. 1) Na-EDTA Normal: 3g Na-EDTA, 3 hr IV infusion, supplements: 15g vitamin C, vitamin B6, magnesium chloride, and heparin. 2) Na-EDTA Mini: 1g Na-EDTA, 1.5 hr IV infusion, supplements: vitamin B6, and magnesium chloride. 3) Ca-EDTA Normal: 3g Ca-EDTA, 1 hr IV infusion, supplements: 15 to 25 g vitamin C, vitamin B6, and magnesium chloride. 4) Ca-EDTA Mini: 0.10 g Ca-EDTA, 10 min IV infusion, supplements: vitamin B6, and magnesium chloride. Regarding the supplements used, vitamin B6 was used to prevent "chelation headaches" while magnesium chloride was used to prevent cramps associated with magnesium deficiency. Vitamin C is thought to assist in lead removal, and heparin was to prevent clotting at the infusion site. Patients were given ten or more treatments, with mineral levels being assays before and after each treatment.

Before and after the first treatment, body burden of heavy metals, including lead, was determined by collecting urine over a twenty-four hour period for analysis. Aluminium, lead, cadmium and manganese levels in urine were measured by the use of Perkin-Elmer 4100ZL AAS with graphite furnace by standard protocols. Calcium, magnesium, iron, copper and zinc were also analyzed on a Perkin-Elmer ICP-AES (Model 3300 DV).

Statistics

Data from the various protocols were compared statistically as follows. A Kolmogorov-Smirnov test was used to determine if the data were normally distributed. If not, Wilcoxon test was used for comparing groups. If analysis supported the normal distribution of data, data were analyzed using Student's t-test. P-values of 0.05 or less are considered statistically significant.

In Vitro Studies

We conducted in vitro experiments using human endothelial cells, lung fibroblasts, and peripheral blood mononuclear cells (PBMCs). HUVEC endothelial cells (Cascade Biologics) were grown in medium M-200 (Cascade Biologics) supplemented with 2% fetal bovine serum (FBS), hydrocortisone, human epidermal factor, fibroblast growth factor, and heparin. CCD 18lu cells (ATCC) were grown in DMED medium (ATCC) with 10% FBS. Peripheral blood mononuclear cells were separated from whole blood by centrifugation in Ficoll-Paque. They are grown in RPMI medium supplemented with 10% FBS.

Method for measurements of the lead in cells was based on the method described in study [35]. After exposure to lead, cells were counted and after centrifugation the cell pellet was dried several hours in an oven at 100ºC. Once dried, cells were digested overnight using 200 µL concentrated nitric acid. The digested cells were diluted with 1.8 ml of matrix modifiers. To prepare matrix modifier in 500 ml volumetric flask were added 2.5 ml of 10% TX-100, 1 g ammonium phosphate monobasic, and 1 ml concentrated nitric acid.

Cellular ATP levels were determined using The CellTiter-GloLuminescent cell Viability Assay kit (Promega). The assay generates a glow type signal produced by luciferase reaction, which is proportional to the amount of ATP present in cells.

To measure mitochondrial potential, cells were harvested from experimental samples at concentrations of $0.5x10^6$ cells per mL. Cells were stained in PBS with 2.5 µg/ml 5,5′,6,6′,-tetrachloro-1,1′,3,3′-tetraethylbenzimidazolylcarbocyanine iodide (JC-1) (Invitrogen) at room temperature for 15-20 min in the dark. JC-1 selectively enters mitochondria of intact cells and forms aggregates that emit at 585 nm (orange-red). Emission was measured by fluorometer (SPEX Instruments, Inc).

To measure intracellular ROS production, cells were loaded with 10 uM CM-DCFH diacetate (Invitrogen) for 15 min at room temperature in dark. CM-DCFH was chosen because it exhibited better retention in cells than other derivatives of DCF. CM-DCF fluorescence was measured at an excitation wavelength of 480 nm and an emission wavelength of 520 nm by fluorometer and as green fluorescence in FL1 channel by flow-cytometer.

RESULTS

Clinical Studies

Urine mineral levels before and after treatments by 3 g of sodium calcium edetate (Ca- EDTA) or edetate disodium (Na-EDTA) are shown in Table 1. For both chelating agents, urine lead and aluminium levels increased over two-fold after chelation.

Table 1: Mineral levels in urine 24 hours before or after chelation using the "Normal" chelation protocols for Na-EDTA and Ca-EDTA. Data are average values and standard deviations.

	Na-EDTA (3g)		Ca-EDTA (3g)	
Metal	Pre	Post	Pre	Post
Al (ug/24h)	13 ± 11	30 ± 23	17 ± 23	48 ± 34
Cd (ug/24h)	4.4 ± 27	5.5 ± 4.4	2.7 ± 1.5	5.8 ± 2.6
Ca (mg/24h)	170 ± 106	299 ± 107	161 ± 108	330 ± 114
Cr (ug/24h)	1.5 ± 2	2.3 ± 3.1	1.4 ± 1.1	3.8 ± 3.1
Cu (ug/24h)	20 ± 25	23 ± 25	14 ± 9	16 ± 14
Fe (ug/24h)	165 ± 214	507 ± 295	76 ± 68	457 ± 380
Pb (ug/24h)	17 ± 20	36 ± 22	15 ± 6	34 ± 16
Mg (mg/24h)	120 ± 54	159 ± 67	145 ± 89	160 ± 101
Mn (ug/24h)	1.4 ± 1.7	25 ± 14	1.3 ± 1.6	29 ± 20
Zn (ug/24h)	690 ± 470	18600 ± 6500	580 ± 590	16600 ± 5200

Urine levels of essential minerals, however, also increased substantially after chelation: zinc and manganese levels were an order of magnitude higher after chelation, urine iron levels increased three-fold, and urine calcium levels increased two-fold. We saw no statistically significant changes in urine cadmium, chromium, copper, or magnesium.

Figure 1 compares urinary lead and aluminium levels in patients treated with 3 g Na-EDTA with those in patients undergoing chelation therapy with 3 g Ca-EDTA using the "Normal" protocols.

We did not observe a significant difference in post-chelation values of lead between patients treated with Ca-EDTA and those treated with Na-EDTA. The aluminium excretion after chelation by two different forms of EDTA with the same dosage demonstrated more effective chelation by Ca-EDTA in comparison with Na-EDTA ($p<0.01$). Distributions of the post- and pre-chelation values of urine aluminium for two antidotes are shown in Fig. 1(b).

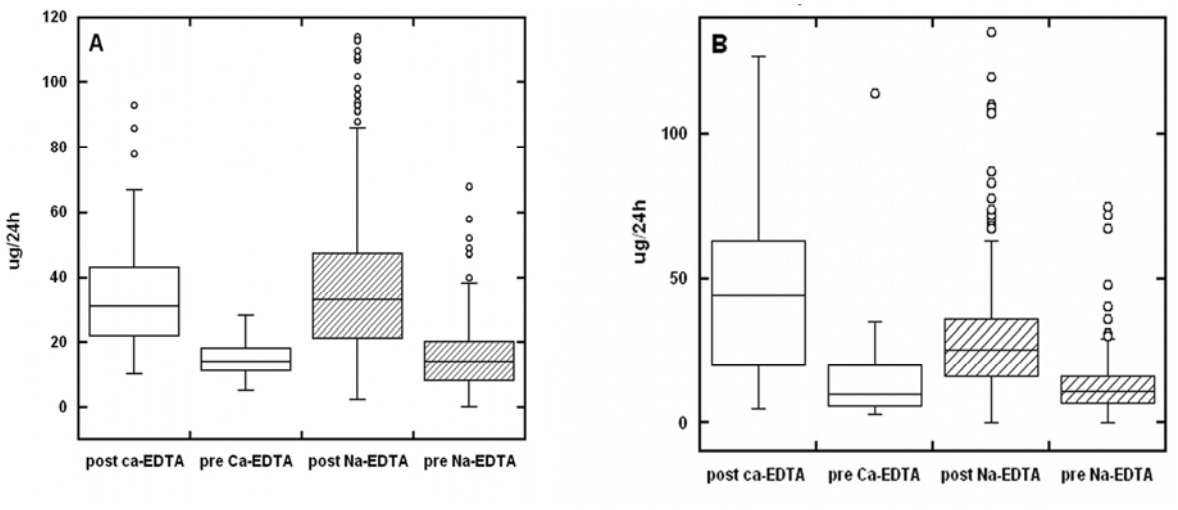

Fig. 1A-B: Effect of Ca-EDTA and Na-EDTA on lead and aluminium chelation Distributions of urine lead (A) and aluminium (B) levels in patients before and after chelation therapy with either Na-EDTA or Ca-EDTA ("Normal" protocols).

We were interested to see if the "Normal" chelation protocol, with 3 g chelation agent doses, was significantly more effective than a "Mini" chelation protocol with lower agent doses. The percent gain in metal excretion attained by using the higher dose (as compared to the "mini" protocol) is shown in Fig. 2 for chelation with Na-EDTA.

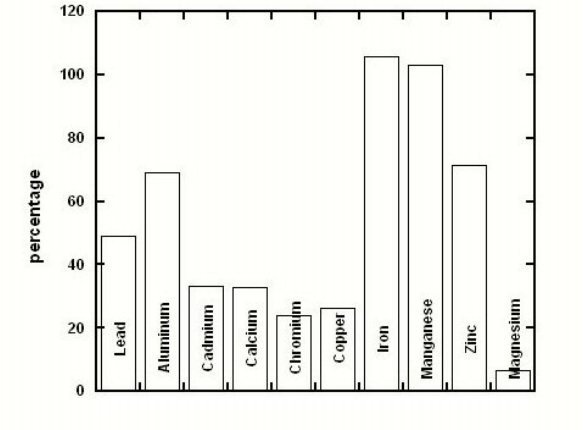

Fig. 2: Effect of Ca-EDTA and Na-EDTA on lead and aluminium chelation Distributions of urine lead (A) and aluminium (B) levels in patients before and after chelation therapy with either Na-EDTA or Ca-EDTA ("Normal" protocols).

Lead excretion, for example, was fifty percent higher using the "Normal" protocol (3g Na-EDTA) compared to the "Mini" protocol (1g Na-EDTA). Aluminium excretion was seventy percent higher, and calcium excretion was significantly but not dramatically higher (thirty percent) when higher Na-EDTA doses were used. This, combined with the more dramatic increases in iron, manganese, and zinc excretion, suggests that the "Mini" protocol may provide the best strategy for treating with Na-EDTA. Similar trends were observed in comparing the "Normal" chelation protocol to the "Mini" chelation protocol with Ca-EDTA. Lead excretion was increased by twenty percent (comparing "Normal" to "Mini") while aluminium chelation was increased seventy percent. The presented data demonstrate that there was no linear relation between dosage of antidote and the level of excreted toxic metals and increased dosage of chelating agent significantly increased the release of essential metals.

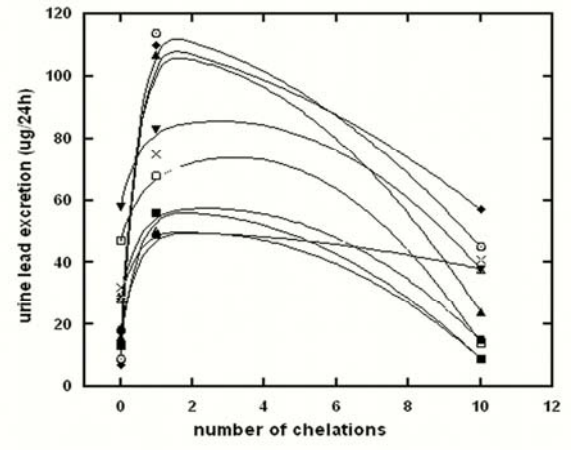

Fig. 3: Lead chelation over the series of chelation therapy. Examples of the measured levels of spontaneous excretion before chelation, and after first and tenth treatments for several patients.

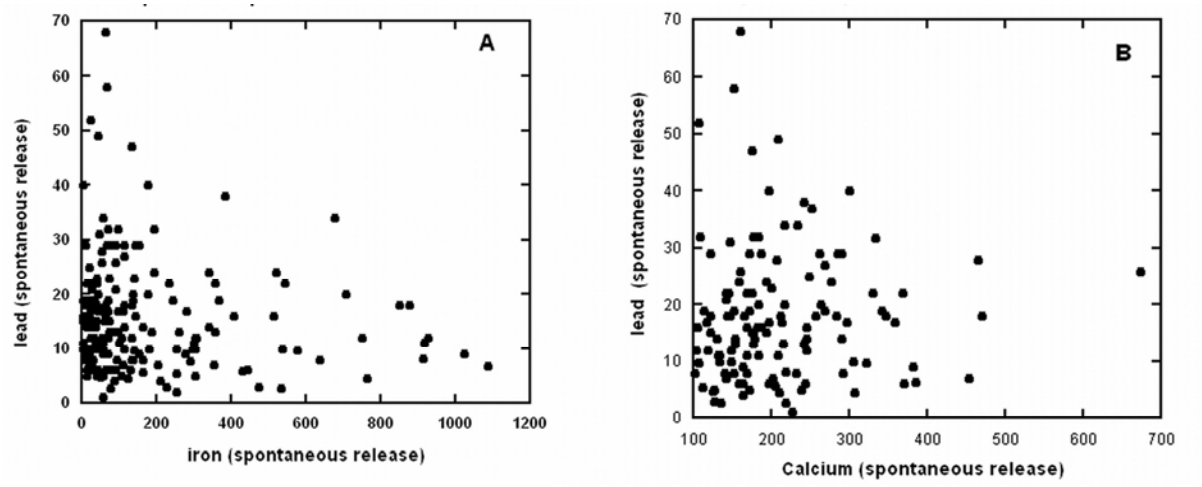

Fig. 3A-B: Relation between levels of iron and calcium and lead absorption . Urine lead levels prior to chelation therapy are plotted against urine iron levels (A) or calcium levels (B).

In addition, we looked at changes in urinary lead levels over the course of several rounds of chelation therapy. For most patients, ten rounds of chelation therapy were sufficient to return urinary lead levels to their levels of spontaneous release. Results of the measurements for several patients are shown in Figure 3. The data show the level of spontaneous excretion of lead before chelation, after the first chelation and ten chelations. Our clinical experience demonstrated that for 90% of patients the level of lead release in urine was decreased to spontaneous release. There were exceptional cases, however, where lead levels remained high (nearly three times the pre-chelation values) even after forty treatments.

We also examined the correlation between pre-chelation lead levels and the pre-chelation levels of iron, zinc and calcium. Results for iron and calcium are shown in Figure 4. While there is variability from patient to patient, the highest pre-treatment lead excretions were observed in patients who showed reduced urine iron, zinc or calcium levels. If one assumes that the levels of essential metals in the urine are proportional to those in the blood, this could indicate that deficiencies in essential minerals enhance the uptake of toxic metals.

In Vitro and *Ex Vivo* Results

On our in vitro experiments, we analyzed intrinsic toxicity of antidotes by measuring level of energy metabolism after exposure to chelating agents. ATP production rates of various cell types were assessed after twenty-four hours exposure to various Ca-EDTA concentrations. Results for endothelial cells are shown in Figure 5. In serum free medium, there is a concentration dependent decrease in ATP production with increasing EDTA levels. Serum supplementation provided a protective effect. Similar results were attained using fibroblasts and PBMCs.

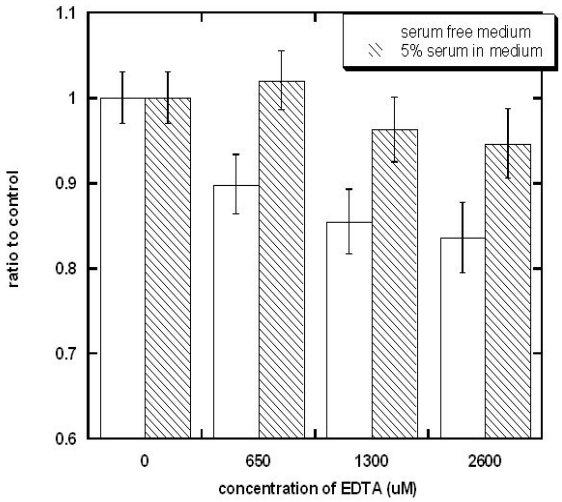

Fig. 5: Effect of EDTA on cell energy metabolism. Effect of EDTA concentration on ATP production rates in endothelial cells. Cells were exposed to EDTA for 24 hours.

Toxicity of lead (II) acetate and lead (II) chloride in cells exposed to the reagents in either serum free media or 10% serum are shown in Figure 6. There seemed to be no difference between the organic (acetate) and inorganic (chloride) forms in their effects on cell ATP levels. For both lead salts, serum provided a protective effect. This is likely due to the

effect of serum levels on lead uptake. Measurements of cellular lead uptake in vitro showed that serum greatly reduced uptake: for example, cells exposed to lead in serum free medium took up fifty times as much of the metal as those treated in medium supplemented with 10% serum. Lead toxicity in our experiments was associated with mitochondrial dysfunction. Figure 7 shows effects of lead concentration on mitochondrial potentials in endothelial cells. Lead reduced mitochondrial potential in a concentration dependent fashion.

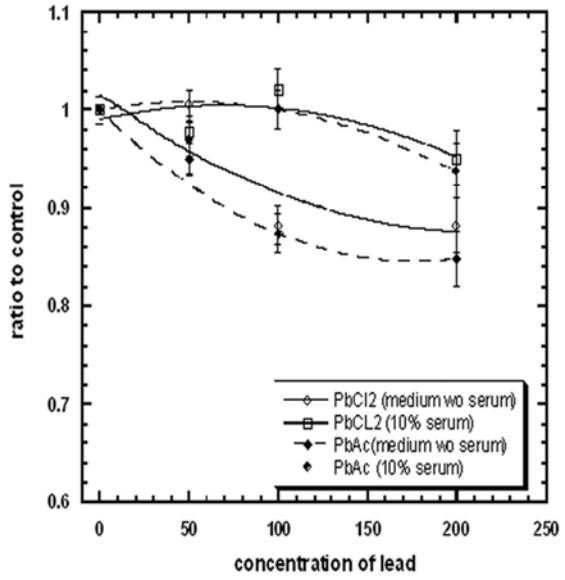

Fig. 6: Effect of serum concentration on lead toxicity. Effect of lead concentration (24 hr exposure) on cellular ATP production rates relative to controls.

Finally, we examined the effect of Ca-EDTA on cellular lead uptake. Figure 8 shows data for fibroblasts simultaneously exposed to lead and EDTA for twenty-four hours. For all lead concentrations measured, EDTA decreased lead uptake rates in a concentration dependent fashion. For example, 100 μM EDTA reduced lead uptake by ten percent, while 400 μM EDTA reduced lead uptake by forty percent. EDTA seemed to be more effective on a relative basis (in terms of reducing uptake ratio to control) when lead concentrations were higher. However, when cells were pre-loaded with lead and then exposed to EDTA, the chelating agent did not enhance the efflux of lead from the cells.

DISCUSSION

In the present manuscript, we analyze chelation therapy data from six-hundred patients without a history of occupational exposure or acute intoxication. The initial treatment with either Ca-EDTA or Na-EDTA leads to increased urine levels, and thus increased clearance, of lead, aluminium, calcium, iron, magnesium, manganese, and zinc. We did not observe increases in urinary cadmium, copper, or chromium. The efficacy in chelating lead is consistent with this metal's high affinity EDTA [36], while the lack of success chelating cadmium is probably due to this metal being tightly bound to metallothionein in liver and kidneys. While urine lead levels after the initial chelation treatment were two to three times higher than those observed prior to therapy, urine zinc and manganese levels were increased over twenty-fold. Other essential metals, such as iron and calcium, were excreted to the urine during chelation therapy; this suggests that clearance of essential minerals during chelation needs to be monitored, or supplements are needed to compensate.

We also compared different dosing regiments (a "Normal" protocol with 3 grams EDTA plus intravenous vitamin C and a "Mini" protocol with 1 gram or less of EDTA) and different cations (Ca-EDTA versus Na-EDTA). With the "Normal" dosing protocol, Ca-EDTA and Na-EDTA were equally effective in chelating lead, but Ca-EDTA was more effective in chelating aluminium. The effect of chelating agent dose on metal clearance was non-linear. For example, increasing the dose of Na-EDTA from 1g to 3 g increased the excretion of lead by fifty percent and aluminium by seventy percent.

Multiple rounds of chelation therapy were used for patients. For most subjects, ten courses of EDTA therapy were sufficient to return urine lead levels to their pre-treatment levels. For some patients, however, urinary lead levels remained high even after forty treatments. In animal studies, EDTA therapy mobilizes metal deposits from hard tissues and redistributes those to soft tissues [36], though there is no effect of chelation therapy on lead concentrations in the brain [37]. EDTA cannot pass though cell membranes (hence, the lacks of efflux observed in our in vitro studies with lead pre-loaded cells). Hence, we suspect that for patients with persistently high urinary lead levels that the metal had accumulated in bone or other intracellular stores where EDTA may not be able to gain rapid access.

Our data also indicate that lead and aluminium accumulation in the body is most severe when other

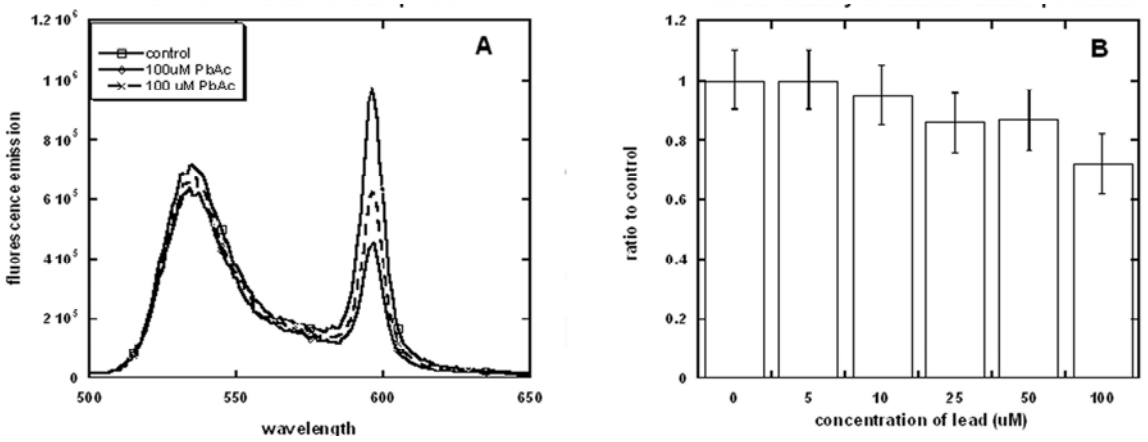

Fig. 7: Reduction of mitochondrial activity after exposure to lead. Effect of lead on mitochondrial potential in endothelial cells. Spectroscopy data are shown in (A) with mitochondrial potential relative to controls in (B).

essential minerals are deficient: urinary lead levels above 30 µg only occurred when calcium levels fell below 350 µg, iron levels fell below 700 µg, or zinc levels fell below 1500 µg. Similar relationships are seen with aluminium. This is consistent with observations from the literature. For instance, lead competes with calcium to inhibit release of neurotransmitters and adversely affects functioning of cellular calcium channels [20]. This contributes to the detrimental effects of calcium (and iron or zinc) deficiency on cognitive and behavioural development [38,39]. Deficiency in essential minerals can enhance dietary lead toxicity, interfering with kidney reabsorption [40], leading to elevated blood and tissue lead concentrations [41], and increasing lead absorption in the gastrointestinal tract [42]. Iron and zinc deficiencies are also associated with increased gastrointestinal lead absorption [43-45].

Our in vitro data shed light on the extent to which EDTA treatments may be toxic to normal cells. In the presence of serum (5%), normal cells (fibroblasts, endothelial cells, PBMCs) are insensitive to EDTA concentrations below 600 µM. EDTA inhibits cellular lead uptake in vitro in a dose dependent fashion, with lead uptake being inhibited by nearly forty percent at an EDTA concentration of 500 µM. Thus, EDTA can limit cellular lead uptake at non-toxic concentrations. The toxicity of lead is confirmed in our studies: lead concentrations above 100 µM adversely affect cell metabolism (ATP production) and inhibit mitochondrial potentials. Serum provides a protective effect, reducing EDTA toxicity at high concentrations while reducing lead uptake and lead toxicity. Our data demonstrated the

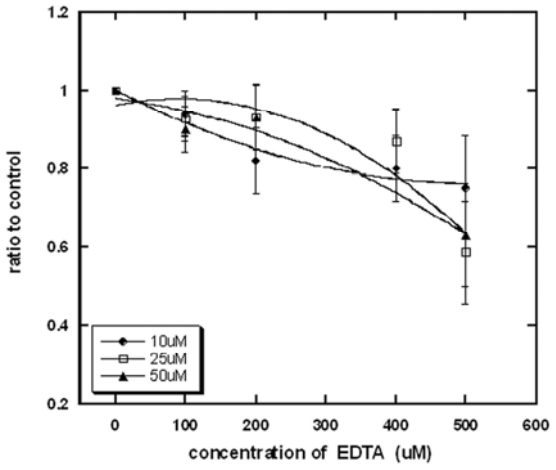

Fig. 8: Dependence of lead chelation on EDTA concentration. Effect of Ca-EDTA on lead uptake (relative to controls) rates of fibroblasts (24 hr exposure). The experiment was performed at three different lead concentrations (see legend).

same efficacy of both antidotes with dosages 3g in the chelation of lead. Ca-EDTA was more effective than Na-EDTA in enhancing the urinary excretion of aluminium.

In summary, Ca-EDTA and Na-EDTA are equally effective, at a 3-gram dose, in chelating lead, while Ca-EDTA is more effective than Na-EDTA in chelating aluminium. Comparing low doses ("mini" protocol with doses of 1 gram or less) with high doses ("normal" protocol with doses of 3 grams) shows diminishing returns. For example, the increase in lead extraction attained by increasing the Ca-EDTA dose from 0.1 grams per treatment to 3

grams per treatment was only twenty percent. Tripling the Na-EDTA dosage (from 1 gram to 3 grams per treatment) increased lead extraction by fifty percent. The issue of EDTA nephrotoxicity has been described in the literature. In ours in vitro studies, EDTA concentrations above 1 mM inhibited endothelial and white blood cell ATP production rates and adversely affected mitochondrial potentials. Based on our data, we proposed that low dosages of chelating agents might be recommended for patients with no–occupational exposure, as the benefit of chelation is not linear with dosage and risk of exposure to antidotes is increasing with increased dosages.

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