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Original Article

Effects of antioxidant and chelation therapy in coronary artery disease patients with elevated lead or cadmium levels

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Abstract: Chelation therapy may decrease the amount of heavy metals in the body, thus decrease the side effects of these toxic materials in affecting the health of our body. Thirty coronary artery disease (CAD) patients with elevated either lead or cadmium level were studied for 6 months to assess whether antioxidant and ethylenediamine tetra-acetic acid (EDTA) chelation therapy have beneficial impacts in adiponectin level, endothelial progenitor cell (EPC) numbers, and ultrasonic endothelial function. Results revealed that lead and cadmium levels in blood and urine were reduced significantly after 3-month of antioxidant and chelation therapy ($P<0.001$). It decreased further after 6-month therapy ($P<0.001$). The adiponectin level increased significantly after 3-month treatment ($P<0.005$), it increased further after 6-month treatment ($P<0.05$ compared with those of 3-month data). The study patients exhibited a significant increase in the EPC numbers of the 3-month (3.57×10^5) and 6-month (4.13×10^5) data compared to that of baseline (2.99×10^5) ($P<0.001$) after antioxidant and chelation therapy. The ultrasonic endothelial function analysis also revealed a progressive increase in the flow increase during reactive hyperemia (FIRH) and flow mediated dilation (FMD) after 3- and 6-month of treatments ($P<0.05$ compared with baseline data). In conclusion, this study demonstrated that antioxidant and chelation therapy is capable of increasing the adiponectin level, EPC numbers, and ultrasonic parameters of FIRH and FMD, which suggest that this therapy may have favorable impacts in CAD patients with elevated heavy metal levels.

Keywords: Anti-oxidant, chelation therapy, coronary artery disease, endothelial function, endothelial progenitor cells, heavy metal

Introduction

Previous studies have shown that blood lead and cadmium, at levels well below current safety standards, are associated with several health outcomes, including peripheral arterial disease, impaired renal function, and elevated blood pressure [1-3]. Menke et al. has reported that blood lead level was significantly associated with both myocardial infarction and stroke mortality even with lead level below 0.48 μmol/L (10 μg/dL) [4]. Food and Drug Administration (FDA) had approved ethylenediamine tetra-acetic acid (EDTA) treatment for heavy metal detoxification more than 50 years ago, which have prompted many physicians to use EDTA as an alternative medicine for many categories of patients [5]. EDTA chelation therapy for patients with coronary heart disease (CAD) has been used in hundreds of thousands patients worldwide. In 2005, Chappel et al. reported 220 patients with documented vascular disease treated with intravenous EDTA chelation therapy had a 93.6% lesser need for angioplasty and a 62.5% reduced need for coronary artery bypass grafting (CABG). Administration of EDTA chelation therapy resulted in fewer subsequent cardiac events than primary treatment with CABG, angioplasty or conventional medical therapy [6]. Recently,

the Trial to Assess Chelation Therapy (TACT) was designed to evaluate whether EDTA and high dose oral vitamins therapy could offer beneficial clinical effect in patients with a previous myocardial infarction (MI). Results revealed that patients received intravenous chelation regimen with disodium EDTA, compared with placebo, had significant reduction of the risks of cardiovascular outcomes including recurrent myocardial infarction, stroke, coronary revascularization, or hospitalization for angina (HR, 0.82; 95% CI, 0.69-0.99; P = 0.035) [7]. Administration of iv EDTA on weekly basis appears to be a sufficient and nontoxic protocol for treating patients with suspected overload and toxicity of heavy metals especially Pb and Cd [5]. Lin and his colleagues have used a new chelation agent - the calcium disodium EDTA, in patients with chronic kidney disease. They found that repeated chelation therapy may improve renal function and slow the progression of renal insufficiency in patients with chronic renal disease [8, 9]. This study planned to use the calcium disodium EDTA as the chelation agent for removal of heavy metals which was different from that used in TACT trial [7] but was the same as that used in Lin and his colleagues' reports [8, 9].

Adiponectin is a fat-derived adipokine known to be down regulated in obesity and diabetes. A review found that adiponectin has insulin-sensitizing effects as well as antiatherogenic properties. Lifestyle modifications and some drug therapies to treat atherosclerosis, hypertension, diabetes, and CAD have important effects in increasing adiponectin levels, decreasing insulin resistance, and improving endothelial dysfunction [10]. Adiponectin possesses anti-inflammatory and antiatherogenic properties that may be related to its ability to stimulate production of nitric oxide from vascular endothelium [10]. Ouchi and his colleagues have reported that endothelium-dependent vasodilation in response to acetylcholine is significantly reduced in adiponectin-knockout mice when compared with wild type mice [11]. Their study suggests that hypoadiponectinemia is associated with impaired endothelium-dependent vasorelaxation. The measurement of plasma adiponectin level may be helpful as a marker of endothelial dysfunction [11].

Endothelial progenitor cells (EPCs) derived from bone marrow circulate in the peripheral blood and have been implicated in neo-angiogenesis after tissue ischemia has occurred [12, 13]. EPCs are capable of proliferating and differentiating into endothelial cells and are therefore ideal candidates for vascular regeneration [14]. Levels of circulating EPCs have been shown to be associated with endothelial function and cardiovascular risk factors [15]. Decreased levels of circulating EPCs independently predict cardiovascular events, suggesting an important role for endogenous vascular repair of EPCs in modulating the clinical course of CAD [16, 17]. This study test the hypothesis that antioxidant agent and chelation therapy using calcium disodium EDTA not only may decrease lead and cadmium levels but also have beneficial impacts in adiponectin level, EPC numbers, and ultrasonic endothelial function in patients with CAD.

Materials and methods

Study subjects

This study enrolled 30 stable coronary artery disease subjects including 29 men and 1 woman with age 53-78 (64.9±6.1) years. This study was approved by the Ethics Commission of Kaohsiung Veterans General Hospital. All participants signed an informed consent to participate in this research. Inclusions of patients were those with two- or multi-vessel coronary artery disease who had abnormal lead and cadmium levels. Since there were no clinical criteria of heavy metals in Taiwan, our patients had significantly high levels of lead or cadmium in blood compared to those of USA population [18], i.e. patient was enrolled if one of their blood lead or cadmium levels greater than 14.50 μg/L or 0.41 μg/L, respectively. All patients had an elevated blood lead level and 21 of them had an elevated blood cadmium level. The study patients had stable coronary artery disease without prior history of stroke. Eighteen (60%) of them had old myocardial infarction (MI) history (MI history for more than 6 weeks); 8 (26.7%) patients received CABG, 22 cases received percutaneous coronary intervention (PCI) with stents at least one vessel. All of them had already quitted smoking and would not receive any PCI or CABG procedure in the coming one year. Exclusion of criteria included: 1) Known allergy to any components of solutions or vitamins; 2) Carotid or coronary revascularization within 6 months, or planned

revascularization; 3) Symptomatic heart failure (HF), or HF hospitalization within 6 months; 4) Uncontrolled hypertension (blood pressure >160/100); 5) No venous access; 6) Serum creatinine >2.0 mg/dL; 7) Baseline platelets <100,000/cumm; 8) Cigarette smoking within 6 months.

Study protocol

The CAD patients would receive intravenous calcium disodium ethylenediaminetetraacetic acid ($CaNa_2EDTA$) chelation therapy weekly for at least 6 months during the study period. The injection solution was made of 500 ml 5% dextrose in water (D5W), which containing 1 g calcium disodium EDTA ($CaNa_2EDTA$). Each treatment solution also contained 750 mg of magnesium sulfate, 5 g of ascorbic acid (Mega C), and 5 g sodium bicarbonate (titrated to physiologic pH) in the D5W. All infusion solutions were prepared following manufacturers' instructions and were administered immediately after mixing. The infusion solution was administered over 2 hours to minimize the potential side effect due to rapid infusion. All patients received treatments weekly for at least 6 months and also took oral gingko biloba 120 mg daily, and multivitamin therapy 1 tablet three times daily as tolerated. The components of infusion solution were as follows: vitamin A 4,000 IU, vitamin E 65 IU, vitamin C 400 mg, vitamin B1 20 mg, vitamin B2 5 mg, vitamin B6 15 mg, vitamin B12 25 μg, niacin 5 mg, niacinamide 5 mg, pantothenic acid 50 mg, folic acid 0.04 mg, biotin 10 μg, choline 72.5 mg, inositol 5 mg, L-arginine 54 mg, methionine 24 mg, magnesium 40 mg, potassium 40 mg, manganese 0.5 mg, zinc 3 mg, chromium 20 μg, and selenium 25 μg. Since we have regularly administered oral gingko biloba and L-arginine infusion, both materials have antioxidant property [19-22], which might have beneficial effect against the oxidative stress. Furthermore, previous report has described that the dietary intake of Vitamin B6 was associated with a reduced risk of coronary heart disease among middle-aged non-multivitamin supplement users. Dietary folate and vitamin B12 were also suggested to be protective factors for coronary heart disease [23]. Hence, the vitamin B6, folic acid, and severe other vitamins are also added to our patients.

All patients were followed up regularly at the Kaohsiung Veterans General Hospital. They were followed weekly for >6 months or until the occurrence of any cardiac events. The number of endothelial progenitor cells and inflammation markers including adipolectin at baseline, and every 3 months after the first visit were evaluated until the program finished. The conventional treatment of patients' CAD, hypertension, hyperlipidemia, or diabetes all were continued as usual. The evaluation time points included the baseline, after treatment for 3-month, and 6-month. The clinical evaluations of the frequency of chest pain and nitroglycerine use and the laboratory evaluations of creatinine, fasting glucose, hemoglobin A1c, blood urea nitrogen, alanine transaminase (ALT) and aspartate transaminase (AST) and cholesterol, endothelial progenitor cells, adiponectin, heavy metals in urine and blood, as well as the ultrasonic endothelial function studies at different time points were performed in all patients.

Measurement of body burden of lead and cadmium

Fasting venous blood specimens were drawn and placed into heparinized blood-collecting tubes (Becton-Dickinson, New Jersey, USA) according to standard guidelines for venipuncture before $CaNa_2EDTA$ infusion. The lead and cadmium levels in urine and blood were determined with an Elan 6100 DRC Plus inductively coupled plasma mass spectrometry (Perkin Elmer, Waltham, USA). All the calibrators and samples were placed in the auto-sampler-tray. The lead body burden was to determine urinary lead level using the modified protocol of EDTA mobilization tests developed by Emmerson and modified by Batuman et al. [24]. Subjects were instructed to eat breakfast before $CaNa_2EDTA$ infusion. Each case received a slow intravenous infusion of 1 gram of $CaNa_2EDTA$ that was pre-mixed with 250 mL of normal saline solution for 2 hours. The 24-hour urine samples were collected in 2-L bottles to assess the body burden of lead. The urine samples were collected by spontaneous voiding. The total daily urine amount of each subject was recorded. Ten mL of urine was obtained after well mixture of the total daily urine before examination.

Assay for adiponectin

Blood samples for laboratory assays were obtained at the early morning following overnight

fasting at the baseline, every three months of chelation therapies and at the end of the study. These blood samples were immediately coded so that investigators performing laboratory assays were blinded to subject identity. Assays for plasma adiponectin were performed in duplicate by ELISA (R&D Systems, Inc., Minneapolis, Minnesota) similar to previous method [25].

Assay of circulating EPC numbers

Assessment of the circulating EPCs by flow cytometry was performed similar to our previous method [26]. In brief, a volume of 40 mL peripheral blood was obtained and incubated for 30 minutes in the dark with monoclonal antibodies against human KDR (R&D, Minneapolis, MN, USA) followed by Allophycocyanin (APC)-conjugated secondary antibody, with the fluorescein isothiocyanate (FITC)-labeled monoclonal antibodies against human CD45 (Becton Dickinson, Franklin Lakes, NJ, USA), and with FITC-conjugated monoclonal antibodies against human CD34 (Becton Dickinson Pharmingen, USA). After incubation, cells were lysed, washed with phosphate-buffered saline (PBS), and fixed in 2% paraformaldehyde. The numbers of circulating EPCs were gated with monocytes and defined as CD34+CD45 low, then evaluated using flow cytometry analysis. Each analysis included 150,000 events. EPCs at baseline, 3-month, and 6-month after antioxidant and chelation therapy were assessed and compared. Our previous report had tested the reproducibility of EPC measurements, a very good reproducibility had been demonstrated ($r = 0.90$, $P<0.001$) [26].

Ultrasound endothelial function

A recently described technique employing high-resolution ultrasound to measure brachial artery diameter were used to study the vasodilator responses induced by reactive hyperemia after 5 min of upper arm occlusion [27]. Assessments of brachial artery endothelial function were performed at baseline, after completion of 30 chelation treatments, and at the end of study by a previously validated technique [28, 29]. Patients would undergo each of three brachial artery ultrasound studies after an overnight fast (12 h). All vasoactive medications, including angiotensin-converting enzyme (ACE) inhibitors, beta-blockers, calcium-channel blockers, long-acting nitrites, and statins will be stopped for 24 h. Studies were performed in a quiet clinical laboratory with the temperature maintained at 21 to 25°C. A 7.5 MHz linear phase-arrayed ultrasound transducer attached to a Hewlett-Packard SONOS 7500 (SONOS 7500 imaging system, Philips Medical Systems, Andover, MA) ultrasound machine was used. Pulsed-wave Doppler is used to record brachial artery velocity for each of the interventions. Three sequential systolic frames (taken at the end of the T-wave on the electrocardiogram) were studied using a built-in software of the echocardiographic machine. The average diameter (100 points) over the operator selected segment was calculated. Flow mediated dilation (FMD) induced by reactive hyperemia was expressed as actual FMD (the diameters as reactive hyperemia-baseline diameter = FMD mm) and as relative change from baseline [(FMD mm/baseline diameter) × 100% = FMD%]. The ultrasonic studies and the measurements were performed by a single technician in order to decrease the interobserver bias. The method of calculation of the flow increase during reactive hyperemia (FIRH) of the brachial artery. Briefly, the peak systolic velocities (cm/s) at baseline and during reactive hyperemia immediately after forearm pressure cuff release were measured. The maximal diameters (D) of brachial artery at baseline and during reactive hyperemia were also recorded. The cross-sectional area was calculated by $0.785\times D^2$ (cm^2). Brachial artery flow was calculated as the product of velocity and cross-sectional area. The difference of peak blood flow at baseline and during reactive hyperemia was initially obtained. The blood flow difference divided by the baseline peak blood flow was referred to the FIRH (%). We assessed reproducibility of our measurement in 20 cases. Intraobserver correlation coefficients for measurement of diameter at baseline and during hyperemia were 0.97 and 0.96, respectively. The average differences between 2 determinations were 0.06 mm and 0.08 mm at baseline and during hyperemia, respectively. The intraobserver correlation coefficients for measurement of peak systolic velocity at baseline and during hyperemia were 0.95 and 0.91, respectively. The average differences between 2 measurements were 5.1 cm/s and 11.4 cm/s at baseline and during hyperemia, respectively.

Table 1. Comprising of blood and urine levels of lead and cadmium at three stages

Concentrations(ug/L)		Baseline	3-month		6-month		
		Mean ± SD	Mean ± SD	*P* value	Mean ± SD	*P* value	*P*1 value
Blood	Lead	21.19±7.61	8.08±4.06	<0.001	2.79±1.73	<0.001	<0.001
	Cadmium	0.48±0.20	0.34±0.20	<0.001	0.21±0.15	<0.001	<0.001
Urine	Lead	11.29±6.29	5.74±4.23	<0.001	2.89±2.54	<0.001	<0.001
	Cadmium	1.50±0.78	1.29±0.74	<0.001	1.16±0.67	<0.001	<0.01

P values were compared with the baseline data to 3-month and 6-month data, respectively. *P*1 values were compared with the 3-month data to 6-month data.

Table 2. Comparison of biochemistry data at three stages after antioxidant and chelation therapy

	Baseline	3-month		6-month	
	Mean ± SD	Mean ± SD	*P* value	Mean ± SD	*P* value
BUN (mg/dL)	18.87±7.46	17.26±4.75	0.31	19.27±5.27	0.82
Crea. (mg/dL)	1.37±0.73	1.32±0.44	0.75	1.35±0.25	0.90
Na (mEq/L)	140.97±3.78	141.87±1.94	0.24	141.87±1.93	0.19
K (mEqL)	4.28±0.34	4.20±0.41	0.40	4.32±0.42	0.71
AST (U/L)	29.35±8.80	29.32±10.59	0.99	25.77±10.82	0.08
ALT (U/L)	31.29±12.62	32.68±16.98	0.72	29.81±14.93	0.67
FBG (mg/dL)	111.23±30.90	100.97 ±18.30	0.12	107.97±22.98	0.64
HbA1C (%)	6.54±1.51	6.13±1.06	0.22	6.25±1.04	0.37
TG (mg/dL)	160.87±91.12	156.81±60.26	0.84	170.77±81.66	0.65
TC (mg/dL)	185.84±31.08	191.06±30.06	0.45	188.61±27.39	0.70
HDL-C (mg/dL)	39.77±9.56	38.87±6.75	0.67	39.77±8.58	1.00
LDL-C (mg/dL)	100.39±24.04	103.35±25.13	0.60	109.10±24.70	0.18
UA (mg/dL)	7.10±1.41	6.84±2.10	0.56	7.06±1.46	0.92

BUN = blood urea nitrogen, Crea. = creatinine, Na = sodium, K = potassium, GOT = aspartate aminotransferase, GPT = alanine aminotransferase, FBG = blood glucose of fasting, HbA1C = glycated hemoglobin, TG = triglyceride, TC = total cholesterol, HDL-C = high density lipoprotein cholesterol, LDL-C = low density lipoprotein cholesterol, UA = uric acid. *P* values were compared with baseline data.

Statistical analysis

All data were expressed as mean ± SD and analyzed by SPSS software (versions 20). A *p* value less than 0.05 was considered to be statistically significant. Comparisons of data on each variable at the baseline, after treatment at 3 month, and at 6 month were initially studied using "one way ANOVA (paired)", if there was a significant difference in each pair; post analysis was assessed using the paired Student's t-test with Bonferroni correction.

Results

The data of blood and urine lead and cadmium levels of CAD patients at baseline, after treatment for 3- and 6-month were shown in **Table 1**. The data of blood lead levels were reduced significantly after 3-month of anti-oxidant and chelation therapy (21.19±7.61 vs 8.08±4.06 μg/L, P<0.001). It decreased further after 6-month of therapy (2.79±1.73 μg/L, P<0.001 compared to those of baseline and 3-month data, respectively). Similarly, the data of blood cadmium were reduced significantly after 3-month (0.48±0.20 vs 0.34±0.20, P<0.001) and 6month of antioxidant and chelation therapy (0.21±0.15, P<0.001 compared to those of baseline and 3-month data, respectively). Significant decrease in the daily urine lead excretion were also noted at 3-month and 6-month data compared to that of the baseline value (P<0.001 in both comparisons). Similar effect of decrease in the cadmium excretion was also noted at 3- and 6-month data.

During the whole course of therapy period, there was no angina or any discomfort during infusion of chelation agent in all of the study

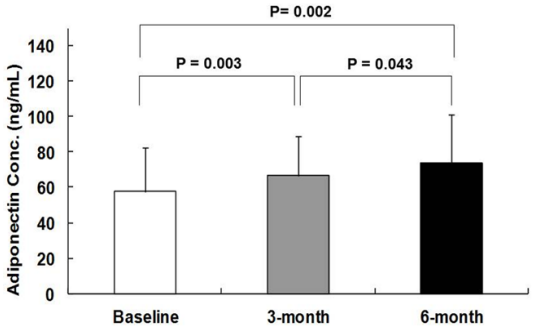

Figure 1. Comparing the adiponectin level at baseline, 3-month data and 6-month after antioxidant and chelation therapy.

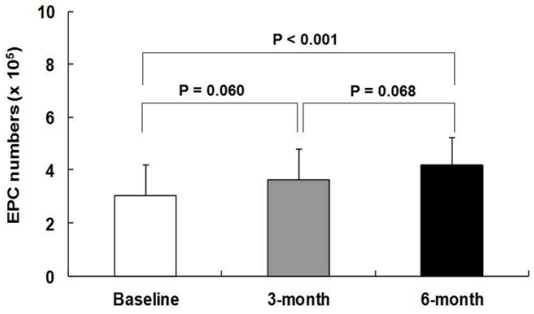

Figure 2. Comparing the EPC numbers at baseline, 3-month, and 6-month after antioxidant and chelation therapy.

patients. **Table 2** showed that there were no significant changes of CBC or biochemistry data during the whole course of antioxidant and chelation therapy. There was a significant increase in the adiponectin level of the 3-month data (baseline vs 3-month: 56.56±25.23 vs 65.24±23.98 ng/mL, P = 0.003) after antioxidant and chelation therapy. The adiponectin level increased further after 6-month therapy (74.63±28.08 ng/mL, P=0.002 and P=0.043 compared to those of baseline and 3-month data, respectively) after treatment (as shown in **Figure 1**).

For the EPCs studies, **Figure 2** exhibited a trend of increase in the EPC numbers of the 3-month value (3.57×10^5) compared to that of baseline value (2.99×10^5). Further increase in the EPC numbers after 6-month treatment (4.13×10^5) compared to that of 3-month value was also noted, but the changes had not reach statistical significance. However, significant increase in the EPC numbers was found after 6-month treatment compared to that of the baseline value (P<0.001).

For the brachial ultrasound measurements, there was a trend of significant increase in the peak blood flow of brachial artery after reactive hyperemia from baseline data (91.75±15.39 mL/s) to those of 3-month (101.18±16.99 mL/s, P = 0.028) and 6-month (109.11 ±17.92 mL/s, P<0.001) after treatment. The trend of significant increase in the mean FMD of brachial artery after reactive hyperemia was also noted from baseline (8.36±4.78%) to those of the 3-month (12.00±5.81%, P = 0.010) and 6-month (13.67±5.27%, P<0.001) after treatment. Similarly, there was a significant flow increase after reactive hyperemia (FIRH) of the brachial artery from baseline (101.80±21.74%) to that of the 3-month after treatment (121.23±20.70%, P=0.001). The FIRH increased further after 6-month of multivitamins and chelation therapy (137.95±27.26%, P = 0.010 compared to 3-month data) (**Table 3**).

Discussion

This study demonstrated that besides the reduction of body lead and cadmium levels, the antioxidant and chelation therapy had significant impacts in increasing the adiponectin level, EPC numbers, and ultrasonic parameters of FIRH and FMD. To the best of our knowledge, there was no previous study assess the impact of chelation therapy to the changes in the adiponectin level and the EPC numbers. This study demonstrated that there was a significant increase in the adiponectin level after 6 months treatment (P = 0.002). Adiponectin appears to attenuate atherogenesis through multiple anti-inflammatory actions on macrophages [30, 31] and vascular endothelial cells [32, 33]. Increase in the adiponectin level may be considered to have the beneficial impact in the anti-inflammation and anti-atherogenesis effects. The results of present study suggested that the antioxidant and chelation therapy was useful in suppression the inflammatory and atherogenesis reaction besides removal of heavy metals beyond the active infusion phase.

This study also demonstrated that the study patients exhibited a significant increase in the EPC numbers after 6-month treatment compared to those of baseline data (*P*<0.001).

Table 3. Comparison on brachial artery measurements at three stages after multivitamins and chelation therapy

	Baseline	3-month		6-month		
	Mean ± SD	Mean ± SD	*P* value	Mean ± SD	*P* value	*P1* value
D1 (mm)	3.45±0.55	3.37±0.57	0.990	3.56±0.54	0.786	0.826
Flow1 (ml/s)	45.90±8.78	46.05±8.57	0.947	46.21±8.27	0.886	0.940
D2 (mm)	3.72±0.52	3.78±0.60	0.317	3.81±0.56	0.216	0.801
Flow2 (ml/s)	91.75±15.39	101.18±16.99	0.028	109.11±17.92	<0.001	0.084
FMD (%)	8.36±4.78	12.00±5.81	0.010	13.67±5.27	<0.001	0.251
FIRH (%)	101.80±21.74	121.23±20.70	0.001	137.95±27.26	<0.001	0.010

D1 = baseline diameter; Flow1 = baseline peak blood flow; D2 = diameter after reactive hyperemia; Flow2 = Peak blood flow after reactive hyperemia; FMD = flow-mediated dilation; FIRH = flow increase after reactive hyperemia; *P* values were compared the baseline data with 3-month data and 6-month data, respectively; *P*1 value was compared the 3-month data with 6-month data.

Previous articles reported that decreased circulating EPC levels independently predict cardiovascular events, which suggest an important role for endogenous vascular repair of EPCs in modulating the clinical course of CAD [16, 17]. Indeed, the level of circulating endothelial progenitor cells was a better predictor of vascular reactivity than was the presence or absence of conventional risk factors [34]. This study demonstrated that anti-oxidant and chelation therapy increase in the EPC numbers, which suggested that chelation therapy may have a beneficial effect for endothelial dysfunction in CAD patients.

Given the widespread usage of EDTA chelation therapy, an assessment of its safety is crucial. With proper dose control and assessment of kidney function, EDTA chelation therapy has been considered to be safer than coronary bypass surgery [35]. Lin et al. have reported that chelation therapy using calcium disodium EDTA may improve renal function, thus slowing the progressing of renal insufficiency in patients with high-normal lead body burden. Lin and his colleagues have reported that the $CaNa_2EDTA$ is very safe and without significant side effect or life threatening complications [8, 9, 36]. The chelator used in this study was the same as that used in Lin's report [8, 9, 36], which did not induce any side effect throughout the course of experiment. We believe that the currently used $CaNa_2EDTA$ is much safer than previously used disodium EDTA.

The FMD and FIRH represent the vascular endothelial function in response to reactive hyperemia. By analysis the FMD and FIRH, we and others have found that the endothelial dysfunction was presented in patients without angiographic or ultrasound evidence of structural CAD, or in apparently healthy aging subjects [37, 38]. Anderson et al. have reported that using multivitamins and $CaNa_2EDTA$ chelation therapy did not showed an improvement of the ultrasonic endothelial function in CAD patients [39]. While our study demonstrated a significant improvement of the ultrasonic endothelial function by measurement of FMD and FIRH at the 3-month and 6-month data compared with those of baseline (**Table 3**). Our study used the $CaNa_2EDTA$ as the chelator, which is different from the Na_2EDTA in Anderson's report [39]. In addition, our patients also received l-arginine infusion weekly and took Ginkgo biloba every day. Besides the beneficial effect of l-arginine, ginkgo biloba extract has recently been shown to have protective effects on endothelial cells injury or endothelial dysfunction induced by lipid peroxide and chemical hypoxia [40]. Chen et al. demonstrated that Ginkgo biloba extract could augment EPCs number, promote EPCs proliferation, migration, and in vitro vasculogenesis capacity [41]. Given the well-established role of EPCs participating in neovascularization and reendothelialization, stimulation of EPCs by Ginkgo biloba extract may contribute to the clinical benefit of Ginkgo biloba extract therapy in patients with CAD [40, 41]. We speculate that the difference in the administered agents may cause the positive impact found in our study but not in Anderson's report.

Limitations of this study

This study has several limitations. First, we just measured the adiponectin level, EPC numbers and function, ultrasonic endothelial function

and clinical evaluations of the frequency of chest pain and nitroglycerine use and the biochemistry data in our patients. This study had not assessed other hard end clinical outcomes. Future study enrolling more patients and assessing other hard outcomes including the rates of recurrent myocardial infarction, mortality, heart failure or angina needs hospitalization, strokes, or coronary revascularization are necessary to further document whether there is a beneficial impact of antioxidant and chelation therapy to CAD patients. Second, we only evaluated 30 persons; the numbers of study subjects are relatively small. Further investigations involving more cases are needed.

Conclusions

This study demonstrates that antioxidant and chelation therapy not only reduce body lead and cadmium levels, but also has significant effects in increasing the adiponectin level, EPC numbers, improving the ultrasonic endothelial function. These findings may have clinical implications in future studies in CAD patient who have abnormal heavy metals.

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Disclosure of conflict of interest

None.

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